14 December 2023
EMA/6332/2024
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Casgevy

International non-proprietary name: exagamglogene autotemcel

Procedure No. EMEA/H/C/005763/0000

Note

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

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List of abbreviations

ABS	Acrylonitrile Butadiene Styren
AET	Analytical Evaluation Threshold
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
AO	Acridine Orange
BCL11A	B-Cell Lymphoma/Leukemia 11A
BET	Bacterial endotoxin test
BFU-E	burst forming unit erythroid
BM	bone marrow
BSC	Bio safety cabinet
Cas9	CRISPR associated 9 nuclease
CBC	Complete blood count
CCV	Cell Count and Viability
CD	cluster of differentiation
CFU	Colony forming unit
CFU-GEMM	Colony forming unit-granulocyte/erythrocyte/monocyte/ megakaryocyt
CFU-GM	Colony forming unit granulocyte/macrophage
CH	Chromatography
CLR	Charles River Laboratories
CMP/MEP	Common Myeloid Progenitor/Megakaryote-Erythrocyte Progenitor
COC	Chain of custody
COI	Chain of identity
CPP	Critical Process Parameter
CQA	Critical Quality Attributes
CRF	Controlled rate freezer
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CSI	Cell Size Increase
DIN	Donation identification number
DMSO	Dimethyl sulfoxide
DP	Drug Product
DS	Drug Substance
DSB(s)	double-strand break(s)
dsODNs	double-stranded oligodeoxynucleotides
DTT	Dithiothreitol
EBMT	European Society for Blood and Marrow Transplantation
EGFP	enhanced green fluorescent protein
EP	Electroporation
ESI-MS	Electrospray ionization mass spectrometry
FACT	Foundation for the Accreditation of Cellular Therapy
Flt-3L	Fms-like tyrosine kinase-3Ligand
FM	Frequency modulation spectroscopy
FMO	Fluorescence minus one
FTA-ABS	Fluorescent treponemal antibody absorption
FT-IR	Fourier-transform infrared spectroscopy
GC-FID	Gas Chromatography, Flame Ionization Detection
G-CSF	Granulocyte-colony stimulating factor

GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GPR161	G protein-coupled receptor 161
gRNA	guide RNA
GUIDE-seq	Genome-wide Unbiased Identification of Double-stranded breaks Enabled by sequencing
HbF	fetal hemoglobin
HD	Healthy Donor
HEPES	N-(2-Hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid)
hHSPCs	human hematopoietic stem and progenitor cells
HPFH	Hereditary Persistence of Fetal Hemoglobin
HSA	Human Serum Albumin
HSC	hematopoietic stem cell
HSPCs	hematopoietic stem and progenitor cells
IEX-HPLC	Ion exchange – high performance liquid chromatography
indels	insertions and deletions
IPC	In Process Control
IPT	Isopropyl ß-D-1-thiogalactopyranoside
IV	intravenous
LAL	Limulus Amebocyte Lysate
LOD	Limit of detection
LOQ	Limit of quantitation
LR-PCR	long-range polymerase chain reaction
LT-HSC	long-term hematopoietic stem cell
MAF	Minor allele frequency
Mbp	Mega base pair
MCB	Master cell bank
MDS	Myelodysplastic syndrome
NGS	next generation sequencing
NHEJ	non-homologous end joining
NLT	Not less than
NMT	Not more than
NOD	non-obese diabetic
NOR	Normal Operating Range
NSG	NOD/SCID/IL2Rγnull
OQ	Operational Qualification
PAM	protospacer adjacent motif
PAR	Proven Acceptable Range
PBMC	Peripheral blood mononuclear cells
PEI	Polyethyleneimine
PPQ	Process Performance Qualification
PQ	Process Qualification
PVAC	Process Validation Acceptance Criteria
qPCR	quantitative polymerase chain reaction
qRT-PCR	quantitative reverse transcription – polymerase chain reaction
RNP	ribonucleoprotein
RT	Room Temperature
SCID	severe combined immunodeficiency
SCD	sickle cell disease
SCF	Stem cell factor

SEC	Size Exclusion Chromatography
SNP	single nucleotide polymorphism
SR	Sickling Reduction
TDT	transfusion-dependent β-thalassemia
TFF	Tangential Flow Filtration
TIDE	Tracking of Indels by Decomposition
TMEFF2	transmembrane protein with EGF like and two follistatin like domains 2
TPO	Thrombopoeitin
TSB	Tryptic Soy Broth
UDF	Ultradiafiltration
URS	User requirement specification
VCC	Viable Cell Count

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Vertex Pharmaceuticals (Ireland) Limited submitted on 29 December 2022 an application for marketing authorisation to the European Medicines Agency (EMA) for Casgevy, 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 17 September 2020.

Casgevy was designated as an orphan medicinal product EU/3/19/2210 on 17 October 2019 in the following condition: for the treatment of beta-thalassaemia intermedia and major.

Casgevy was designated as an orphan medicinal product EU/3/19/2242 on 9 January 2020 in the following condition: for the treatment of sickle cell disease.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Casgevy as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: https://www.ema.europa.eu/en/medicines/human/EPAR/casgevy

The applicant applied for the following indications:

β-thalassemia

Casgevy is indicated for the treatment of transfusion-dependent β-thalassemia (TDT) in patients 12 years of age and older for whom a human leukocyte antigen (HLA)-matched related haematopoietic stem cell (HSC) donor is not available.

Sickle cell disease

Casgevy is indicated for the treatment of sickle cell disease (SCD) in patients 12 years of age and older with recurrent vaso-occlusive crises who have the βS/βS, βS/β+ or βS/β0 genotype, for whom a human leukocyte antigen (HLA)-matched related haematopoietic stem cell (HSC) donor is not available.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that exagamglogene autotemcel was considered to be a new active substance.

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

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included EMA Decisions P/0548/2021 and P/0549/2021 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIPs EMEA-002730-PIP03-21 and EMEA-002730-PIP04-21-M01 were not yet completed as all measures were deferred.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

1.5. Applicant's request(s) for consideration

1.5.1. Conditional marketing authorisation

The applicant applied initially for a full marketing authorisation, but during the assessment, in response to CAT and CHMP concerns on the comprehensiveness of the data, requested consideration of its application for a conditional marketing authorisation in accordance with Article 14-a of Regulation (EC) No 726/2004.

1.5.2. New active Substance status

The applicant requested the active substance exagamglogene autotemcel contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

1.6. PRIME

Casgevy was granted eligibility to PRIME on 17 September 2020 in the following indication: Treatment of sickle cell disease.

Eligibility to PRIME was granted at the time in view of the following:

- At the time of granting, 3 main treatments of sickle cell disease were considered; hydroxyurea (HU), transfusion therapy combined with iron chelation, and allogeneic hematopoietic stem cell transplantation (allo-HSCT). Allo HSCT is a curative therapy but is only available for less than 20% of eligible patients with sickle cell disease who have a matched related sibling donor; also, some patients can be affected by significant GVHD; an unmet medical need was agreed.
- Non-clinical data have been generated to establish the pharmacodynamics and proof of mechanism of exagamglogene autotemcel for the treatment of SCD.
- Treatment with exagamglogene autotemcel increased γ globin expression, resulting in pancellular increases in HbF and total haemoglobin, the percentage of circulating RBCs containing HbF (F-cells), and seem to correlate with VOC (SCD) and transfusion (TDT) avoidance. These data also provided early evidence that the increase in HbF levels could lead to substantial improvements in clinically meaningful endpoints. Notably, SCD Subject 1 and Subject 2 have not had VOCs since exagamglogene autotemcel infusion (over 11 months for Subject 1 and over 2 months for Subject 2 from the data cut-off date).

Upon granting of eligibility to PRIME, Jan Mueller-Berghaus was appointed by the CHMP as Rapporteur.

A kick-off meeting was held on 03/02/2021. The objective of the meeting was to discuss the

development programme and regulatory strategy for the product. The applicant was recommended to address the following key issues through relevant regulatory procedures:

- Comparability strategy for transfer of process between manufacturing sites, including approach for analytical testing between the manufacturing sites, approach for off-target analysis for newly synthesized sgRNA batches, general suitability of DP release criteria, potency assessment approach and general suitability of stability program
- Overall data package to support the MAA
- The approach for demonstrating significant benefit for maintenance of Orphan Designation.

Casgevy was granted eligibility to PRIME on 22 April 2021 in the following indication: Treatment of transfusion-dependent β-thalassemia.

Eligibility to PRIME was granted at the time in view of the following:

- Due to the limitations of available therapies there remains a need for curative therapies with novel mechanism of action for the treatment of TDT and also for patients who do not have a β0/β0 genotype.
- The nonclinical pharmacology studies demonstrated high editing efficiency, editing specificity, differentiation potential, induction of HbF, and persistence of editing post-engraftment. Hence, the proof of principle could be agreed.
- Clinical data comes from Study 111 which is a single-arm, open-label, multisite, single-dose Phase 1/2/3 study currently enrolling subjects 12 to 35 years of age with β0/β0 and non-β0/β0 TDT. All TDT subjects have been transfusion independent (TI) for 2 months after exagamglogene autotemcel infusion. One of the subjects has been transfusion independent for 4.1 months after exagamglogene autotemcel infusion. Two subjects each have a genotype which results in disease severity similar to β0/β0. One subject has been transfusion independent for 22.8 months, and the other subject has been TI for 5.9 months. At last follow-up, total Hb levels were 13.3 g/dL, 12.9 g/dL, and 11.0 g/dL for the respective subjects.
- Overall, access to the PRIME scheme was granted based on the promising efficacy results. Long term efficacy data would be needed in order to assess the potential curative effect.

1.7. Protocol assistance

The applicant received the following Protocol Assistance on the development relevant for the indication subject to the present application:

Date	Reference	SAWP co-ordinators
17 September 2020	EMEA/H/SA/4534/1/2020/PA/ADT/III	Peter Mol, Johanna Lähteenvuo
17 September 2020	EMEA/H/SA/4534/2/2020/PA/ADT/II	Peter Mol, Johanna Lähteenvuo
22 July 2021	EMA/SA/0000061377	Jens Reinhardt, Rune Kjeken
11 November 2021	EMA/SA/0000069761	Ferran Torres, Elena Wolff-Holz
16 December 2021	EMA/SA/0000071786	Elena Wolff-Holz, Paolo Foggi

24 March 2022	EMA/SA/0000077616	Elena Wolff-Holz, Johanna Lähteenvuo

The Protocol Assistance pertained to the following quality, non-clinical, and clinical aspects:

EMEA/H/SA/4534/1/2020/PA/ADT/III (17/09/2020) - Nonclinical and clinical development (TDT)

- The adequacy of the nonclinical data package to support a MAA that includes the TDT and SCD indications.

- The proposed primary and secondary efficacy endpoints in support of the TDT indication.

- The use of the efficacy and safety data from Study 121 (SCD) to support the benefit/risk profile assessment of exagamglogene autotemcel for the treatment of TDT.

EMEA/H/SA/4534/2/2020/PA/ADT/II (17/09/2020) - Clinical development (SCD)

- The proposed primary and secondary efficacy endpoints in support of the SCD indication.

- The use of the efficacy and safety data from Study 111 (TDT) to support the benefit/risk profile assessment of exagamglogene autotemcel for the treatment of SCD.

EMA/SA/0000061377 (22/07/2021) - Quality development (SCD)

- The strategy to demonstrate comparability between DP manufacturing sites.
- The approach to establishing the commercial drug product release specification.
- Whether exagamglogene autotemcel is eligible for exemption from the requirement to perform batch release testing in an EU laboratory.
- Adequacy of the planned genomics package to assess the potential for off-target editing.
- The proposed Process Validation plan.
- The plan to introduce new bulk lots of SPY101 sgRNA into clinical and commercial manufacturing of exagamglogene autotemcel.
- Adequacy of the proposed Healthy Donor DP stability studies, supported by existing stability data, to support submission of a MAA and establishment of a commercial shelf-life for exagamglogene autotemcel.
- Whether the exagamglogene autotemcel drug substance is the same for the SCD and TDT indications.

EMA/SA/0000069761 (11/11/2021) - Clinical development (SCD, TDT)

- The proposed registry-based study using EBMT and CIBMTR to evaluate long-term safety and effectiveness of exagamglogene autotemcel in patients with transfusion dependent β thalassemia and severe sickle cell disease.

EMA/SA/0000071786 (16/12/2021) - Quality development (SCD)

- The proposed plan to demonstrate comparability between (commercial) manufacturing sites.

EMA/SA/0000077616 (24/03/2022) - Quality development (SCD, TDT)

- Whether the exagamglogene autotemcel active substance is the same for the sickle cell disease and β-thalassemia indications.

1.8. Steps taken for the assessment of the product

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

CAT Rapporteur: Jan Mueller-Berghaus CAT Co-Rapporteur: Heli Suila

The application was received by the EMA on	29 December 2022
The procedure started on	24 January 2023
The CAT Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	19 April 2023
The CAT Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	17 April 2023
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	2 May 2023
The CAT agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	17 May 2023
The applicant submitted the responses to the CAT consolidated List of Questions on	10 July 2023
The following GMP inspection was requested by the CHMP and its outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	
– GMP inspection at a manufacturing site in the USA in June 2023. The outcome of the inspection carried out was issued on.	25 October 2023
The CAT Rapporteur circulated the Joint Assessment Report on the responses to the List of Questions to all CAT and CHMP members on	22 August 2023
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	31 August 2023
The CAT agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	8 September 2023
The applicant submitted the responses to the CAT List of Outstanding Issues on	29 September 2023
The CAT Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CAT and CHMP members on	24 October 2023
The CAT agreed on a 2nd list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	31 October 2023
The applicant submitted the responses to the 2nd CAT List of Outstanding Issues on	8 November 2023
The CAT Rapporteurs circulated the Joint Assessment Report on the responses to the 2nd List of Outstanding Issues to all CAT and CHMP members on	29 November 2023
The CAT, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting	8 December 2023

a marketing authorisation to Casgevy on	
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 Casgevy on	14 December 2023
The CAT and CHMP adopted a report on similarity of Casgevy with Oxbryta and Reblozyl	8/14 December 2023
Furthermore, the CAT and CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product	8/14 December 2023

2. Scientific discussion

2.1. Problem statement

Haemoglobinopathies are disorders caused by genetic mutations that affect the production or function of haemoglobin (Hb) molecules. Two such haemoglobinopathies are β-thalassemia and sickle cell disease (SCD), which are both rare diseases. Hb is a tetramer composed of 2 α-globin and 2 β-globin chains. Both β-thalassemia and SCD are caused by mutations in the β-globin gene. These mutations result in reduced or no expression of β-globin in β-thalassemia and in the formation of abnormal sickle Hb (HbS) in SCD. Each of these diseases is associated with considerable morbidity and mortality.

These disorders are endemic in the Mediterranean, African and Asian regions; through mobility and migration flows, they are common rare diseases of genetic origin in Europe (Pereira *et al.* 2014).

Transfusion-dependent thalassemia

2.1.1. Disease or condition

β-thalassemia is an inherited autosomal recessive disorder caused by genetic mutations that reduce or eliminate the expression of β-globin, which results in an α- to non-α-globin chain imbalance and decrease in adult haemoglobin (HbA) tetramers in red blood cells (RBCs).

Unpaired α-globin chains precipitate inside RBCs, leading to the destruction of erythroid precursors in the bone marrow, known as ineffective erythropoiesis, and destruction of RBCs in circulation, known as haemolysis.

Transfusion-dependent β-thalassemia (TDT), a rare disease, is the most severe form of β-thalassemia, which is characterized by severe anaemia requiring regular lifetime transfusions of RBCs, which lead to a number of serious complications, including multi-organ damage.

2.1.2. Epidemiology

β-thalassemia is one of the most common autosomal recessive disorders worldwide with high prevalence in populations in the Mediterranean (5% to 15%), Middle-East and West Asia (2% to 5%), South-East Asia (up to 10%), and South Asia (up to 18%) (Colah et al., 2010). Due to population migration, β-

thalassemia is also found in Northern Europe, North and South America, Caribbean, and Australia. Currently, the worldwide living population of β-thalassemia major patients is estimated to be 200,000 that are registered and receiving treatment.

2.1.3. Aetiology and pathogenesis

β-thalassemia is caused by a spectrum of mutations that result in reduced or absent production of adult haemoglobin (HbA). Different forms of Hb are produced during different stages of development. Foetal haemoglobin (HbF) is the predominant Hb prior to birth and extending into the newborn period. HbF is a tetrameric globin protein containing 2 γ-globin and 2 α-globin chains (α2γ2). After the newborn period, the main form of Hb is HbA, a heterotetramer comprised of 2 β-globin and 2 α-globin chains (α2β2). HbA normally accounts for >95% of the total Hb in the blood of adults. The degree of impaired HbA production, resulting from the extent of incomplete (β+) or absent (β0) β-globin expression, determines the severity of β-thalassemia.

2.1.4. Clinical presentation, diagnosis

Reduction in β-globin production results in an accumulation of excess, uncomplexed α-globin in erythroblasts. The clinical implications of this α-globin/β-globin imbalance are (Thein, 2005) haemolysis leading to a lack of sufficient erythrocytes and Hb to effectively transport oxygen throughout the body

1) Oxidative damage of the cell membrane, thereby resulting in apoptosis of erythrocyte precursors and therefore ineffective erythropoiesis.
2) Ineffective erythropoiesis which leads to morbidities such as splenomegaly, bone marrow expansion, concomitant bone deformities, and iron overload.

2.1.5. Management

The **recommended treatment** for TDT is **lifelong regular blood transfusions**, usually administered every two to five weeks, **to maintain the pre-transfusion haemoglobin level 9.5-10.5 g/dl**. A higher target pre-transfusion haemoglobin level of 11.0-12.0 g/dl may be appropriate for patients with heart disease, clinically significant extramedullary haematopoiesis or other medical conditions, and for those patients who do not achieve adequate suppression of bone marrow activity at the lower haemoglobin level (2021 Guidelines for the management of TDT, TFI).

Though chronic blood transfusion regimens are effective at preventing the hallmark symptoms and physical manifestations of disease, they introduce a large iron overload (Cao et al., 1996) that may lead to mortality through iron associated heart and liver toxicity (Vichinsky et al., 2005) To prevent this, iron overload must be managed with iron chelation regimens that are usually initiated at an early age (Saliba et al., 2015). Poor compliance with chelation regimens remains a key challenge. Despite the improvements with current therapies, there is poor quality of life and overall survival until the age of 30 years is only 55% (Modell et al., 2000; Delea et al., 2007).

Currently, the only **curative treatment options** for TDT are allogeneic haematopoietic stem cell transplant (allo-HSCT) and a lentiviral gene therapy (betibeglogene autotemcel; Zynteglo), whose EU marketing authorization had been withdrawn upon request of the marketing authorization holder. There are significant risks associated with allo-HSCT such as serious infections, graft failure and graft-versus-host disease (GvHD), some of which can be fatal. As such, transplants are infrequently performed, and are offered primarily to subjects who have available human leukocyte antigen (HLA)-matched sibling donors, who are young (<16 years of age), and who do not have significant iron overload. Because of

the need of an HLA-matched sibling donor, allo-HSCT is available to only <25% of eligible patients with remainder of the patients requiring lifelong transfusions and chelation. Transplants using alternative donor sources such as unrelated cord blood and haploidentical donors remain experimental due to higher risk of engraftment failure and GvHD (Mathews et al., 2014).

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

Gene-based therapies are promising approaches that have the potential to provide a functional cure in patients with severe β-thalassemia.

2.1.6. Feedback from patients' organisations

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

The received feedback indicated the most meaningful would be new medicines / therapies that will require less frequent blood transfusions, so that the risk of iron overload is reduced (and therefore fewer co-morbidities e.g. heart, liver, endocrine diseases).

Sickle-cell disease

2.1.7. Disease or condition

SCD is caused by a single-nucleotide substitution resulting in valine replacing glutamic acid at position 6 of the β-globin chain leading to HbS. In the deoxygenated state, HbS polymerizes producing abnormal, sickle-shaped RBCs with limited flexibility, increased adhesive and inflammatory properties, and a predisposition to haemolysis.

Sickle RBCs result in painful vaso-occlusive crises (VOCs), chronic anaemia, inflammation, stroke, organ failure, and early mortality. VOCs result from blockages in small- to medium-sized blood vessels that deprive downstream tissues of nutrients and oxygen resulting in tissue infarction and ischemia/reperfusion injury. These events culminate in progressive tissue damage in multiple end-organs leading to their dysfunction, and ultimately failure.

2.1.8. Epidemiology

The incidence and prevalence of SCD are geographically variable. A recent systemic literature review (SLR) on global epidemiology found that birth prevalence of SCD (children ≤1 year old), mostly measured through new-born screening, was highest in several sub-Saharan Africa countries (500-2000/100,000), South America and Caribbean Islands (20-1000/100,000); in USA and European countries found in the SLR, birth prevalence was ≤500/100,000 (Colombatti et al., 2022).

In Europe, SCD occurs at disproportionately high rates among individuals of African descent and, to a lesser extent, among individuals of Middle Eastern, Mediterranean, Indian, and Asian descent. In Europe, SCD prevalence has increased rapidly and is expected to increase further as a result from ongoing migration. The applicant stated that at least 34,000 individuals are estimated to have SCD in Europe and among these approximately 40% or 11,000 patients are estimated to be eligible for treatment with exa-

cel. Although the numbers might be outdated; a study estimated "SCD prevalence in France at between 19,800 and 32,400 patients in 2016" alone (Leleu et al., 2021).

2.1.9. Aetiology and pathogenesis

The most severe and prevalent form of SCD, referred to as sickle cell anemia (SCA), is an autosomal recessive disease due to homozygous mutations in which a valine replaces a glutamic acid at position 6 in the β-globin protein, which leads to red blood cell (RBC) sickling.

2.1.10. Clinical presentation, diagnosis

SCD is a chronic disease, characterized by recurrent acute vaso-occlusive crises (VOCs), which lead to acute pain, chronic haemolysis, anaemia, progressive tissue injury, and organ dysfunction. The disease affects multiple organs causing acute and chronic complications such as acute chest syndrome (ACS), stroke, priapism, splenic sequestration, osteonecrosis, renal failure, pulmonary hypertension, liver disease, bone damage, limited growth, increased susceptibility to infections, fatigue, and progressive cognitive decline.

About 90% of children born with SCD in the US or EU will survive into adulthood, but their lifespan is shortened by 2 to 3 decades compared to the general population, with a median age of death of approximately 40 to 50 years.

2.1.11. Management

Approved therapies to prevent complications of SCD include hydroxyurea (HU) in the US and EU and L-glutamine oral powder, crizanlizumab, and voxelotor in the US.11-15 These therapies reduce complications of SCD; however, patients can still have breakthrough VOCs. However, HU is not effective in all patients, is not well tolerated, nor is it curative, and has carcinogenic and teratogenic risks. Allogeneic hematopoietic stem cell transplantation (HSCT) is the only known cure for SCD, but HSCT is only available to about 20% of patients who have a matched donor16, and graft-versus-host disease (GvHD) is a known risk. Therefore, there is significant unmet medical need for the treatment of SCD.

2.1.12. Feedback from patients' organisations

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

Feedback received was "*Any treatment that will keep patients out of the hospital and are readily/easily available to have some freedom professionally and that eradicate the root cause of pain, will be appreciated. The acceptability of side effects depends on the type of side-effects and their impact on daily life but also in the long-run*. […] *The minimization of painful episodes (or their complete eradication) is the number one priority of patients, and can only be achieved through the production of Haemoglobin F in the body. Therefore, any new treatment developed with this in mind will bear substantial consideration by patients.*"

2.2. About the product

Casgevy (exagamglogene autotemcel or exa-cel) is an Advanced Therapeutic Medicinal Product (ATMP) and was classified as a gene therapy medicinal product containing genetically modified cells. Its active substance is a genetically modified autologous CD34+ cell enriched population that contains human hematopoietic stem and progenitor cells (HSPCs) edited ex vivo by CRISPR/Cas9 at the erythroid-specific enhancer region of the BCL11A gene. (CRISPR/Cas9 is "clustered regularly interspaced short palindromic repeats-associated 9 nuclease.")

Exa-cel was developed as a one-time treatment leading to a functional cure for patients with transfusion-dependent β-thalassemia (TDT) and severe sickle cell disease (SCD). The current pharmacotherapeutic group is other haematological agents, ATC code: B06AX05.

The mechanism of action, irrespective of indication, is a targeted and permanent genetic modification to hHSPCs that leads to an increase in fetal hemoglobin (HbF) protein levels. In detail, the permanent, irreversible, and precise edits created with the ribonucleoprotein (RNP) complex composed of Cas9 and the highly specific guide RNA, SPY101, target a critical binding site of the transcription factor GATA1 in the non-coding erythroid lineage-specific enhancer region of the BCL11A gene on chromosome 2.1-3 Repair of these breaks by nonhomologous end joining produces insertions and deletions (indels) in the DNA that disrupt GATA1 binding, thereby lowering BCL11A transcription in erythroid cells only, while preserving the normal function of BCL11A in other cell types. BCL11A codes for a transcriptional repressor of γ-globin.3 The reduction of BCL11A gene transcription and subsequent decrease in BCL11A protein level leads to concomitant increases in γ-globin expression, and, upon erythroid differentiation, increased levels of HbF.

It is intended for autologous use and should only be administered once after myeloablation via intravenous infusion. A single autologous dose consists of at least 3 million CD34+ cells per kg of patient weight, suspended in CryoStor CS5 cryopreservation medium containing 5% DMSO at approximately 10 million cells/mL. In clinical studies, doses up to 19.7×10^6 CD34+ cells/kg have been administered. The minimum recommended dose is the same for adults and adolescents 12 years of age and older (see Summary of Product Characteristics [SmPC]).

It must be administered in an authorised treatment centre by a physician(s) with experience in HSC transplantation and in the treatment of patients with β-hemoglobinopathies (see section 4.2 of the SmPC).

2.3. Type of Application and aspects on development

The clinical development programme presented for this MAA consists of a single, ongoing First-in-Human pivotal phase 1/2/3 study per sought indication and a third, ongoing long-term follow-up study for both indications. During the assessment, additional ongoing and planned clinical trials investigating exa-cel in one or both indications were disclosed, to be part of the SOBs.

Scientific advices

The applicant received several scientific advices from CHMP (see section 1.6).

In addition, feedback on revised approaches following scientific advices received was given during a PRIME Meeting on 15/10/2021, and during the pre-submission meeting on 05/05/2022.

With respect to quality, for most of the aspects the applicant has taken the recommendations given by CHMP into consideration. There were deviations with respect to the advice given for the drug product release specifications regarding the panel of potency testing (see Discussion on Clinical Efficacy).

In addition to the scientific advice received in September 2020 on adequacy of the nonclinical data package, adequacy of the genomic package to assess the potential for off-target editing has been addressed in the scientific advice received in July 2021.

Scientific advice on clinical matters were partly neglected. In particular, primary efficacy endpoints and timing of assessments were changed in the ongoing open-label studies despite discouragement, with implications on the methodologic assessment in the context of single pivotal trial MAAs (see Discussion on Clinical Efficacy).

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as a dispersion for infusion containing 4-13 x 10e6 cells/mL of viable CD34+ enriched cell population of exagamglogene autotemcel as active substance suspended in a cryopreservative solution. Other ingredients are: CryoStor CS5 (containing 5% dimethyl sulfoxide [v/v] and dextran 40).

The product is available in cryopreservation vials made of cyclic olefin copolymer. Each vial contains 1.5 ml to 20 ml of Casgevy.

Casgevy is shipped from the manufacturing facility to the treatment centre storage facility in a cryoshipper. One cryoshipper may contain multiple cartons, which may contain multiple vials, all intended for a single patient.

2.4.2. Active Substance exagamglogene autotemcel

2.4.2.1. General information

The International Non-Proprietary Name (INN) of the active substance is exagamglogene autotemcel. The active substance exagamglogene autotemcel (exa-cel) contains autologous CD34+ hematopoietic stem and progenitor cells (HSPCs) edited by CRISPR-Cas9 technology to permanently disrupt GATA1 binding and thereby to reduce BCL11A expression which is known as a repressor of γ-globin gene expression, resulting ultimately in the increase of γ-globin and foetal haemoglobin (HbF) protein production in erythroid cells to treat sickle cell disease (SCD) and transfusion-dependent β-thalassemia (TDT) patients.

Exa-cel is made from a continuous manufacturing process without an isolated active substance. Throughout this report, exa-cel active substance will be used to refer to the cells in cell culture medium that have been edited with ribonuclease protein complex. The ribonuclease protein complex is based on the two critical starting materials Cas9 (nuclease) and SPY101 (guide RNA). The guide RNA enables CRISPR-Cas9 to make a precise DNA double stranded break at the critical transcription factor binding site (GATA1) in the erythroid specific enhancer region of the BCL11A gene.

Figure 1 provides an overview of critical regions of exa-cel including the GATA1 binding site and DNA cut site by SPY101 performed by exa-cel.

Figure 1: Overview of Critical regions of Exa-cel

For a clearer overview, the manufacturing of the starting materials is discussed in the section following the finished product (as opposed to the section on control of materials).

2.4.2.2. Manufacture, process controls and characterisation

Description of manufacturing process and process controls

The active substance is manufactured from autologous hematopoietic stem and progenitor cells procured via apheresis from patients with sickle cell disease (SCD) or transfusion dependent ß-thalassemia (TDT) to normally produce 2 leukopak bags of HSPCs, which are considered the starting material for manufacturing of exagamglogene autotemcel. Due to a continuous manufacturing process no active substance is defined and tested for release.

The manufacturing process consists of the enrichment of CD34+ cells from the leukopak starting material, pre-electroporation culture of the enriched CD34+ cells, electroporation of the cells with a ribonucleoprotein complex consisting of the Cas9 nuclease and the SPY101 sgRNA starting materials, post-electroporation culture, harvest, and cryopreservation of the cells.

The CD34+ cell enrichment step is performed using a CliniMACS Prodigy system. Eventually, the cells coupled with the CD34 antibody beads are eluted. The enriched cells are then centrifuged and resuspended in culture media, transferred into flasks and cultured. For electroporation, the cells are centrifuged, resuspended in electroporation buffer, combined with the Cas9-SPY101 ribonucleoprotein (RNP) complex, and electroporated for uptake of the RNP complex into the cells. After a recovery period, the cells are cultured, pooled, centrifuged and resuspended in CryoStor CS5 cryopreservation medium. Before filling, the cells are filtered and sampled for release testing. Samples for safety testing are taken post-filling. The filled vials are then frozen and stored in a liquid N2 vapor container. Reprocessing has not been indicated for any operation.

For leukopak production the patients undergo stem cell mobilization and apheresis on two or three subsequent days. Leukopak collection data are provided from the respective collection centre and are used for setting up the Prodigy predefined program for manufacturing. The minimal final finished product dose is 3x10e6 CD34+ Casgevy cells per patient kg. In order to maximise CD34+ cell collection, a total of at least 20x10e6 CD34+ cells/kg is recommended in the summary of product characteristics (SmPC) and the authorized treatment centre (ATC) manual for product manufacturing. This value is based on the cell number available for clinical batch manufacturing that resulted in a sufficient patient dose. In addition, at least 2x10e6 cells/kg unmodified rescue cells need to be collected for back-up.

Leukapheresis starting material collection and testing sites are located in the EU/EAA and meet standards of quality and safety regarding donation, procurement and testing of the starting material in accordance with relevant EU legislation, i.e., Directive 2004/23/EC or 2002/98/EC and other relevant requirements. Donor testing is described to be performed according to Directive 2004/23/EC. The minimum tests performed to screen infectious disease (HIV1, HIV2, hepatitis B, hepatitis C, syphilis) are in line with the requirements in Directive 2006/17/EC. As the process is continuous from active substance to finished product, the manufacturing sites are detailed under Finished Product.

The RNP complex is prepared by mixing the sgRNA SPY101 and the Cas9 protein and is then added to the CD34+ cells at a final concentration. The cell/RNP mixture is subsequently subjected to electroporation. The electroporated CD34+ cells are subjected to post-electroporation culture.

Concerning the batch size (or rather the batch size range), the applicant considers the size to be highly dependent on donor variability in respect to number of CD34+ cells in the incoming leukopaks, and on the fact that the dose is administered to patients dependent on patient weight. It has been demonstrated that donor variability does not extensively impact the finished product quality attributes. Minimum batch size has been defined as a certain number of viable cells after all required release sampling. The maximum batch size has been defined as the largest batch size manufactured during development. The release for batch sizes larger than maximum will be based on QP risk assessment. Minimal CD34+ cell number before harvest has been defined as an IPC, the definition of the minimal batch size should be adapted accordingly. This point is recommended to the applicant as a quality recommendation. The ATC will receive a respective clinical technical requirement document.

Moreover, the patient may need to undergo additional cycle(s) of mobilization and apheresis. In addition, a back-up collection of patient cells is instructed to be collected and cryopreserved prior to myeloablative conditioning and infusion with exa-cel for rescue treatment under certain conditions.

Control of materials

Starting materials:

Due to the complexity of manufacturing of the starting materials Cas9 (nuclease), SPY101 (gRNA) and the RNP formed from those, information regarding their quality is given after the active substance section. Information on the cellular starting material is provided above.

Raw materials:

The material qualification program consists of raw material risk assessment, material qualification, supplier qualification and monitoring. An overview of critical raw materials and materials containing human derived components used in exa-cel manufacturing is provided. The materials are indicated either as clinical grade / licensed products, CE-marked / produced under ISO 13485, or GMP compliant / meeting ISO 20399 "Biotechnology — Ancillary materials present during the production of cellular therapeutic products and gene therapy products" standard. Raw materials of biological origin are defined as critical raw materials.

Product-contact materials have been listed and product certificates are provided. All materials are single-use and supplied sterile. The method of sterilisation is indicated in the provided certificates of analysis (CoA) / Certificates of compliance (CoC). The materials are accepted into exa-cel manufacturing based on review of supplier's CoA/CoC, and sterility certification, where available.

Control of critical steps and intermediates

A comprehensive overview of critical in-process controls and critical in-process tests performed throughout the active substance manufacturing process is provided. Acceptable information has been

provided on the control system in place to monitor and control the active substance manufacturing process with regard to critical, as well as non-critical operational parameters and in-process tests.

Process validation

Exa-Cel is manufactured at the two different sites Roslin Cell Therapies, BioCube 2, Edinburgh, UK (BioCube) and Charles River Laboratories Inc, Memphis, TN (CRL). In each contract manufacturing organization, the same or equivalent equipment, critical materials, and reagents are used for manufacturing.

The process qualification at BioCube includes starting material derived from healthy donors and patients. Besides the finished product batch release results, data for potency are provided for the PPQ batches. In addition, characterization data on process related impurities (percentual depletion of Cas9, SPY101 gRNA, FLT-3L and SCF), product related cellular impurities, and the proportion of %LT HSC enriched and editing frequency in these cells are available and within the calculated historical range. The results for critical process parameters (CPPs) and selected non-critical process parameters (non-CPPs) are provided and assessed by process validation acceptance criteria (PVAC). The applicant used the process proven acceptable range (PARs) as process validation acceptance criteria (PVAC) for the CPPs in the process performance qualification (PPQ) runs at both manufacturing sites. In line with ICH guideline Q8(R2) (EMA/CHMP/ICH/167068/2004), the PAR is a characterized range of a process parameter for which operation within this range, while keeping other parameters constant, will result in producing a material meeting relevant quality criteria. Therefore, compliance of process parameters with PARs only is not necessarily considered sufficient. All CRL PPQ lots are, however, within the normal operating ranges (NORs). For BioCube, the duration of CD34+ cells in electroporation (EP) buffer and duration of the finished product cells in CryoStor CS5 at room temperature (RT) were both out of NORs (but within PAR) for two of the PPQ runs. Overall, this slight prolongation of the cell durations in electroporation buffer and CS5 solution is not considered to result in non-acceptable PPQ runs from a scientific point of view. The use of PARs as PVACs for the CPPs in the PPQ runs at both manufacturing sites has not been justified, but no further questions are raised as the PPQ data are within an acceptable range of the NOR.

During the procedure the applicant provided the data for all non-critical process parameters and not only selected ones for all PPQ runs from BioCube and CRL, but also for batches that have been manufactured from more than two leukopaks and more than one mobilization cycle. This included data on the leukopak which is considered to be a critical starting material. From the provided data it became obvious that especially for SCD patient material the number of WBC and the number of mobilised CD34+ cells is lower, compared to TDT patients. This often triggers the need for a repeated mobilisation in this patient population. The relative process yield at the different manufacturing steps is overall very similar for both indications, but higher for SCD patient batches with respect to the absolute cell numbers needed for release testing or as back-up cells. Overall, the % yield for the process is sufficient for the manufacturing of the finished product for TDT patients. In view of SCD patient material, the overall % yield would need to be higher in order to manufacture the finished product from one mobilization cycle. In this respect, it should be noted that no improvement of the manufacturing process has been performed throughout clinical development. As a part of the life cycle management and continuous process improvements, the applicant committed to assess options to increase the overall %CD34+ cell yield and if applicable introduce respective changes into the manufacturing process, respectively. This point is recommended to the applicant as a quality recommendation. In line with this, the IPC at harvest will have to be adapted according to the improvements made for CD34+ yield. This point is recommended to the applicant as a quality recommendation.

The applicant confirmed that the manufactured BioCube and CRL PPQ batches have been manufactured consecutively. All process qualification batches at BioCube are within the defined specification for finished product release. Moreover, results for the intended commercial process) are within a similar range. The results for the biological assays showed a broader range within the specification and are likely related to inter-donor variability. The amount of process related impurities is within a slightly wider range for Cas9 nuclease, but otherwise consistent (for the healthy donor (HD) runs used for PPQ).

The PPQ campaign was conducted with runs in every manufacturing suite. Like for the PPQ lots manufactured at BioCube, the batches for Cas9 and the sgRNA Spy101 are representative for the commercial batches. In the end, the assessment of this case as performed by the applicant is considered sufficient. The applicant also established a monitoring protocol as a preventive action.

All remaining process qualification batches are within the specification for finished product release. Moreover, results are within a similar range. The results for the biological assays show a boarder range within the specification and are likely related to inter donor variability.

The applicant stated that additional PPQ lots will be performed in order to demonstrate effective process and analytical control following implementation of these corrective actions to fulfil requirements for PPQ at CRL. These data have been provided during the procedure, which included four additional batches manufactured from HD material and two additional patient batches. The applicant committed to provide an annual update on the continuous process verification (CPV) for the manufacturing sites BioCube and CRL. This point is recommended to the applicant as a quality recommendation. In addition, the qualification status of three days cell collections for procurement of the starting material for both manufacturing sites might need to be substantiated by complementary data.

Manufacturing process development

The manufacturing process development is based on process characterization studies that are designed based on a product and process risk assessment, considering the defined Quality Target Product Profile (QTPP) and the Critical Quality Attributes (CQAs). A list of CQAs with criticality justification is provided. Criticality assessment of other quality attributes (e.g. product- and process-related impurities) has been presented.

The leukopak is collected from patients suffering from SCD or TDT or healthy donors (used for process characterization). For comparison, the parameters: total Whole Blood Cells (WBC), WBC concentration, total CD34+ amount, %CD34 per leukopak, and volume per leukopak upon receipt are analysed for the three donor types. The suitability of healthy donor material to be used as a surrogate starting material in process development was analysed by providing differences in means together with 90% confidence intervals. The data indicate that despite slight differences, the healthy donor material is considered as an acceptable surrogate for the starting material to be used in the characterization studies.

After apheresis, the leukopak is shipped at 5±3 °C in a temperature-controlled procedure to the manufacturing site and kept at 5±3 °C until the isolation process (enrichment) starts. Each leukopak is enriched for CD34+ on the day of its arrival; the CD34+ cells are kept at 5±3°C. The impact of the pre-enrichment and post-enrichment hold times on the exa-cel finished product CQAs, have been analysed.

After CD34+ enrichment, cells of the first leukopak are stored overnight; the CD34+ derived from the second leukopak of the same patient are directly cultivated after enrichment. The post-enrichment time with a PAR is defined for the process. The maximal hold-times are based on a study using healthy donor material.

For electroporation the cell cultures are harvested and washed, counted, and individually resuspended in electroporation buffer before the cells are pooled. The ribonucleoprotein complex (RNP) is prepared *in situ* just prior to electroporation, mixing the SPY101 and the Cas9 protein. The RNP is then transferred to the cells resuspended in EP buffer to a final concentration and mixed with the cells. Overall, the defined NOR and PAR for the pre-electroporation process parameters are considered acceptable.

Electroporation Duration has not been included in characterisation studies but has been based on early process development of electroporation protocol, including results from several recommended electroporation pre-programmed algorithms. Data is considered adequate to describe how the electroporation duration time has been defined.

The post-electroporation duration is also important at the time point of finished product release, whereas the is detected post electroporation. The applicant provided data based on three healthy donors that have been analysed at different time points after electroporation. Based on this data a model on specialized non-linear curve was calculated. Based on the modelled non-linear fitted curve a proven acceptable range for post electroporation culture has been defined, considering the commercial release specification. This approach is in general considered acceptable, however, the non-linear fitted curve will be different for a batch that shows a lower overall editing efficiency, for example. The lower side of the PAR for the post electroporation duration is therefore still not considered acceptable. This data will be provided as a post-marketing commitment. This point is recommended to the applicant as a quality recommendation.

Based on the process characterisation studies and statistical evaluation, CPPs were identified. Hold times have been evaluated. Overall, the data provided support the proposed ranges and the control strategy appears appropriate. The applicant considers all listed product- and process-related impurities as having no safety concerns and have thus classified them as non-critical. Acceptable justifications for the limits of the currently proposed IPCs and the testing strategy are provided.

Comparability BioCube and CRL with SCRM

The process for exa-cel was developed at RoslinCT-SCRM, UK (SCRM) and has been transferred to BioCube, which is under the same organization. For the technology transfer, there were no changes in the process between the facilities. The transfer has been considered accomplished based on three successful manufacturing runs.

For comparability between Roslin CT-SCRM and BioCube, historical batch data and split run data have been analysed. Split-run studies have been conducted by processing material from the same donor at both sites in parallel.

Taken together, the historical batch analysis and the split-run analysis do support that BioCube and SCRM show a considerable overlap of the data for all analysed parameters and support sufficient comparability.

For the Technology Transfer, there were no changes in the exa-cel process between RoslinCT SCRM and Charles Rivers Laboratories (CRL). The applicant stated that the equipment for exa-cel were comparable or functionally the same. Except for the changed, all reagents and ancillary materials were the same. All methods at were transferred to and equivalently qualified. Based on the provided data it can be concluded that the technology transfer was successful.

For comparability between RoslinCT SCRM and CRL split run and historical batch data have been analysed. Split run studies have been conducted with each manufacturing site processed the split bag received within a similar time frame using the same lots of Cas9 and SPY101, reagents, and manufacturing procedures. Data on from seven runs based on healthy donor material are provide. The

dossier has been updated to include information on the qualification status of the analytical methods used.

For the split runs, for most parameters tested nearly no site difference was observed. Some lots show a trending towards a slightly lower Cas9 impurity-profile but not for the other process related impurities. Besides the results from the split analysis, a statistical comparison of all runs conducted is provided. The split run data on indicates nearly no site difference. To further substantiate comparability, the applicant has provided a comparison of CPPs and IPCs with predefined comparability acceptance criteria. The provided data support comparability.

Characterisation

Exagamglogene autotemcel is made from continuous processing without an isolated active substance. The product characterization data are provided for exa-cel finished product and are described in the respective finished product section.

2.4.2.3. Specification

No data on control of active substance are available as there is no active substance release testing in place. The manufacturing of the active substance is continuous and moves directly to the finished product. After the electroporation step the cells are formulated into the finished product and the release testing is conducted on the finished product.

2.4.2.4. Stability

The active substance hold time and release testing are not applicable since the active substance is not filled in a container before finished product formulation and the active substance is not stored. Therefore, no stability data have been collected for the active substance, but data are provided at the level of the finished product.

2.4.3. Starting Material Cas9

2.4.3.1. General Information

The *Streptococcus pyogenes*-derived Cas9 nuclease is considered as critical starting material. In combination with the sgRNA SPY101, the Cas9 protein mediates a sequence specific DNA-double strand break in the BCL11A gene in CD34+ target cells. The Cas9 nuclease protein does include a C- and N-terminal sv40 large T antigen nuclear localization sequences (NLS).

2.4.3.2. Manufacture, process controls and characterisation

Manufacturing process and controls:

The Cas9 protein is manufactured as bulk material, which is transported for sterile fill. The fermentation volume and target batch sizes are stated. The provided documentation for sites that are involved in Cas9 manufacturing is considered sufficient to confirm that the manufacturing follows the principles of GMP, which is in line with EU guideline on GMP for ATMP, part IV.

Cas9 is produced in the *E. coli*. The production of the plasmid expression vector was subcontracted. The manufacturing starts from a bacterial MCB. A stability protocol for the MCB has been provided, with data available. The applicant confirmed to provide the results on the MCB stability data when

available and to update the information on the supported shelf life. This point is recommended to the applicant as a quality recommendation. The applicant intends to implement a tiered banking strategy with a WCB, which is endorsed in view of the continuity of the cell bank. Overall, testing of the MCB is compliant with Ph. Eur. 5.14. The indicated test regime is in line with the requirements of Ph. Eur. 5.14. and acceptable. The applicant stated that data on the EoP cells are currently not available but will be available and provided end 2024. This point is recommended to the applicant as a quality recommendation.

Quality information of raw materials used in the manufacture of Cas9 is provided. Most of the raw materials are of Ph. Eur. quality grade. Based on the material risk assessment, there are no critical raw or product contact materials. Release testing at the time of receipt or prior to use in manufacturing are considered adequate to ensure the consistency of the material. Examples of CoAs and/or COCs with manufacturer information for each material have been provided.

The process for Cas9 manufacturing is well monitored and each step is controlled by meaningful in-process controls. A summary of the process controls is provided describing CPPs and IPCs for Cas9 bulk manufacturing. Maximal process hold-times are implemented at all manufacturing steps.

The resin lifetime was evaluated for performance using IPC measurements of height equivalent to a theoretical plate (HETP) and asymmetry factor prior to each of the four lots.

For final filling, the Cas9 bulk is transported, thawed and processed through a filter into sterile vials. Shipping performance was verified through real-world performance qualification (PQ). Successful OQ and PQs demonstrated that shipping temperatures will be consistently maintained for the stated duration.

The sterile filtration-fill step is adequately subjected to aseptic process validation (APV) with simulation runs using tryptic soy broth (TSB) in place of product component and considering worst-case conditions. All initial process simulation runs were successfully completed with no microbial growth observed. Post-incubation media fertility is ensured via growth promotion testing using six acceptable reference strains. Requalification is confirmed to be conducted with an interval of at least 6 months. This point is recommended to the applicant as a quality recommendation.

The initial clinical Cas9 batch has been manufactured by a supplier that was subsequently changed. The supplier manufactured one non-clinical, one clinical and one clinical reference batch. The provided comparability data for Cas9 manufactured by the initial and subsequent supplier support the comparability of the Cas9 batches used in clinical development and for commercial purpose.

Characterisation:

Characterization studies include data analysing the presence of product related variants based on truncations, oxidation, deamidation and the formation of high-molecular-weight species.

The applicant provided data on the biological activity of the respective Cas9 batches and concludes that the presence of the described oxidations and deamidations to not impact Cas9 function. The applicant scientifically justified that the risk for an increase of these product specific impurities is low and therefore the absence of batch release criteria for this product related impurity is considered acceptable.

2.4.3.3. Specification, analytical procedures, reference standards, batch analysis, and container closure

Specification, analytical procedures, batch analysis:

For the Cas9 bulk and the final fills of sterile Cas9 batches a comparable testing strategy is in place. The Cas9 batches are tested for identity, quantity, purity, host cell protein (HCP), monomers, functionality, endotoxin and bioburden or sterility, respectively. The process related impurities are analysed on Cas9 bulk level only, which is considered acceptable as the sterile filtration and filling steps will not increase the impurity levels. The process related impurities on residual DNase and RNase are provided as characterization data and are depleted to levels below the assay's detection level. Based on the provided characterization batch data it is considered acceptable that those are not included for batch release. For the Cas9 bulk, a bioburden is defined and the final sterile fill Cas9 is tested according to Ph. Eur. 2.6.1 for sterility. Stability specifications for Cas9 Sterile Fill include the same CQAs and acceptance criteria as the release specification except for test for identity. In addition, CCIT (Dye Ingress) test is included as stability specification. The release and stability specifications and their justifications are considered appropriate.

Compendial analytical methods (pH, Endotoxin, Bioburden, Sterility) used for bulk and sterile fill Cas9 release testing are considered appropriately verified for use. Non-compendial analytical methods used for bulk and sterile fill Cas9 release and stability testing include concentration, identity test, purity, HCP, HCDNA, Potency, Visual Inspection for Appearance, and Dye Ingress for CCIT. Validation of non-compendial methods for CAS9 has been performed. Overall, appropriate analytical method descriptions have been provided. The non-compendial analytical methods have, in general, been validated according to ICHQ2(R1). All non-compendial assays have been described sufficiently.

The HCP impurity is measured using a commercially available HCP-ELISA kit. According to Ph. Eur. 2.6.34, data confirming the coverage of the used assay have been provided.

Based on the set specification, the amounts of HC-DNA and HCP that might be transferred to the active substance manufacturing process of exa-cel are stated. For both impurities, a certain clearance during the cultivation process was assumed. In general, the provided calculation-based depletion from the final finished product and the risk estimation are considered acceptable. The HC-DNA impurity is assessed critical in view of finished products which are manufactured on eukaryotic cell lines which have a tumorigenic potential like continuous cells lines (e.g. HEK293T or HeLa) used in the manufacturing of other starting materials. This is not considered applicable for the HC-DNA derived from prokaryotes as for the Cas9 starting material. Nevertheless, these impurities should be kept as low as feasible by the process. The Cas9 batch specifications for HC-DNA and HCP are defined. The batch results presented in the dossier are for all batches within the set specifications.

Reference standard:

Currently a specific Lot is qualified as the primary reference standard (PRS). The applicant intends to implement a 2-tiered reference standard program, including a working reference standard (WRS). Overall, this approach is considered acceptable. The applicant confirmed that every new WRS is validated against the PRS, to avoid a drift in the potency over the Cas9 life cycle.

Container Closure:

The Cas9 bulk is filled into bottles and the Cas9 sterile fill is stored in vials. The respective certificates have been provided for both container systems, including a statement of Ph. Eur. Compliance. Data on extractables and leachables are provided for the vial and cap and vial with stopper.

2.4.3.4. Stability

Cas9 bulk:

The proposed shelf-life for bulk Cas9 is currently defined as 24 months at -75 °C. The stability study is based on supportive and PPQ batches. Data for month 24 and 48 are available for the supportive batches with an intended study length of 60 months. Moreover, data for time point 0 are provided for the PPQ batches, with an intended study length of 36 months. The overall stability protocol, including the testing time points is following the ICH stability guidance ICH Q1A (R2) and Q5C and is considered acceptable. The batches are monitored for stability using the proposed commercial testing strategy and specifications, with additional testing for bioburden. The 48 months data for the supportive Cas9-bulk lots are considered acceptable in order to support currently defined shelf life of 24 month at -75 °C. During the procedure all available data of the stability program have been requested to confirm the data with batches of the commercial manufacturing process. Provided data supports to proposed shelf life and storage conditions.

Cas9 sterile filled:

The proposed shelf-life for the sterile filled Cas9 is currently 24 months at -75 °C. The stability study is based on supportive batches, PPQ batches and additional commercial batch. For all batches the study duration is 60 M. The overall stability protocol, including the testing time points is following the ICH stability guidance ICH Q1A (R2) and Q5C and is considered acceptable. The batches are assessed for stability using the commercial analytical tests and specifications, with additional testing for bioburden. The data provided from the supportive batches support the intended stability of 24 months at -75 °C. Here, data on Cas9 concentration, purity and functionality are available. The applicant provided comparability studies for Cas9-bulk and sterile filled Cas9 stored in vials (storage at recommended accelerated storage conditions). Based on the results of the release tests it has been demonstrated that there is a high comparability between the two different vials. The applicant provided a commitment to implement an ongoing stability program for Cas9 bulk and Cas9 sterile filled. This point is recommended to the applicant as a quality recommendation. Provided data supports to proposed shelf life and storage conditions.

2.4.4. Starting Material SPY101

2.4.4.1. General Information

SPY101 sgRNA is a synthetic oligonucleotide (100-mer) with characteristics of duplexes of A-form and B-form DNA and is supplied as lyophilized powder. It targets the BCL11a gene and directs Cas9 nuclease to cleave both strands of DNA. The phosphorothioate linkage is a mixture of diastereomers since the coupling and oxidation step are not carried out in a stereospecific manner. Methylated 2’ ribosyl hydroxyl group as well as the thiolated phosphate linkages have been incorporated at both terminal ends to inhibit the degradation by nucleases.

2.4.4.2. Manufacture, process controls and characterisation

SPY101 is manufactured as a lyophilized bulk powder, stored at -20 °C, and shipped under frozen, controlled-temperature conditions, for reconstitution and sterile fill manufacturing. Finished sterile fill SPY101 vials are stored at -70°C and shipped under frozen, controlled-temperature conditions, for the finished product manufacturing. Information on the testing sites has been provided. A valid proof of GMP compliance has been provided for sites involved in manufacture of SPY101 starting material.

Based on the product and process understanding, a defined set of controls are employed for each process step to ensure consistent process performance and product quality. Critical Process Parameters (CPPs) have characterized Normal Operating Range (NOR) and Proven Acceptable Range (PAR).

No reprocessing steps are utilized for the SPY101 manufacture.

In summary, the manufacturing process has been described in sufficient detail. Sufficient information on the manufacturing facilities and microbial contamination control has been provided.

The starting materials are phosporamidites. Specifications and information on the structure of these starting materials have been provided. A justification for the classification of starting materials and information on the suppliers of the starting materials has been provided. It has been confirmed that in the case of additional suppliers a variation will be filed. Information on the analytical methods for the control of the starting materials, the impurity profiles of the starting materials including a criticality assessment has been provided.

No materials of human or animal origin are used in the production of SPY101. A list of all materials including product components has been included in the dossier and BSE/TSE statements have been provided.

The information on control of critical steps is sufficient and has been deduced from manufacturing process development studies.

The SPY101 sterile fill manufacturing process was successfully validated in accordance with a three-stage approach (process design, process qualification and continued process verification). PPQ runs for SPY101 bulk were executed. PPQ runs for sterile fill (SF) SPY101 were executed using bulk lots SPY101. The sterile filtration-fill step is adequately subjected to APV with simulation runs using TSB in place of product component and considering worst-case conditions. All initial process simulation runs were successfully completed with no microbial growth observed. Post-incubation media fertility is ensured via growth promotion testing using six acceptable reference strains. Requalification is confirmed to be conducted with an interval of at least 6 months. This point is recommended to the applicant as a quality recommendation.

All PPQ studies were performed based on pre-approved protocols. There were no critical deviations. Information on the major deviations has been provided. Multiple validation assessments or studies including aseptic process simulation (media fill), sterile fill homogeneity study, filter validation (including compatibility and extractables), and container closure integrity were conducted in support of the process performance qualification. In all, the pre-defined success criteria for the PPQ campaign were met and successful process validation has been demonstrated. Microbial hold time study has been conducted as part of PPQ.

Sufficient information on pre-clinical, early clinical development and late-stage clinical development has been included in the dossier. Process optimisations have been adequately described and comparability between processes has been demonstrated.

Following identification of CQAs, risk assessments were performed to determine what material attributes and process parameters potentially impact CQAs. The risk assessment was a combination of Subject Matter Expertise (SME) judgment and a FMEA-tool. The outcome of the risk assessment populated a comprehensive list of materials and process steps where variability was investigated as part of the SPY101 process characterization studies. Characterization studies considered the CQAs and other process attributes, incoming materials and equipment capability, when designing studies to be performed. The process characterization experiments identified by risk assessment formed the basis of the overall control strategy for the bulk and sterile fill manufacturing processes. Following the characterization studies, NOR and PAR were confirmed for the parameters of interest and resulted the final control strategy, which was designed to mitigate risk through adjustment of control ranges, process controls and release criteria and stability specifications. Overall, the control strategy is acceptable.

Extended characterization of SPY101 was performed to evaluate its structure, physicochemical characteristics, and biological properties using a variety of analytical techniques. For each technique, experimental data including e.g. spectra have been provided.

The ability of SPY101 to guide Cas9 to the on-target editing site to cleave double-stranded DNA is demonstrated through the functionality assay.

The organic impurities of SPY101 can be formed during the manufacturing process and/or storage. The process optimizations have increased the purity of SPY101. The increased purity is attributed to a reduction of the late-eluting impurities by the optimization of synthesis parameters and a reduction in early-eluting impurities by optimization of the purification parameters.

Comprehensive information on the impurity profile has been provided.

Sufficient information on elemental impurities and residual solvents has been provided.

2.4.4.3. Specification, analytical procedures, reference standards, batch analysis, and container closure

The specification for bulk and sterile fill SPY101 have been presented and they are considered acceptable.

The commercial specification setting strategy focused on batch analysis and stability data generated from clinical, commercially representative, and Process Performance Qualification (PPQ) batches for bulk and sterile fill SPY101.

The specifications are acceptable based on the available data. The applicant has committed to re-evaluate the specifications for bulk and sterile fill SPY101 when data from five additional batches of each are available. This point is recommended to the applicant as a quality recommendation. Potency acceptance criteria for both SPY101 bulk and sterile fill should be re-evaluated when data for at least five additional batches of bulk and sterile fill each are available. This point is recommended to the applicant as a quality recommendation. Additionally, acceptance criteria for relevant impurities for bulk and sterile fill SPY101 should be established and justified once data from five additional bulk and sterile fill lots is available. This point is recommended to the applicant as a quality recommendation.

Compendial analytical methods used for SPY101 bulk and sterile fill release testing are conducted as described by relevant sections of Ph. Eur. and have been verified for use. For non-compendial analytical methods used for SPY101 bulk and sterile fill release appropriate analytical method descriptions have been provided.

Analytical procedures validation/verification and full validation protocols/reports have been provided. The analytical methods are suitable for their intended use.

Batch analysis data for bulk SPY101 batches and Sterile Fill SPY101 batches have been provided. All results are within the specifications valid at the time of release.

Sufficient information on reference standards and materials and the container closure system have been provided. Bulk SPY101 is stored in a HDPE bottle with PP cap. The container closure system for sterile fill SPY101 consists of a vial, sealed with a stopper, and crimped with a flip-off cap. Specifications for all components and certificates of analyses and compliance have been provided. The information on the sterilisation of the packaging components is sufficient.

2.4.4.4. Stability

The proposed shelf-life for bulk SPY101 is 18 months when stored in the intended container closure system at -20°C. The stability studies include supportive and registration stability studies in accordance with ICH Q1. Up to 48 months data are available for the supportive stability batches. 12- and 24-months data are available for the registration batches. 6 months stability data are available for the PPQ batches. No overall negative trends have been observed in any stability lots to date.

In addition, the stability of bulk SPY101 stored in the intended container closure system has also been monitored at 5°C with PPQ lots and additional registration batch. One batch has been stored at 25°C for six months.

The proposed shelf-life for sterile fill SPY101 is 18 months when stored in the intended container closure system at -70°C. Stability data have been provided for supportive batches (data for up to 36 months available), registration batches (12 months) and PPQ batches (6 months).

All results are within the specifications and no negative trend has been observed in the stability studies. In addition, the stability of sterile fill SPY101 stored in the intended container closure system has been monitored at -20°C with PPQ Lots. One additional batch has been stored at 25°C/60% RH.

Changes between processes, methods and specifications have been adequately described in the dossier.

The proposed shelf-life for bulk SPY101 and sterile fill SPY101 is acceptable.

2.4.5. Finished Medicinal Product

2.4.5.1. Description of the product and pharmaceutical development

A single dose of exagamglogene autotemcel consists of at least 3x10e6 CD34+ cells per kg of patient weight, suspended in CryoStor CS5 cryopreservation medium containing 5% DMSO, at approximately 4-13 x 10e6 cells/mL. Composition of the finished product is provided in Table 1.

Table 1: Composition of the finished product

Components	Function	Quality Standard	Quantity per lot
CRISPR-Cas9-mediated gene-edited autologous CD34+ HSPCs	Drug Substance	Please refer to Section 3.2.P.5.1 for specification	4-13 $x10^6$ cells/mL[1]
CryoStor® CS5	Excipient	Please refer to Section 3.2.P.4.1 for excipient specification	Target to 1 mL for every 10 million cells

[1] 4-13 $x10^6$ cells/mL As this is an autologous formulation, batch quantity is dependent on number of cells processed

The target concentration for exa-cel finished product is defined. Edited cells from more than one vial and more than one batch may be used to provide a complete patient dose through intravenous administration. The fill volume is proposed to have a rather broad range from 1.5 mL – 20 ml filled into 20 mL vials. The applicant justified the proposed broad ranges of cell concentration and fill volume in 20 mL vials by clinical experience and the nature of the autologous cell therapy which is associated with a high variability in the number of cells available from each mobilization cycle. A description of the strategy chosen for filling of vials with exa-cel finished product has been provided. It is aimed to fill the minimum number of vials with similar volumes across all vials. This was supported by listing the fill volume per each vial and the final number of vials applied for dosing.

Commercially available cryopreservation medium, CryoStor CS5 containing 5% DMSO has been chosen based on published literature. No other excipient has been used for formulation of exa-cel during pharmaceutical development.

Following identification of QTTP and CQAs, risk assessments were performed to determine what material attributes and process parameters potentially impact CQAs. For material risk assessment, all material attributes used in the manufacturing were evaluated. To identify a potential impact from variability of a given material, Subject Matter Expertise (SME) on the product and process properties was utilised, as well as historical experience and characterization of exa-cel performance. For the process risk assessment, the Failure Mode and Effects Analysis (FMEA) was employed to assess an effect of variability in process parameters on CQAs. The risk score was assigned by SME based on frequency, detectability and severity of a potential failure effect. Characterization studies considered the potential CQAs, incoming materials and equipment capability, when designing studies to be performed. Following the characterization studies, criticality of the parameters was statistically evaluated and determined the final control strategy, which was designed to mitigate risk through adjustment of control ranges, process controls and release criteria and stability specifications.

Manufacturing process development

The applicant has performed manufacturing process development studies to evaluate the impact of the finished product cell concentration on formulation and product hold time on exa-cel finished product quality attributes.

The process control strategy has been presented including IPCs, CPPs and non-CPPs. Impact of proposed NORs and PARs for CPPs on CQAs have been evaluated individually for each manufacturing step. Compliance with finished product release specifications for cumulative maximum intended NORs of culture durations and hold times has been demonstrated by data from exa-cel finished product batches that have been manufactured with extended in-process hold times above NORs at all unit operations except the leukopak shipping time.

Container closure system (CCS)

Exa-cel finished product is filled and stored in 20 ml ready-to-fill closed vials. The fill volume range for exa-cel finished product is 1.5 – 20.0 mL. The CCS is pre-assembled and sterilised prior to the finished product fill. The material used for the manufacturing of the vial complies with Ph. Eur. 3.1.3 and the material used for the manufacture of the vial stopper complies with Ph. Eur. 3.2.9. The sterilisation of the closed vials (vials and stoppers) is performed by gamma irradiation according to Ph. Eur. 5.1.1. at certified sterilisation providers. A description of the paperboard cryo-storage box used for shipping of the exa-cel final finished product filled in vials has been provided.

The vial closure system has been used throughout clinical development and is intended for commercial use. Compatibility of the container closure system with the dosage form has been evaluated by extractable and leachable studies, container closure integrity (CCI) testing and long-term stability studies. Stability studies were performed with containers of the same material and reduced size compared to the CCS used in commercial manufacture. The performed CCI tests met acceptance criteria, however, only limited time point data is available from the oxygen head space method. CCI testing data for 12 and 24-month timepoints are pending. A post-marketing commitment is made to provide 12 and 24-months data from on-going exa-cel CCI studies with oxygen head space method when available. This point is recommended to the applicant as a quality recommendation.

Compatibility

For administration, exa-cel finished product is thawed, withdrawn from the vial through an 18 µm filter using a syringe and infused directly to the patient within 20 minutes of thaw. Compatibility data

obtained from a study conducted with administration IV sets consisting of commonly used infusion set materials polyurethane, silicone, polyvinyl chloride (PVC)), and an 18-micron filter has been provided and found acceptable.

2.4.5.2. Manufacture of the product and process controls

Manufacturers

Roslin Cell Therapies, BioCube 2, Edinburgh, UK and Charles River Laboratories Inc, Memphis, TN, USA are indicated as manufacturers of the Exa-cel active substance / finished product. Vertex Pharmaceuticals, Ireland performs QP batch certification for EU.

For the Roslin sites, GMP compliance has been confirmed by provision of GMP certificates covering gene therapy products. For Charles River Laboratories Inc, Memphis, USA an inspection has been conducted in June 2023 by HPRA. The GMP certificate covering gene therapy products and the EudraGMDP reference number have been provided. For all facilities, provided documentation to confirm release testing and batch certification in compliance with GMP of exa-cel is adequate.

Manufacturing process

The manufacturing process from CD34+ enrichment of the leukopak starting material to exa-cel finished product is continuous with no isolated active substance or intermediate. No reprocessing is performed for manufacture of the finished product. The shippers are validated and are designed for thermal control and protection from transport hazards as well as the safe shipment of material without the risk of spilling liquid nitrogen phase (LN2).

Potential effects on exa-cel CQAs by filtration and freezing in CRF were evaluated. No significant impact was observed for evaluated conditions.

The applicant has applied for an exemption from retesting upon importation to EU. The provided justification is based on limited amount of material available for retesting and is considered sufficient.

Controls of critical steps and intermediates

No IPC testing is performed from harvest to cryopreservation. For CPPs target, NORs and PARs have been established based on characterisation studies presented in the dossier. These were found to be acceptable.

Process validation and/or evaluation

The manufacturing process has been validated. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner.

Shipping

Process validation and qualification for the shipping of the leukopak and exa-cel finished product has been performed. The Leukopak and finished product shipping routes were identified, mapped and a risk assessment was conducted on those routes. Based on this risk assessment, transport simulation studies were conducted at worst-case conditions for the exa-cel finished product but not for the leukopak. Operational qualification (OQ) and real-world performance qualification (PQ) studies were conducted to evaluate the maintenance of thermal control during the transport of leukopaks and the finished product. The liquid nitrogen shipper Operational Qualification (OQ) was performed in accordance with ISTA Standard 20 for small parcel and palletized exposures with focus on Thermal OQ (ISTA 7D or 7E) and Physical OQ (ISTA 3A and/or 3B). Respective required temperatures were maintained for evaluated durations. For the transport of leukopaks, thermal PQ qualification was

performed at a temperature range of 5-8 °C. In addition, real-world validation data for shipping of the leukopak starting material has been provided. Transport was performed on international and continental USA shipping routes at 2-8 °C up to 72 h. The proposed total shipment duration of 48 hours for the leukopak is covered. For transport of the exa-cel finished product, it was indicated that the total shipping duration cannot exceed the study duration shipping times specific to the shipper name/model.

2.4.5.3. Product specification, analytical procedures, batch analysis

Finished Product Specifications

The finished product specifications include attributes and methods relevant for this type of product and include specifications for: appearance, identity, purity, potency, quantity/content, safety (sterility, mycoplasma and endotoxin).

Stability specifications for the finished product include the same CQAs and acceptance criteria as the finished product release specification except for tests for identity, mycoplasma, and endotoxin.

A series of unique patient identifiers are created and tracked throughout the supply chain by visual and electronic system.

Description and validation of the analytical procedure as well as GMP certificates for sites involved in testing have been provided and are deemed acceptable. The same release and stability acceptance limit has been defined for both indications. Suitability of the specification for both TDT and SCD indications was adequately justified.

During clinical development, two further assays have been performed to evaluate the biological activity of the exa-cel finished product. Both assays will not be performed as routine characterization testing but will be included as extended characterization tests for commercial exa-cel manufacturing process changes.

Off-target editing has been studied as part of non-clinical safety evaluations and included in the non-clinical part of this report.

Analytical methods

Descriptions of analytical procedures have been provided for all release tests. Compendial analytical methods used for Exa-cel finished product release testing are sterility and endotoxin (Chromogenic LAL method). The methods are conducted as described by relevant sections of Ph. Eur. and have been verified for use.

Overall, appropriate analytical method descriptions have been provided. All non-compendial assay descriptions for the finished product have been revised during the procedure with respect to criticality of reagents and equipment. Reagents and equipment that would require a method optimization and/or partial to full revalidation upon a change are deemed as "Do Not Substitute".

Validation of analytical procedures

Validation reports have been provided for analytical procedures. Overall, performed validation studies comply with ICH Q2 (R1).

Validation has been performed at several sites. For non-compendial analytical procedures validation (except appearance) comprised analysis of specificity, linearity, accuracy, precision (repeatability, intermediate or overall; varies for analytical procedures), limit of quantitation (LOQ), range and

robustness. For analytical procedures conducted by more than one facility, operator precision or reproducibility were evaluated. Tabulated summaries of validation results as well as harmonised validation outcomes have been provided. Overall validation results including reproducibility were within predefined acceptance criteria.

Batch analysis

Batch data presented in the dossier include 3 finished product batches used in non-clinical GLP studies, 71 finished product batches used for 54 TDT patients (including PPQ batches), and 99 finished product batches used for 49 SCD patients (including PPQ batches). The manufacturing process of these batches is representative of the commercial process. All batches met the acceptance criteria in place at the time of testing. Results of the three non-clinical batches were within ranges of clinical and PPQ lots. Information with respect to exa-cel finished product batches presented in Section P.5.4 of the dossier comprises patient IDs, finished product batch numbers, numbers of Cas9 and SPY101 batches used for the manufacture of the clinical exa-cel batches, dates of manufacture, manufacturing sites, batch sizes and information on the clinical study number where each exa-cel batch has been used is provided.

Characterisation of impurities

Product-related impurities are indirectly controlled by the specifications. The absence of specification for cellular impurities is supported by phenotypic characterisation data. Based on the characterisation results and considering that this is an autologous product, and it is assumed that the cell composition is determined by patient-to-patient variability, it is acceptable that no acceptance criteria for specific cellular impurities are in place. Likewise, characterisation data are provided to demonstrate clearance of process-derived impurities.

The results of simulation study on vials and stopper with CS5 cryoprotectant demonstrate no presence of Class 1, 2A, 2B, 3 elemental impurities above a ICH Q3D (R1) permitted daily exposure (PDE).

The applicant has provided a risk assessment concerning the presence of nitrosamine impurities in exa-cel finished product taking into account the findings and principles outlined in the Article 5(3) referral on nitrosamine impurities in human medicinal products CHMP assessment report (EMA/369136/2020) and the respective EMA Q&A document (EMA/409815/2020). The CryoStor CS5 is the only excipient identified in the category of high risk due to its proprietary formulation and use in the finished product formulation. Since CS5 has a neutral pH and is only used at ambient temperature, the risk to form a nitrosamine impurity was deemed low. Results for confirmatory testing conducted for material identified with medium risk or high risk for the presence of nitrosamines have been provided. 1 out of 10 assessed nitrosamines (namely NDMA) was detected in the formulation buffer (CryoStor CS5) syringe/filter combination. The applicant detected a NDMA nitrosamine impurity in the CryoStor CS5 excipient. Based on a risk assessment, where the applicant also considers that the patient will only receive a single i.v. dose administration of the medicinal product, the applicant considers the detected levels of NDMA in CryoStor CS5 buffer to pose a low and acceptable risk to the patient. A control strategy for NDMA in the CryoStor CS5 is proposed. This includes investigation of the root cause for NDMA and CAPA implementation. As interim solution, all incoming batches of CS5 will be tested for the presence of NMDA with an acceptance limit based on the toxicology assessment provided. The applicant commits to provide the outcome of the initiated investigation with respect to root cause analysis of detection of NDMA in the CryoStor CS5 buffer, implemented CAPA and potential revisions to the commercial control strategy as a post-marketing commitment. This point is recommended to the applicant as a quality recommendation. Based on the confirmation from the CHMP Non-Clinical Working Party (NcWP) that an incidental single exposure of 525 ng NDMA could be accepted from a safety point of view, and the feedback from the applicant that a tighter limit for NDMA in CS5 cannot be implemented at this time as this would limit the availability of the product, the above approach is considered acceptable.

Justification of Specification

Acceptance criteria of specifications have been established based on clinical batch release data from TDT and SCD patients and stability data from healthy donors. Suitability of the same proposed commercial specifications for both TDT and SCD patient material has been justified. Some open issues remain with respect to proposed acceptance limits of specifications that were requested to be addressed as post-marketing commitments by the applicant. This point is recommended to the applicant as a quality recommendation. Colour is currently not included in the appearance specification of the exa-cel finished product. The applicant commits to revise the appearance specification for the inclusion of coloration assessment considering Ph. Eur. 2.2.2 "Degree of coloration of liquids". This point is recommended to the applicant as a quality recommendation.

Reference standards

Exagamglogene autotemcel finished product is an autologous cellular product and therefore does not have a reference standard.

2.4.5.4. Stability of the product

Stability studies for the finished product are performed at real-time conditions for a storage in vapor phase liquid nitrogen (≤-135 °C) up to 24 months which corresponds to the proposed shelf-life of the finished product. In addition, finished product stability stored at intermediate storage conditions at -80±10 °C is monitored for a duration up to 3 months. Freeze-thaw and in-use stability studies are presented. Stability of the thawed finished product for an in-use time of 20 minutes at RT has been demonstrated. The stability studies have been performed with healthy donor (HD) material, which is considered appropriate. In addition, supportive stability data with respect to potency has been provided from bracketing endpoint-stability studies conducted with patient-derived retain samples.

Stability batches have been manufactured at three different sites: RoslinCT-SCRM, CRL and Roslin BioCube. All batches are manufactured using the same process for clinical and proposed commercial use. Comparability of finished product from these three manufacturing sites has been described and few issues have been raised which have been adequately resolved. The applicant committed that after completion of ongoing real-time stability studies, comparability with respect to stability of finished product manufactured at both commercial manufacturing sites will be evaluated. This point is recommended to the applicant as a quality recommendation. In addition, it is committed to place at least one healthy donor batch of the finished product in an annual stability study per production year at each active manufacturing site.

Stability studies have been performed in accordance with current ICH/CHMP guidelines. The testing frequency is according to ICH Q5C. The finished product is filled and stored in vials and stoppers. The fill volume range for exa-cel finished product is 1.5-20.0 mL. A scaled-down container closure model was used in the stability studies. Long term stability data is available for supportive batches (24 months), registration batches (12-18 months) and PPQ and registrational batches (9 months). All stability studies for registration and PPQ batches are ongoing. All available data met specifications and no overall negative trends have been observed. Some of the tests have been included only for the registration batches. However, as mentioned above, additional supportive stability data with respect to potency has been provided from bracketing endpoint-stability studies conducted with patient-derived retain samples at a beginning point T0 and an endpoint T1. Presented data at T0 and T1 were within their respective specifications and fall within ranges of release testing results from clinical lots. Additional stability data from patient derived material with respect to viability are not available currently. The applicant commits that additional stability studies with final product derived from TDT and SCD patient material will be performed in case sufficient finished product material is left-over in

addition to the retain samples, release control samples, and actual dose as a post-marketing commitment. This point is recommended to the applicant as a quality recommendation.

Based on provided data from on-going real-time stability studies as well as supportive stability data from bracketing endpoint-stability studies the proposed exa-cel finished product shelf-life of 24 months when stored at ≤ 135 °C is deemed acceptable.

In-use stability

For administration of exa-cel, each vial is thawed individually, as described in the SmPC, over 15 minutes in a water bath at 37°C. Once thawed, the finished product is infused within 20 minutes of completion of thawing and is handled at RT. The proposed in-use stability was demonstrated by in-use stability studies. The provided data support the in-use stability of 20 minutes at room temperature (20°C – 25°C).

2.4.5.5. Adventitious agents

The adventitious agents risk assessment for the finished product has considered non-viral and viral agents and was implemented in accordance with relevant guidance. Ensuring the safety of a cell and gene therapy product from both viral and non-viral adventitious agents is a complex process, as viral purification and viral inactivation steps would adversely impact the biological activity of the finished product. Therefore, neither viral purification nor viral inactivation steps are included in the finished product manufacturing process. However, these steps are discussed in the lines of mitigating potential risk from ancillary materials which are of biologic origin and used in the manufacturing process of the finished product. The details of control of adventitious agents include raw material control, process controls, facility and engineering controls, release testing and measures to prevent contamination.

Raw materials (discussed in the raw materials section of the active substance part) used in the manufacturing process are accepted based on appropriate microbial, viral and mycoplasma testing as stated on the supplier Certificate of Analysis. The safety testing performed on exa-cel finished product are provided in the dossier and considered acceptable.

Testing of Cas9 Master Cell Bank (MCB) (*E. coli*) and testing of adventitious agents (endotoxin, bioburden, sterility, CCIT) for both Cas9 and SPY101 components is adequately presented in the dossier and considered sufficient. Certificate of analysis has been provided for one batch of sterile fill Cas9 and SPY101.

Based on the controls in place and assessments of risk posed by materials of animal origin used in finished product manufacturing, it can be concluded that the BSE/TSE and adventitious virus risk arising from the use of these materials in the finished product manufacturing process is negligible.

The only excipient in the finished product is CryoStor CS5 containing contains 5% DMSO. BSE/TSE certificate for CryoStor CS5 has been provided. As per the certificate, the entire product line is free from materials at risk for BSE (Bovine Spongiform Encephalopathy) and TSE (Transmissible Spongiform Encephalopathy). CryoStor CS5 is 100% synthetic in that it is a serum-free, protein-free, animal-origin-free product.

The microbial and mycoplasma testing performed on the final product include sterility (Ph. Eur. 2.6.1), mycoplasma and endotoxin testing (Ph. Eur. 2.6.14).

Both manufacturing sites follow microbial control strategy procedures to minimize the risk of introducing adventitious agents.

The presented holistic control of the risk of contamination of adventitious agents is considered adequate.

2.4.5.6. GMO

The finished product is a cellular product consisting of autologous CD34+ human hematopoietic stem and progenitor cells (hHSPCs) modified by CRISPR/Cas9-mediated gene editing. As the finished product is considered a GMO, a separate GMO environmental risk assessment report was provided to estimate the risk of the finished product to third parties and the environment. This is further discussed in section 2.5.5. Ecotoxicity/environmental risk assessment of this report.

2.4.6. Discussion on chemical, pharmaceutical and biological aspects

Quality Development

Starting material Cas9 and Spy101

Separate 3.2.S modules have been provided for Cas9 nuclease protein and SPY101 single guide RNA starting materials. The information provided on the Cas9 and SPY101 starting materials are, in general, appropriate.

The *Streptococcus pyogenes*-derived Cas9 nuclease is considered as critical starting material. In combination with the sgRNA SPY101, the Cas9 protein mediates a sequence specific DNA-double strand break in the BCL11A gene in CD34+ target cells.

The Cas9 protein is manufactured as bulk, which is finally sterile filled. Overall, the Cas9 manufacturing process is well defined.

The Cas9 batches contain different product related impurities (protein deamidation, oxidation and truncation), which have been characterized and do not reduce the biological activity of Cas9. Overall, the product related impurities are consistent between batches.

For bulk SPY101 and sterile fill SPY101 no major objections have been raised. The other concerns have been adequately addressed.

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

At the time of the CHMP opinion, there were a number of minor unresolved quality issues. These points are put forward and agreed as recommendations for future quality development.

Active substance/finished product

In general, Module 3 of the exagamglogene autotemcel (exa-cel) dossier is of good quality and clearly and concisely written. Major objections on missing GMP certificates and the potency assays were resolved during the procedure, and other issues are all resolved. A number of post-approval recommendations are agreed with the applicant.

The finished product is manufactured at two alternative manufacturing sites. Ireland performs QP batch certification for EU. Valid proof of GMP compliance covering gene therapy medicinal products has

been provided by submission of the respective GMP certificate and EudraGMDP reference. This was sufficient to resolve the raised major objection.

The manufacturing process of the finished product is continuous without an isolated active substance or intermediate. The manufacturing process, process controls and process validation have been appropriately described.

The required finished product dose is 3x10e6 CD34+ exagamglogene autotemcel per patient kg. The aim should be, that the finished product dose can be manufactured within one mobilization cycle. In those cases, in which the finished product dose cannot be achieved a repeated CD34+ mobilization(s) is/are initiated, until the patient dose is received.

As a part of the life cycle management and continuous process improvements the applicant committed to assess options to increase the overall %CD34 yield and introduce changes into the manufacturing process, respectively. In line with the manufacturing improvements, the IPC of a minimal number of cells at harvest as well as the definition for the minimum batch size should be further adapted.

For the leukopak a total of at least 20x10e6 CD34+ cells/kg is recommended in the SmPC for product manufacturing. This value is based on the cell number available for clinical batch manufacturing that resulted in a sufficient patient dose. In addition, at least 2x10e6 cells/kg unmodified rescue cells are needed to be collected for back-up.

Process validation and qualification for the shipping of the leukopak and the finished product has been performed. Real-world shipping data for the leukopak has been provided.

Characterisation of the finished product has been performed using appropriate methods.

Test parameters proposed to be included in the finished product specifications are considered appropriate. An adequate number of finished product batch results for both the TDT and SCD indication manufactured at both manufacturing facilities have been presented. All results were within specification. Impurities were adequately described. For detection of the nitrosamine NMDA in the CryoStor CS5 excipient, a root cause analysis is ongoing, and a control strategy has been developed. Justification of specifications are based on provided batch release and stability data. Suitability of the same proposed commercial specifications for both TDT and SCD patient material has been adequately justified. Based on currently available stability data the proposed finished product shelf life of 24 months at ≤-135°C is adequately justified.

2.4.7. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.4.8. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CAT recommends the following points for investigation: Further review and analysis of data from ongoing stability studies and commitments for additional studies, re-evaluation of current specification limits based on additional manufacturing experience, additional characterization studies, evaluation of potential process improvements as additional information is gathered through continued process verification, and additional GMP commitments.

2.5. Non-clinical aspects

2.5.1. Introduction

The nonclinical testing programme has been adapted to the ex vivo genome editing approach of autologous CD34$^+$ hHSPCs used for the generation of exa-cel, which aims at lowering *BCL11A* gene expression in erythroid cells in order to reactivate HbF expression.

The CRISPR-Cas9 editing components, i.e., the SPY101 gRNA bound to the Cas9 nuclease as a ribonucleoprotein (RNP) complex (SPY101-Cas9 RNP), targets a critical binding site of the transcription factor GATA1 in the non-coding erythroid lineage-specific enhancer region of the *BCL11A* gene. Repair of the introduced double strand breaks (DSBs) by nonhomologous end joining (NHEJ) produces indels in the DNA that disrupt GATA1 binding, thereby lowering *BCL11A* expression in erythroid cells only, while preserving the normal function of BCL11A in other cell types. The reduction of BCL11A in developing erythroid precursors leads to increased γ-globin mRNA expression and HbF reactivation.

The overall nonclinical testing strategy is intended to support marketing authorization in both TDT and SCD. Non-clinical data have been generated to establish the pharmacodynamics and proof of mechanism of exa-cel for both indications; the biodistribution and toxicology testing are also applicable to both disease indications.

The non-clinical programme includes *in vitro* evaluation of on-target editing in CD34$^+$ hHSPCs from healthy donors, β-Thalassemia and SCD patients, *in vitro* evaluation of allelic editing (genotype) and γ-globin expression (phenotype) using single-cell clonal analysis, confirmation of on-target editing in long-term repopulation HSCs, and *in vitro* and *in vivo* multilineage differentiation of SPY101-RNP edited CD34$^+$ hHSPCs.

No *in vivo* proof-of-concept studies were conducted as no relevant animal disease model for TDT and SCD exists that could be used for evaluation of exa-cel. Instead, *in vivo* studies were conducted in sub-lethally irradiated NOD/SCID/IL2Rγnull (NSG) xenotransplant mouse model in order to evaluate the long-term engraftment of exa-cel and persistence of editing of exa-cel for up to 20 weeks.

A GLP biodistribution and persistence study was performed to assess the potential of CRISPR/Cas9-edited human cells to engraft in target tissues as well as migrate to, accumulate, and persist in non-target organs of NSG mice. Moreover, combined GLP toxicity and tumorigenicity study has been conducted in NSG mice with a duration of 20 weeks. Studies of longer duration in NSG mice are hampered by the risk of graft-versus-host disease. Both GLP studies were conducted as single-dose studies using the intravenous (IV) route reproducing the clinical use of exa-cel. The test material used in these studies are representative of test material used in the clinic and derived from healthy donors.

In addition, specialized genotoxicity studies were conducted for exa-cel to evaluate on-target editing, off-target editing, and the risk for chromosomal translocations. Cells from healthy donors as well as CD34$^+$ hHSPCs from β-Thalassemia and SCD patients sourced from clinical trials were included in the off-target editing evaluation.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

Several *in vitro* studies confirmed successful gene editing at the target site in SPY101-RNP-treated CD34$^+$ hHSPCs and demonstrated an increase in γ-globin expression upon erythroid differentiation in cells derived from healthy donors and from β-Thalassemia and SCD patients.

Three studies were conducted with research lots from healthy donors using research-grade reagents and process. These studies include assessment of functional potential of SPY101-RNP-edited cells in up-regulation of γ-globin expression, analysis of genotype-to-phenotype correlation, and HbF upregulation. Three additional *in vitro* studies were conducted in order to characterize the three lots used in the pivotal GLP studies and generated from healthy donors.

Editing frequency, γ-globin expression and fetal haemoglobin upregulation:

Mean editing frequencies in research lots of healthy donors ranged from 64% to 78%. On Day 15 after *in vitro* erythroid lineage differentiation, these editing frequencies resulted in an increase of the mean γ/α-globin mRNA ratio from 0.32 in the control cells (EGFP-RNP-edited cells) to 0.74 in the SPY101-RNP-edited cells of Study CTxSR-010, and from 0.02 in the control cells (EGFP-RNP-edited cells) to 0.14 in the SPY101-RNP-edited cells of Study CTxSR-021. Similarly, the mean γ/(γ+β)-globin mRNA ratio increased from 0.39 to 0.71 and from 0.05 to 0.32, respectively. The mean γ/α-globin protein ratio measured using LC-MS on Day 15 increased from 0.17 in the EGFP-RNP-edited control cells to 0.49 in the SPY101-RNP-edited cells (Study CTxSR-010) and from 0.11 to 0.5 measured on Day 18 (Study CTxSR-021), while the mean γ/(γ+β)-globin protein ratio measured by LC-MS increased from 0.26 to 0.53 and from 0.28 to 0.65, respectively. The mean percentage of HbF relative to total hemoglobin (HbF/HbF+HbA) was additionally assessed by ion-exchange – high performance liquid chromatography (IEX-HPLC) in Study CTxSR-021 and an increase from 2% to 16% was observed at Day 18.

In the GLP tox batches, the mean editing frequency was 76%. Upon editing, the mean γ/α-globin mRNA ratio increased on Day 13 post-differentiation from 0.15 in untreated control cells to 0.39 in SPY101-RNP-edited cells. The mean γ/(γ+β)-globin mRNA ratio increased from 0.29 in untreated control cells to 0.56 in SPY101-RNP-edited cells. Mean percentage of HbF/(HbF+HbA) measured by IEX-HPLC increased from 14% in untreated cells to 32% in the SPY101-RNP-edited cells as measured after 18 days of *in vitro* erythroid differentiation.

Genotype-to-phenotype correlation in single cell-derived erythroid colonies:

For the genotype-phenotype correlation single cell-derived erythroid colonies from SPY101-RNP-edited cells (research lots) were generated and analysed after 15 days of erythroid differentiation in order to define the precise indel found in each allele. Subsequently, α-globin, β-globin, γ-globin expression was analysed for 164 individual clones. This study revealed that a high percentage of clones had bi-allelic indels (76%), and that more than 90% of the single cell colonies had at least one allele modified. Thereby, most indels disrupted the GATA 1 binding site sequence within the erythroid enhancer of *BCL11A* in intron 2, which resulted in upregulation of γ-globin expression. This study also demonstrated that the dose-dependency is driven by the number of edited alleles, as colonies with bi-allelic edits had higher γ-globin mRNA (median γ/α-globin ratio of 0.74 and a median γ/(γ+β)-globin ratio of 0.51) as compared to mono-allelic edits (median γ/α-globin ratio of 0.35 and a median γ/(γ+β)-globin ratio of 0.31), or colonies without edited alleles (median γ/α-globin ratio of 0.09 and a median γ/(γ+β)-globin ratio of 0.11).

The indel distribution of the single cell-derived erythroid colonies derived from one donor revealed three major indel species detected at high frequencies: -15 (15 bp deletion, 18.9% of 318 alleles), +1 (1 bp insertion, 18.6% of 318 alleles) and -13 (13 bp deletion, 10.7% of 318 alleles). The same indel species were also observed in the bulk population of the $CD34^+$ HSPCs prior to single cell sorting. The increased γ-globin expression in the single cell colonies with these major indel species was evaluated and confirmed. As a similar indel spectrum with the same major indel species was observed in cells from 2 additional healthy donors and 3 β-Thalassemia patients (-15, ranging from 18.5% to 24.2%; +1, ranging from 28.3% to 35.1%; -13, ranging from 17% to 23.2%) and in cells from SCD patients, this indel spectrum seems to be generally observed in SPY101-RNP-edited cells.

Viability and erythroid differentiation potential:

The SPY101-RNP-edited CD34$^+$ cells of the three lots used in the pivotal GLP studies were further evaluated for viability, the distribution of the CD34$^+$ cell populations, and the differentiation potential of edited CD34$^+$ hHSPCs. Thereby, SPY101-RNP-treatment revealed either no effect on viability of edited CD34$^+$ cells or a slight and transient decrease of viability shortly after thawing the SPY101-RNP-edited cells. Moreover, there was no difference in the percentage of live cells between SPY101-RNP-edited cells and untreated cells across the investigated CD34$^+$ subpopulations which also included long-term hematopoietic stem cell (LT-HSC). In addition, the editing efficiencies across the CD34$^+$ subpopulations (ranging from 78% to 85%) were comparable to the CD34$^+$ bulk population (76%). During erythroid differentiation there were no changes between SPY101-RNP-edited and unedited cells with regard to the erythroid surface marker analysed. Finally, the methylcellulose colony forming unit (CFU) assay did also not reveal any effects of SPY101-RNP treatment on hematopoietic differentiation, as the potential of SPY101-RNP-edited CD34$^+$ cells to generate CFU-GM and BFU-E colonies corresponding to myeloid and erythroid progenitors remained unaffected in this assay.

Editing frequency, γ-globin expression, fetal haemoglobin upregulation and differentiation potential of SPY101-edited CD34$^+$ cells from β-Thalassemia and SCD patients:

While most of the *in vitro* studies were performed with CD34$^+$ cells from healthy donors, the applicant confirmed that the gene editing frequencies in CD34$^+$ cells from β-Thalassemia and SCD patients do reach similarly high levels as observed with cells from healthy donors. SPY101-RNP treatment of CD34$^+$ cells from two β^+/β^+ donors and one β^0/β^0 donor resulted in an editing frequency of >90% as compared to ~85% editing frequency in healthy donor CD34$^+$ cells. During erythroid differentiation of edited cells from healthy donors and from β-Thalassemia patient staining of different surface biomarkers revealed only one difference. At late stages of erythroid differentiation, the expected decrease of CD71 expression is more pronounced in edited cells from healthy donor as compared to patient cells. However, this difference is rather a hallmark of the disease, as mainly the EGFP-RNP-edited cells revealed this difference. In SPY101-RNP-edited cells, the difference between healthy donor and patient cells seems to be lower. Increases in the γ/α-globin mRNA ratio and increases of HbF protein expression were also evaluated in edited CD34$^+$ hHSPCs from β-Thalassemia patients. Thereby, when comparing the γ/α-globin mRNA ratio between EGFP-RNP- and SPY101-RNP-edited cells, the healthy donor cells revealed an increase from 0.08 to 0.42 and from 0.06 to 0.36, respectively. The cells from the two β^+/β^+ donors revealed an increase from 0.19 to 0.58 and from 0.1 to 0.42, respectively, while the cells from the β^0/β^0 donor revealed an increase from 0.21 to 0.41. The percentage HbF of total Hb (HbF/(HbF+HbA)) measured by IEX-HPLC revealed an increase from untreated cells to SPY101-RNP-edited cells from 10% to 30% and from 7% to 29% in healthy donors. The SPY101-RNP-edited cells from the two β^+/β^+ donors revealed an increase from 50% to 79% and from 24% to 73%, respectively. The SPY101-RNP-edited cells from the β^0/β^0 donor revealed an increase from 68% to 92%. The high percentage of HbF in the unedited or EGFP-RNP-edited patient cells is due to the low levels of HbA in the patient samples.

For evaluating the gene editing frequencies in CD34$^+$ from SCD patients, the experimental conditions have been optimized across the four experiments conducted. PBMCs from 8 SCD patients and 3 healthy donors or purified CD34$^+$ cells isolated from non-mobilized PBMCs of 1 SCD patient or 1 healthy donor were evaluated. The use of unpurified PBMCs resulted in a higher variability of editing frequencies including several samples with editing frequencies at ~ 60% and one sample at ~ 40%. However, other samples including samples from SCD patients reached high editing frequencies of ~ 80%. While all samples analysed revealed a clearly increased γ/(γ+β)-globin mRNA ratio, the editing frequencies and the increase in the γ/(γ+β)-globin mRNA ratios do not always demonstrate a close correlation. However, this is likely a consequence of using mostly unpurified PBMCs in this study. The percentage HbF of total Hb (HbF/Hb; total Hb correspond to HbF +HbA in healthy donors and HbF +HbS in SCD patient samples) measured by IEX-HPLC revealed an increase from EGFP-RNP-edited cells to SPY101-RNP-edited cells from 4% to 25% and from 8% to 28% in healthy donors. In cells from three SCD patients, the percentage

HbF of total Hb in SPY101-RNP-edited cells compared to EGFP-RNP-edited cells increased from 8% to 30%, 18% to 42%, and 11% to 48%, respectively.

In vivo pharmacodynamic studies:

Due to the lack of a relevant disease animal model, the applicant just evaluated engraftment of SPY101-edited human $CD34^+$ cells in the NSG xenograft model for up to 20 weeks. In addition, the editing persistence and the erythroid differentiation potential of SPY101-edited human $CD34^+$ cells into B-cells, T-cells and myeloid cells were confirmed in this model. There were no differences observed between SPY101-RNP-edited cells, mock-electroporated cells, or untreated cells.

In a second engraftment study, the engrafted cells were collected for ex vivo culture and erythroid differentiation experiments at 16 weeks post-injection. In addition, cells from blood, bone marrow (BM), and spleen were collected and evaluated for on-target editing. This study revealed higher engraftment of the purified, unmanipulated $CD34^+$ hHSPCs as compared to all other groups. Increased engraftment of unmanipulated cells as compared to cultured hHSPCs has also been observed by others. Apart from the better engraftment observed for unmanipulated $CD34^+$ hHSPCs, no differences were observed between the other groups with regard to overall engraftment of the $CD34^+$ hHSPCs and differentiation into B-cells, T-cells and myeloid cells. However, when comparing the percentage of engrafted $hCD45RA^+$ cells in whole blood at Week 8 and Week 16, a significant drop of $hCD45RA^+$ cells became apparent in all groups.

As human erythroid differentiation is not efficiently supported in NSG mice, *in vitro* methylcellulose CFU assays were conducted with cells that were collected from the BM of transplanted mice. This evaluation confirmed that the SPY101-RNP-edited $CD34^+$ hHSPCs collected from transplanted NSG mice are still able to differentiate *in vitro* towards the erythroid lineage at 16 weeks post-engraftment. Thereby, the SPY101-RNP colony frequency turned out to be slightly decreased as compared to the EGFP-RNP colony frequency. However, this difference was not statistically significant (Two-tailed Student t-test, P=0.2672) and the applicant indicated that the BFU-E output from both test groups was quite variable across the samples evaluated, suggesting that sample heterogeneity and/or the quality of the cells post-thaw might have influenced the results.

Finally, the long-term editing persistence and the diversity of indel sequences was evaluated at 16 weeks post-engraftment and compared with the indel sequence diversity observed in the input cells.

2.5.2.2. Secondary pharmacodynamic studies

No secondary pharmacodynamic studies were performed nor are required, as exa-cel is not expected to have any effect on non-target physiological systems.

2.5.2.3. Safety pharmacology programme

Separate safety pharmacology studies were not performed, as the mechanism of action of exa-cel does not predict any non-target effect on physiological systems and organs including CNS, cardiac, respiratory, renal or gastrointestinal systems. However, selected safety pharmacology parameters, were investigated in the GLP toxicity study and no effects on CNS, cardiac, respiratory, renal or gastrointestinal systems were observed.

2.5.2.4. Pharmacodynamic drug interactions

No dedicated pharmacodynamic drug interaction studies were conducted.

2.5.3. Pharmacokinetics

The non-clinical pharmacokinetic investigation focused on the *in vivo* engraftment and persistence of the edited human cells.

In the pivotal biodistribution and persistence study the same batches as used in the pivotal toxicology study were evaluated. Engraftment and persistence of SPY101-RNP-edited $CD34^+$ hHSPCs was evaluated at Week 8 and Week 20 following engraftment in NSG mice and compared to the engraftment of unedited $CD34^+$ hHSPCs. Presence of human cells was evaluated in the hematopoietic system as well as in non-hematopoietic tissues and included hCD45+ qPCR analysis, flow cytometry (using markers for $hCD45RA^+$ and $hCD45^+$) and immunohistochemistry for $hCD45^+$ cells. Amplicon next generation sequencing (NGS) for SPY101-RNP-specific edits was also conducted using cells from spleen and BM.

There was a low number of mortalities (1.7%) that occurred in this study which were indicated as being likely related to the irradiation conditioning of the NSG mice.

Engraftment of human cells in whole blood, spleen, and BM was observed at Week 8 and Week 20 for all of three lots evaluated. Engraftment was highest into the BM and spleen at Week 8. However, there were quite large donor-to-donor differences in terms of engraftment and chimerism observed in whole blood and spleen between the three donors. In addition, there was a general trend that % chimerism in whole blood, spleen, and BM was lower at Week 20 than Week 8 for all donors, treatment groups and both sexes. Statistically significant differences between engraftment of unedited control cells and SPY101-RNP treated cells were observed in males at Week 20 in whole blood (percent chimerism based on $hCD45RA^+$ cells), spleen (percent chimerism based on $hCD45RA^+$ cells), and BM (both absolute cell counts and % chimerism for $hCD45RA^+$ and $hCD45^+$ cells) upon flow cytometry. qPCR analysis revealed lower % chimerism in hematopoietic tissues as compared to the % chimerism determined by flow cytometry, for which the applicant provided an acceptable explanation based on calculation differences. Moreover, qPCR data revealed a very low % chimerism in non-hematopoietic tissues, which is expected based on the nature of the product and the ex vivo genome editing approach used. Highest percent chimerism in non-hematopoietic tissues were still low (~ 1-2%) and observed in highly vascularized tissues such as the kidney, liver and lung. Thus, it is likely that these signals extend from remaining blood in the vasculature. In hematopoietic tissues % chimerism was comparable between animals treated with unedited control cells and animals treated with SPY101-RNP-treated cells. The only exception were blood samples form male recipient mice at Week 8, as male mice treated with unedited cells revealed a statistically significant lower average % chimerism as compared to the males treated with SPY101-RNP-treated cells. However, at Week 20 blood samples from male recipient mice of both groups revealed similar % chimerism suggesting that the difference observed at Week 8 might be an outlier.

In addition to the % chimerism, also the average indel percentage and the editing spectrum were evaluated in spleen and BM samples of the recipient mice at Week 8 and Week 20 post-engraftment. The average indel percentage revealed no significant differences between the input cells and the samples collected from the recipient mice after 8 and 20 weeks of engraftment, respectively.

2.5.4. Toxicology

The toxicity of exa-cel has been evaluated in an *in vivo* combined GLP toxicity and tumorigenicity study with a duration of 20 weeks in NSG mice. In addition, a battery of specialized genotoxicity studies consisting of in silico and *in vitro* safety assessments were conducted to evaluate the potential risks associated with off-target editing and chromosomal translocations. Finally, induction of innate immune responses upon treatment of primary $CD34^+$ hHSPCs with SPY101 gRNA and Cas9 protein has been evaluated *in vitro*.

2.5.4.1. Single dose toxicity

The single dose GLP toxicology study with an observation period of 20 weeks revealed that the single IV injection of 1 x 10^6 cells/animal of unedited or SPY101-RNP-edited $CD34^+$ hHSPCs into non-lethally irradiated NSG mice was well-tolerated. There were only minor changes associated with $CD34^+$ hHSPC administration, such as non-adverse hematological changes in red cell parameters (females) and minimal to moderate infiltration/hyperplasia of round cells in the white pulp of the spleen and the thymus observed in the majority of mice that received either unedited or edited $CD34^+$ hHSPCs. Overall, there is no evidence for an increased incidence of toxicologically relevant findings and/or tumorigenic events in edited or unedited groups compared to the vehicle control group. In addition, no toxicologically relevant differences were found between animals treated with cells derived from the different donors.

2.5.4.2. Repeat dose toxicity

A repeat-dose toxicity study was not performed and is not required as exa-cel is intended for single-dose administration only.

2.5.4.3. Genotoxicity

On-target editing:

Larger insertions and deletions at the on-target site as well as translocations were evaluated using long range PCR (LR-PCR) and hybrid capture sequencing. LR-PCR is amplifying a ~10kb region of the BCL11A locus and is therefore able to identify large indels including their size at the on-target site, while hybrid capture sequencing is able to identify translocations and large indels at the on-target site without discriminating between the two and without determination of the size of large insertions. By comparing the rate of insertions greater than 30bp determined by the two methods, the applicant determined the risk of translocations. Thereby, the applicant determined a mean large insertion rate of 4.0% by LR-PCR and a mean large insertion rate of 4.7% by hybrid capture sequencing, respectively. This difference turned out to be not statistically significant. In contrast, there was a statistically significant difference observed in the frequency of large deletions of more than 30bp, which mean rate was 6.3% for LR-PCR and 4.9% for hybrid capture sequencing. This difference was explained by the propensity for fragment with large deletions to be amplified with greater efficiency during the LR-PCR amplification step.

The size of deletions included up to ~ 7.5 kb, while the size of insertions included up to 5.5 kb.

Evaluation of chromosomal aberrations was complemented by karyotype analysis conducted on 240 metaphase cells, of which 60 cells were treated with SPY101-RNPs. Thereby, 5 cells showed chromosomal aberrations. Four of these cells were hypoploid cells that lost either one or two chromosomes. Thereby, chromosomal losses occurred in control cells without electroporation (one cell with loss of chromosome 9 and chromosome 18; one cell with loss of chromosome 3) and in cells treated with SPY101-RNPs (one cell with loss of chromosome 14; one cell with loss of chromosome 20). These 4 chromosomal aberrations are likely artifacts of the karyotype slide making process and the reported frequencies are within background levels. In addition, one cell treated with SPY101-RNPs revealed a partial loss of chromosome 2 (p10).

Off-target editing:

Potential off-target editing of the SPY101 gRNA has been assessed using a two-step process, including site nomination analysis as a first step and site confirmation analysis as a second step. For the site nomination step, the applicant conducted two orthogonal approaches to identify candidate off-target sites: a genome-wide computational homology searches for identification of candidate off-target sites based on sequence similarity to the on-target region (in silico analysis) and an empirical, cell-based,

homology-independent assay, called Genome-wide Unbiased Identification of Double-stranded breaks Enabled by sequencing (GUIDE-seq).

Three published computational tools have been used for the in silico analysis: CCTop, CRISPOR, and COSMID. For CCTop analysis, five mismatches were considered with regard to the most active protospacer adjacent motifs (PAMs), NGG and NAG. For other less optimal PAMs (NGA, NAA, NCG, NGC, NTG, NGT) up to 4 mismatches were considered. CRISPOR is a tool that implements many different published CRISPR on- and off-target scoring functions and uses a match algorithm that slightly differs from the algorithm of CCTop. The COSMID algorithm can detect off-target sites with indels (limited to up to 2 mismatches and 1 indel) also referred to as bulges. Thus, the COSMID algorithm complements the search done with CCTop and CRISPOR in this regard.

The homology off-target search was expanded to include common genetic variants that would increase homology to the on-target site if present. This analysis was based on candidate off-target regions with up to three mismatches, or up to two mismatches plus one gap homology to the SPY101 gRNA on-target sequence and a PAM of either NGG, NAG, NGA, NAA, NCG, NGC, NTG, or NGT. Thereby, single nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) > 10% and later with a MAF > 1% based on samples from the 1000 Genomes Project were considered.

For the homology-independent search, GUIDE-Seq analysis has been conducted. GUIDE-seq is an approach in which a double-stranded oligodeoxynucleotide (dsODN) is co-administered with the gRNA and the Cas9 nuclease into cells. Upon DSB, which may be a result of either Cas9 cleavage activity or another biological process beyond Cas9 cleavage, the short GUIDE-seq dsODN may then be incorporated into the site of the DSB. Since the dsODN contains a region that is distinct from all known endogenous sequences of the human genome, sites harbouring dsODN incorporations may be specifically amplified and subsequently sequenced using NGS.

GUIDE-Seq was initially performed in $CD34^+$ cells from one healthy donor. Thereby, dsODN concentrations > 1µM revealed signs of cytotoxicity as well as increased numbers of identified DSB sites. Despite the increased cytotoxicity observed at 2.5 and 5µM dsODN, all 857 sites detected in this study were included in the initial site confirmation step. Subsequently, a dose titration experiment was conducted and a dsODN concentration of 0.5µM dsODN resulting in at least 70% viability of $CD34^+$ cells was defined and used in an additional GUIDE-seq experiment carried out on cells from two additional donors. A set of 21 candidate off-target regions were identified in this study. All of these candidate regions were within intronic or intergenic regions. Remarkably, no overlap was observed between these 21 candidate off-target regions and the sites identified by GUIDE-seq in the initial study at 1µM dsODN concentration. Moreover, also no overlap was observed between the 21 regions identified by GUIDE-Seq and the 5,007 sites identified by in silico analyses. One site out of the 21 regions overlapped with a published list of low-complexity regions and was not included in the subsequent site confirmation analysis. Altogether, 52 regions identified by GUIDE-seq analyses were broken down to a total of 142 candidate cleavage sites and included in the subsequent site confirmation step.

In the site confirmation step, definitive confirmation of off-target editing at the candidate sites nominated by the nomination step is performed using hybrid capture based sequencing on SPY101-RNP treated and untreated control $CD34^+$ cells. Thereby, probes for the candidate off-target regions are used to selectively enrich for these regions during construction of DNA sequencing libraries. Subsequently, the libraries are deeply sequenced. The threshold of the hybrid capture deep sequencing with a median coverage of > 2,500 was set at 1% in initial studies and at 0.2% in subsequent studies using ultra-deep sequencing (> 10,000x coverage of candidate off-target sites). However, for ultra-deep sequencing the set of candidate off-target regions identified by in silico analyses were restricted. Instead of up to five mismatches, only up to three mismatches, or up to two mismatches plus one gap homology to the SPY101 gRNA on-target sequence and a PAM of either NGG, NAG, NGA, NAA, NCG, NGC, NTG, or NGT were considered. Altogether 223 sites were included in ultra-deep sequencing conducted on $CD34^+$

hHSPCs from four healthy donors edited with SPY101-RNP. Nine candidate sites were located within exons or exon/intron junctions, and two of them, transmembrane protein with EGF like and two follistatin like domains 2 (TMEFF2) and G protein-coupled receptor 161 (GPR161), are associated with tumorigenicity in gastric and breast cancer, respectively. However, TMEFF2 and GPR161 do not have a role in hematopoietic cancer and the site confirmation analysis did not confirm these sites as off-target sites. Indeed, hybrid capture deep sequencing did not detect any off-target editing.

In addition to the off-target evaluation conducted in cells from healthy donors, $CD34^+$ hHSPCs from SCD and TDT patients sourced from clinical trials sites from 6 different countries were also evaluated for off-target editing. GUIDE-seq was performed on SPY101-edited $CD34^+$ cells from three TDT and three SCD patients to identify candidate off-target sites. From the 64 candidate off target regions identified in this study, no common candidate GUIDE-seq regions were identified across all patient samples. However, three regions overlapped between particular pairs of patient samples and two regions overlapped with one of the 52 candidate GUIDE-seq regions identified previously with $CD34^+$ cells from healthy donors. One candidate off-target region was located in an exon sequence (BCAS2 Pre-mRNA Processing Factor). All other candidate off-target regions were located either in intronic or intergenic sequences. The newly identified candidate off-target regions of each individual TDT and SCD patient and the 9 candidate off-target regions from the analysis of SNP with an allele frequency > 10% based on samples from the 1000 Genomes Project, were evaluated together with the previously identified and evaluated 223 candidate sites in a hybrid capture ultra-deep sequencing study conducted on samples from the TDT and SDC patients. This study revealed four candidate off-target regions, of which two were nominally significant at the 0.2% threshold. However, the applicant does not consider these sites as real off-target sites due to the following reasoning: the regions lacked homology to the on-target site, the regions showed evidence of DSBs or indels in untreated controls, and the regions did not exhibit a pattern of edits consistent with a single CRSIPR/Cas9 cleavage site (indels that line up at a single cleavage site). Moreover, both regions were located in a ~ 500-bp intergenic window in a centromeric satellite repeat region of chromosome 3 that is prone to show naturally-occurring DSBs.

Finally, also 7 GMP Process qualification lots were included in on- and off-target evaluation using hybrid capture sequencing with no evidence of off-target editing.

Hybrid capture deep sequencing did not confirm any off-target editing in any of the studies conducted.

2.5.4.4. Carcinogenicity

A pilot study has been conducted to establish the HL-60 positive control in NSG mice. Thereby, a dose level of 0.2×10^6 HL-60 cells/animal and a study duration of 40 days (~ 6 weeks) has been defined for the positive control group of the combined GLP toxicity and tumorigenicity study.

SPY101-RNP-edited $CD34^+$ hHSPCs were administered at a dose of 1×10^6 cells/animal, while 1×10^6 unedited $CD34^+$ hHSPCs/animal and injection buffer only were used as negative controls. Engraftment of cells from all three donors used for establishing the three toxicology batches was confirmed by flow cytometry. Unedited and SPY101-RNP-edited cells of the same donor revealed comparable engraftment in whole blood, spleen, and BM after 20 weeks post-engraftment. While palpable masses and the presence of atypical cells in blood smears were observed in mice that received 0.2×10^6 HL-60 cells, no signs of tumorigenic events were observed in mice administered SPY101-RNP-edited or unedited $CD34^+$ hHSPCs.

2.5.4.5. Reproductive and developmental toxicity

No stand-alone reproductive or developmental toxicity studies were performed or planned for exa-cel. This is acceptable considering the absence of toxicity in reproductive organs in the 20-week study in

NSG mice and the type of the product. Also, the potential risk of inadvertent germline transmission is generally considered low for genetically modified cells.

2.5.4.6. Toxicokinetic data

Toxicokinetic data are not applicable for this type of product.

2.5.4.7. Local tolerance

Local tolerance at the injection site has been assessed as part of the combined toxicity and tumorigenicity study. No test-related findings were observed at microscopical examination of the injection site.

2.5.4.8. Other toxicity studies

In vitro evaluation of induction of type I interferon innate immune response upon SPY101-RNP-treatment of $CD34^+$ hHSPCs did not reveal an innate immune response.

2.5.5. Ecotoxicity/environmental risk assessment

Exa-cel is a cellular product consisting of autologous CD34+ human hematopoietic stem and progenitor cells (hHSPCs) modified by CRISPR/Cas9-mediated gene editing. As exa-cel is considered a GMO, a GMO environmental risk assessment was done to estimate the risk of exa-cel to third parties and the environment.

Manufacturing of exa-cel does not require a viral vector for genetic modification. Exa-cel is manufactured ex vivo via the transient introduction of the CRISPR-Cas9 editing system comprising the SPY101 gRNA and the Cas9 nuclease protein. As the editing complex is not present in the drug product, there is no risk of editing the cells of a non-target human in the event of accidental transfer.

As exa-cel is a human-derived cellular product, protective measures on handling and administration, disposal, and accidental exposure are in place in the SmPC. The protective measures aim at avoiding potential transmission of infectious diseases.

Overall, the clinical use of exa-cel provides a negligible risk for the environment and for the third parties.

2.5.6. Discussion on the non-clinical aspects

Pharmacology:

In vitro and *in vivo* studies confirmed successful gene editing at the target site in $CD34^+$ hHSPCs and demonstrated an increase in γ-globin and HbF expression upon erythroid differentiation in cells derived from healthy donors as well as in cells from β-Thalassemia and SCD patients. In addition, the applicant could demonstrate that the viability of the edited cells as well as their differentiation potential remains largely unaffected when compared to appropriate control cells.

Healthy donor hHSPCs were collected using multiple mobilisation methods such as G-CSF and Dual (G-CSF+Plerixafor). No differences in on-target editing frequency or γ-globin upregulation in cells was observed when comparing used mobilisation methods.

When comparing data across different *in vitro* studies, there was quite a large variability in the reported γ/α-globin mRNA ratios and γ/(γ+β)-globin mRNA ratios. This variability has been explained by the applicant by donor-to-donor variability in baseline γ-globin expression.

In order to support the mode of action of exa-cel, the applicant provided additional non-clinical data demonstrating both a correlation between the percentage of edited cells and percentage of BCL11A downregulation and a correlation between percentage of BCL11A downregulation and percentage of γ/α-globin increase.

Regarding the indel pattern with the three major indel species -15, +1, and -13 at the on-target site in SPY101-RNP-edited $CD34^+$ hHSPCs, the applicant clarified that the indel pattern seen is non-random, unique to the target, and consistent across donors. Thereby, the +1-thymidine insertion observed at position 20 with a frequency of 30%–35% in edited cells from three different donors, represents an insertion that is commonly seen upon NHEJ following an RNA guided Cas9 nuclease induced DNA DSB when a thymine "T" nucleotide is at the -4-position upstream of PAM.

The frequencies of some unique indels observed in input cells changed considerably during an engraftment period of 16- or 20-weeks in NSG mice. According to the applicant such changes are expected due to (a) the oligoclonal nature of reconstitution in the mouse and (b) the fact that only a small fraction of the input cell population represents LT-HSC that contributed to long-term reconstitution after engraftment.

Pharmacokinetics:

The biodistribution study demonstrated that exa-cel engrafted to the hematopoietic system in NSG mice, and in lesser extend to the non-hematopoietic tissues.

Engraftment of human cells was highest into the bone marrow (47 - 74 chimerism %) and spleen (25 - 81 chimerism %) after 8 Weeks of exa-cel administration and was persisting at least 20 Weeks. Thereby, large donor-to-donor differences of human engraftment were commonly seen, which were most likely due to differences in the frequency of LT-HSC present within the $CD34^+$ cell population used for engraftment.

At 20 Weeks, the engraftment %/persistence was generally lower than at Week 8. Especially in blood, engraftment of exa-cel resulted in only 1 - 8% $CD45^+$ cells after 20 Weeks. Decreases in human engraftment over time in the humanized NSG mouse model are expected and explainable at least partially by differences in mouse compared to human growth factors in the BM niche, which renders the conditions suboptimal for human HSPC self-renewal and differentiation. Usually, higher levels of human cell engraftment was observed in the spleen and BM, while a low rate of % chimerism (hCD45/$CD45RA^+$ leukocytes) was seen amongst blood cells. This observation is in line with published data.

Generally, the editing process did not affect engraftment, biodistribution, or persistence of hHSPCs. The only statistically significant difference in chimerism % between edited and unedited controls was observed in one study in male mice using flow cytometry. Unlike flow cytometry, qPCR did not reveal a statistically significant difference in the same study, and also the 20-week toxicity and tumorigenicity study (Study 1016-2465) conducted with the same donor cells did not show statistically different engraftment in male mice at Week 20 between unedited and SPY-101 edited cells.

Finally, the list of selected organs and tissues analysed in the biodistribution study using qPCR to detect human cells in non-target tissues in NSG mice (Study 1016-2475) has been provided and sufficiently justified.

Toxicology:

The pivotal single dose toxicity and tumorigenicity study has been conducted using lots from three healthy donors. The lots were manufactured under non-GMP conditions in a non-GMP facility using the

same process and same manufacturer as for GMP material. In addition, the source for SPY101 bulk was different between the pre-clinical material and the clinical material. However, the applicant performed comparison of release test data for lots manufactured with SPY101 from the different laboratories. Thereby, no differences in the functional performance or quality between the toxicology and process qualification lots became evident. Thus, the material used in the single dose toxicology study is considered adequate.

The dose level of 1 x 10^6 $CD34^+$ hHSPCs/mouse administered in the single dose toxicity study is acceptable, despite the fact that there is only a 1.6-fold safety margin as compared to the max. human dose level of 20 x 10^6 cells/kg. However, the main safety concern of exa-cel is associated with editing at critical potential off-target sites and associated genotoxicity that might induce tumorigenic events. This risk is addressed mainly in the non-clinical genotoxicity evaluation, which included evaluation of both on-target and off-target editing.

On-target editing evaluation was conducted using LR-PCR and hybrid capture sequencing for determining the frequency and size of large insertions and deletions of more than 30bp. The same two methods were also used for an indirect determination of the risk of chromosomal translocations by comparing the rates of large insertions determined by the two methods. While the data of this comparison are not indicative of a significant translocation rate, determination of the actual translocation rate is more difficult using such an indirect approach. Both differences in the mean large insertion rates determined by LR-PCR and hybrid capture sequencing and differences observed between the individual samples of the same donor seem to be within the variability of the methods used as implicated by the reproducibility assessment of the two methods. Thus, small percentages of chromosomal translocations might occur that remain undetected by the chosen indirect approach. However, as the likelihood of detecting chromosomal translocations using other methods such as CAST-Seq is expected to be low, omission of additional chromosomal translocation studies is acceptable. As exa-cel did not reveal off-target editing, chromosomal translocations would most likely occur at the on-target site. If not eliminated by apoptosis, HSCs with a disrupted BCL11A expression due to chromosomal translocations would be expected to undergo negative selection, as the loss of BCL11A expression has been shown to impair the ability of HSCs to repopulate the BM (Luc et al., 2016, Cell Rep. 16:3181-94.). Thus, the potential oncogenic risk of exa-cel that could be associated with induction of chromosomal translocations and aberrations is expected to be low.

Karyotype analysis might, in principle, also be used to evaluate chromosomal translocation and aberrations. However, even the applicant indicated that the method is relatively insensitive based on the fact that rare aberrations and aberrations that are smaller than 5 Mb in size cannot be detected. Thus, the sensitivity of the karyotype analysis remains elusive. In addition, the results of the karyotype analysis are not easy to interpret, as artifacts seem to be quite common. Thus, karyotype analysis is not considered as a suitable method for evaluating the frequency of chromosomal translocations and aberrations induced by SPY101-RNP-treatment. Apart from these fundamental difficulties, karyotype analysis revealed a partial loss of chromosome 2 upon editing using SPY101-RNPs. This partial loss was observed only once and located >30 mega base pair (Mbp) away from the *BCL11A* gene at 2p16.1. It was concluded that this chromosomal aberration is non-clonal and that no high-frequency recurrent chromosomal aberrations were observed from CRISPR/Cas9-edited $CD34^+$ hHSPCs after 5 days in culture.

The potential cellular impact of large deletions and insertions at the on-target site has been discussed by evaluating whether or not such indels could affect the coding sequence of BCL11A. As the target site of SPY101 is located >25 kb from the nearest exon, it is not expected that expression of BCL11A will be affected in other cell types than erythrocytes. In addition, the applicant clarified that the only known regulatory sequences located close to the SPY101 target site are regulatory sequences involved in HbF expression in erythroid lineage cells.

For determination of potential off-target sites, two orthogonal methods (in silico analysis in combination with an unbiased cell-based) were used, which is quite standard and acceptable for identification of potential off-target sites.

The number of mismatches considered has been indicated for all in silico analyses (CCTop, CRISPOR, and COSMID) and no additional threshold or filters beside the 3 mismatches or 2 mismatches and 1 gap used in the second and third off-target assessments (RES-IND-042 and R264) were introduced.

Pairwise Jaccard similarity between each pair of the three methods (CCTop, CRISPOR, and COSMID) revealed only low similarities ranging from 0.0048 to 0.1532. However, since the three methods were chosen in order to complement each other and to capture the full homology space relative to the SPY101 protospacer sequence, the low similarities are acceptable.

For considering the potential of genetic variants to impact off-targeting editing activity, SNPs with a MAF > 1% based on samples from the 1000 Genomes Project were considered. Thereby, 50 additional sites were nominated that were validated using samples from 3 SCD and 3 TDT patients. While validation of these additional sites is endorsed, it is likely that only a small portion of SNPs with a frequency of 1% will be represented in the samples from these 6 patients. Therefore, the applicant provided an annotated list of the sites identified through the variant-aware homology search and tested all 50 sites for proximity to the 49 genes listed in the Washington University Pathology Services MyeloSeq™-HD panel (https://pathologyservices.wustl.edu/items/myeloseq/) that are known to be recurrently mutated in myeloid neoplasms. Thereby, no overlap was identified by the applicant. Indeed, the vast majority of the sites identified through the variant-aware homology search may be considered uncritical. Some uncertainties remain with regard to a nominated off-target site within an intronic sequence of *DLC1* and a nominated off-target site at an exon/intron boundary (with an intronic predicted cut-site) of *ATM,* as both genes have been associated with hematological malignancies (Ullmannova-Benson et al., 2009 Leukemia 23:383-390; Gumy-Pause et al., 2004, Leukemia 18: 238-242). However, it is neither known whether these potential off-target sites would actually result in off-target effects in the presence of the respective SNP, nor whether off-target effects at these sites would indeed predispose to hematological malignancies.
Remaining uncertainties with regard to potential off-target effects due to genetic variants have been addressed by including "gene editing-related oncogenesis" as an important potential risk in the RMP and by collecting adverse events of malignancies in clinical trials and in the post-marketing setting.

The use of a cell-based assay such as GUIDE-seq instead of a biochemical assay is justifiable based on the fact that exa-cel is relying on an ex-vivo gene editing approach and that $CD34^+$ hHSPCs have been evaluated in the GUIDE-seq. Biochemical assays use purified chromatin-free DNA and therefore usually reveal larger numbers of candidate off-target sites as compared to GUIDE-seq. However, since the chromatin state of the DNA is not considered in biochemical assay, it is likely that the number of false-positive sites is increased by biochemical assays (Bao et al., 2021, Nat Protoc. 16:10-26).

Overall, the number of different samples and donors used in GUIDE-seq analyses for the nomination of candidate off-target sites and in hybrid capture deep or ultra-deep sequencing for the confirmation of candidate sites is considered adequate.

It is recognized that hybrid capture deep sequencing did not detect any off-target editing in any of the studies conducted. However, some regions of the human genome are not able to reliably be assessed by hybrid capture sequencing. Therefore, regions that for example have high/low % GC, or contain highly repetitive regions or regions that are otherwise difficult to be sequenced, have been excluded from the analysis. While the applicant has set criteria on the exclusion of such sites, and provided justifications on each of these criteria, it is a matter of fact that not all of the potential off-target sites could be included in the validation phase of the analysis. Although, the excluded sites were generally not the most homologous sites and thus, do not have the highest likelihood for off-target effects, some potential off-target sites might have been missed in the confirmation step.

Moreover, only up to 3 mismatches, or up to 2 mismatches plus 1 gap homology to the SPY101 gRNA on-target sequence and a PAM of either NGG, NAG, NGA, NAA, NCG, NGC, NTG, or NGT were considered in the hybrid capture sequencing analysis using ultra-deep sequencing. While this decision is understandable from a technical point of view, as it allows to cut down the number of sites from the previously 5,007 sites identified to 173 candidate sites, it does leave out any potential off-target sites that are expected to occur at lower frequencies due to a higher number of mismatches. It could be argued that candidate sites with up to 3 mismatches have a higher likelihood to be confirmed as off-target sites, and as not detected by hybrid captured ultra-deep sequencing, also sites with more than 3 mismatches are unlikely to be confirmed as off-target sites. For further justifications on the number of mismatches considered in the validation phase, the applicant indicated that an exploratory analysis with an indel frequency threshold of 0.2% was conducted in the initial hybrid capture deep sequencing experiment considering sites with up to 5 mismatches (CTxSR-015), which did also not detect any off-target editing. Moreover, the applicant indicated that the GUIDE-Seq is expected to detect any real off-target sites including sites with >3 mismatches. In addition to the discussion, the applicant also conducted an additional homology search starting with the 49 genes listed in the Washington University Pathology Services MyeloSeq™-HD panel and investigated the homology major transcript of each gene to the SPY101 gRNA protospacer sequence in order to find the site with the minimal number of mismatches and an NGG PAM relative to the SPY101 protospacer sequence. Genetic variants with a global MAF ≥1% from the 1000 Genomes Project were accounted for when calculating sequence similarity. A total of 7 genes were identified, mapping to 10 intronic regions with 6 mismatches, while no sites with 6 or fewer mismatches were identified due to genetic variants. No genes with less than 6 mismatches were identified. Although this additional homology search was restricted in several ways including (a) evaluation of genes involved in MDS and AML only, and (b) evaluation of homology between the SPY101 gRNA protospacer sequence and intronic and coding sequences of the major transcript of each gene, the totality of all data addressing potential off-target editing indicate a low likelihood of exa-cel for inducing off-target editing including off-target editing at critical sites.

With regard to the evaluation of tumorigenicity in the NSG mice, the lack of tumorigenic signs in mice treated with exa-cel is acknowledged. However, the validity of the animal model used is questionable. While the 20-week study duration supports using the positive control cell line HL-60, which efficiently induces tumors and a median survival of 4-8 week when administered to NSG mice, the limited duration of the study is likely too short to allow rare events of critical off-target editing or translocations to develop to palpable masses and/or present atypical cells in blood smears or other tumorigenic signs. However, the study duration can hardly be increased due to the graft-versus-host disease, which is expected to start 24 weeks after administration of $CD34^+$ hHSPCs. Moreover, such a study has to use SPY101-RNP-edited $CD34^+$ hHSPCs in immunosuppressed animals, as any other surrogate gRNA and/or surrogate cells would be considered as not relevant. In order to prove such a model as valid, a positive control harboring rare events of targeted editing at a critical site, such as a tumor suppressor gene, would be required. However, whether validation of such a model could be successful and proved to be sensitive enough for detecting critical off-target editing occurring at a low or very low frequency is uncertain. Thus, the potential genotoxic/tumorigenic risk of exa-cel is solely judged by the *in vitro* studies investigating off-target gene editing and chromosomal translocations.

Environmental risk assessment:

Exa-cel is not expected to pose a risk to the environment.

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

2.5.7. Conclusion on the non-clinical aspects

Overall, the primary pharmacodynamic studies provided adequate evidence that potential beneficial levels of genome editing and HbF expression can be reached, despite the lack of a relevant disease animal model. In addition, the viability of the SPY101-RNP-edited cells as well as their differentiation potential remains largely unaffected when compared to appropriate control cells.

The pharmacokinetic investigation of exa-cel focused on the *in vivo* engraftment and persistence of the edited human cells in sub-lethally irradiated NSG mice, the most relevant animal model for non-clinical efficacy and safety evaluation of exa-cel. The pharmacokinetic study confirmed that exa-cel is able to engraft and persist in hematologic tissues in a comparable way as unedited human $CD34^+$ HSPCs. In addition, the overall percent editing remained similar over time.

The GLP-compliant toxicity and tumorigenicity study of exa-cel did not raise any safety concerns. However, the significance of the *in vivo* model used for addressing the tumorigenic risk associated with potential off-target editing and/or chromosomal translocations induced during the editing process of the $CD34^+$ hHSPCs is limited, as the validity of the model remains questionable. Thus, the tumorigenic risk of exa-cel needs to be addressed primarily by the evaluation of the potential of SPY101-RNP to induce off-target editing and chromosomal aberrations, which has been sufficiently addressed using in silico and *in vitro* approaches.

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

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

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

- **Tabular overview of clinical studies**

Study Identifier; Type of Study; Country; Study Status	**Study Design; Type of Control**	**Study Objectives**	**Test Product(s); Dosage Regimen; Route of Administration**	**Number of Subjects Dosed With Study Drug[a]; Population**	**Duration of Treatment**
CTX001-111; Phase 1/2/3 (safety and efficacy); United States, United Kingdom, Italy, Germany, Canada; Ongoing	Single-arm, open-label	Primary • To evaluate the safety and efficacy of a single dose of autologous CRISPR/Cas9 modified CD34+ hHSPCs (exa-cel) in subjects with TDT Secondary • To quantify percentage of edited alleles in peripheral blood leukocytes and CD34+ cells of the bone marrow • To assess the production of HbF post-exa-cel infusion • To assess the effects of infusion of exa-cel on	Exa-cel; single dose administration; minimum recommended dose of exa-cel is 3×10^6 CD34+ cells/kg; Intravenous use	48 subjects; Males and females with TDT aged 12 to 35 years (inclusive)	Single dose

Study Identifier; Type of Study; Country; Study Status	Study Design; Type of Control	Study Objectives	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects Dosed With Study Drug[a]; Population	Duration of Treatment
		disease-specific events and clinical status			
CTX001-121; Phase 1/2/3 (safety and efficacy); United States, United Kingdom, Italy, Germany, Canada, Belgium, France; Ongoing	Single-arm, open-label	Primary • Evaluate the safety and efficacy of a single dose of exa-cel in subjects with severe SCD Secondary • Assess the effects of infusion of exa-cel on disease-specific events and clinical status • Quantify gene editing efficiency	Exa-cel; single dose administration; minimum recommended dose of exa-cel is 3×10^6 CD34+ cells/kg; Intravenous use	35 subjects; Males and females with severe SCD aged 12 to 35 years (inclusive)	Single dose
VX18-CTX001-131; Long-term follow-up in subjects with either TDT or severe SCD; United States, United Kingdom, Germany, Canada; Ongoing	Single-arm, open-label	Primary • Evaluate long-term safety up to 15 years after exa-cel infusion in subjects who received a single dose of exa-cel for treatment of TDT or SCD Secondary • To evaluate efficacy of exa-cel up to 15 years after exa-cel infusion in subjects who received exa-cel for treatment of TDT or severe SCD	No study drug administration	No study drug administration; Subjects who complete or discontinue the parent study (CTX001-111 [TDT]; CTX001-121 [SCD]) after exa-cel infusion	No study drug administration
Study 151; Phase 3 (safety and efficacy); United States, United Kingdom, Germany, Italy; Ongoing	Single-arm, open-label	Primary • Evaluate the efficacy of a single dose of exa-cel in pediatric subjects with severe SCD Secondary • Evaluate the safety and tolerability of a single dose of exa-cel • Assess the effects of infusion of exa-cel on disease-specific events and clinical status • Quantify gene editing efficiency	Exa-cel; single dose administration; minimum recommended dose of exa-cel is 3×10^6 CD34+ cells/kg; Intravenous use	A minimum of 10 subjects dosed with exa-cel; males and females with severe SCD 2 through 11 years of age; as of 11 September 2023, 9 enrolled, one dosed	Single dose
Study 161; Phase 3b (efficacy and safety); United States, Germany, Italy, planned in Kingdom of Saudia Arabia; Ongoing	Single-arm, open-label	Primary • HbF and Hb levels over time. Secondary • Evaluate efficacy and safety of a single dose of exa-cel in adolescent and adult subjects with either TDT or severe SCD • Assess the effects of infusion of exa-cel on disease-specific events and clinical status • Quantify gene editing efficiency	Exa-cel; single dose administration; minimum recommended dose of exa-cel is 3×10^6 CD34+ cells/kg; Intravenous use	Approx. 26 subjects; Males and females with TDT or severe SCD 12 to 35 years of age (inclusive); as of 11 September 2023, 7 subjects with TDT and 10 subjects with SCD have enrolled and 0 subjects have been dosed	Single dose
Study 171; Phase 3 (efficacy and safety); United Kingdom, and Italy;	Single-arm, open-label	Primary • Evaluate the efficacy of a single dose of exa-cel Secondary	Exa-cel; single dose administration; minimum recommended dose of exa-cel	Approx. 16 subjects; Males and females with severe SCD 12 through 35 years of age	Single dose

Study Identifier; Type of Study; Country; Study Status	Study Design; Type of Control	Study Objectives	Test Product(s); Dosage Regimen; Route of Administration	Number of Subjects Dosed With Study Drug[a]; Population	Duration of Treatment
Planned		• Safety of exa-cel • Assess the effects of infusion of exa-cel on disease-specific events and clinical status • Quantify gene editing efficiency	is 3 × 10^6 CD34+ cells/kg; Intravenous use	(inclusive), βS/βC genotype	

Abbreviations: Cas9: CRISPR-associated protein 9; CRISPR: clustered regularly interspaced short palindromic repeats; exa-cel: exagamglogene autotemcel (formerly CTX001); HbF: fetal hemoglobin; hHSPCs: human hematopoietic stem and progenitor cells; SCD: sickle cell disease; TDT: transfusion-dependent β-thalassemia.

[a] Number of subjects dosed as of 06 September 2022 (Study 111) and 16 September 2022 (Study 121).
[b] No data for studies 151, 161, and 171 have been submitted for the MAA, but these studies are relevant for the SOBs for TDT and SCD or SCD alone, respectively.

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

No dedicated clinical pharmacology studies were conducted given that exa-cel is a cell and gene therapy.

Exa-cell is an autologous HSCT requiring mobilisation of CD34+ cells, myeloablation, infusion of exa-cel, as well as engraftment and persistence of (edited) cells.

Busulfan was used as a single agent for myeloablative bone marrow conditioning in the clinical studies. In Studies 111 and 121, busulfan was administered intravenously (IV) daily for 4 consecutive days and the dose was individually adjusted based on PK monitoring. Key PK data on the busulfan mono-based myeloablative regimen and their relationship to neutrophil and platelet engraftment has been submitted. For TDT, mean administered busulfan dose was 3.54 mg/kg/day for the q6h regimen and 3.87 mg/kg/day for the qd regimen. For SCD, the mean (SD) administered busulfan dose was 2.98 mg/kg/day for the q6h regimen and 3.30 mg/kg/day for the qd regimen. For both indications, myeloablation as performed resulted in profound neutropenia and engraftment of edited cells in all evaluable patients. No clinically relevant effects of age at screening, sex, or genotype on busulfan cAUC were observed. No clinically relevant effects of race were apparent. There were no clinically relevant effects of busulfan cAUC or busulfan dose regimen (q6h or qd) on time to neutrophil or platelet engraftment.

In the evaluable patients, i.e. n=54 for TDT and n=43 for SCD, all patients achieved neutrophil engraftment. Time to neutrophil engraftment in median (range) was 29 (12 to 56) days for TDT and 27.0 (15 to 40) days in SCD patients. Of the 97 subjects (54 subjects with TDT and 43 subjects with SCD) across both studies who achieved neutrophil engraftment, all patients also achieved platelet engraftment. The respective time to platelet engraftment in median (range) was 44 (20 to 200) days for TDT and 35.0 (23 to 126) days for SCD. 1 subject with TDT was pending platelet engraftment at the time of the data cutoff date (16 April 2023), but was confirmed to have achieved platelet engraftment with the timeframe given above.

For autologous HSCT, the minimal number of CD34+ cells is ≥2.0 x 10^6 CD34+ cells/kg bodyweight (BW) (The EBMT Handbook, 2018). In the pivotal studies, the protocol-defined **dose** was a minimum of 3.0 × 10^6 CD34+ cells/kg and a maximum of 20 × 10^6 CD34+ cells/kgBW. All subjects were infused per protocols. During the studies, an adjustment was made to the exa-cel drug product calculation to account for the density coefficient of the final formulation medium and doses were recalculated. Recalculation of doses of already infused patients found that three patients in study 121 received an infusion of

2.9 × 10^6 CD34+ cells/kgBW, for who efficacy (including PD) were reported "indistinguishable from those who received the protocol-defined minimum dose or higher", i.e. ≥3.0 x 10^6 CD34+ cells/kgBW.

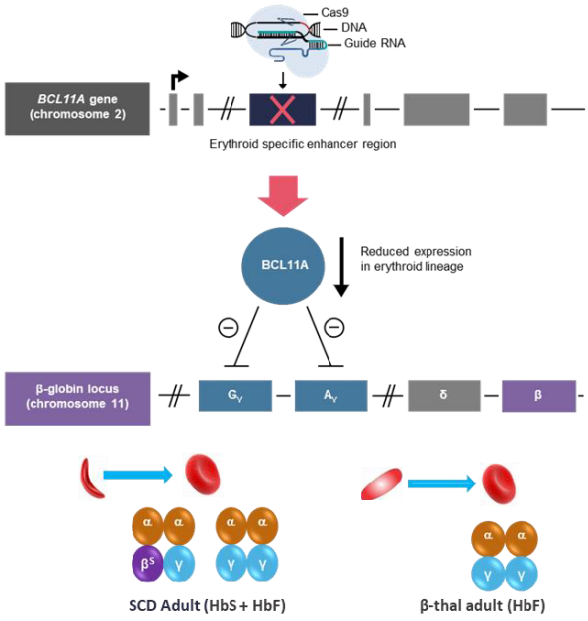
The recommended dose of exa-cel is the same for both indications (TDT and SCD) regardless of age, sex, or genotype: a single, minimum IV dose of at least 3.0 × 10^6 CD34+ cells/kgBW.

2.6.2.2. Pharmacodynamics

Mechanism of action

The *ex vivo* genetic modification of the CD34+ cells is expected to result in reduction of BCL11A gene transcription and subsequent decrease in BCL11A protein level with concomitant increases in γ-globin expression, and, upon erythroid differentiation, increased levels of HbF *in vivo*.

Figure 3 Mode of Action of BCL11A Suppression and Resultant γ-globin De-repression



Source: Modified from Canver and Orkin, 2016[32]
β-thal: β-thalassemia; CRISPR: clustered regulatory interspace short palindromic repeats; Cas9: CRISPR-associated 9 nuclease; HbF: fetal hemoglobin; HbS: sickle hemoglobin; SCD: sickle cell disease

Primary and Secondary pharmacology

Pharmacodynamic parameters were evaluated both in the pivotal study for each sought indication (studies 111 and 121), as well as the LTFU study 131.

Erythropoiesis occurs primarily in bone marrow. Determination of intracellular BCL11A protein levels in erythroid precursor cells as a primary pharmacology parameter is not feasible. Subsequent to BCL11A protein decrease, there is a γ-chain-mRNA and -protein synthesis.

Haemoglobin formation depends on availability of α- and non-α-chains. Haemoglobin of α- and γ-chain composition is HbF, which is quantifiable in peripheral blood in (mature) erythrocytes and thus considered a PD parameter.

Presence of intended genetic modification

The proportion of alleles with intended genetic modification were determined in the exa-cel product (see also quality section), in peripheral blood (leukocytes) over time, and in the CD34+ cells of the bone

marrow (BM) over time. Per protocols, bone marrow sampling was scheduled for months 6, 12 and 24 after exa-cel infusion, while blood sampling for allelic editing determination is more frequent, monthly for Month 1 through Month 12 and then every 6 months through Month 24.

Commercial kits are used for genotyping, DNA extraction from whole blood, CD34+ isolation from bone marrow and in the DNA extraction from CD34+ cells.

For **TDT**, the available data (n=44) reports a mean (SD) of intended genetic modification in the CD34+ cells in BM at month 6 post-treatment of 78.48% (11.39%), that "remained stable for each subject for the duration of follow-up through Month 48". For **SCD**, the available data (n=37) reports a mean (SD) of intended genetic modification in the CD34+ cells in BM 6 months post-treatment of 86.12% (7.54%), that "remained stable for the duration of follow-up (up to Month 24[…])".

For TDT, expected "distributions of the percent difference in bone marrow $CD34^+$ Percent Editing from Percent Editing in the Drug Product at Months 6, 12, and 24 following exa-cel" was provided, indicating, the majority of patients have up to 25% less editing in bone marrow; the upper and lower range are minus 50% and ~40%, "partly explained by the difference between the editing of the long-term HSC and editing of other progenitor-cell populations contained in the $CD34^+$ cells". The respective data for SCD indicated that all patients have less than a 25% difference between bone marrow and drug product editing.

HbF concentration

HbF is determined prior to and at least three-monthly after treatment. HbF was considered attributable to exa-cel treatment starting 60 days after the last RBC transfusion (a washout period based on the half-life of a circulating RBC). Following exa-cel treatment, HbF increased in all evaluable patients.

For **TDT**, the available data is summarized as "Mean (SD) HbF levels were 7.8 (2.9) g/dL at Month 3, and were maintained with mean ≥10.9 g/dL from Month 6 onward.

For **SCD**, the available data is summarized as mean (SD) HbF levels were 4.5 (1.4) g/dL at Month 3, "being stable over time, including after Months 24".

Total Hb concentration and HbF/Hb ratio

Total Hb is a composition of HbF and other haemoglobins, i.e. HbA, HbS, HbE. Total Hb, HbF and HbF/Hb ratio were considered attributable to exa-cel treatment starting 60 days after the last RBC transfusion (a washout period based on the half-life of a circulating RBC). Hb has (age-) and sex-specific reference ranges.

As the transfusion threshold is based on total Hb, total Hb is a relevant parameter for TDT. For TDT, the target Hb is indicated as ≥9 g/dL (see primary endpoint); notably, transfusion thresholds are sex-independent, but may be adapted based on co-morbidities and/or outer conditions. For SCD, the clinical development is based on the expectation that 20% HbF is protective and eliminates VOCs, extrapolating experience in SCD patients with hereditary persistence of foetal haemoglobin. Thus, for SCD the proportion of HbF to total Hb is relevant.

Total Hb for TDT and HbF/Hb ratio for SCD are detailed in the section clinical efficacy.

2.6.3. Discussion on clinical pharmacology

In principle, treatment of TDT and SCD with exa-cel is an autologous HSCT. *Ex vivo* genome editing of autologous CD34+ aims at reduction of BCL11A gene transcription and subsequent decrease in BCL11A protein level with concomitant increases in γ-globin expression, and, upon erythroid differentiation, increased levels of HbF *in vivo*. Increased levels of HbF are expected to result in increased endogenous

total Hb in TDT and decreased HbS in SCD, thus alleviating transfusion burden in TDT and reduction of VOCs in SCD. A possible impact of the higher oxygen affinity of HbF on erythropoietin secretion and HbS levels over time has been discussed by the applicant; it was concluded that exa-cel results in substantial increases of total Hb, which off-sets the predicted increase in O2 affinity associated with HbF and therefore is not expected to result in any deficit of tissue oxygen.

Due to the nature of exa-cel, standard approaches to PK (ADME) do not apply; the respective statement in SmPC section 5.2 is agreed. Still, the engraftment of ex vivo edited CD34+ cells to demonstrate viability, cellular function and differentiation potential, the extent of intended genetic modification, and HbF as measurable clinical readout of the intended genetic modification are evaluable.

The pivotal studies in both indications are ongoing; n=59 have been enrolled into study 111 (TDT), n=63 in study 121 (SCD). Submitted was data of an interim analysis with varying numbers of patients evaluable for pharmacology information over time with September 2022 data cut, updated with data as of 16-Apr-2023.

Mobilisation of CD34+ and myeloablation as well as (temporary) discontinuation of standard-of-care medication are required steps before exa-cel infusion. SmPC section 4.5 includes respective language. The high number of CD34+ cells starting material required for exa-cell manufacturing has been scrutinized in the quality assessment. The number of patients having discontinued for not wanting to undergo an addition apheresis, or insufficient number of manufactured cells despite multiple collections resulted in a special warning on risk of insufficient mobilisation/apheresis in patients with SCD. Additionally, a minimum target number of CD34+ cells to be collected has been included in SmPC section 4.2.

Information was provided on the busulfan-mono myeloablation regimen applied in the clinical studies, which is also reflected in SmPC section 5.1. Regarding safety of mobilisation and myeloablation regimen, see clinical safety section.

The recommended dose of exa-cel is at least 3.0×10^6 CD34+ cells/kgBW, administered intravenously. This dose exceeds the minimum number of CD34+ recommended for autologous HSCT. In the clinical studies, doses of $\geq 3.0 \times 10^6$ CD34+ cells/kgBW in TDT and $\geq 2.9 \times 10^6$ CD34+ cells/kgBW in SCD resulted in all evaluable patients in neutrophil and platelet engraftment, as well as induction or increase of HbF expression in all patients.

The SmPC cautions that platelet engraftment times are longer than those for allo-HSCT, with details on platelet engraftment times observed in the clinical studies included in the SmPC.

Differing allelic editing in BM compared to product batches was explained by a difference between the editing of the long-term hematopoietic stem cells (LT-HSC) and the editing of other progenitor cell populations contained in the CD34+ cells. Regarding TDT patients with HbF data being substantially lower than in other patients with similar allelic editing, a [remarkably] slow overall haematopoietic recovery was argued. Cases of overall slower haematopoietic recovery in TDT patients are reflected in the SmPC. In addition, monitoring of complete blood counts and transfusion needs is detailed in SmPC section 4.2 on standard procedures for patient monitoring.

2.6.4. Conclusions on clinical pharmacology

Absence of standard ADME information is acceptable given the nature of exa-cel. Provided information supports adequacy of the applied busulfan-mono myeloablation regimen, and adequacy of the proposed dose of $\geq 3.0 \times 10^6$ CD34+ cells/kgBW for engraftment of the edited cells as demonstrated by neutrophil and platelet engraftment. Available clinical data confirms an increase in HbF attributable to exa-cel.

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

2.6.5. Clinical efficacy

Transfusion-dependent thalassemia

2.6.5.1. Dose response study

No dose-response studies have been conducted, see also section clinical pharmacology.

2.6.5.2. Main study

Study CTX001-111: A Phase 1/2/3 Study of the Safety and Efficacy of a Single Dose of Autologous CRISPR-Cas9 Modified CD34+ Human Hematopoietic Stem and Progenitor Cells (hHSPCs) in Subjects With Transfusion-dependent β-Thalassemia

Study 111 is a single-arm, open-label, multi-site, single dose, Phase 1/2/3 study in subjects 12 to 35 years of age who have TDT. Patients had to have a documented homozygous β-thalassemia or compound heterozygous β-thalassemia including β-thalassemia/hemoglobin E (HbE). Transfusion dependence was defined as a history of at least 100 mL/kg/year or 10 units/year of packed red blood cell (RBC) transfusions in the 2 years before signing the ICF.

The study is ongoing; at time of latest data cut, n=59 patients have been enrolled, and n=54 had been dosed (data cutoff date: 16 April 2023).

Methods

- **Study Participants**

Inclusion criteria

Subjects must meet all the following inclusion criteria to be eligible for enrolment into the study:

1. Subjects 12 to 35 years of age, inclusive, on the date of informed consent.
2. Subject (or their legally authorized representative or guardian) will sign and date an informed consent form (ICF) and, where applicable, an assent form.
3. Diagnosis of transfusion-dependent β-thalassemia (TDT) as defined by:
   a. Documented homozygous β-thalassemia or compound heterozygous β-thalassemia including β-thalassemia/hemoglobin E (HbE). Subjects can be enrolled based on historical data, but a confirmation of the genotype using the study central laboratory will be required before busulfan conditioning. The β0 and non β0 genotypes are defined using the HbVar Database.
   b. A history of at least 100 mL/kg/year or 10 units/year of packed RBC transfusions in the prior 2 years before signing the consent or the last rescreening for patients going through rescreening.
4. Karnofsky performance status of ≥80% for subjects ≥16 years of age. Lansky performance status of ≥80% for subjects <16 years of age.
5. Eligible for autologous stem cell transplant as per investigator's judgment.
6. Access to detailed medical records on packed RBC transfusions, including units and estimated volumes (latter if available) of packed RBCs and associated pre-transfusion Hb values, weight, and in-patient hospitalizations, for at least the 2 years prior to consent. Pretransfusion body weight and Hb values should be collected (if available) for every transfusion.

7. Female subjects of childbearing potential (postmenarcheal, has an intact uterus and at least 1 ovary, and is less than 1 year postmenopausal) must agree to use acceptable method(s) of contraception from consent through at least 6 months after exa-cel infusion.
8. Male subjects of reproductive capacity must agree to use effective contraception from start of mobilization through at least 6 months after exa-cel infusion.
9. Willing and able to comply with scheduled visits, treatment plan, laboratory tests, contraceptive guidelines, and other study procedures.
10. Willing to participate in an additional long-term follow-up study (Study VX18-CTX001-131) after completion of this study.

Exclusion criteria

Subjects meeting any of the following criteria are not eligible for enrolment in the study:

1. A willing and healthy 10/10 Human Leukocyte Antigen (HLA)-matched related donor is available per investigator's judgement.
2. Prior allo-HSCT.
3. Subjects with associated α-thalassemia and >1 alpha deletion, or alpha multiplications.
4. Subjects with sickle cell β-thalassemia variant.
5. Clinically significant and active bacterial, viral, fungal, or parasitic infection as determined by the investigator.
6. White blood cell (WBC) count $<3 \times 10^9$/L or platelet count $<50 \times 10^9$/L not related to hypersplenism, per investigator judgment.
7. History of a significant bleeding disorder.
8. History of any illness or any clinical condition that, in the opinion of the investigator, might confound the results of the study or pose an additional risk in administering study drug to the subject. This may include but is not limited to: immediate family member with a known family cancer syndrome, history of relevant drug allergies; history of cardiovascular or central nervous system disease; history or presence of clinically significant pathology; history of uncontrolled seizure disorders, or history of psychiatric disorders.
9. Any prior or current malignancy or myeloproliferative disorder or a significant immunodeficiency disorder.
10. Advanced liver disease, defined as:
    a. Aspartate transaminase (AST), alanine transaminase (ALT) >3 × the upper limit of normal (ULN), or direct bilirubin value >2.5 × ULN, or:
    b. Baseline prothrombin time (International Normalized Ratio; INR) >1.5 × ULN, or
    c. History of cirrhosis or any evidence of bridging fibrosis on a prior liver biopsy, if available
    d. Subjects with active hepatitis infection (see criterion 16).
    e. Subjects with history of chronic hepatitis infection are also excluded unless liver biopsy within 3 months prior to or at screening shows no evidence of bridging fibrosis or cirrhosis.
    f. Liver iron content (LIC) ≥15 mg Fe/g dry weight on R2 MRI of liver, unless liver biopsy within 3 months prior to or at screening shows no evidence of bridging fibrosis or cirrhosis.
11. A cardiac T2* <10 ms by MRI or left ventricular ejection fraction (LVEF) <45% by echocardiogram.
12. Baseline estimated glomerular filtration rate <60 mL/min/1.73 m2.
13. Diffusing capacity of the lungs for carbon monoxide (DLco) <50% of predicted (corrected for hemoglobin and/or alveolar volume).
14. Prior treatment with gene therapy/editing product.
15. Intolerance, contraindication, or known sensitivity to plerixafor, granulocyte colony stimulating factor (G-CSF) products (e.g., filgrastim), or busulfan. Prior anaphylaxis with excipients of exa-cel product (Dimethyl sulfoxide [DMSO], Dextran).

16. Positive for the presence of human immunodeficiency virus-1 (HIV-1) or human immunodeficiency virus-2 (HIV-2) (positive for both antigen/antibody AND nucleic acid tests [NAT]), hepatitis B virus (HBV) (positive for hepatitis B core antibody [HBcAb] or positive hepatitis B surface antigen [HBsAg] AND for NAT tests), syphilis (positive screening AND positive confirmatory tests), or hepatitis C virus (HCV; positive for both antibody [HCAb] and for NAT tests). Additional infectious disease markers should be obtained and tested as required by the local authority for the collection and processing of cellular therapy products. These additional tests (e.g., HTLV-1, HTLV-2, malaria, tuberculosis, toxoplasmosis, Trypanosoma cruzi, or West Nile virus) will be evaluated to determine overall impact to the patient and manufacturing of exa-cel.
17. Participation in another clinical study with an investigational drug within 30 days of screening or fewer than 5 half-lives of the investigational agent, whichever is longer from screening.
18. An assessment by the investigator that the subject would not comply with the study procedures outlined in the protocol.
19. Pregnant or breastfeeding females.

- **Treatments**

A single minimum intravenous dose of exa-cel of at least 3.0×10^6 CD34+ cells/kgBW following the standard for autologous HSCT, i.e. mobilization, apheresis, and myeloablation.

Mobilization consisted of a combination of G-CSF products (e.g., filgrastim) and plerixafor. G-CSF product (e.g., filgrastim) was administered subcutaneously or intravenously at a dose of 5 µg/kg/dose approximately every 12 hours (q12h) for 5 to 6 days. The dose was based on body weight taken within 5 days of the first day of mobilization. Plerixafor was administered after the subject had received G-CSF (e.g., filgrastim) for 4 days. Plerixafor was administered via subcutaneous injection; the recommended dose was 0.24 mg/kg administered approximately 4 to 6 hours before planned apheresis. Dose was based on the body weight taken within 5 days before the first day of mobilization.

Subjects underwent apheresis for 2 or 3 consecutive days to collect CD34+ hHSPCs for exa-cel manufacturing. The targeted CD34+cell collection was at least 15×10^6 CD34+ cells/kg in order to facilitate manufacturing of the drug product.

Busulfan was administered intravenously through a central venous catheter daily at a starting dose of 3.2 mg/kg/day for 4 consecutive days, with a target AUC of 74 mg·h/L (target range: 59 to 89) for the q6h regimen and 82 mg·h/L (target range:74 to 90) for the qd regimen. Qd dosing was the preferred schedule, but the busulfan dose could be adjusted to be given every 6 hours (q6h) per site's standard practice. The single dose of exa-cel was given at least 48 hours and within 7 days after the last busulfan dose.

If exa-cel infusion did not occur within 7 days after the last dose of busulfan, subjects were to receive the backup $CD34^+$ stem cells. If engraftment did not occur by Day 21 after exa-cel infusion, G-CSF (e.g., filgrastim) could have been administered following discussion with the medical monitor.

- **Objectives**

The *primary objective* was to demonstrate safety and efficacy of a single dose of autologous CRISPR/Cas9 modified CD34+ hHSPCs (exa-cel) in subjects with TDT.

Secondary objectives

- To quantify percentage of edited alleles in peripheral blood leukocytes and CD34+ cells of the bone marrow
- To assess the production of HbF post-exa-cel infusion
- To assess the effects of infusion of exa-cel on disease-specific events and clinical status

- **Outcomes/endpoints**

Primary efficacy endpoint

Proportion of subjects achieving TI12, defined as maintaining weighted average Hb ≥9 g/dL without RBC transfusions for at least 12 consecutive months any time after exa-cel infusion. The evaluation of TI12 starts 60 days after last RBC transfusion for post-transplant support or TDT disease management.

Key secondary efficacy endpoint

Proportion of subjects achieving TI6, defined as maintaining weighted average Hb ≥9 g/dL without RBC transfusions for at least 6 consecutive months any time after exa-cel infusion. The evaluation of TI6 starts 60 days after last RBC transfusion for post-transplant support or TDT disease management.

Secondary efficacy endpoints

i. Proportion of subjects achieving at least 95%, 90%, 85%, 75%, 50% reduction from baseline in annualized transfusions up to 24 months starting 60 days after exa-cel infusion.
ii. Relative change from baseline in transfusions up to 24 months starting 60 days after exa-cel infusion
iii. Duration of transfusion free in subjects who have achieved TI12
iv. Proportion of alleles with intended genetic modification present in peripheral blood leukocytes over time. intended genetic modifications are indels that modify the sequence of the erythrocyte-specific enhancer in intron 2 of BCL11A.
v. Proportion of alleles with intended genetic modification present in CD34+ cells of the bone marrow over time.
vi. Fetal hemoglobin concentration (pre-transfusion) over time.
vii. Total hemoglobin concentration (pre-transfusion) over time
viii. Change in patient reported outcomes (PROs) over time using EuroQol Quality of Life Scale (EQ-5D-5L) for subjects ≥18 years old, EQ-5D-Y for subjects <18 years old, functional assessment of cancer therapy-bone marrow transplant (FACT-BMT) for subjects ≥18 years old, and Pediatric Quality of Life Inventory (PedsQL) for subjects <18 years old.
ix. Change in parameters of iron overload, including:
    - Liver iron concentration (LIC) from baseline as assessed by R2 magnetic resonance imaging (MRI) and cardiac iron content (CIC) from baseline as assessed by T2* MRI.
    - Change in serum ferritin level from baseline over time

i. Proportion of subjects receiving iron chelation therapy over time

- **Sample size**

With a total of 45 subjects dosed, 3 IAs could be performed following a group sequential testing procedure in the study to allow for early evaluation of efficacy. This sample size provided at least 95% power to rule out a response rate of 50% when the true response rate is 80% for both the primary and key secondary efficacy endpoint with 1-sided alpha of 2.5%.

- **Randomisation and Blinding (masking)**

This was a non-randomised, open-label, single-arm study

- **Statistical methods**

Planned methods of analyses were provided in the statistical analysis plan, exploratory biomarker analysis plan and the clinical pharmacology analysis plan. These plans were finalized before performing the data cut for the prespecified IA2.

Key details of the planned methods of analyses are summarized in the following paragraphs:

Analysis sets

The following analysis sets were defined: Enrolled Set (all subjects who provided informed consent and were eligible), Safety Analysis Set (all subjects in the Enrolled set who started mobilization regimen), Full Analysis Set (FAS; subjects in the Enrolled set who received exa-cel infusion; used for demographics and baseline characteristics), and Primary Efficacy Set (PES; Subset of the FAS which included subjects with ≥ 16 months FU after exa-cel infusion and ≥ 14 months FU after RBC transfusion for post-transplant support or TDT disease management). Subjects who completed the 24 months of follow up in the study after exa-cel infusion were included in the PES, with the exception of those who received RBC transfusions between Month 10 and Month 12 but have less than 14 months (including up to 2 months in Study 131) additional follow up time. In addition, subjects who died or discontinued the study due to AEs considered related to exa-cel and had less than 16 months follow-up after exa-cel infusion, or continuously received RBC transfusions for more than 12 months after exa-cel infusion were also be included in this set and counted as non-responders.

The Confirmatory Efficacy Set (CES) is a subset of the PES that includes subjects who have not reached the end of evaluation period for the primary endpoint at the time of protocol amendment v6.0 (EUR) finalization. Supportive analyses will be conducted based on the CES for the primary efficacy endpoint at IA3 and the final analysis.

Efficacy analyses

At the interim analyses, the analysis of the primary efficacy endpoint was to be based on the Primary Efficacy Set. The proportion of subjects achieving TI12 were to be provided, with one-sided P value (against a null hypothesis of 50% response rate) and two-sided 95% exact Clopper-Pearson CI. In the final analysis, the analysis of the primary efficacy endpoint will be based on the FAS.

A subject will be a responder if he/she meets the response criteria any time during the response evaluation period. If a subject has died or discontinued the study before achieving TI12 starting 60 days after the last RBC transfusion for post-transplant support or TDT management post exa-cel infusion, due to *reasons other than exa-cel-related adverse events*, the transfusion free status and weighted average Hb starting from 60 days after the last RBC transfusion of the subject will be carried forward up to 24 months post exa-cel infusion.

Subjects who die, discontinue the study *due to exa-cel-related adverse events* and have less than 16 months follow-up post exa-cel infusion, or continuously receive RBC transfusion for post-transplant support or TDT management after 12 months post exa-cel infusion will be considered non-responders for TI12. Subjects who receive RBC transfusions between Month 10 and Month 12 will be considered responders if they have a subsequent 14-month RBC transfusion free period, which could include up to 2 months in long-term follow-up Study 131, and meet the requirement on Hb.

Subgroup analyses of the primary efficacy endpoint will be performed with point estimate and 95% confidence interval for each of the following subgroups, during interim and final analyses. Other subgroup analyses may be performed as appropriate. Descriptive summary will be considered if a subgroup has a sample size < 5. Subgroup analysis refers to age at screening (<18, and ≥18 years) and genotype (β0-like, i.e. β0/β0, IVS-I-110/IVS-I-110, or β0/IVS-I-110; non-β0-like: all other genotypes).

Multiplicity

Multiplicity was considered with respect to testing the null hypothesis for the primary and the key secondary efficacy endpoints across 3 IAs and the final analysis. The familywise type I error rate was/will be controlled by an alpha spending approach for tests at interim and final analyses and a sequential testing of the primary and key secondary efficacy endpoints (i.e., the key secondary efficacy endpoint was/will be tested only if the primary efficacy endpoint has crossed an efficacy boundary). The first/second/third IA may be conducted when the primary efficacy set consists of approximately 17/24/30 subjects. The actual alpha spending will be based on the number of subjects in the primary efficacy set

at the IA. If an IA was not conducted, the alpha planned for this IA would be recovered in the subsequent analysis.

The efficacy boundaries for the primary efficacy endpoint (TI12) and key secondary efficacy endpoint (TI6) were specified to control the type I error at 1-sided 2.5% across multiple looks, based on the exact binomial distribution are summarized in Table 6.

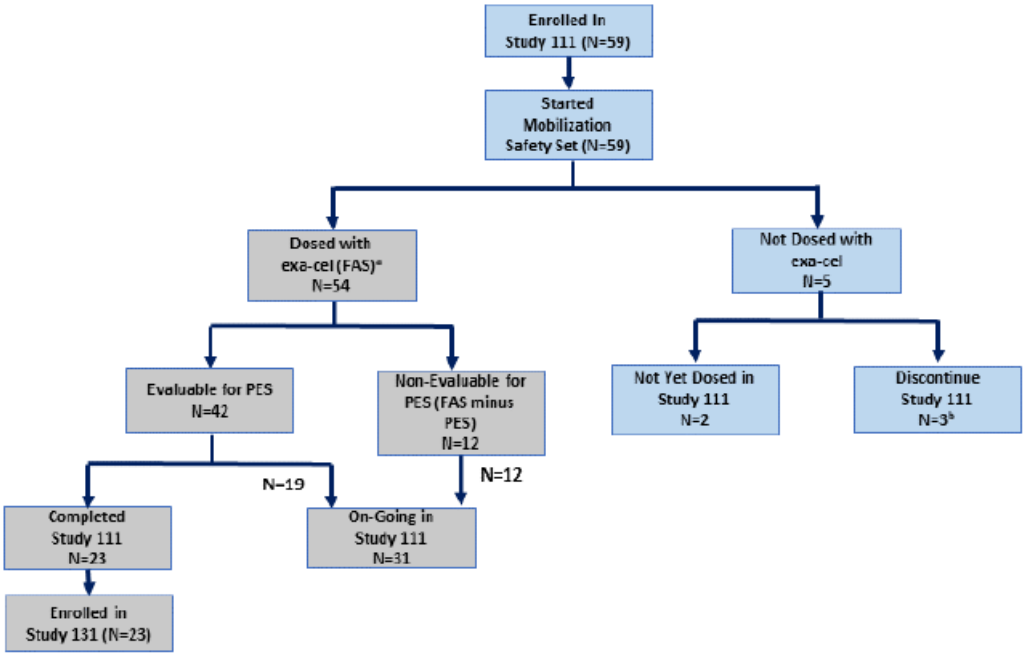
Table 6. Efficacy Boundaries and Probabilities of Crossing Efficacy Boundary Under Null Hypothesis for the Primary and Key Secondary Efficacy Endpoints at the Interim and Final Analyses

Analysis Time Point	Efficacy Boundary	One-sided Alpha Spent at Each IA or Final Assuming a Response Rate of 50%[a]
IA 1: N = 17	14/17	0.636%
IA 2: N = 24	18/24	0.780%
IA 3: N = 30	22/30	0.275%
Final: N = 45	31/45	0.417%
Overall α Spending	--	2.109%

IA1 was not conducted; the type I error assigned to IA1 was recovered for subsequent analyses.

Results

- **Participant flow**



- **Recruitment**

Patients were recruited from 13 sites in the United States, Canada, United Kingdom, Germany, and Italy.

Study start date: 10 September 2018

CSR Data (IA 2) cutoff date: 06 September 2022, efficacy and safety update 16 April 2023

- **Conduct of the study**

There were 4 global amendments and 14 country/region-specific amendments to the study protocol. There was 1 protocol addendum to allow for remote source data verification in Germany. Table 9-6 [CSR of study 111] lists the protocol versions, amendment dates, and key changes in study conduct specified in each amendment. See also clinical ARs.

With EU Amendment 6.0 (08 Jan 2021) the study was upgraded to a phase 1/2/3 study, and primary, key secondary and secondary efficacy endpoints were updated. This was reflected in the US with Amendment 6.6 (20 July 2021).

Study participants recruited under the initial protocol version(s) with different study objectives, different in- and exclusion criteria were also used for the final assessment of efficacy and safety.

- **Baseline data**

For the 54 subjects in the FAS, 19 (35.2%) subjects ≥12 and <18 years of age. The majority of subjects were Asian (42.6%) or White (33.3%). Subjects were balanced by sex.

The majority of subjects (33 [61.1%] subjects) had β0/β0-like genotypes (β0/β0, β0/IVS-I-110, or IVS-I-110/IVS-I-110). Thirty-eight subjects had an intact spleen. Baseline median (range) annualized units of TDT-related RBC transfusions per year was 35.3 (11.0 to 71.0) units per year and the baseline median (range) annualized volume of TDT-related RBC transfusions was 205.7 (48.3, 330.9) mL/kg per year for the prior 2 years before screening. Overall, the population of subjects enrolled in the study was representative of patients with TDT.

No clinically relevant differences were observed between the subgroups by age, genotype, or sex. As expected, subjects with β0/β0-like genotypes had more baseline annualized RBC transfusion episodes than subjects with non-β0/β0-like genotypes.

Baseline characteristic data for the PES showed no clinically relevant differences from the FAS. For the 42 subjects in the PES, with 13 subjects ≥12 and <18 years of age. The majority of subjects were Asian (38.1%) or White (40.5%). Subjects were balanced by sex. Twenty five subjects (59.5%) had β0/β0-like genotypes. Thirty subjects had an intact spleen. The baseline median (range) annualized units of TDT-related RBC transfusions per year was 35.0 (20.5 to 71.0) units and the baseline median (range) annualized volume of TDT-related RBC transfusions was 201.0 (115.2, 330.9) mL/kg per year for the prior 2 years before screening.

- **Numbers analysed**

Disposition/Reason	Total n (%)
Enrolled Set[a]	59
Safety Analysis Set[b]	59
Started the conditioning regimen	54
FAS[c]	54
PES[d]	42
Never dosed with any study drug[e]	0
Never dosed with exa-cel[f]	3
Started exa-cel infusion	54 (91.5)
Completed exa-cel infusion	54 (100.0)
Not completed exa-cel infusion	0
On Study 111 and not yet dosed with exa-cel[g]	2 (3.4)
On Study 111 and dosed with exa-cel	31 (52.5)
Completed Study 111[h]	23 (39.0)
Completed Study 111 and enrolled in long-term follow-up study	23 (100.0)
Discontinued Study 111 after exa-cel infusion	0
Discontinued Study 111 after exa-cel infusion and enrolled in long-term follow-up study	0

Sources: Study 111/Table 14.1.1 and Study 131/Table 14.1.1a (data cutoff date of 16 April 2023)

AE: adverse event; exa-cel: exagamglogene autotemcel; FAS: Full Analysis Set; IA3: interim analysis 3; n: size of subset; PES: Primary Efficacy Set; RBC: red blood cell; TDT: transfusion-dependent β-thalassemia; TI12: maintained a weighted average Hb ≥9 g/dL without RBC transfusions for at least 12 consecutive months any time after exa-cel infusion

Notes: Percentages were calculated relative to the number of subjects in the Enrolled Set, unless otherwise specified. Percentages of subjects who completed or did not complete exa-cel infusion (and percentages of reasons for not completing exa-cel infusion) were calculated relative to the number of subjects who started exa-cel infusion. Percentages of subjects who completed the study and enrolled in the long-term follow-up study were calculated relative to the number of subjects who completed the study. Percentages of subjects who discontinued the study after exa-cel infusion and enrolled in the long-term follow-up study were calculated relative to the number of subjects who discontinued the study after exa-cel infusion. Percentages for reasons for discontinuing the study after exa-cel infusion were calculated relative to the number of subjects who received exa-cel infusion.

[a] Enrolled Set included all enrolled subjects who signed informed consent and met the eligibility criteria.

[b] Safety Analysis Set included all subjects who started the mobilization regimen.

[c] FAS included all subjects who received exa-cel infusion.

[d] PES included all subjects who have been followed for at least 16 months post exa-cel infusion and for at least 14 months after completion of the RBC transfusions for post-transplant support or TDT management. Subjects who completed the 24 months of follow up in the study post exa-cel infusion were included in this set. In addition, subjects who died or discontinued the study due to exa-cel-related adverse events and had less than 16 months follow-up post exa-cel infusion, or continuously received RBC transfusions for more than 12 months post exa-cel infusion were also included in this set. For a full definition of the PES refer to Study 111/SAP Version 4.3.

[e] Never dosed with any study drug included all subjects who discontinued the study and did not receive any study drugs for mobilization, conditioning, and exa-cel infusion.

[f] Never dosed with exa-cel included all subjects who discontinued the study and did not receive exa-cel infusion.

[g] On study included all subjects who enrolled and had not yet completed (or discontinued) the study.

[h] Completed study included all subjects who completed the Month 24 visit.

- **Outcomes and estimation**

The main analyses of all efficacy endpoints were based on the PES (n=42). […] descriptive summaries of most secondary endpoints were presented based on the FAS (n=54). The key secondary endpoint TI6 is omitted, as it is not considered to carry relevant information in addition to the primary endpoint TI12.

TI12

Following infusion with exa-cel, 39 of 42 (92.9%) subjects in the PES achieved TI12 (95% CI: 80.5%, 98.5%; 1-sided P<0.0001 [against a 50% response rate]).

Subgroup analyses of the primary efficacy endpoint by age at screening (≥12 and <18 years of age and ≥18 and ≤ 35 years of age), genotype (β 0/ β 0-like and non- β 0/ β 0-like), and sex were generally consistent with the results from the primary analysis.

Duration of transfusion free period

The transfusion free duration for subjects who achieved TI12 is summarized in **Table** . All subjects in the PES who met the primary endpoint remained transfusion independent for all subsequent follow-up; the mean (SD) duration of transfusion independence was 23.6 (7.8) months, ranging from 13.5 to 48.1 months.

Table 7. Summary of duration of RBC transfusion free while maintaining weighted Hb ⩾9 g/dL for subjects who achieved TI12 (PES)

Category	Studies 111 and 131 Total N = 42
Subjects who achieved TI12, N1	39
Duration of RBC transfusion free while maintaining weighted hemoglobin ≥9 g/dL for subjects who achieved TI12 (months)	
n	39
Mean (SD)	23.6 (7.8)
Median	22.3
Min, max	13.5, 48.1

Source: Study 131/Table 14.2.4a (data cutoff date of 16 April 2023)

EAC: Endpoint Adjudication Committee; exa-cel: exagamglogene autotemcel; Hb: hemoglobin; max: maximum; min: minimum; N: total sample size; n: size of subsample; N1: N1: subjects who achieved TI12; PES: Primary Efficacy Set; RBC: red blood cell; TDT: transfusion-dependent β-thalassemia; TI12: maintained a weighted average Hb ≥9 g/dL without RBC transfusions for at least 12 consecutive months any time after exa-cel infusion

Notes: The post CTX001 infusion follow-up periods in both Studies 111 and 131, if any, were included in this analysis The evaluation of duration of transfusion free while maintaining a weighted average Hb ≥9 g/dL in subjects who achieved TI12 started 60 days after the last RBC transfusion for post-transplant support or TDT disease management. Duration of RBC transfusion free while maintaining a weighted average Hb ≥9 g/dL (months) = (the day before the start date of first RBC transfusion after achieving TI12 or the day of the last assessment before weighted Hb <9 g/dL after achieving TI12 or data cut date whichever was earlier – start date of TI12 + 1)/30. For Studies 111 and 131, if there were multiple transfusion free periods, the longest transfusion free period was used in the summary. For post-baseline, only RBC transfusions adjudicated by an EAC as meeting the purpose of post-transplant support or TDT disease management were included.

Transfusion independence is complemented by information on time to last RBC transfusion in subjects who achieved TI12 in the PES. The median (range)time to the last RBC transfusion for subjects who achieved TI12 was 28.0 (11 to 91)days from exa-cel infusion.

Total Hb

Total Hb and HbF concentrations over time after exa-cel infusion are summarised in the dossier as follows: In the [TDT]FAS, increased mean levels of Hb and HbF were achieved early (by Month 3) after exa-cel treatment and were maintained at consistent levels from Month 6 through the duration of follow-up of Month 48:

- Mean (SD) total Hb levels were 11.4 (2.2) g/dL at Month 3 and were maintained with mean ≥12.2 g/dL from Month 6 onward.
- Mean (SD) HbF levels were 7.8 (2.9) g/dL at Month 3 and were maintained with mean ≥ 10.9 g/dL from Month 6 onward.

Subgroup analyses by age at screening (≥12 and <18 years of age and ≥18 and ≤35 years of age), genotype (β0/β0-like and non-β0/β0-like), and sex in the FAS were performed for the following

secondary efficacy endpoints: total Hb and HbF concentration over time, proportion of alleles with intended genetic modification, and the proportion of F-cells.

These analyses demonstrated that for adolescents (≥12 to <18 years of age; N = 19) and adults (≥18 to ≤35 years of age; N = 35) the values of Hb and HbF concentrations, and F-cells at each time point were consistent with the overall results from the main analyses. Similarly, there were no clinically meaningful differences based on genotype (β0/β0-like and non β0/β0-like), race, or sex. Overall, these data demonstrate that efficacy is consistent across of age, genotype, race, or sex.

Findings for the subgroup analyses on total haemoglobin are included in the safety and efficacy update as Table 14.2.8.2a, in which median total Hb was lower in female patients than in male patients starting month 5 after treatment.

Summary of main efficacy results

The following table summarises the efficacy results from the main studies 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).

Table 8. Summary of Efficacy for trial CTX001-111

Title: A Phase 1/2/3 Study of the Safety and Efficacy of a Single Dose of Autologous CRISPR-Cas9 Modified CD34+ Human Hematopoietic Stem and Progenitor Cells (hHSPCs) in Subjects With Transfusion dependent β-Thalassemia			
Study identifier	Protocol CTX001-111 EudraCT number: 2017-003351-38		
Design	Single-arm, open-label, multi-site, single dose, Phase 1/2/3 study in subjects 12 to 35 years of age (inclusive) who have transfusion-dependent β-thalassemia (TDT).		
	Duration of main phase:		Stage 1 (screening and pre-mobilization): approximately 1 to 3 months; Stage 2 (mobilization, autologous CD34+ stem cell collection, exa-cel manufacture and disposition): approximately 2 to 3 months; Stage 3 (myeloablative conditioning and infusion of exa-cel): approximately 1 month; Stage 4 (follow-up after exa-cel infusion): approximately 2 years.
	Duration of Run-in phase:		Not applicable
	Duration of Extension phase:		Not applicable
Hypothesis	Null hypothesis of 50% response rate		
Treatments groups	N/A		Single arm
Endpoints and definitions	Primary endpoint	Transfusion independence (TI)12	Proportion of subjects who achieved TI12, defined as maintained weighted average Hb ≥9 g/dL without red blood cell (RBC) transfusions for at least 12 consecutive months any time after exa-cel infusion.[a]
	Secondary endpoint	Duration of transfusion free	Duration of RBC transfusion free while maintaining weighted Hb ≥9 g/dL for subjects who achieved TI12

	Secondary endpoint	Hemoglobin (Hb) concentration	Total Hb over time.	
Database lock	16 April 2023			
Results and Analysis				
Analysis description	**Primary Analysis**			
Analysis population and time point description	Primary Efficacy Set (PES): a subset of the Full Analysis Set (FAS) that included all subjects who were followed for at least 16 months after exa-cel infusion and for at least 14 months after completion of the RBC transfusions for post-transplant support or TDT disease management.[b]			
Descriptive statistics and estimate variability	Treatment group	N/A; all subjects received a single dose of autologous CRISPR-Cas9 modified CD34+ hHSPCs		
	Number of subjects	42		
	TI12 n (%) 2-sided 95% exact Clopper-Pearson confidence interval (CI)	39 (92.9) (80.5, 98.5)		
	Duration of RBC transfusion free (months), median (min;max)	n = 39, 22.3 (13.5;48.1)		
	Total Hb (g/dL) Mean (SD);	Month 3: 11.4 (2.2) Mean Hb levels were maintained with mean ≥12.2 g/dLfrom Month 6 onward		
Effect estimate per comparison	Primary endpoint: TI12	Comparison groups	50% response rate	
		P-value (1-sided against a 50% response rate)	<0.0001[c]	

Notes	No subjects discontinued the study after exa-cel infusion. Three subjects discontinued from the study after the start of mobilization but before conditioning: subject did not want to undergo a second apheresis procedure (N = 1), subject had concerns with continued study participation (N = 1), and subject withdrew consent (N = 1); no subject discontinued due to an adverse event (AE). [a] The evaluation started 60 days after last RBC transfusion for post-transplant support or TDT disease management. Analyses were based on RBC transfusions adjudicated by the EAC as for the purpose of post-transplant support or TDT disease management. [b] Subjects who had completed the 24 months of follow-up in the study after exa-cel infusion were also included except for those who received RBC transfusions between Month 10 and Month 12 but have less than 14 months (including up to 2 months in Study 131) additional follow-up time. This set also included subjects who died or discontinued the study due to AEs related to exa-cel and had less than 16 months of follow-up after exa-cel infusion, or continuously received RBC transfusions for more than 12 months after exa-cel infusion. [c] This endpoint is considered as statistically significant in the reference of 1-sided alpha = 0.01416.

2.6.5.3. Clinical studies in special populations

The eligibility criteria for the single (pivotal) study define the age range as 12 to 35 years (inclusive at the date of informed consent). N=19/54 patients in the FAS were adolescents.

Abnormal liver function or advanced liver disease (e.g., hepatic cirrhosis), abnormal cardiac function, and abnormal renal function were some of the exclusion criteria.

	Age 65-74 (Older subjects number /total number)	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
Controlled Trials	0	0	0
Non Controlled Trials	0	0	0

2.6.5.4. In vitro biomarker test for patient selection for efficacy

Not applicable.

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

Not applicable.

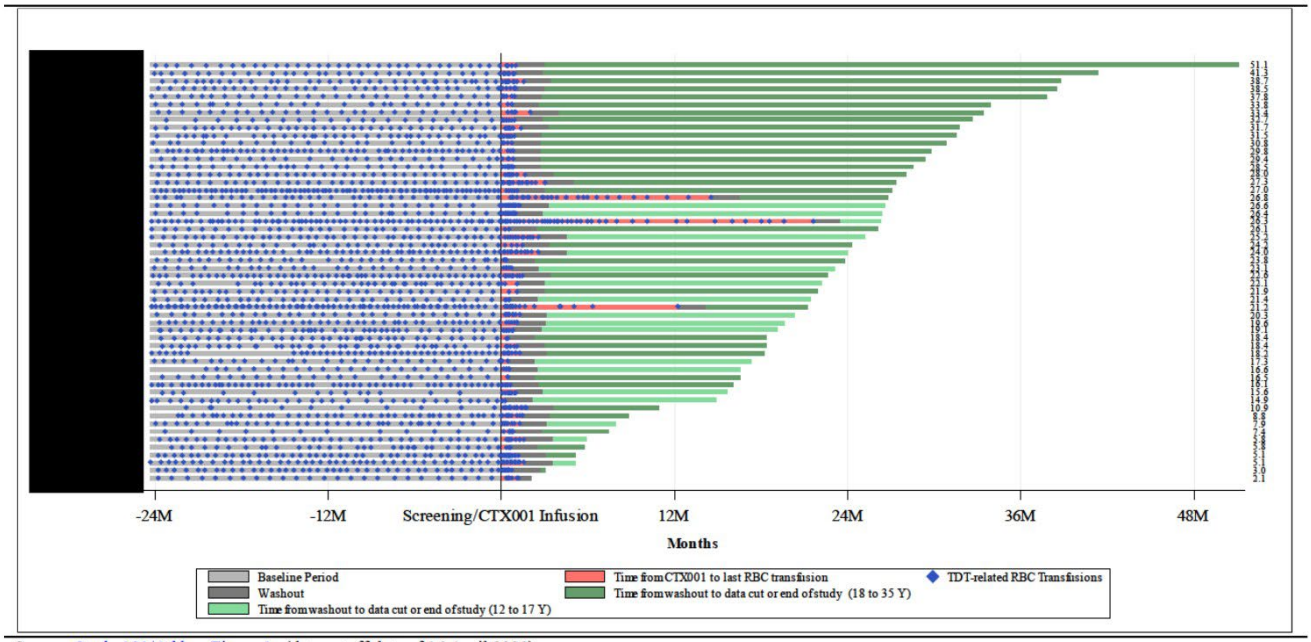
2.6.5.6. Supportive study

A Long-term Follow-up Study of Subjects With β-thalassemia or Sickle Cell Disease Treated with Autologous CRISPR-Cas9 Modified Hematopoietic Stem Cells (exa-cel), study 131

This study is a multi-site, open-label, rollover study designed to evaluate the long-term safety and efficacy of exa-cel in subjects who received exa-cel in a parent study, completed or discontinued said parent study, and are followed in study 131 for a total follow-up of 15 years after exa-cel infusion. For the purpose of this assessment, the endpoint duration of transfusion free is of interest to address sustainability of the effect.

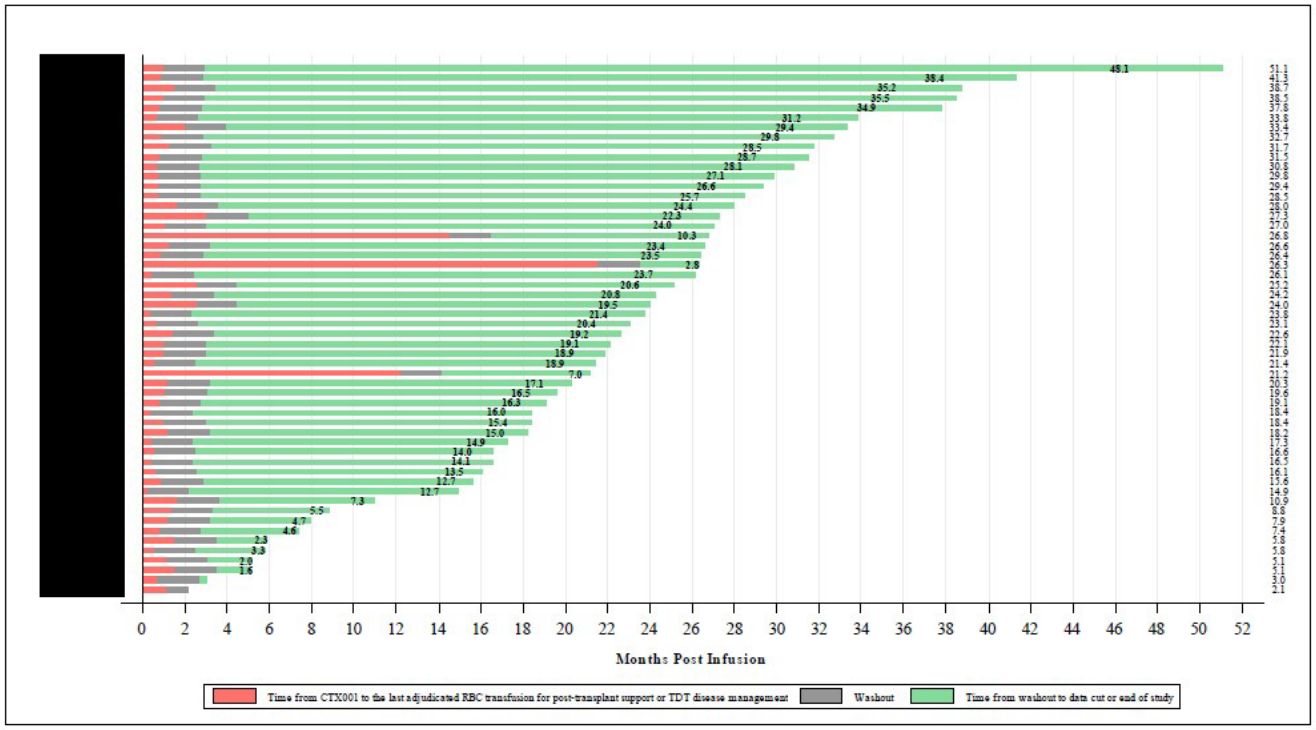
At time of data cut, 23 patients from study 111 have been enrolled into study 131, longest FU is >48 months post treatment. Duration of period free from transfusions combined for studies 111 and 131 is given below.

Figure 4. Duration of Period Free From Transfusions (Studies 111 and 131 (TDT) FAS)



Source: Study 131/Ad hoc Figure 1a (data cutoff date of 16 April 2023)
EAC: Endpoint Adjudication Committee; exa-cel: exagamglogene autotemcel; FAS: Full Analysis Set; PES: Primary Efficacy Set; RBC: red blood cell; TDT: transfusion-dependent β-thalassemia
Notes: Investigator-reported TDT-related historical RBC transfusions were included for the baseline period, which was defined to be the 2 years before signing of the ICF or the latest rescreening for subjects going through rescreening. Only RBC transfusions that were adjudicated by EAC as post-transplant support or TDT disease management were displayed for post exa-cel infusion period. The number on the right end is the duration of total follow-up in month. The number after subject ID is age at screening.
- *: subjects in the [TDT]PES who achieved TI12.
- **: subjects in the [TDT]PES who did not achieve TI12.

Figure 5. Duration of Period Free From Transfusions (Studies 111 and 131 (TDT) FAS)



Only RBC transfusions that are adjudicated by EAC as post-transplant support or TDT disease management are included.
The number on the right end is the duration of total follow-up.
*: subjects in the Primary Efficacy Set.

Sickle-cell disease

2.6.5.7 Dose-response studies

No dose-response studies have been conducted, see also section clinical pharmacology.

2.6.5.8 Main study

A Phase 1/2/3 Study to Evaluate the Safety and Efficacy of a Single Dose of Autologous CRISPR-Cas9 Modified CD34+ Human Hematopoietic Stem and Progenitor Cells (exa-cel) in Subjects With Severe Sickle Cell Disease (Protocol CTX001-121)

Study 121 is a single-arm, open-label, multi-site, single dose, Phase 1/2/3 study in subjects 12 to 35 years of age who have severe SCD. Severe SCD was defined by the occurrence of at least 2 of the following events each year during the 2-year period before screening, while receiving appropriate supportive care (e.g., pain management plan, hydroxyurea if indicated):

- Acute pain event that required a visit to a medical facility and administration of pain medications (opioids or intravenous [IV] non-steroidal anti-inflammatory drugs) or red blood cell (RBC) transfusions
- Acute chest syndrome, as indicated by the presence of a new pulmonary infiltrate associated with pneumonia-like symptoms, pain, or fever

- Priapism lasting >2 hours and requiring a visit to a medical facility
- Splenic sequestration, as defined by an enlarged spleen, left upper quadrant pain, and an acute decrease in hemoglobin (Hb) concentration of ≥2 g/dL

The study is ongoing; at time of latest data cut (16 April 2023), n=63 patients have been enrolled, but not all had been treated yet.

- **Methods**

Study Participants as per protocol version 6.8 (DE), dated 18 August 2021

Inclusion criteria

Subjects must meet all the following inclusion criteria to be eligible for enrolment into the study:

1. Subject, or their legally authorized representative or guardian, will sign and date an informed consent form (ICF) and, where applicable, an assent form.
2. Subjects 12 to 35 years of age, inclusive, on the date of informed consent.
3. Documented βS/βS, βS/β0, or βS/β+ genotype. Subjects can be enrolled based on historical genotype results, but confirmation of genotype is required before busulfan conditioning. The β0 genotypes are defined using the HbVar Database.
4. Subjects with **severe SCD**. Severe SCD is defined by the occurrence of at least 2 of the following events per year during the 2-year period before screening, while receiving appropriate supportive care (e.g., pain management plan, HU):
    - Acute pain events that requires a visit to a medical facility and administration of pain medications (opioids or intravenous [IV] non-steroidal anti-inflammatory drugs [NSAIDs]) or RBC transfusions
    - Acute chest syndrome, as indicated by the presence of a new pulmonary infiltrate associated with pneumonia-like symptoms, pain, or fever
    - Priapism lasting >2 hours and requiring a visit to a medical facility
    - Splenic sequestration, as defined by an enlarged spleen, left upper quadrant pain, and an acute decrease in hemoglobin concentration of ≥2 g/dL.

Historical severe VOCs will be adjudicated by the Endpoint Adjudication Committee (EAC).

5. Normal transcranial Doppler (TCD) velocity (time-averaged mean of the maximum velocity [TAMMV] <170 cm/sec for non-imaging TCD and <155 cm/sec for imaging TCD) in the middle cerebral artery (MCA) and the internal carotid artery (ICA) for subjects 12 to 16 years of age
6. Karnofsky performance status of ≥80% for subjects ≥16 years of age or Lansky performance status of ≥80% for subjects <16 years of age.
7. Eligible for autologous stem cell transplant as per investigator's judgment.
8. Female subjects of childbearing potential (postmenarcheal, has an intact uterus and at least 1 ovary, and is less than 1 year postmenopausal) must agree to use acceptable method(s) of contraception from consent through at least 6 months after exa-cel infusion.
9. Male subjects of reproductive capacity must agree to use effective contraception from start of mobilization through at least 6 months after exa-cel infusion.

10. Willing and able to comply with scheduled visits, treatment plan, laboratory tests, contraceptive guidelines, and other study procedures.
11. Willing to participate in the long-term follow-up study (Study VX18-CTX001-131), after completion of this study.

Exclusion criteria

Subjects meeting any of the following criteria are not eligible for enrolment:

1. An available 10/10 human leukocyte antigen (HLA)-matched related donor.
2. Prior HSCT.
3. Clinically significant and active bacterial, viral, fungal, or parasitic infection as determined by the investigator.
4. White blood cell (WBC) count $<3 \times 10^9$/L or platelet count $<50 \times 10^9$/L, not related to hypersplenism per investigator judgment.
5. Treatment with regular RBC transfusions that, in the opinion of the investigator, cannot be interrupted after engraftment.
6. Subjects with history of alloimmunization to RBC antigens and for whom the investigator anticipates that there will be insufficient RBC units available for the duration of the study.
7. More than 10 unplanned hospitalizations or emergency department visits related to SCD in the 1 year before screening, that in the opinion of the investigator, are consistent with significant chronic pain rather than acute pain crises.
8. HbF level >15.0%, irrespective of concomitant treatment with HbF-inducing treatments such as HU.
9. History of abnormal TCD (TAMMV ≥200 cm/sec for non-imaging TCD and ≥185 cm/sec for imaging TCD) for subjects 12 to 18 years of age.
10. History of untreated Moyamoya disease or presence of Moyamoya disease at Screening that in the opinion of the investigator puts the subjects at the risk of bleeding.
11. History of a significant bleeding disorder.
12. History of any illness or any clinical condition that, in the opinion of the investigator, might confound the results of the study or pose an additional risk to the subject. This may include, but is not limited to: history of relevant drug allergies; history of cardiovascular or central nervous system disease; history or presence of clinically significant pathology; history of mental disease; or history of familial cancer syndrome.
13. Any prior or current malignancy or myeloproliferative disorder or a significant immunodeficiency disorder.
14. Advanced liver disease, defined as
    a. Alanine transaminase (ALT) >3 × the upper limit of normal (ULN) or direct bilirubin value >2.5 × ULN, or
    b. Baseline prothrombin time (PT) (international normalized ratio [INR]) >1.5 × ULN, or
    c. History of cirrhosis or any evidence of bridging fibrosis, or active hepatitis on liver biopsy
15. Baseline estimated glomerular filtration rate <60 mL/min/1.73 m2.

16. Lung diffusing capacity for carbon monoxide (DLco) <50% of predicted value (corrected for hemoglobin and/or alveolar volume).
17. Left ventricular ejection fraction (LVEF) <45% by echocardiogram.
18. Prior treatment with gene therapy/editing product.
19. Intolerance, contraindication, or known sensitivity to plerixafor or busulfan. Subject must not have any risk factors in the opinion of the investigator that would increase the likelihood of busulfan-related toxicities. Prior anaphylactic reaction with excipients of exa-cel product (dimethylsulfoxide [DMSO], dextran).
20. Positive for the presence of human immunodeficiency virus-1 (HIV-1) or human immunodeficiency virus-2 (HIV-2) (positive for both antigen/antibody AND nucleic acid tests [NAT]), hepatitis B virus (HBV) (positive for Hepatitis B core antibody [HBcAb] or positive hepatitis B surface antigen [HBsAg] AND NAT tests), syphilis (positive screening AND positive confirmatory tests), or hepatitis C virus (HCV; positive for both antibody [HCAb] AND for NAT tests). Additional infectious disease markers should be obtained and tested as required by the local authority for the collection and processing of cellular therapy products. These additional tests (e.g., HTLV-1, HTLV-2, malaria, tuberculosis, toxoplasmosis, Trypanosoma cruzi, or West Nile virus) will be evaluated to determine overall impact to the subject and manufacturing of exa-cel.
21. Participation in another clinical study with an investigational drug/product within 30 days of screening or fewer than 5 half-lives of the investigational agent, whichever is longer from screening.
22. Subjects who are not able to comply with the study procedures outlined in the protocol as judged by the investigator.
23. Pregnancy or breastfeeding.

- ***Treatments***

A single minimum intravenous dose of exa-cel of at least 3.0×10^6 $CD34^+$ cells/kgBW following the standard for autologous HSCT, i.e. mobilization, apheresis, and myeloablation.

For mobilisation, study participants received plerixafor at a dose of 0.24 mg/kg via subcutaneous injection 2 to 3 hours before the start of apheresis. The dose was based on body weight taken within 5 days before the first day of mobilization.

Subjects underwent apheresis for 2 or 3 consecutive days to collect CD34+ hHSPCs for exa-cel manufacturing. The targeted CD34+cell collection was at least 15×10^6 CD34+ cells/kg in order to facilitate manufacturing of the drug product.

Busulfan was administered intravenously through a central venous catheter daily at a starting dose of 3.2 mg/kg/day for 4 consecutive days, with a target AUC of 74 mg·h/L (target range: 59 to 89) for the q6h regimen and 82 mg·h/L (target range:74 to 90) for the qd regimen. Qd dosing was the preferred schedule, but the busulfan dose could be adjusted to be given every 6 hours (q6h) per site's standard practice. The single dose of exa-cel was given at least 48 hours and within 7 days after the last busulfan dose.

If exa-cel infusion did not occur within 7 days after the last dose of busulfan, subjects were to receive the backup $CD34^+$ stem cells. If engraftment did not occur by Day 21 after exa-cel infusion, G-CSF (e.g., filgrastim) could have been administered following discussion with the medical monitor.

- ***Objectives***

The *primary objective* was to demonstrate safety and efficacy of a single dose of autologous CRISPR/Cas9 modified CD34+ hHSPCs (exa-cel) in subjects with severe SCD.

Secondary Objectives

- Assess the effects of infusion of exa-cel on disease-specific events and clinical status
- Quantify gene editing efficiency

- ***Outcomes/endpoints***

Primary efficacy endpoint

Proportion of subjects who have not experienced any severe VOCs for at least 12 consecutive months (**VF12**) after exa-cel infusion. The **evaluation of VF12 starts 60 days after last RBC transfusion for post-transplant support or SCD disease management**.

Key Secondary Efficacy Endpoint

Proportion of subjects free from inpatient hospitalization for severe VOCs sustained for at least 12 months (**HF12**) after exa-cel infusion. The evaluation of HF12 starts 60 days after last RBC transfusion for post-transplant support or SCD disease management

Secondary Efficacy Endpoints

i. Proportion of subjects with reduction in annualized rate of severe VOCs at the time of analysis from baseline by at least 90%, 80%, 75%, 50% up to 24 months after exa-cel infusion. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.

ii. Relative change from baseline in annualized rate of severe VOCs up to 24 months after exa-cel infusion. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.

iii. **Duration of severe VOC free** in subjects who have achieved VF12

iv. **Relative change from baseline in rate of inpatient hospitalizations for severe VOCs** up to 24 months after exa-cel infusion. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.

v. Relative change from baseline in annualized duration of hospitalization for severe VOCs up to 24 months after exa-cel infusion. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.

vi. **Proportion of subjects with sustained HbF ≥20%** at the time of analysis for at least 3 months, 6 months, or 12 months. The evaluation starts 60 days after last RBC transfusion for posttransplant support or SCD disease management.

vii. Change in number of units of RBCs transfused for SCD-related indications over time

viii. HbF concentrations over time

ix. Hb concentrations over time

x. Change from baseline in reticulocyte count (percent reticulocytes and absolute reticulocyte count) over time

xi. Change from baseline in indirect bilirubin over time

xii. Change from baseline in haptoglobin over time

xiii. Change from baseline in lactate dehydrogenase over time

xiv. Proportion of alleles with intended genetic modification present in peripheral blood leukocytes over time

xv. Proportion of alleles with intended genetic modification present in CD34+ cells of the bone marrow over time

xvi. Change in patient reported outcomes (PROs) over time in adults (≥18 years) using;

- Pain-scale:11-point numerical rating scale (NRS)
- Functional assessment of cancer therapy-bone marrow transplant (FACT-BMT)
- Adult Sickle Cell Quality of Life Measurement System (ASCQ-Me)
- EuroQol Quality of Life Scale (EQ-5D-5L)

xvii. Change in PROs over time in adolescents (12 to <18 years of age) using;

- Pain-scale: 11-point NRS
- Pediatric Quality of Life Inventory (PedsQL Teen self-report and parent proxy
- versions)
- PedsQL SCD module (Teen self-report and parent proxy versions)
- EQ-5D-Youth (EQ-5D-Y self-report and parent proxy version)

The Endpoint Adjudication Committee (EAC) was composed of an independent, external group of experts with appropriate clinical and scientific background to evaluate VOCs. The EAC adjudicated historical VOCs (during the 2 years before screening) and on-study VOCs to ensure that the events met the study definition of a severe VOC.

- **Sample size**

With a total of 45 subjects dosed, 3 IAs could be performed following a group sequential testing procedure in the study to allow for early evaluation of efficacy. This sample size provided at least 95% power to rule out a response rate of 50% when the true response rate is 80% for both the primary and key secondary efficacy endpoints with 1-sided alpha of 2.5%.

- **Randomisation and blinding (masking)**

This was a non-randomised, open-label, single-arm study.

- **Statistical methods**

Planned methods of analyses were provided in the statistical analysis plan and the clinical pharmacology analysis plan. These plans were finalized before performing the data cut for the prespecified IA2.

Key details of the planned methods of analyses are summarized in the following paragraphs:

Analysis sets

The following analysis sets were defined: Enrolled Set (all subjects who provided informed consent and were eligible), Safety Analysis Set (all subjects in the Enrolled set who started mobilization regimen), Full Analysis Set (FAS; subjects in the Enrolled set who received exa-cel infusion; used for demographics and baseline characteristics), and Primary Efficacy Set (PES; Subset of the FAS which

included subjects with ≥ 16 months FU after exa-cel infusion and ≥ 14 months FU after RBC transfusion for post-transplant support or SCD management). Subjects who completed the 24 months of follow up in the study after exa-cel infusion were included in the PES, with the exception of those who received RBC transfusions between Month 10 and Month 12 but had less than 14 months (including up to 2 months in Study 131) additional follow up time. In addition, subjects who died or discontinued the study due to AEs considered related to exa-cel and had less than 16 months follow-up after exa-cel infusion, or continuously received RBC transfusions for more than 12 months after exa-cel infusion were also included in this set and counted as non-responders.

The Confirmatory Efficacy Set (CES) is a subset of the primary efficacy set that includes subjects who have not reached the end of the evaluation period for the primary endpoint at the time of protocol amendment v6.0 (EUR) finalization. Supportive analyses will be conducted based on the CES for the primary efficacy endpoint at IA3 and the final analysis.

Efficacy analyses

At the interim analyses, the analysis of VF12 will be based on the PES. The proportion of subjects who meet the primary efficacy endpoint will be provided, with the one-sided P value (against a 50% response rate) and the two-sided 95% exact Clopper-Pearson CI. In the final analysis, the analysis of VF12 will be based on the FAS.

A subject will be a responder if he/she meets the response criteria any time during the response evaluation period. If a subject has died or discontinued the study before achieving VF12 starting at least 60 days after the last RBC transfusion for post-transplant support or SCD management due to reasons other than exa-cel-related adverse events, then the VOC free status of the subject will be carried forward up to 24 months post exa-cel infusion.

Subjects who die or discontinue the study due to exa-cel-related adverse events and have less than 16 months follow-up post exa-cel infusion, or continuously receive RBC transfusion for post-transplant support or SCD management post exa-cel infusion after Month 12 will be considered non-responders for VF12. Subjects who receive RBC transfusions between Month 10 and Month 12 will be considered responders if they have a subsequent 14-month severe VOC free period, which could include up to 2 months in the long-term follow-up Study 131.

Subgroup analyses of the primary efficacy endpoint will be performed with point estimate and 95% confidence interval for each of the following subgroups, during interim and final analyses. Other subgroup analyses may be performed as appropriate. Descriptive summary will be considered if a subgroup has a sample size < 5. Subgroup analysis refers to age at screening (<18, and ≥18 years), genotype, and number of VOCs/year for the prior 2 years at baseline (<3 vs. ≥3).

Multiplicity

Multiplicity was considered with respect to testing the null hypothesis for the primary and key secondary efficacy endpoints across 3 IAs and the final analysis. The familywise type I error rate was/will be controlled by an alpha spending approach for tests at interim and final analyses and a sequential testing of the primary and key secondary efficacy endpoints (i.e., the key secondary efficacy endpoint will be tested only if the primary efficacy endpoint has crossed an efficacy boundary).

The following efficacy boundaries for the primary and key secondary efficacy endpoints are specified to control the type I error at one-sided 2.5% across multiple looks, based on the exact binomial distribution (Table 9).

Table 9. Efficacy Boundaries and Probability of Crossing Efficacy Boundary under Null Hypothesis for the Primary Efficacy Endpoint at Interim and Final Analyses

Analysis Time Point	Efficacy Boundary	Alpha Spent at Each IA or Final Assuming a Response Rate of 50% [a]
IA1: N=10	9/10	1.074%
IA2: N = 17	14/17	0.366%
IA3: N=30	22/30	0.540%
Final: N = 45	31/45	0.440%
Overall α Spending		2.420%

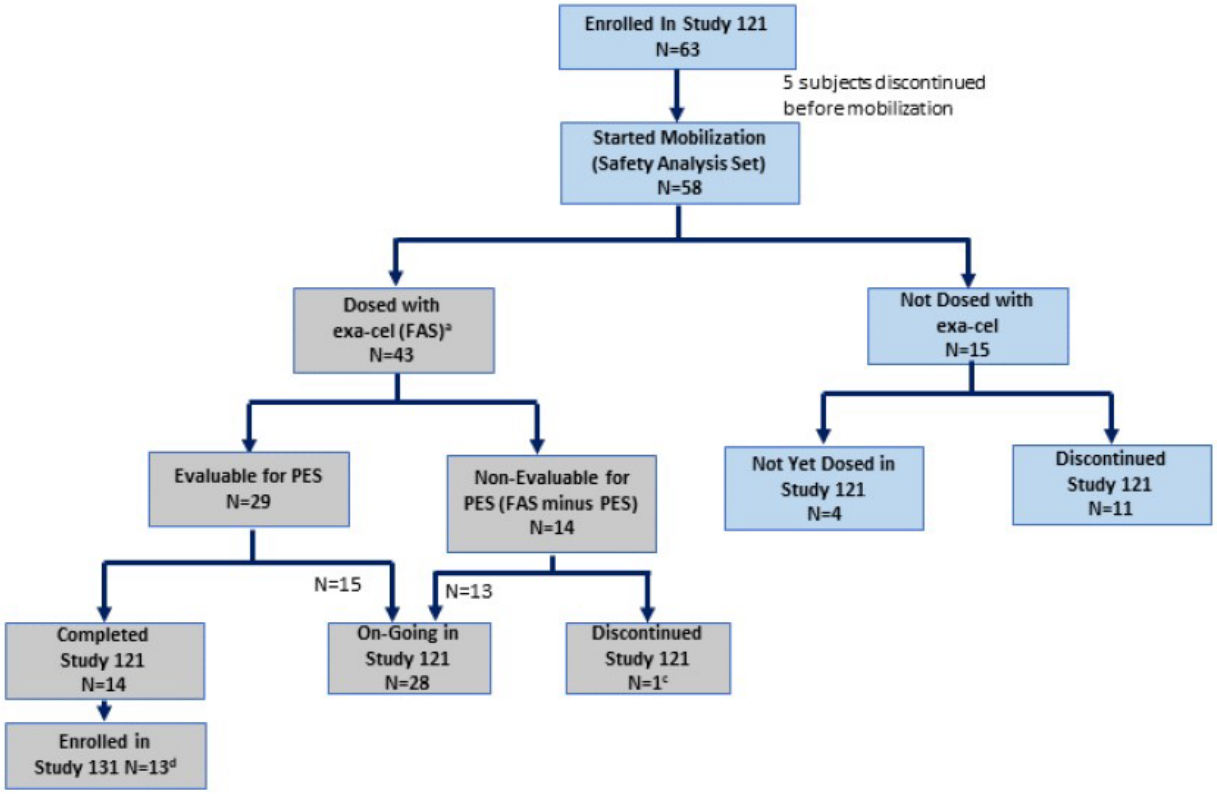
Source: Appendix 16.1.9/SAP Version 4.3 EUR//Table 9-1
IA: interim analysis; N: number of subjects
[a] IA1 was not conducted, the alpha planned for this IA was recovered for the subsequent analysis and the primary and key secondary endpoints will be considered as statistically significant if the corresponding 1-sided *P* value is <0.0144.

IA1 was not conducted; the type I error assigned to IA1 was recovered for subsequent analyses.

Results

Participant flow

Figure 6. Disposition for Studies 121 and 131, Enrolled Set and (SCD) Enrolled Set



Sources: Study 121/Table 14.1.1 and Study 131/Table 14.1.1b (data cutoff date of 16 April 2023)
exa-cel: exagamglogene autotemcel; FAS: Full Analysis Set; N: total number of subjects; PES: Primary Efficacy Set
Notes: Subjects listed as non-evaluable for PES are included in the FAS minus PES data set. For the 4 subjects not yet dosed in Study 121 as of the data cutoff date, 1 subject has since been dosed and 3 are planned to be dosed by Q4 2023.

[a] The FAS included all subjects who received exa-cel infusion.

[b] Reason for discontinuation after exa-cel: death due to [redacted] and was not related to exa-cel.

[c] Reason for discontinuation after starting mobilization: inadequate cell collections (6 subjects), no longer met eligibility criteria for renal function (1 subject), non-compliance (1 subject), withdrew consent (2 subjects), and [redacted] (1 subject).

[d] One subject enrolled in Study 131 after the data cutoff date.

Sixteen subjects discontinued the study before exa-cel infusion: 5 subjects before mobilization and 11 subjects after the start of the mobilization but before start of conditioning. Of the 11 subjects who discontinued during mobilization, six subjects discontinued due to inadequate cell collections, one subject discontinued due to no longer meeting eligibility criteria for renal function, one subject discontinued due to non-compliance, two subjects withdrew consent, and one subject discontinued due to reasons provided in the dossier ; no subject discontinued due to an AE. One subject discontinued the study after exa-cel infusion as a result of death, not related to exa-cel.

Recruitment

Patients were recruited from 16 sites in the United States, Canada, United Kingdom, France, Belgium, Germany and Italy.

Study start date: 27 November 2018

CSR Data (IA 2) cutoff date: 16 September 2022, efficacy and safety update 16 April 2023

Conduct of the study

There were 4 global amendments and 16 country/region-specific amendments to the study protocol. There was 1 protocol addendum to allow for remote source data verification in Germany. Table 9-6 [CSR of study 121] lists the protocol versions, amendment dates, and key changes in study conduct specified in each amendment.

With EU Amendment 6.0 (14 Dec 2020) the study was upgraded to a phase 1/2/3 study, and primary and (key) secondary endpoints were updated, implemented in France in March 2021 and Germany in January 2021. This change was reflected in North America with Amendments 6.9 in the US and 6.10 in Canada (both 22 Sep 2021).

Baseline data

For the 43 subjects in the FAS, 12 (27.9%) subjects were ≥12 and <18 years of age. The majority of subjects were Black or African American (86%). Subjects were approximately balanced by sex.

Of the 43 subjects in the FAS, the majority of subjects had βS/βS genotype. The baseline median (range) annualized rate of VOCs was 3.5 (2.0 to 18.5) per year, and baseline median (range) annualized rate of inpatient hospitalizations for severe VOCs was 2.5 (0.5 to 9.5) each year over the prior 2 years before enrollment.

The baseline mean (range) annualized units of RBC transfusions (simple and/or exchange) was 11.6 (0.0 to 86.1) per year over the prior 2 years before enrollment.

Overall, the population of subjects enrolled in the study was representative of patients with SCD who would be eligible for treatment with exa-cel.

Baseline characteristic data for the PES (N = 29) are representative of the FAS population.

Numbers analysed

Disposition/Reason	Total n (%)
Enrolled Set[a]	63
Safety Analysis Set[b]	58
Started the conditioning regimen	43
FAS[c]	43
PES[d]	29
Never dosed with any study drug[e]	5
Never dosed with exa-cel[f]	16
Started exa-cel infusion	43 (68.3)
Completed exa-cel infusion	43 (100.0)
Not completed exa-cel infusion	0
On Study 121 and not yet dosed with exa-cel[g]	4 (6.3)
On Study 121 and dosed with exa-cel[g]	28 (44.4)
Completed Study 121[h]	14 (22.2)
Completed Study 121 and enrolled in long-term follow-up study[i]	13 (92.9)
Discontinued Study 121 after exa-cel infusion	1 (1.6)
Discontinued Study 121 after exa-cel infusion and enrolled in long-term follow-up study	0
Reason for discontinuing study after exa-cel infusion	
Death	1 (2.3)

Sources: Study 121/Table 14.1.1 and Study 131/Table 14.1.1b (data cutoff date of 16 April 2023)

AE: adverse event; EES: Early Efficacy Set; exa-cel: exagamglogene autotemcel; FAS: Full Analysis Set; n: size of subsample; PES: Primary Efficacy Set; RBC: red blood cell; SCD: sickle cell disease

Notes: Percentages were calculated relative to the number of subjects in the Enrolled Set, unless otherwise specified. Percentages of subjects who completed or did not complete the exa-cel infusion (and percentages of reasons for not completing exa-cel infusion) were calculated relative to the number of subjects who started the exa-cel infusion. Percentages of subjects who discontinued the study after exa-cel infusion and enrolled in the long-term follow-up study were calculated relative to the number of subjects who discontinued study after exa-cel infusion. Percentages of reasons for discontinuing study after exa-cel infusion were calculated relative to the number of subjects who received exa-cel infusion.

[a] Enrolled Set included all enrolled subjects who signed informed consent and met the eligibility criteria.

[b] Safety Analysis Set included subjects who started the mobilization regimen.

[c] FAS included all subjects who received exa-cel infusion.

[d] PES included subjects who had been followed for at least 16 months after exa-cel infusion and for at least 14 months after completion the RBC transfusions for post-transplant support or SCD management. Completion of the (initial) RBC transfusions was determined when all those transfusions for post-transplant support or SCD management had finished followed by 60 days without transfusion. Subjects who completed the 24 months of follow-up in the study post exa-cel infusion were included in this set. In addition, subjects who died or discontinued the study due to exa-cel-related adverse events and had less than 16 months follow-up post exa-cel infusion, or continuously received RBC transfusions for more than 12 months post exa-cel infusion will also be included in this set. For a full definition of the PES refer to Study 121/SAP Version 4.3.

[e] Never dosed with any study drug included all subjects who discontinued the study and did not receive any study drugs for mobilization, conditioning, and exa-cel infusion.

[f] Never dosed with exa-cel included all subjects who discontinued the study and did not receive exa-cel infusion.

[g] On study included all subjects who enrolled and had not yet completed (or discontinued) the study.

[h] Completed study included all subjects who completed the Month 24 Visit.

[i] One subject rolled over into Study 131 after the data cutoff date of 16 April 2023.

Outcomes and estimation

The main analyses of all efficacy endpoints were based on the PES (n=29). […] descriptive summaries of most secondary endpoints were presented based on the FAS (n=43).

VF12

Following infusion with exa-cel, 28 of 29 (96.6%) subjects in the PES achieved VF12 (95% CI: 82.2%, 99.9%; P < 0.0001 [against a 50% response rate]).

Supportive Analysis: Maintenance of VF12

A supportive analysis showed that 27 of the 28 subjects in the PES who achieved VF12 remained VOC free thereafter (i.e., up to data cut date). One subject achieved VF12 and was VOC free for ~22.7 months after exa-cel infusion, then had a single event adjudicated as a VOC by the Endpoint Adjudication Committee (EAC).

HF12

Following infusion with exa-cel, 100% of the 29 subjects in the PES achieved HF12 (95% CI: 88.1%, 100.0%; P<0.0001 [against a 50% response rate]).

•

Total Hb, (HbF) and %HbF of total Hb

In the [SCD]FAS, increased mean Hb levels and HbF (%) were achieved early (by Month 3) after exa-cel infusion and were generally maintained over time from Month 6 through Month 42:

- Mean (SD) total Hb levels were 12.0 (1.5) g/dL at Month 3 and were maintained with mean ≥ 11.1 g/dL from Month 6 onward
- Mean (SD) proportion of total Hb comprised by HbF (HbF %) was 37.3% (9.0%) at Month 3 and was maintained at generally ≥40% from Month 6 over the duration of follow-up.

Proportion of Subjects With Sustained HbF Percentage ≥20%

All 29 (100%) subjects in the PES had sustained HbF ≥20% for at least 12 consecutive months.

Summary of main efficacy results

Title: A Phase 1/2/3 Study to Evaluate the Safety and Efficacy of a Single Dose of Autologous CRISPR-Cas9 Modified CD34+ Human Hematopoietic Stem and Progenitor Cells (exa-cel) in Subjects With Severe Sickle Cell Disease		
Study identifier	Protocol CTX001-121 EudraCT number: 2018-001320-19	
Design	Single-arm, open-label, multi-site, single-dose, Phase 1/2/3 study in subjects 12 to 35 years of age (inclusive) who have severe sickle cell disease (SCD).	
	Duration of main phase:	Stage 1 (screening and pre-mobilization): approximately 2 to 4 months; Stage 2 (mobilization, autologous CD34+ stem cell collection, exa-cel manufacture and disposition): approximately 2 to 4 months; Stage 3 (myeloablative conditioning and infusion of exa-cel): approximately 1 month; Stage 4 (follow-up after exa-cel infusion): approximately 2 years.
	Duration of Run-in phase:	Not applicable
Hypothesis	Null hypothesis of 50% response rate	

Treatments groups	N/A		Single arm	
Endpoints and definitions	Primary endpoint	VF12	Proportion of subjects who achieved VF12, defined as absence of any severe vaso-occlusive crisis (VOC) for at least 12 consecutive months after exa-cel infusion.[a]	
	Key secondary endpoint	HF12	Proportion of subjects who achieved HF12, defined as free from inpatient hospitalization for severe VOCs for at least 12 months after exa-cel infusion.[a]	
	Secondary endpoint	Hemoglobin (Hb) concentration	Total Hb and fetal hemoglobin (HbF) over time.	
	Secondary endpoint	N (HbF ≥20%)	Proportion of subjects with sustained HbF ≥20% for at least 3 months, 6 months, or 12 months.	
Database lock	16 April 2023			
Results and Analysis				
Analysis description	**Primary Analysis**			
Analysis population and time point description	Primary Efficacy Set (PES): a subset of the Full Analysis Set (FAS) that included all subjects who were followed for at least 16 months after exa-cel infusion and for at least 14 months after completion of the red blood cell (RBC) transfusions for post-transplant support or SCD management.[b]			
Descriptive statistics and estimate variability	Treatment group		N/A; all subjects received a single dose of autologous CRISPR-Cas9 modified CD34+	
	Number of subjects		29	
	VF12 n (%)		28 (96.6)	
	2-sided 95% exact Clopper-Pearson confidence interval (CI)		(82.2%, 99.9%)	
	HF12 n (%)		29 (100.0)	
	2-sided 95% exact Clopper-Pearson CI		(88.1%,100.0%)	
	HbF (% of total Hb) Mean (SD)		Month 3: 36.8% (7.9%) Mean HbF % was maintained at generally ≥40% from Month 6 over the duration of follow-up	
	N (HbF ≥20%)		29 (100.0)	
Effect estimate per comparison	Primary endpoint: VF12		Comparison groups	50% response rate

		P-value (1-sided against a 50%	<0.0001[c]
	Key secondary endpoint: HF12	Comparison groups	50% response rate
		P-value (1-sided against a 50%	<0.0001[c]
Notes	One subject discontinued the study after exa-cel infusion as a result of death, not related to exa-cel. Sixteen subjects discontinued the study before exa-cel infusion: 5 subjects before mobilization and 11 subjects after the start of the mobilization but before start of conditioning. Of the 11 subjects who discontinued during mobilization, 6 subjects discontinued due to inadequate cell collections, 1 subject discontinued due to no longer meeting eligibility criteria for renal function, 1 subject discontinued due to non-compliance, 2 subjects withdrew consent, and 1 subject discontinued due to reasons provided in the dossier; no subject discontinued due to an adverse event (AE). [a] The evaluation of the endpoint for a subject started 60 days after last RBC transfusion for post-transplant support or SCD management. The last RBC transfusion refers to that in the period of initial RBC transfusions for post-transplant support or SCD management. [b] Completion of the (initial) RBC transfusions was determined when all those transfusions for post-transplant support or SCD management finished followed by 60 days without transfusion. Subjects who completed the 24 months of follow-up in the study after exa-cel infusion were included in this set, with the exception of those who received RBC transfusions between Month 10 and Month 12 and had less than 14 months (including up to 2 months in Study 131) additional follow-up time. This set also included subjects who died or discontinued the study due to AEs considered related to exa-cel and had less than 16 months follow-up after exa-cel infusion, or continuously received RBC transfusions for more than 12 months after exa-cel infusion.		

2.6.5.9 Clinical studies in special populations

The eligibility criteria for the single (pivotal) study define the age range as 12 to 35 years (inclusive at the date of informed consent). In the updated primary efficacy set, six patients were adolescents.

Abnormal liver function or advanced liver disease (e.g., hepatic cirrhosis), and abnormal cardiac function, and abnormal renal function were some of the exclusion criteria.

2.6.5.10 In vitro biomarker test for patient selection for efficacy

N/A

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

N/A

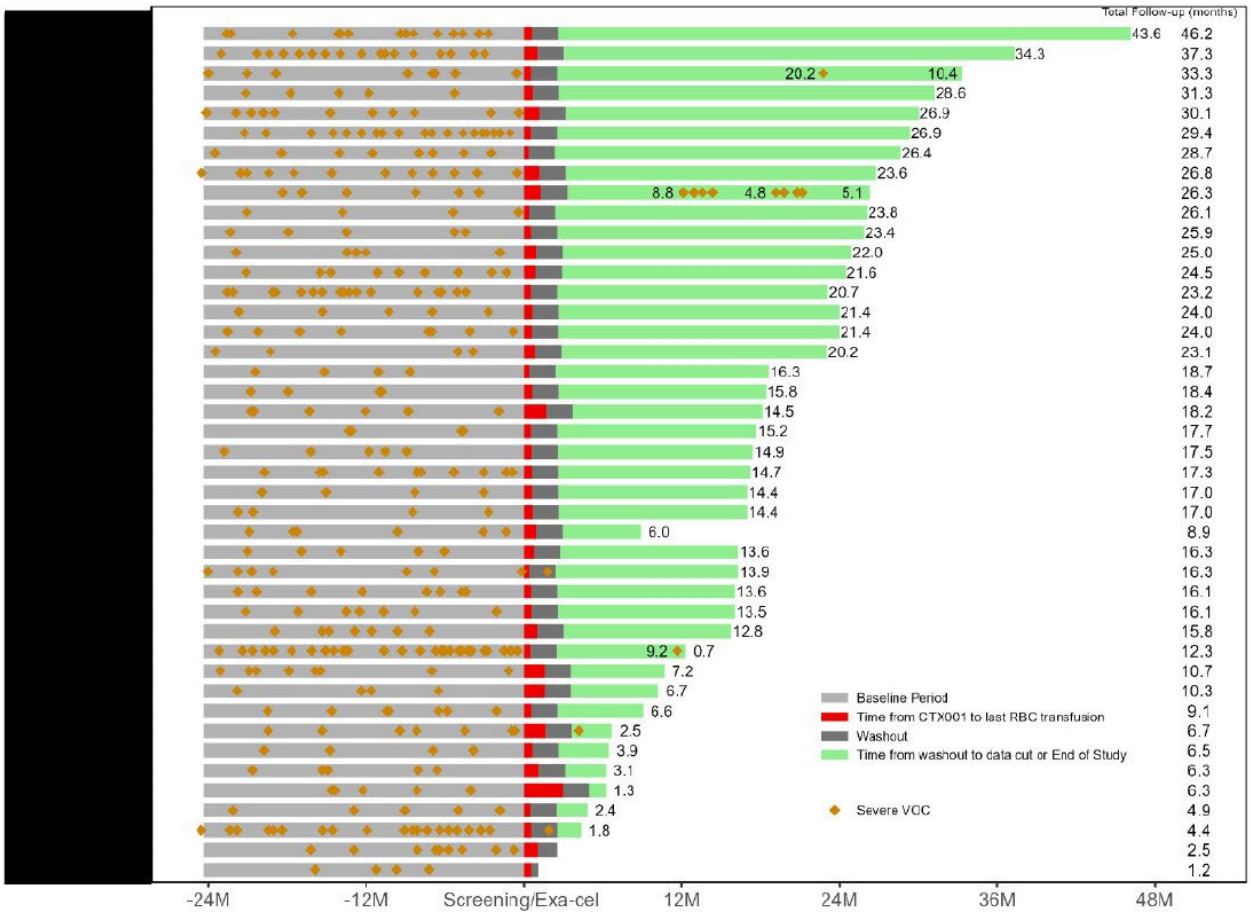
2.6.5.12 Supportive study

A Long-term Follow-up Study of Subjects With β-thalassemia or Sickle Cell Disease Treated with Autologous CRISPR-Cas9 Modified Hematopoietic Stem Cells (exa-cel), study 131

This study is a multi-site, open-label, rollover study designed to evaluate the long-term safety and efficacy of exa-cel in subjects who received exa-cel in a parent study for a total follow-up of 15 years after exa-cel infusion. For the purpose of this assessment, the endpoint duration of severe VOC free is of interest to address sustainability of the effect.

At time of data cut, 13 patients from study 121 have been enrolled into study 131, longest FU is 46.2 months post treatment. Duration of severe VOC free period combined for studies 121 and 131 is given in the Figure below.

Figure 7. Historical and After Exa-cel Severe VOCs and Severe VOC Free Duration (Studies 121 and 131 [SCD]FAS)



Notes: Only severe VOCs that were adjudicated by the EAC as meeting the protocol criteria were displayed for both the baseline period and the post exa-cel infusion period. Baseline period is the 2 years prior to most recent screening. The number on the right end is the duration of total follow-up in month. Last RBC transfusion refers to the last RBC transfusion for post-transplant support or SCD management during the initial RBC transfusion period.
- *: subjects in the [SCD]PES who achieved HF12.
- **: subjects in the [SCD]PES who did not achieve HF12.
- #: subject who died during the study.

2.6.6. Discussion on clinical efficacy

Casgevy (exagamglogene autotemcel or exa-cel) is an autologous $CD34^+$ hHSPCs modified by CRISPR/Cas9-mediated gene therapy medicinal product with editing of the erythroid enhancer region of the BCL11A gene. In addition, it is a new active substance that has not been previoulsy approved for any indication in the EU.

This MAA covers two indications. The clinical development programme presented for MAA consists of 2 single, ongoing FIH pivotal phase 1/2/3, single-arm studies (study 111 in TDT patients, study 121 in SCD patients) and a third, ongoing joint LTFU study (131) for both indications. Of note, for both indications the study started as exploratory phase 1/2 study with different primary efficacy endpoints, restricted to adult patients.

Based on CPMP/EWP/2330/99, in single pivotal trial approaches the study has to be exceptionally compelling, and special attention is to be paid to methodology and clinical relevance. In addition, exa-cel is considered a new pharmacological principle, for which more than one phase 3 study might be expected.

As discussed in the clinical pharmacology section, the relevant pharmacology data is considered to have been replicated within the clinical development programme of exa-cel, thus methodology and clinical relevance remain to be critically discussed. The similarity of the methodology of the clinical studies allows for combined discussion of the methodological issues; the clinical relevance is afterwards discussed per indication.

Design and conduct of clinical studies

Acceptability of the single-arm trial with intra-patient comparison as well as the primary efficacy endpoints to support MAA were agreed in EMA scientific advices for the studied patient populations. Changing the primary endpoint and timing of efficacy assessment in an ongoing open label study that is intended to support an MAA, however, was discouraged in a scientific advice as this might jeopardize the trial integrity (EMEA/H/SA/4534/1/2020/PA/ADT/III and EMEA/H/SA/4534/2/2020/PA/ADT/II).

Despite these recommendations, the exploratory FIH phase 1/2 studies were repeatedly revised, including the aforementioned primary efficacy endpoints and timing of efficacy assessment, but also revision of eligibility criteria. The major revision including upgrade to a phase 1/2/3 study was implemented with protocol amendment 6.0 (EUR) rather late (08 Jan 2021). The applicant clarified that only one TDT subject and 1 SCD subject had reached the primary endpoint by the time of this amendment and hence changes could not be data driven. This is, however, only partially agreed, as data from subjects who had not been followed ≥ 16 months still might show first indications of efficacy and safety or a lack thereof. Taken together, the numerous changes in the pivotal studies raise concerns related to the internal validity of the trials. However, while there could be a possibility that some information on the efficacy (and safety) could have been available at the time the Version 6.0 of the study was implemented, the impact to the overall study integrity is not expected to be large. Therefore, these changes are overall considered acceptable.

Regarding statistical methods, deficiencies were identified for both studies. Efficacy boundaries and multiplicity correction are not fully comprehensible. The design was not well explained and (if one follows the plan) would not fully exploit the available significance level, i.e. would be conservative. At the same time, it might even exceed the significance level and no longer control the type 1 error rate as analyses might have been conducted in a data driven way (no adherence to planned timing of IAs). Furthermore, dropping an interim and simply recycling its significance level would not be an appropriate approach to control the T1E in a group sequential design with alpha spending function. It is noted, though, that there

was no alpha spending approach (other than suggested by the applicant) to update the significance levels. The nominal significance levels at the interim analyses were seemingly defined by splitting the overall significance level. This hence would potentially allow the cumulation of the significance level after an interim analysis was dropped. It is not a common approach and, again, might have been data driven. Dropping the first interim analysis was reported to be based on recommendations from EMA on 17 September 2020 and 03 February 2021. The group sequential design was modified after receiving the advice with SAP v2.0 (01 September 2021) to include three rather than four interim analyses but did not take the advice into account. Hence, the rationale provided by the applicant appears to be a post hoc justification, which was considered inadequate. This all resulted in concerns on the appropriateness of the study design and the conduct of the study. However, it seems unlikely that they impact the outcome of the study in a meaningful way as results were compellingly clinically relevant and statistically highly significant. Furthermore, the trials seemingly would have been significant as well if the applicant would have followed the analysis plan to conduct IA1.

The primary estimand (using LOCF) was not endorsed and the applicant was asked to define an adequate estimand and strategies to handle intercurrent events and/or missing values for future analyses, in line with the discussions presented in the draft RP on single arm trials (EMA/CHMP/564424/2021). The applicant agreed to provide all enrolled patients with non-responder imputation for all dropouts in the SmPC. This reflects the rather high number of early treatment discontinuations, which are of relevance especially for SCD.

For TDT, the use of a weighted Hb average instead of a total Hb constantly being above the pre-defined threshold was questioned. Sensitivity analyses provided upon request, requiring all Hb measurements to remain ≥ 9 g/dl, show that this did not impact the number of responders. This is reassuring.

Study participants recruited under the initial protocol version(s) with different study objectives, potentially different acceptance ranges for the DP, different in- and exclusion criteria were also used for the final assessment of efficacy and safety. It is noted that a "Confirmatory Efficacy Set" was defined, i.e. a subset of the Primary Efficacy Set that includes subjects who have not reached the end of the evaluation period for the primary endpoint at the time of protocol amendment v6.0 (EUR) finalization, which might address some of the methodological concerns. However, no data for this set is (yet) available.

Initially, there were also concerns on representability of the PESs due to low or absence of subject per (age/genotype) sub-groups; with updated data as of 16-Apr-2023 these concerns no longer exist.

The number of patients expected to be evaluable patients for the final analysis and confirmatory efficacy sets were unclear. With the updated information as of 16 April 2023, the applicant provided educated estimates on evaluable patients for these analyses, which is for the confirmatory efficacy set n=55 for TDT, and n=45 for SCD, while for the final analysis it is n=56 for TDT, and n=46 for SCD, respectively. These numbers clarify the extent of patients who already underwent mobilization and/or apheresis but were not treated afterwards.

For TDT, this issue is of less concern, since only three subjects discontinued after start of mobilization but prior to conditioning. All three subjects withdrew consent. In contrast, the number of subjects who could not be treated is substantial for SCD patients, with implications on the benefit/risk (see section 3 Benefit/Risk). Four out of the 5 patients who discontinued before mobilization withdrew consent or were non-compliant, and 1 patient had a delayed hemolytic transfusion reaction. Of the 11 subjects who discontinued after mobilization the minimum dose could not be achieved in 6 subjects (9.5% of all enrolled subjects, 10.3% of all mobilized subjects), 3 withdrew consent or were non-compliant, 1 discontinued due to reasons provided in the dossier, and 1 subject was no longer eligible due to decline in renal function. Of note, of the 16 SCD patients who were not treated, 15 were above 18 years of age

and only one was between 12 and 18 years. The discontinuation rate in adults was 30% (15/50), while it was only 7.7% (1/13) in adolescents.

Table 10. Responder rates considering patients who did not proceed to exa-cel infusion

	Trial 111 (TDT)	Trial 121 (SCD)
Enrolled set	59	63
Subjects who discontinued before mobilization	0	5
Subjects who discontinued after start of mobilization but before conditioning	3	11
Not yet dosed with exa-cel	2	4
Death after infusion but before primary analysis time point	0	1*
FAS	54	43
PES	42	29
Responder rate in PES (including only subjects who were successfully treated) n/N (%; 95% CI)	39 / 42 (92.9%; 80.5%, 98.5%)	28 / 29 (96.6%; 82.2%, 99.9%)
Responder rate in all enrolled and mobilized subjects [a,b] n/N (%; 95% CI)	39 / 45 (86.7%; 73.2%, 94.9%)	28 / 40 (70.0%; 53.5%, 83.4%)
Responder rate in all enrolled subjects[c,d] n/N (%; 95% CI)	39 / 45 (86.7%; 73.2%, 94.9%)	28 / 46 (60.9%; 45.4%, 74.9%)

* One death
[a] Subjects who were enrolled and mobilized and have not yet reached the primary analysis time point or failed prior to reaching the primary analysis time point were excluded from this analysis
[b] Non-responder imputation for dropouts after mobilization regardless of reason for drop-out was used
[c] Subjects who were enrolled and have not yet reached the primary analysis time point or failed prior to reaching the primary analysis time point were excluded from this analysis
[d] Non-responder imputation for all early dropouts regardless of reason for drop-out

Absence of additional supportive studies

For this MAA, only data generated in the pivotal studies 111 and 121 or their joint long-term follow-up study was submitted, but no additional supportive data. During the assessment, the applicant informed the CAT and CHMP of additional ongoing and planned clinical trials. These trials are expected to address some limitations regarding the comprehensiveness of the data and thus were considered for the SOBs.

Transfusion-dependent thalassemia

The proposed indication for MA is *"Casgevy is indicated for the treatment of transfusion dependent β thalassemia (TDT) in patients 12 years of age and older for whom haemopoietic stem cell (HSC) transplantation is appropriate and a human leukocyte antigen (HLA) matched related haematopoietic stem cell (HSC) donor is not available."*

Clinical data is being generated in the ongoing interventional study 111 and the respective long-term follow-up study 131. Patients were recruited from EU and USA. Therefore, the representativeness of the TDT patient population for the European one is agreed.

For MAA, initially an interim analysis of the primary efficacy set with n=27/59 patients has been submitted, updated to n=42/59 with data cut-off date 16-Apr-2023. The primary efficacy set consists of all patients who were followed for at least 16 months after exa-cel infusion and for at least 14 months after completion of the RBC transfusions for post-transplant support or TDT disease management. Subjects who completed the 24 months of follow-up after exa-cel infusion were also included in this set,

except for those who received RBC transfusions between Month 10 and Month 12 but have less than 14 months (including up to 2 months in Study 131) additional follow up time. This set also includes subjects who died or discontinued the study due to AEs related to exa-cel and had less than 16 months of follow-up after exa-cel infusion, or continuously received RBC transfusions for more than 12 months after exa-cel infusion.

The patients were TDT patients, 12 to 35 years of age, eligible for HSCT, but lacking an available suitable donor. Patients with β0/β0-like and non-β0/β0-like genotypes were included. β0/β0-like genotype group included β0/β0, IVS-I-110/IVS-I-110, or β0/IVS-I-110. Non-β0/β0-like genotype included all other genotypes.

The inclusion and exclusion criteria are in general considered adequate. The study population consisted of all most important genotypes of TDT.

All genotypes and both adolescents and adults were represented in the PES. With respect to the study patient population the age group >35 years of age has not been studied, which is reflected in the SmPC.

Efficacy data and additional analyses

Transfusion independence and durability of effect

Relevant endpoints are the primary endpoint TI12 and the secondary endpoint "RBC transfusion free". In study 111, 39 of 42 (92.9%) subjects in the Primary Efficacy Set (PES) achieved TI12. All subjects in the PES who met the primary endpoint remained transfusion free for the duration of follow-up; the mean (SD) transfusion free duration while maintaining weighted Hb ≥9 g/dL was 23.6 (7.8) months, starting 60 days after the last RBC transfusion.

N=39/42 patients in the PES achieved transfusion independence, that has been sustained through the end of follow up. Combining data of studies 111 and 131, the total duration of transfusion free ranged from 13.5 to 48.1 months.

There were three patients who did not reach TI12. As of the April 2023 data cut-off, all 3 subjects have stopped receiving RBC transfusions 14.5 months, 12.2 months, and 21.6 months after exa-cel infusion and have been transfusion free through the time of this data cut-off date for 10.3 months, 7.0 months, and 2.8 months (starting 60 days after the last RBC transfusion), respectively.

While the early efficacy results are promising, for a comprehensive data set especially longer FU is needed to demonstrate that the benefit of the treatment is maintained in the long term. This is reflected by considering a conditional instead of a full approval, with generation of respective long-term follow-up data post-authorisation as part of specific obligations.

Total Hb

The mean (SD) total Hb levels were 11.4 (2.2) g/dL at Month 3 and were maintained with mean ≥12 g/dL from Month 6 onward. Maintaining a weighted average haemoglobin (Hb) ≥9 g/dL was part of the transfusion independence definition; Hb of 9 g/dL is a conceivable, sex-independent threshold for RBC transfusions.

Mean total Hb levels for a mixed female/male population might be impacted by physiologically lower Hb levels in women. Thus, the total Hb is to be considered per sex. In study 111, starting month 6 the total Hb in median (range) was 12.3 (7.9-14.0) g/dL for female, and 12.7 (6.5-16.4) g/dL for male patients, including the patients who did not achieve TI12. The given reference range for Hb in women is 12.6-15.9 g/dL, while it is 14.8-17.8 for men. With respect to reference ranges, starting month 6 over time Hb of 51% female, but 78.3% male patients were below the lower limit, with implications for SmPC and

patient information. Both the SmPC section 5.1 and the patient information include information on Hb below WHO age and sex dependent reference thresholds.

In addition, the applicant provided data on relative reduction in annualized volume of RBC transfusions for 3 of the 42 subjects in the [TDT]PES that had not achieved TI12 at the time of the data cut and have not rolled over into Study 131. This analysis is excluded from efficacy assessment, as delayed onset of transfusion independence while not having achieved T12 (yet) has been reported. The latter is adequately reflected in the SmPC.

Additional expert consultation

Patients' organisation was invited to comment on any aspects that are of particular importance to patients/carers. The received feedback indicated the most meaningful would be new medicines / therapies that will require less frequent blood transfusions, so that the risk of iron overload is reduced (and therefore fewer co-morbidities e.g. heart, liver, endocrine diseases).

Assessment of paediatric data on clinical efficacy

The mechanism of action is identical across the studied patient populations/ages, and no factor impacting extrapolatability of results in adults to results in adolescents was identified.

In the available primary efficacy set (n=42), n=13 adolescents have been included, of which twelve achieved T12. The one adolescent who has not achieved T12, has stopped transfusions at approximately 21.6 months and subsequently been transfusion free for 2.8 months (starting 60 days after the last RBC transfusion).

Additional efficacy data needed in the context of a conditional MA

Overall the dataset is not considered to be comprehensive.

There are patients with overall slow haemotopoietic recovery, that have not yet achieved transfusion independence as defined in the ongoing pivotal trial. Longer follow up of these patients is expected to better characterise the effect of exa-cel on TDT patients. Regarding the concern on durability of the effect over time in an aging population with the potential risk of loss of efficacy due to clonal haematopoiesis, the ongoing study 131 to cover up to 15 years post-treatment is expected to generate respective long-term follow-up data.

Final results from the Study 111 should be submitted no later than 31 August 2026 and is subject to a specific obligation laid down in the MA (SOB-1).

Interim results from the study 161 should be submitted no later than 31 December 2027 and is subject to a specific obligation laid down in the MA (SOB-4).

Interim results of a study based on data from a registry, according to an agreed protocol, should be submitted no later than 31 December 2027 and is subject to a specific obligation laid down in the MA (SOB-6).

Interim reports from study 131 should be submitted no later than 31 August 2026 and 31 August 2029 and are subject to a specific obligation laid down in the MA (SOB-7).

Sickle-cell disease

The proposed indication for MA is "*Casgevy is indicated for the treatment of severe sickle cell disease (SCD) in patients 12 years of age and older with recurrent vaso-occlusive crises for whom haemopoietic stem cell (HSC) transplantation is appropriate and a human leukocyte antigen (HLA) matched related haematopoietic stem cell (HSC) donor is not available*".

Clinical data is being generated in the ongoing interventional studies 121, 151, 161, and 171 and their respective long-term follow-up study 131. Only clinical data generated in studies 121 and 131 is available for MAA. Patients are recruited from EU and USA. Therefore, the representativeness of the SCD patient population for the European one is agreed.

For MAA, initially an interim analysis of the primary efficacy set with n=17/63 patients has been submitted, updated to n=29/63 patients with data cut-off date 16-Apr-2023. The primary efficacy set consists of all patients who were followed for at least 16 months after exa-cel infusion and for at least 14 months after completion of the RBC transfusions for post-transplant support or SCD management. Subjects who completed the 24 months of follow-up after exa-cel infusion were also included in this set, except for those who received RBC transfusions between Month 10 and Month 12 but have less than 14 months (including up to 2 months in Study 131) additional follow up time. This set also includes subjects who died or discontinued the study due to AEs related to exa-cel and had less than 16 months of follow-up after exa-cel infusion, or continuously received RBC transfusions for more than 12 months after exa-cel infusion.

Patients in study 121 were 12 to 35 years of age (inclusive, at time of informed consent) with documented βS/βS, βS/β0, or βS/β+ genotype and with severe SCD. While the study population consisted of all most important genotypes of SCD, only four 4 non βS/βS subjects (3 βS/β0 subjects and 1 βS/β+subject) have been dosed with exa-cel. The proportion of subjects for each of the genotypes is reflected in SmPC section 5.1, as is the addition that (currently) no data is available for patients with other genotypes. However, the MoA of exa-cel is genotype-agnostic and available HbF trajectories are similar across genotypes; β^S/β^C genotype is enrolled in study 171, and the β^S/β^E genotype is expected to be covered by the planned PASS.

Thus, the study patient population in general supports the proposed indication; the age group >35 years of age has not been studied, which is reflected in the SmPC.

Efficacy data and additional analyses

Absence of severe VOC and maintenance of effect

Of relevance are the primary endpoint VF12 and the secondary endpoint "duration of VOC free". 28 of 29 (96.6%) subjects in the Primary efficacy set (PES) achieved VF12, i.e. absence of severe VOCs for at least 12 consecutive months; Twenty seven of 28 (96.4%) subjects who achieved VF12 remained VOC free for the duration of follow-up in Studies 121 and 131, up to 43.6 months after exa-cel infusion demonstrating the durability of treatment effect.

Absence of hospitalization for severe VOC

Being free of hospitalizations for severe VOCs for at least 12 consecutive months was the key secondary endpoint, HF12. All (100%) subjects in the PES achieved HF12.

%HbF/total Hb

In the [SCD]FAS, the mean (SD) proportion of total Hb comprised by HbF (%) was 37.3% (9.0%) at Month 3 and were maintained at ≥40% from Month 6 onward.

Additional expert consultation

Patients' organisation was invited to comment on any aspects that are of particular importance to patients/carers. The received feedback indicated the most meaningful would be minimization or complete eradication of painful episodes for SCD, respectively. In addition, hospitalizations were identified as particular burden for SCD patients.

Assessment of paediatric data on clinical efficacy

The mechanism of action is identical across the studied patient populations/ages, and no factor impacting extrapolatability of results in adults to results in adolescents was identified.

At the 16 April 2023 data cut-off date, 6 of 29 (20.7%) subjects in the PES were adolescents. 6 out of 6 (100%) adolescent subjects in the PES achieved VF12 and HF12 and have been VOC free for up to 16.3 months. Adolescent subjects treated with exa-cel had rapid, robust, and durable increases in levels of HbF (%) in Studies 121 and 131, similar to what was observed in adult patients. All adolescent subjects (n = 12) with available Month 6 HbF (%) data (N = 10), had HbF (%) levels of ≥20% by Month 6.

Additional efficacy data needed in the context of a conditional MA

For SCD the longer-term information generated with study 131 not only on duration of severe VOC free periods/maintenance of absence of VOCs, but circumstances of VOC events despite %HbF/total Hb being above the predefined threshold is expected to better characterise the effect of exa-cel on severe SCD.

A high number of subjects discontinued the study early (most importantly due to inability to successfully manufacture drug product). Underlying patient characteristics to prospectively select only patients with high probability of successfully manufactured product besides the underlying disease and implications for mobilisation are currently unknown, but these patients impact the effect size. Therefore, additional data on the extent of patients for which no product can be produced is expected with the ongoing and planned studies 151, 161, and 171.

Thus, the dataset is not considered to be comprehensive.

The final results from study 121 should be submitted no later than 31 August 2026 and is subject to a specific obligation laid down in the MA (SOB-2).

The final results from the study 151 should be submitted no later than 31 December 2027 and is subject to a specific obligation laid down in the MA (SOB-3).

The interim results from the study 161 should be submitted no later than 31 December 2027 and is subject to a specific obligation laid down in the MA (SOB-4).

The final results from the Study 171 should be submitted no later than 30 June 2032 and is subject to a specific obligation laid down in the MA (SOB-5).

Interim results of a study based on data from a registry, according to an agreed protocol, should be submitted no later than 31 December 2027 and is subject to a specific obligation laid down in the MA (SOB-6).

Interim reports from study 131 should be submitted no later than 31 August 2026 and 31 August 2029 and are subject to a specific obligation laid down in the MA (SOB-7).

2.6.7. Conclusions on the clinical efficacy

Transfusion-dependent thalassemia

An updated analysis of a single pivotal, SAT, FIH phase 1/2/3 study (111) of n=42/59 patients has been submitted as pivotal data, supported by an interim analysis of study 131 with longer-term follow-up data.

The clinical evidence covers TDT patients of β0/β0-like (β0/β0, β0/IVS-I-110, IVS-I-110/IVS-I-100) and nonβ0/β0-like genotype, 12 to 35 years of age, eligible for HSCT, but for whom no suitable donor was available. The proposed indication is in line with the studied population.

Despite the limited sample size of the patients evaluable for the primary endpoint of study 111, the efficacy as demonstrated by transfusion independence as primary endpoint and being durable for the follow-up period(s) in n=39/42 patients, of which n=12 were adolescents, is compelling, outweighing methodological issues with this ongoing single FIH/pivotal, single-arm study.

Regarding total Hb levels, Hb levels above accepted, sex-independent transfusion thresholds still might be below "normal", which is reflected in the SmPC and patient information.

Sickle-cell disease

An updated analysis of a single pivotal, SAT, FIH phase 1/2/3 study (121) of n=43/63 patients has been submitted for MAA, supported by an interim analysis of study 131 with longer-term follow-up data.

The clinical evidence covers βS/βS and βS/β0 patients with severe SCD, 12 to 35 years of age, eligible for HSCT, but for whom no suitable donor was available. Enrolled patients of βS/β+ genotype have not been evaluable.

However, as the mechanism of action of exa-cel is genotype-agnostic and available HbF trajectories are similar across genotypes, the proposed labelling is supported by the studied population.

Despite the limited sample size of the patients evaluable for the primary endpoint of study 121, the efficacy as demonstrated by absence of severe VOCs for at least 12 consecutive months after exa-cel infusion in n=28/29 patients, maintained through end of available follow-up in n=27/28 patients, is compelling, outweighing methodological issues with this ongoing single FIH/pivotal, single-arm study.

The one patient not meeting the primary endpoint had a clinical benefit as demonstrated by absence of hospitalizations for severe VOCs; absence of hospitalizations for severe VOCs for at least 12 months has been achieved by n=29/29 patients in the PES.

The number of SCD patients who could not be treated is substantial (see Table 9); details on patients enrolled and patients infused has been included in section 5.1 of the SmPC. For comprehensiveness clinical trial data of a larger number of patients is warranted, as is more data on association of successful product manufacturing with patient characteristics, e.g. age. Respective data is expected from the ongoing or planned clinical trials 121, 151, 161, and 171.

The CAT considers the following measures necessary to address the missing efficacy data in the context of a conditional MA:

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) aged 12 years and older, the MAH should submit the

final results from the Study 111.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with severe sickle cell disease (SCD) aged 12 years and older, the MAH should submit the final results from the study 121.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with severe sickle cell disease (SCD) aged 12 years and older, the MAH should submit the final results from the study 151.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) and severe sickle cell disease (SCD) aged 12 years and older, the MAH should submit the interim results from the study 161.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with severe sickle cell disease (SCD) aged 12 years and older, the MAH should submit the final results from the Study 171.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) and severe sickle cell disease (SCD) aged 12 years and older, the MAH should conduct and submit the interim results of a study based on data from a registry according to an agreed protocol. Yearly progress reports shall also be submitted.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) and severe sickle cell disease (SCD) in patients aged 12 years and older, the MAH should submit the interim results from study 131.

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

- In order to further characterise the long-term safety and efficacy of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) and severe sickle cell disease (SCD) aged 12 years and older, the MAH should conduct and submit the results of a study based on data from a registry, according to an agreed protocol.

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

2.6.8. Clinical safety

2.6.8.1. Patient exposure

Safety data are presented from 2 pivotal studies conducted in subjects aged ≥12 to 35 years, inclusive at time of informed consent, with up to 2 years of follow up after exa-cel infusion:

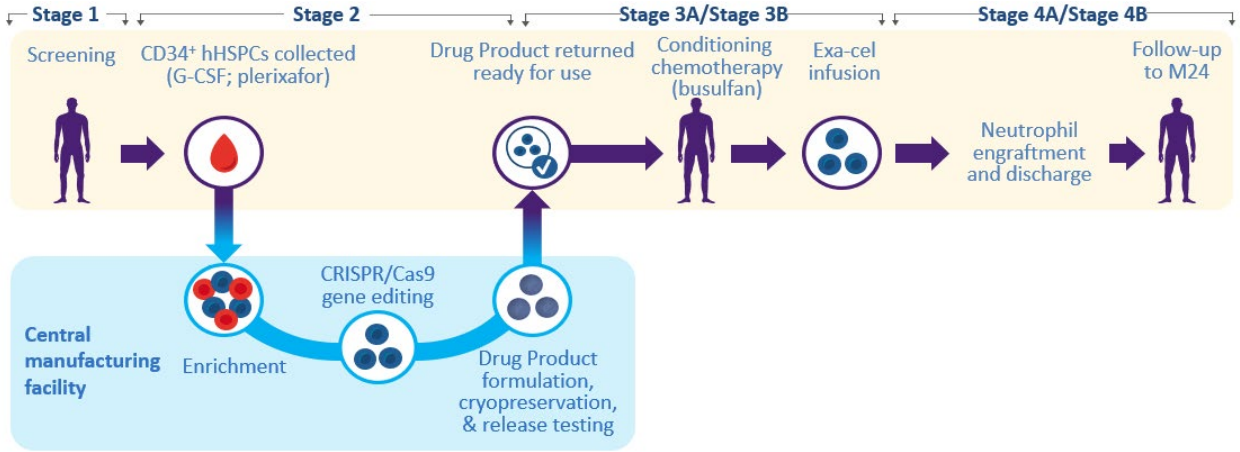
- Study CTX001-111 (Study 111; cutoff date: 16 April 2023): Phase 1/2/3 study in subjects with TDT
- Study CTX001-121 (Study 121; cutoff date: 16 April 2023): Phase 1/2/3 study in subjects with SCD

In addition to the pivotal studies, data are presented for Study VX18-CTX001-131 (Study 131), a long-term follow-up (LTFU) study for both indications (data cutoff date: 16 April 2023 [subjects from Study 111] and 16 April 2023 [subjects from Study 121]).

In both open-label, multi-site studies the minimum recommended dose of exa-cel is 3.0 × 10^6 CD34+ cells/kg administered intravenous (IV) as a single infusion following myeloablative conditioning. In both Study 111 and Study 121, for each subject, the study was conducted in 4 stages:

- Stage 1 included screening and the pre-mobilization period;
- Stage 2 included mobilization, autologous CD34+ stem cell collection, and exa-cel manufacture and disposition;
- Stage 3 included myeloablative conditioning and exa-cel infusion;
- Stage 4 included post-infusion in-hospital follow-up (until neutrophil engraftment and stabilization of major medical issues) and post-discharge follow-up through the Month 24 (M24) study visit (approximately 2 years).

Figure 8. Stages in studies 111 and 121



The following safety endpoints were included in both studies:

- Successful neutrophil engraftment
- Time to neutrophil engraftment
- Time to platelet engraftment
- Safety and tolerability assessments based on AEs, clinical laboratory values, and vital signs
- Incidence of transplant-related mortality (TRM) within 100 days and within 1 year after exa-cel infusion
- All-cause mortality

These endpoints are standardly evaluated following HSCT.

Study 131 is a long-term follow-up study for subjects who have received exa-cel and provides up to 15 years of follow up after exa-cel infusion. Study 131 included the following safety endpoints:

- New malignancies
- New or worsening hematologic disorders (e.g., immune-mediated cytopenias, aplastic anemia, primary immunodeficiencies)
- All-cause mortality
- All serious adverse events (SAEs) occurring up to 5 years after exa-cel infusion

- Adverse events (AEs) and SAEs related to exa-cel.

At the data cutoff, for Study 111, 59 subjects with TDT were enrolled, 59 subjects started mobilization, and 54 were dosed with exa-cel at a median dose of 8.0 (range: 3.0 to 19.7) × 10^6 CD34+ cells/kg. 2 subjects were not yet dosed with exa-cel, 3 patients discontinued the study. The median follow-up duration after exa-cel infusion was 22.8 (range: 2.1 to 51.1) months for subjects with TDT.

At the data cutoff, for Study 121, 63 subjects with SCD were enrolled, 58 subjects started mobilization, and 43 subjects with SCD were dosed with exa-cel at a median dose of 4.0 (range: 2.9 to 14.4) × 10^6 CD34+ cells/kg. 4 subjects were not yet dosed with exa-cel, 11 patients discontinued the study after the start of the mobilization but before start of conditioning. The median follow-up duration after exa-cel infusion was 17.5 (range: 1.2 to 46.2) months for subjects with SCD.

At the data cutoff, for the long-term follow-up Study 131, 36 subjects had enrolled in Study 131 (23 from Study 111 and 13 from Study 121).

Table 11. Follow-up Duration After Exa-cel Infusion through Study 131 (Long-term Follow-Up): FAS

	TDT (Studies 111 + 131) N = 54	SCD (Studies 121 + 131) N = 43
Follow-up duration after exa-cel infusion (month)		
n	54	43
Mean (SD)	22.3 (10.51)	18.6 (9.99)
Median	22.8	17.5
Min, max	2.1, 51.1	1.2, 46.2
Exposure after exa-cel infusion (patient-months)	1205.9	797.9
Exposure after exa-cel infusion (patient-years)	100.5	66.5
Follow-up duration after exa-cel infusion by interval[a], n (%)		
≤3 months	2 (3.7)	2 (4.7)
>3 months to ≤6 months	4 (7.4)	2 (4.7)
>6 months to ≤12 months	4 (7.4)	8 (18.6)
>12 months to ≤24 months	20 (37.0)	16 (37.2)
>24 months[a]	24 (44.4)	15 (34.9)

Sources: Study 131/Table 14.1.10.1a and Study 131/Table 14.1.10.1b (data cutoff date of 16 April 2023)
exa-cel: exagamglogene autotemcel; FAS: Full Analysis Set; N: total sample size; n: size of subsample; SCD: sickle cell disease; TDT: transfusion-dependent β-thalassemia
Notes: Follow-up duration (months) after exa-cel infusion= (Data cutoff date or end date of Study 131 whichever is earlier – exa-cel infusion date + 1)/30. Exposure (patient-months/patient-years) after exa-cel infusion = Sum of the after exa-cel infusion follow-up duration (months/years) from subjects who have received exa-cel infusion in the FAS.
[a] Follow-up duration is not equivalent to study visit (see calculation above). Due to protocol-specified visit windows, a subject in this category may not have completed the Month 24 visit in Study 111 or Study 121, as applicable, thus had not enrolled in Study 131.

Table 12. Subject Demographics and Baseline Characteristics (FAS)

Study 111

Demographics	PES N = 42	FAS N = 54
Sex, n (%)		
Male	21 (50.0)	29 (53.7)
Female	21 (50.0)	25 (46.3)
Childbearing potential[a], n (%)		
Yes	21 (100.0)	25 (100.0)
No	0	0
Age at screening (years)		
n	42	54
Mean (SD)	21.6 (6.4)	21.3 (6.6)
Median	[redacted]	
Min, max		
Age category at screening, n (%)		
≥12 and <18 years	13 (31.0)	19 (35.2)
≥18 and ≤35 years	29 (69.0)	35 (64.8)
Race, n (%)		
White	17 (40.5)	18 (33.3)
Black or African American	0	0
Asian	16 (38.1)	23 (42.6)
American Indian or Alaska Native	0	0
Native Hawaiian or other Pacific Islander	0	0
Not collected per local regulations	5 (11.9)	8 (14.8)
Other	1 (2.4)	2 (3.7)
Multiracial	3 (7.1)	3 (5.6)
Ethnicity, n (%)		
Hispanic or Latino	1 (2.4)	1 (1.9)
Not Hispanic or Latino	38 (90.5)	47 (87.0)
Not Collected per Local Regulations	3 (7.1)	6 (11.1)

Sources: Study 111/Table 14.1.3.1 and Ad hoc Table 14.1.3.3 (data cutoff date of 16 April 2023)
FAS: Full Analysis Set; N: total sample size; n: size of subsample; PES: Primary Efficacy Set
Notes: Percentages were calculated relative to the number of subjects in the FAS or the PES, unless otherwise specified.
[a] Percentages for childbearing potential were calculated relative to the number of females in the FAS or the PES.

Study 121

Demographics	PES N = 29	FAS N = 43
Sex, n (%)		
Male	16 (55.2)	24 (55.8)
Female	13 (44.8)	19 (44.2)
Childbearing potential[a], n (%)		
Yes	13 (100.0)	19 (100.0)
No	0	0
Age at screening (years)		
n	29	43
Mean (SD)	22.2 (6.1)	21.2 (6.1)
Median	[redacted]	
Min, max		
Age category at screening, n (%)		
≥12 and <18 years	6 (20.7)	12 (27.9)
≥18 and ≤35 years	23 (79.3)	31 (72.1)
Race, n (%)		
White	1 (3.4)	3 (7.0)
Black or African American	26 (89.7)	37 (86.0)
Asian	0	0
American Indian or Alaska Native	0	0
Native Hawaiian or other Pacific Islander	0	0
Not collected per local regulations	0	0
Other	2 (6.9)	3 (7.0)
Multiracial	0	0
Ethnicity, n (%)		
Hispanic or Latino	2 (6.9)	2 (4.7)
Not Hispanic or Latino	26 (89.7)	40 (93.0)
Not collected per local regulations	1 (3.4)	1 (2.3)

Sources: Study 121/Table 14.1.3.1 and Ad hoc Table 14.1.3.3 (data cutoff date of 16 April 2023)
FAS: full analysis set; max: maximum; min: minimum; N: total sample size; n: size of subsample; PES: Primary Efficacy Set
Notes: Percentages were calculated relative to the number of subjects in the FAS or the PES, unless otherwise specified.
[a] Percentages for childbearing potential were calculated relative to the number of females in the FAS or the PES.

2.6.8.2. Adverse events

Per protocol, exa-cel infusion occurred between 48 hours and 7 days after completion of myeloablative conditioning with busulfan; therefore, effects of busulfan conditioning are also observed after exa-cel infusion. From exa-cel infusion onward, the investigator assessed relationship of AEs to exa-cel and busulfan individually.

Busulfan has a well-characterized and understood safety profile and numerous important risks, including prolonged myelosuppression (e.g., anemia, thrombocytopenia; 100% of subjects in randomized, controlled clinical trials) and veno-occlusive liver disease (incidence in randomized clinical trials 7% to 12%). In addition the following common side effects (≥10% of subjects in randomized clinical trials): infections and infestations (e.g., cytomegalovirus or Epstein- Barr virus reactivation, bacterial infection, viral infection); blood and lymphatic system disorders (e.g., neutropenia, thrombocytopenia, febrile neutropenia, anemia, pancytopenia); immune system disorders (e.g., allergic reaction); metabolism and nutrition disorders (e.g., anorexia, hypomagnesaemia); psychiatric disorders (e.g., anxiety, insomnia);

nervous system disorders (e.g., headache, dizziness); cardiac disorders (e.g., tachycardia,); vascular disorders (e.g., hypertension, thrombosis), respiratory thoracic and mediastinal disorders (e.g., dyspnea, epistaxis), gastrointestinal disorders (e.g., stomatitis, nausea, vomiting); hepatobiliary disorders (e.g., hepatomegaly); skin and subcutaneous tissue disorders (e.g., rash, pruritus), musculoskeletal and connective tissue disorders (e.g., back pain); renal and urinary disorders: (e.g., hemorrhagic cystitis); general disorders and administration site conditions (e.g., asthenia, chills, fever); and investigations (e.g., bilirubin increased, transaminases increased).

At the data cutoff of 06 September 2022, the incidence of AEs and SAEs after exa-cel infusion was consistent with that expected from myeloablative conditioning with busulfan and autologous HSCT. Overall, most AEs were non-serious and Grade 1 or Grade 2 in severity.

In subjects with TDT (N = 48), no deaths or AEs leading to discontinuation have occurred. All 48 (100.0%) subjects with TDT had at least 1 AE after exa-cel infusion. Most subjects (45 [98.3%]) had at least 1 AE considered possibly related or related the busulfan and fewer subjects (13 [27.1%]) had at least 1 AE considered possibly related or related to exa-cel. Grade 3 or above AEs occurred in a total of 41 (85.4%) subjects, including 40 (83.3%) subjects with an event considered possibly related or related to busulfan and 8 (16.7%) subjects with an event considered possibly related or related to exa-cel. SAEs occurred in 17 (35.4%) subjects, of which 9 (18.8%) subjects had SAE(s) considered possibly related or related to busulfan and 2 (4.2%) subjects had SAE(s) considered possibly related or related to exa-cel.

In subjects with SCD (N = 35), 1 subject died due to reasons provided in the dossier, not related to exa-cel. No other deaths or AEs leading to discontinuation occurred. All 35 (100.0%) subjects with SCD had at least 1 AE after exa-cel infusion. All 35 (100%) subjects had at least 1 AE considered possibly related or related to busulfan and fewer subjects (12 [34.3%]) had at least 1 AE considered possibly related or related to exa-cel. Grade 3 or above AEs occurred in 34 (97.1%) subjects, including 34 (97.1%) subjects with an event considered possibly related or related to busulfan and 7 (20.0%) subjects with an event considered possibly related or related to exa-cel. SAEs occurred in 14 (40.0%) subjects, of which 4 (11.4%) subjects had SAE(s) considered possibly related or related to busulfan anno subject had SAE(s) considered possibly related or related to exa-cel.

Table 13. AEs Related to Exa-cel by PT from Exa-cel Infusion Through M24: iFAS

Preferred Term	TDT (Study 111) n (%)	SCD (Study 121) n (%)	Total TDT + SCD n (%)
Evaluable subjects, N1	48	35	83
Subjects with any AEs related to exa-cel[a]	13 (27.1)	12 (34.3)	25 (30.1)
CD4 lymphocytes decreased	5 (10.4)	8 (22.9)	13 (15.7)
Lymphopenia	2 (4.2)	5 (14.3)	7 (8.4)
Thrombocytopenia	2 (4.2)	1 (2.9)	3 (3.6)
Headache	2 (4.2)	0	2 (2.4)
Neutrophil count decreased	2 (4.2)	0	2 (2.4)
Platelet count decreased	1 (2.1)	1 (2.9)	2 (2.4)
Acute respiratory distress syndrome	1 (2.1)	0	1 (1.2)
Anaemia	1 (2.1)	0	1 (1.2)
Chills	1 (2.1)	0	1 (1.2)
Delayed engraftment	1 (2.1)	0	1 (1.2)
Dermatitis	0	1 (2.9)	1 (1.2)
Epistaxis	1 (2.1)	0	1 (1.2)
Haemophagocytic lymphohistiocytosis	1 (2.1)	0	1 (1.2)
Hypocalcaemia	1 (2.1)	0	1 (1.2)
Idiopathic pneumonia syndrome	1 (2.1)	0	1 (1.2)
Lymphocyte count decreased	1 (2.1)	0	1 (1.2)
Neutropenia	0	1 (2.9)	1 (1.2)
Paraesthesia	1 (2.1)	0	1 (1.2)
Petechiae	1 (2.1)	0	1 (1.2)
Pyrexia	1 (2.1)	0	1 (1.2)
Sinus tachycardia	1 (2.1)	0	1 (1.2)
Tachycardia	1 (2.1)	0	1 (1.2)
White blood cell count decreased	1 (2.1)	0	1 (1.2)

Source: ISS Table 14.3.1.3.6

AE: adverse event; exa-cel: exagamglogene autotemcel; iFAS: integrated Full Analysis Set; n: size of subsample; PT: preferred term; SCD: sickle cell disease; TDT: transfusion-dependent β-thalassemia

Notes:

- MedDRA version 25.0
- Evaluable subjects, N1: The number of subjects in the integrated Safety Analysis Set who were on or after the day of exa-cel infusion (i.e., iFAS)
- percentages are calculated as n/N1*100
- Interval starts from the first day of exa-cel infusion and goes through Month 24.
- When summarizing number and percentage of subjects, a subject with multiple events within a category is counted only once in that category.

[a] "Related", "Possibly Related", and "Missing" are considered as "Related" in this table; related includes events related or possibly related to exa-cel and busulfan, or exa-cel only: 4 (8.3%) subjects with TDT had AEs considered related or possibly related to exa-cel only Study 111 CSR/Table 14.3.1.3.8); 1 (8.3%) subject with SCD had AEs considered related or possibly related to exa-cel only Study 121 CSR/Table 14.3.1.3.8)

Table 14. AEs Related to Busulfan Occurring in ⩾10% of Subjects in Any Category by PT from Start of Busulfan Through M24: iFAS

Preferred Term	TDT (Study 111) n (%)	SCD (Study 121) n (%)	Total TDT + SCD n (%)
Evaluable subjects, N1	48	35	83
Subjects with any AEs related to busulfan	45 (93.8)	35 (100.0)	80 (96.4)
Nausea	23 (47.9)	29 (82.9)	52 (62.7)
Stomatitis	24 (50.0)	24 (68.6)	48 (57.8)
Febrile neutropenia	20 (41.7)	17 (48.6)	37 (44.6)
Platelet count decreased	14 (29.2)	18 (51.4)	32 (38.6)
Vomiting	15 (31.3)	17 (48.6)	32 (38.6)
Abdominal pain	16 (33.3)	14 (40.0)	30 (36.1)
Decreased appetite	10 (20.8)	20 (57.1)	30 (36.1)
Mucosal inflammation	19 (39.6)	10 (28.6)	29 (34.9)
Neutrophil count decreased	12 (25.0)	15 (42.9)	27 (32.5)
Anaemia	13 (27.1)	11 (31.4)	24 (28.9)
Thrombocytopenia	16 (33.3)	7 (20.0)	23 (27.7)
Diarrhoea	8 (16.7)	9 (25.7)	17 (20.5)
Pyrexia	7 (14.6)	10 (28.6)	17 (20.5)
Skin hyperpigmentation	6 (12.5)	11 (31.4)	17 (20.5)
Epistaxis	10 (20.8)	6 (17.1)	16 (19.3)
Headache	8 (16.7)	7 (20.0)	15 (18.1)
Hypokalaemia	8 (16.7)	7 (20.0)	15 (18.1)
CD4 lymphocytes decreased	5 (10.4)	9 (25.7)	14 (16.9)
Fatigue	6 (12.5)	8 (22.9)	14 (16.9)
Neutropenia	6 (12.5)	7 (20.0)	13 (15.7)
White blood cell count decreased	7 (14.6)	6 (17.1)	13 (15.7)
Alopecia	6 (12.5)	6 (17.1)	12 (14.5)
Constipation	5 (10.4)	5 (14.3)	10 (12.0)
Oropharyngeal pain	3 (6.3)	7 (20.0)	10 (12.0)
Dry skin	4 (8.3)	5 (14.3)	9 (10.8)
Gastritis	4 (8.3)	5 (14.3)	9 (10.8)
Skin exfoliation	1 (2.1)	8 (22.9)	9 (10.8)
Alanine aminotransferase increased	6 (12.5)	2 (5.7)	8 (9.6)
Petechiae	8 (16.7)	0	8 (9.6)
Abdominal pain upper	3 (6.3)	4 (11.4)	7 (8.4)
Amenorrhoea	3 (6.3)	4 (11.4)	7 (8.4)
Dyspepsia	3 (6.3)	4 (11.4)	7 (8.4)
Hypomagnesaemia	3 (6.3)	4 (11.4)	7 (8.4)
International normalised ratio increased	3 (6.3)	4 (11.4)	7 (8.4)
Lymphopenia	2 (4.2)	5 (14.3)	7 (8.4)
Pruritus	3 (6.3)	4 (11.4)	7 (8.4)
Venoocclusive liver disease	6 (12.5)	1 (2.9)	7 (8.4)
Weight decreased	1 (2.1)	6 (17.1)	7 (8.4)
Hyperphosphataemia	1 (2.1)	5 (14.3)	6 (7.2)

Hypophosphataemia	5 (10.4)	1 (2.9)	6 (7.2)
Dysuria	1 (2.1)	4 (11.4)	5 (6.0)
Fluid retention	5 (10.4)	0	5 (6.0)

Source: ISS Table 14.3.1.3.5

AE: adverse event; exa-cel: exagamglogene autotemcel; IA2: interim analysis 2; iFAS; integrated Full Analysis Set; M24: Month 24 Visit; n: size of subsample; PT: preferred term; SCD: sickle cell disease; TDT: transfusion-dependent β-thalassemia

Notes:

- Analysis starts from the first day of busulfan conditioning and goes through Month 24; As of the IA2 data cut-off, all subjects who had undergone myeloablation with busulfan had also received exa-cel infusion.
- MedDRA version 25.0
- evaluable subjects, N1: The number of subjects in the integrated Safety Analysis Set who were on or after the day of exa-cel infusion (i.e., iFAS)
- percentages are calculated as n/N1*100
- study interval is from enrollment through Month 24 visit or end of study visit.
- When summarizing number and percentage of subjects, a subject with multiple events within a category is counted only once in that category.
-"Related", "Possibly Related", and "Missing" are considered as "Related" in this table; related includes events related or possibly related to exa-cel and busulfan, or busulfan only.

For subjects with TDT (N = 48), 41 (85.4%) subjects had 1 or more AEs with a maximum severity of Grade 3 or above. Of the Grade 3 or above AEs occurring in >10% of subjects, the majority of these AEs are cytopenias that are non-serious laboratory findings known to occur with busulfan conditioning (e.g., anemia and thrombocytopenia) or other AEs known to occur with busulfan conditioning (e.g., febrile neutropenia, stomatitis, mucosal inflammation, veno-occlusive disease [VOD]).

For subjects with SCD (N = 35), 34 (97.1%) subjects had 1 or more AEs with a maximum severity of Grade 3 or above. Of the Grade 3 or above AEs occurring in >10% of subjects, the majority of these AEs were stomatitis or non-serious laboratory findings known to occur with busulfan conditioning (platelet count decreased, thrombocytopenia, neutrophil count decreased, neutropenia, white blood cell count decreased and anemia). One subject with SCD had a Grade 5 (fatal) SAE not related to exa-cel.

By individual study and across both studies, most Grade 3 or higher AEs occurred within the first 6 months after exa-cel infusion and myeloablative conditioning.

Table 15. Grade 3 or Above AEs Occurring in >10% of Subjects in Any Category by PT for the Exa-cel to M24 Interval: iFAS

Preferred Term	TDT (Study 111) n (%)	SCD (Study 121) n (%)	Total TDT + SCD n (%)
Evaluable subjects, N1	48	35	83
Subjects with any Grade 3 or above AEs	41 (85.4)	34 (97.1)	75 (90.4)
Febrile neutropenia	24 (50.0)	17 (48.6)	41 (49.4)
Stomatitis	19 (39.6)	21 (60.0)	40 (48.2)
Platelet count decreased	15 (31.3)	19 (54.3)	34 (41.0)
Neutrophil count decreased	13 (27.1)	16 (45.7)	29 (34.9)
Decreased appetite	11 (22.9)	16 (45.7)	27 (32.5)
Anaemia	18 (37.5)	8 (22.9)	26 (31.3)
Thrombocytopenia	17 (35.4)	7 (20.0)	24 (28.9)
Mucosal inflammation	14 (29.2)	9 (25.7)	23 (27.7)
Neutropenia	5 (10.4)	7 (20.0)	12 (14.5)
White blood cell count decreased	7 (14.6)	5 (14.3)	12 (14.5)
CD4 lymphocytes decreased	3 (6.3)	5 (14.3)	8 (9.6)
Epistaxis	5 (10.4)	2 (5.7)	7 (8.4)
Headache	3 (6.3)	4 (11.4)	7 (8.4)
Abdominal pain	2 (4.2)	4 (11.4)	6 (7.2)
Venoocclusive liver disease	5 (10.4)	1 (2.9)	6 (7.2)
Constipation	1 (2.1)	4 (11.4)	5 (6.0)
Pruritus	1 (2.1)	4 (11.4)	5 (6.0)
Cholelithiasis	0	4 (11.4)	4 (4.8)
Non-cardiac chest pain	0	4 (11.4)	4 (4.8)

Source: ISS Table 14.3.2.4.1

AE: adverse event; exa-cel: exagamglogene autotemcel; iFAS: integrated Full Analysis Set; M24; Month 24 Visit; n: size of subsample; PT: preferred term; SCD: sickle cell disease; TDT: transfusion-dependent β-thalassemia

Notes:

- MedDRA version 25.0
- evaluable subjects, N1: The number of subjects in the integrated Safety Analysis Set who were on or after the day of exa-cel infusion (i.e., iFAS)
- Percentages are calculated n/N1*100
- exa-cel to M24: Day of exa-cel infusion to Month 24 visit or end of study visit-When summarizing number and percentage of subjects for each study interval, a subject with multiple events within a category and study interval is counted only once in that category and study interval.

At data cutoff date of 16 April 2023, the majority of subjects had at least 1 AE that was considered related or possibly related to busulfan and fewer subjects had at least 1 AE that was considered related or possibly related to exa-cel, as outlined below:

• Fourteen (25.9%) subjects with TDT had at least 1 AE that was considered related or possibly related to exa-cel
  - o AEs occurring in ≥2 subjects with TDT were headache and laboratory-related events (CD4 lymphocytes decreased, neutrophil count decreased, lymphopenia, platelet count decreased, thrombocytopenia, and white blood cell count decreased).
  - o Most AEs considered related or possibly related to exa-cel were also considered related or possibly related to busulfan; 5 (9.3%) subjects had at least 1 AE that was considered related or possibly related to exa-cel only.

• Thirteen (30.2%) subjects with SCD had at least 1 AE that was considered related or possibly related to exa-cel
  - o AEs occurring in ≥2 subjects with SCD were laboratory-related events (CD4 lymphocyte decreased, lymphopenia, and neutropenia).

o Most AEs considered related or possibly related to exa-cel were also considered related or possibly related to busulfan; only 1 non-serious AE of neutropenia was considered related or possibly related to exa-cel only.

The incidence and nature of AEs, including the most common AEs (≥25% of subjects in each study), from exa-cel administration to Month 24, were generally consistent with myeloablative busulfan conditioning, autologous HSCT, and underlying disease (SCD). As reported in the initial MAA, no additional exa-cel specific safety concerns were identified.

Infusion-related reactions:

Overall, no clinically significant infusion-related reactions were observed and the exa-cel infusion was well tolerated. Infusion reactions, which may range from mild to severe reactions including anaphylaxis, are a known risk of dimethylsulfoxide (DMSO) and Dextran 40, which are excipients present in the exa-cel formulation. An analysis of AEs considered related to exa-cel with onset on Study Day 1 (day of exa-cel infusion) was performed to identify infusion reactions. After exa-cel infusion, 2 (3.7%) subjects with TDT and no subjects with SCD had infusion-related AEs on Study Day 1 that were considered related to exa-cel and consistent with common infusion reaction signs and symptoms.
All infusion-related AEs (sinus tachycardia and chills in 1 subject and tachycardia in 1 subject) were considered by the investigator to be related to exa-cel, were Grade 1 in severity, did not require treatment, and resolved on the same day. These AEs were also consistent with known side effects of busulfan, which was administered within the week before exa-cel infusion. No infusion-related AEs were serious. No anaphylactic reactions due to exa-cel occurred at any time point. In addition, no infusion-related AEs resulted in interruption or discontinuation of exa-cel infusion. Overall, no clinically significant infusion-related reactions were observed and the exa-cel infusion was well tolerated.

Febrile neutropenia:

The overall incidence and timing within the first few months following myeloablation and exa-cel infusion of febrile neutropenia observed in both studies is consistent with the literature for subjects undergoing myeloablative conditioning and autologous HSCT with no additional exa-cel specific concerns identified.

At data cutoff date of 16 April 2023, 33 (61.1%) TDT subjects had an AE of febrile neutropenia, of whom 29 (53.7%) had a Grade 3 or 4 AE. In subjects with SCD (N = 43), 23 (53.5%) subjects had an AE of febrile neutropenia, of whom 20 (46.5%) had a Grade 3 or 4 AE. One subject with TDT and 1 subject with SCD had an SAE of febrile neutropenia. None of the AEs or SAEs of febrile neutropenia were considered related to exa-cel. Twenty-five (46.3%) subjects with TDT and 22 (51.2%) subjects with SCD had at least 1 AE of febrile neutropenia that was considered related or possibly related to busulfan. Most AEs of febrile neutropenia had an onset of ≤21 days following exa-cel infusion; all events had an onset within the first 3 months after exa-cel infusion.

Infections:

Neutropenia is associated with increased risk of serious infection following myeloablative conditioning and HSCT, particularly during the period before neutrophil engraftment. Consistent with myeloablative conditioning and HSCT, infection AEs were observed following myeloablation with busulfan and exa-cel infusion. Grade 3 or 4 AEs of infection and SAEs of infection were much less common, as discussed below.
In subjects with TDT, infection AEs occurred in 35 (64.8%) subjects. The most common AE of infection (occurring in ≥10% of subjects) was COVID-19 (14 [25.9%]). Grade 3 or 4 infection AEs occurred in 15 (27.8%) subjects and infection SAEs occurred in 11 (20.4%) subjects. SAEs of pneumonia (3 [5.6%] subjects), COVID-19 (2 [3.7%] subjects), and upper respiratory tract infection (2 [3.7%] subjects) were the only SAEs that occurred in ≥2 subjects. Ten (18.5%) subjects with TDT had at least 1 infection AE that was considered related or possibly related to busulfan. No infection AEs were considered related or possibly related to exa-cel.
In subjects with SCD, infection AEs occurred in 28 (65.1%) subjects. The most common AEs of infection (occurring in ≥10% of subjects) were COVID-19 (11 [25.6%]), oral candidiasis (8 [18.6%]), upper respiratory tract infection (6 [14.0%]), and pneumonia (5 [11.6%]). Grade 3 or 4 infection AEs occurred in 10 (23.3%) subjects and infection SAEs occurred in 9 (20.9%) subjects. SAEs of pneumonia (4

[9.3%]) and sepsis (2 [4.7%]) were the only SAEs that occurred ≥2 subjects. Twelve (27.9%) subjects with SCD had at least 1 infection AE that was considered related or possibly related to busulfan. No infection AEs were considered related or possibly related to exa-cel.
Overall, for subjects with TDT or SCD, the incidence of infection was consistent with that observed after HSCT. There was no association between the incidence of infection AEs and time to neutrophil engraftment.

Bleeding:

Subjects with TDT

In subjects with TDT, bleeding AEs occurred in 37 (68.5%) subjects. The majority of bleeding AEs were Grade 1 or Grade 2 in severity. Grade 3 or 4 bleeding AEs occurred in 11 (20.4%) subjects. The most common bleeding AEs (occurring ≥10% subjects) after exa-cel infusion were epistaxis (20 [37.0%]), petechiae (12 [22.2%] subjects), hematuria (7 [13.0%] subjects), and gingival bleeding (6 [11.1%] subjects). The majority of epistaxis and gingival bleeding events were Grade 1 or Grade 2 and all events of petechiae were Grade 1 or Grade 2 in severity; 3 out of 7 events of hematuria were Grade 1 or Grade 2 in severity. The median duration of bleeding AEs was 3.5 (range: 1 to 48) days. Twenty-six (48.1%) subjects with TDT had at least 1 bleeding AE that was considered related or possibly related to busulfan. Two (3.7%) subjects with TDT had non-serious bleeding AEs (petechiae [1 subject] and epistaxis [1 subject]) assessed by the investigator as related or possibly related to exa-cel and busulfan; both events were non-serious, and resolved. None of the bleeding AEs were considered related to exa-cel only.

Subjects with SCD

In subjects with SCD, bleeding AEs occurred in 18 (41.9%) subjects. The majority of bleeding AEs were Grade 1 or Grade 2 in severity. Grade 3 or 4 bleeding AEs occurred in 3 (7.0%) subjects. The most common bleeding AE (occurring ≥10% subjects) after exa-cel infusion was epistaxis (8 [18.6%]). The median duration of bleeding AEs was 3.0 (range: 1 to 126) days.
Eleven (25.6%) subjects with SCD had at least 1 bleeding AE that was considered related or possibly related to busulfan. None of the bleeding AEs were considered related or possibly related to exa-cel.
One (2.3%) subject with SCD had a bleeding SAE (epistaxis), which the investigator considered possibly related to busulfan and not related to exa-cel. The event occurred before platelet engraftment and resolved within few days.

Overall, for subjects with TDT or SCD, the incidence and severity of clinically significant bleeding events was consistent with that observed after autologous HSCT. There was no association between the incidence of bleeding events and time to platelet engraftment.

Veno-occlusive liver disease:

Veno-occlusive liver disease (VOD) is complication of HSCT. Risk factors for development of VOD include conditioning regimen with busulfan, HSCT (higher risk in autologous than allogeneic, 15% vs 6%, respectively) underlying disease of TDT, very young or very old age, elevated ferritin levels, and history of previous liver disease. Patients with TDT are likely more susceptible to VOD due to liver damage associated with iron overload.

In subjects with TDT, after myeloablation with busulfan and exa-cel infusion, 7 (13.0%) subjects had an AE of VOD, including 2 subjects with a Grade 3 non-serious AE and 5 subjects with SAEs (Grade 2 or Grade 3); all events resolved. None of the VOD events were Grade 4. The time of onset for the events ranged from Study Day 13 to Study Day 32. All events were considered related or possibly related to busulfan; none of the events were considered related to exa-cel. All 7 subjects with VOD events received VOD prophylaxis starting from the time of busulfan conditioning and continuing after exa-cel infusion (ursodeoxycholic acid, defibrotide, or both). There was no apparent increase in risk of VOD associated with different prophylaxis practices (e.g., use of VOD prophylaxis) among sites.

In subjects with SCD, 1 (2.3%) subject had a non-serious, Grade 3 AE of VOD that resolved within 12 days. The subject received VOD prophylaxis starting from the time of busulfan conditioning and continuing after exa-cel infusion and was treated with defibrotide. The event was considered related to busulfan and not related to exa-cel.
The overall incidence and pattern of VOD events is consistent with the literature for subjects with TDT or SCD undergoing busulfan-based myeloablative conditioning and autologous HSCT with no additional exa-cel specific concerns identified.

Hemophagocytic lymphohystiocytosis:

HLH is another well-characterized HSCT complication, and is characterized by macrophage and mononuclear cell activation in the blood and tissues, and hemophagocytosis in the bone marrow and reticuloendothelial organs (e.g., liver, spleen, and lung) that results in cytopenias.

In subjects with TDT, a SAE of HLH occurred, which was considered as Grade 4 in severity and possibly related to exa-cel and not related to busulfan; it was resolved within 191 days. No other events of HLH had been reported.

Engraftment syndrome:

Engraftment syndrome includes a spectrum of complications that can occur in the periengraftment period. Per 2 sets of diagnostic criteria, engraftment syndrome may include noninfectious fever, rash (>25% of body), diarrhea, hepatic dysfunction, renal insufficiency, transient encephalopathy, features consistent with capillary leakage (e.g., pulmonary infiltrates/edema of noncardiogenic origin, hypoxia, and weight gain). Of these complications, skin rash and noninfectious fever within 7 days following HSCT are common (occurring in 59% of patients).

One (1.9%) subject with TDT had a Grade 3 non-serious AE; the event was considered not related to exa-cel or busulfan; the subject achieved neutrophil engraftment on Study Day 12.

Engraftment syndrome typically occurs in the early stages of neutrophil recovery and is more common after autologous HSCT than allogeneic HSCT.

Long-term follow-up study:

A total of 23 subjects with TDT (subjects enrolled from Study 111) and 13 subjects with SCD (subjects enrolled from Study 121) enrolled in Study 131 after completion of the Month 24 visit. The overall duration of follow-up (including follow-up in Study 131) for these subjects ranged from 23.2 to 51.1 months after exa-cel infusion.
No deaths have occurred during Study 131.
One subject with TDT had an SAE of influenza; the event was considered not related to exa-cel. One subject with SCD had an SAE of gastroenteritis norovirus; the event was considered not related to any study drug. Both SAEs resolved within few days.
For subjects with SCD, Study 131 includes specific collection of SCD-related complications. No subjects had AEs of SCD-related complications after the Month 24 Visit.
No AEs of new malignancies have occurred at any time after exa-cel infusion, including after Month 24 in Study 131. No AEs of new or worsening hematologic disorders have occurred during Study 131.
Overall, no new safety findings were observed for subjects enrolled in the long-term follow-up study, including no evidence of new malignancies or new or worsening hematologic disorders.

2.6.8.3. Serious adverse event/deaths/other significant events

SAEs:

Overall, the incidence and nature of the SAEs from exa-cel administration to M24 were generally consistent with myeloablative busulfan conditioning and autologous HSCT. There were no additional exa-cel specific safety concerns identified.

In total, 2 (3.7%) subjects with TDT and no subject with SCD had an SAE considered possibly related or related to exa-cel. In subjects with TDT, 19 (35.2%) subjects had at least 1 SAE. VOD was the most

common SAE occurring in 5 (9.3%) of TDT subjects; these events were considered related to busulfan and not related to exa-cel. VOD is a known risk of busulfan and is a known AE with busulfan myeloablation and autologous HSCT. No other SAEs occurred in ≥10% of subjects.

In subjects with SCD, 16 (37.2%) subjects had at least 1 SAE; no SAE occurred in ≥10% of subjects.

Table 16. Serious Adverse Events Occurring in ⩾2 subjects by PT for the Exa-cel to M24 Interval: iFAS

TDT

Preferred Term[a]	TDT (Study 111) n (%)
Evaluable subjects, N1	54
Subjects with any SAEs	19 (35.2)
Venoocclusive liver disease	5 (9.3)
Pneumonia	3 (5.6)
COVID-19	2 (3.7)
Hypoxia	2 (3.7)
Thrombocytopenia	2 (3.7)
Upper respiratory tract infection	2 (3.7)

Source: Study 111/Table 14.3.2.2.1 (data cutoff date of 16 April 2023)
exa-cel: exagamglogene autotemcel; FAS: Full Analysis Set; M24: Month 24 Visit; n: size of subsample; PT: preferred term; SAE: serious adverse event; SAS: Safety Analysis Set; TDT: transfusion-dependent β-thalassemia
Notes: MedDRA version 26.0. Evaluable subjects, N1: The number of subjects in the SAS who were on or after the day of exa-cel infusion (i.e., FAS). Percentages were calculated as n/N1*100. When summarizing number and percentage of subjects for each study interval, a subject with multiple events within a category and study interval was counted only once in that category and study interval. Table shows exa-cel to M24 study interval: day of exa-cel infusion to Month 24 visit or end of study visit.
[a] All PTs are described in busulfan product information by matching PT or similar medical concept.[8]

SCD

Preferred Term[a]	n (%)
Evaluable subjects, N1	43
Subjects with any SAEs	16 (38.1)
Cholelithiasis	4 (9.3)
Pneumonia	4 (9.3)
Abdominal pain	3 (7.0)
Constipation	3 (7.0)
Pyrexia	3 (7.0)
Abdominal pain upper	2 (4.7)
Non-cardiac chest pain	2 (4.7)
Oropharyngeal pain	2 (4.7)
Pain	2 (4.7)
Sepsis	2 (4.7)
Sickle cell anaemia with crisis	2 (4.7)[b]

Source: Study 121/Table 14.3.2.2.1 (data cutoff date of 16 April 2023)
exa-cel; exagamglogene autotemcel; FAS: Full Analysis Set; M24: Month 24 Visit; n: size of subsample; PT: preferred term; SAE: serious adverse event; SAS: Safety Analysis Set; VOC: vaso-occlusive crisis
Notes: MedDRA version 26.0. Evaluable subjects, N1: the number of subjects in the SAS who were on or after the day of exa-cel infusion (i.e., FAS). Percentages were calculated as n/N1*100. When summarizing number and percentage of subjects for each study interval, a subject with multiple events within a category and study interval was counted only once in that category and study interval. Table shows exa-cel to M24 study interval: day of exa-cel infusion to Month 24 visit or end of study visit.
[a] All PTs are either described in the busulfan product information by matching PT or similar medical concept or are associated with underlying disease (cholelithiasis, sickle cell anemia with crisis).[8, 81]
[b] Corresponds to hospitalizations for VOCs in Subjects [redacted] Additional information is provided in Section 3.3.3.1.

- For the 2 subjects with TDT with SAEs reported related or possibly related to exa-cel, the events are also known complications following myeloablative conditioning with busulfan and autologous HSCT

Deaths:

There were no deaths in the TDT study (Study 111). There was 1 death which occurred SCD study (Study 121), which was due to reasons unrelated to the study treatment.

Other significant events:

At the data cutoff of 06 September 2022, 3 subjects who had not achieved TI12 remained clinically well, including no serious infection or bleeding events, throughout the period after myeloablative conditioning and exa-cel infusion. Two of the subjects had been followed for ≥18 months and the remaining 1 subject had been followed for >12 months after exa-cel infusion at the time of the IA2 data cut. For each subject, neutrophil and platelet engraftment was followed by normalizing neutrophil counts, increasing platelet counts, improvement in cellularity after myeloablation, and improvement in M:E over time. Two of the subjects had comparatively longer times to platelet engraftment (Study Days 213 and 199 compared to Study Day 56), but otherwise no other unique features related to their individual treatment course were identified. All 3 subjects notably had an intact spleen; however, evaluation of subject's medical histories, demographics, and study procedures did not reveal any unique or predisposing characteristics for their not having achieved TI12 at the time of the IA2 data cut.

None of the bone marrow analyses showed dysplasia or blast count abnormalities in any subject. Assessment of karyotyping and FISH, which by protocol are performed at the discretion of the investigator, were negative in 2 subjects; these assessments were not performed for the third subject.

For 1 subject, at Month 12, next generation sequencing (NGS) was performed by the investigator on the bone marrow aspirate given the subject's ongoing RBC transfusion requirement. Results from fluorescence in situ hybridization (FISH) and karyotyping performed at Month 12 were normal and showed no cytogenic evidence of chromosomal abnormalities.

At Month 18, a subsequent bone marrow sample was collected. At this assessment, NGS detected the same which the testing site did not consider to be a significant change compared to the previous result. The investigator considered this of "unclear / unknown" clinical significance given the overall clinical situation of stable to improving blood counts and significant reduction in transfusion requirements. Based on review of the totality of the data, the investigator determined there was no evidence for hematologic concern and no further action was taken. The investigator plans to continue monitoring, including NGS, to evaluate for any changes over time. A review by two independent hematopathologists of the subject's bone marrow morphology and molecular data from baseline through Month 18 was initiated by the sponsor. This review revealed no evidence of myelodysplastic syndrome (MDS) or any other hematologic malignancy.

The applicant provided evidence, that there are no potential off-targets around the site, and evidence indicated that the mutation did not occur as a consequence of off-targeting. Data presented indicate that the mutation was most likely present in the patient's cells before the gene-editing step.

Updated laboratory data as of the data cutoff date of 16 April 2023 include normal neutrophil count of 1.64×10^9/L (lower limit of normal [LLN]: 1.5×10^9/L; Study Day 774) and improved platelet count of 56×10^9/L (Study Day 774); however, the subject remained thrombocytopenic as of the last available assessment (LLN: 150×10^9/L). Fluorescence in situ hybridization (FISH) and karyotyping results remain normal and showed no cytogenic evidence of chromosomal abnormalities at Month 24. Serial bone marrow histology through Month 24 did not show any evidence of MDS or malignancy.

All 3 subjects experienced substantial clinical benefit as evidenced by the reduction in annualized RBC transfusion volume and reduction in frequency of RBC transfusions. As of the data cutoff date of 16 April 2023, all 3 subjects have stopped receiving RBC transfusions and have been transfusion free for 10.3, 7.0, and 2.8 months, respectively, starting 60 days after the last RBC transfusion.

At data cutoff of 16 April 2023, the applicant reported an additional patient where NGS was carried out. NGS analysis was not ordered for cause by the Principal Investigator (PI). Rather, testing was initiated by the hematopathology laboratory per local practice (e.g., when thrombocytopenia is noted in post-HSCT oncology patients). Once this local practice was discovered, it was discontinued by the PI for all study subjects. At Month 6 post exa-cel dosing, the subject's platelet count was 24×10^9/L, which is what triggered the laboratory's automatic NGS testing. For this subject, NGS was performed on Month 6 bone marrow against a panel of genes associated with MDS and did not identify any relevant findings. Month 6 results showed a mutation that was also present at baseline prior to busulfan and exa-cel dosing. No other mutations were detected. The subject is doing well clinically and has been RBC transfusion free for 23.4 months (as of the data cutoff date of 16 April 2023). The subject's platelet count has improved over time and was 95×10^9/L at the last available visit (Month 24).

Safety aspects during mobilization and apheresis

In TDT patients, a dual-agent including granulocyte colony-stimulating factor [G-CSF] therapy and plerixafor was used for mobilization of stem cells. A total of 8 (13.6%) TDT subjects had SAEs that occurred during mobilization and before start of myeloablative busulfan conditioning. SAEs included bacteremia (2 subjects), nausea, vomiting, abdominal pain lower, acute kidney injury, alanine transaminase (ALT) increased, aspartate transaminase (AST) increased, blood bilirubin increased, dehydration, drug hypersensitivity, dysmenorrhea, gastroenteritis viral, hepatic siderosis, hypokalemia, hyponatremia, nephrolithiasis, ovarian cyst torsion, and paranasal cyst (1 subject each). It is acknowledged that G-CSF (filgrastim) has a safety profile, which might be causing many AEs (and SAEs) mentioned above, but also, it must be accomplished that all these AEs and SAEs belong inevitably to the treatment procedure with exa-cel. The applicant states, that the majority of AEs were consistent with the known safety profile of G-CSF or plerixafor and/or apheresis procedure in the context of underlying disease. To further assess whether mobilization and apheresis was affected by underlying disease of TDT, the applicant reviewed the occurrence of hemolysis during the mobilization and apheresis procedure. Based on this analysis, no AEs of hemolysis or similar terms occurred in any subject around the time of mobilization and apheresis procedures. The underlying disease of TDT did not seem to affect the safety profile during mobilization and apheresis, which was similar to approved labels and well tolerated.

Fifty-five (94.8%) subjects with SCD had at least 1 AE during mobilization but prior to conditioning, and 14 (24.1%) subjects had AEs that were considered related, and 19 (32.8%) subjects had AEs that were considered possibly related to plerixafor. A total of 22 (37.9%) SCD subjects had an AE of sickle cell anemia with crisis (VOC), of which a total of 20 (34.5%) subjects had an SAE of sickle cell anemia with crisis (VOC), including 3 (5.2%) subjects who also had ACS (acute chest syndrome). The most common AEs during mobilization (>10% of subjects) were sickle cell anemia with crisis (VOC), nausea, vomiting, abdominal pain, constipation, diarrhea, paresthesia oral, vascular access site pain, hypomagnesemia, hypocalcemia, hypokalemia, headache, paresthesia, pruritus, pain in extremity, back pain, and arthralgia. According to the applicant, the majority of these AEs were consistent with the known safety profile of plerixafor or apheresis procedure and underlying disease. When scrutinizing the SPC of plerixafor, the safety profile does not assume as frequent and variable amount of AEs and SAEs as in subjects with SCD. VOC is unique to the SCD population, and the incidence in Study 121 was comparable to that of other published studies in SCD during mobilization and apheresis and will be included in the ADR section of the exa-cel SmPC. Overall, mobilization and apheresis was well tolerated.

It is of note, that 37.9% of SCD subjects had an AE of sickle cell anemia with crisis (VOC) and 34.5% of SCD subjects had a SAE of sickle cell anemia with crisis during mobilization and apheresis. The collective exposure for subjects (N = 58) from the start of mobilization to the day before myeloablative busulfan conditioning in Study 121 was 530.57 patient-months. The exposure-adjusted rates of severe and serious VOCs were compared between this period and that of pre-mobilization as well as to the period prior to study enrollment. The exposure-adjusted event rates of severe VOCs were similar between pre- and post-mobilization time periods (0.20 vs 0.25 severe VOCs/patient-month), which is also consistent with the median annualized rate of severe VOCs of 3.5 at baseline. The exposure-adjusted event rates of serious VOCs were also similar between pre- and post-mobilization time periods (0.12 vs 0.13 serious VOCs/patient-month), which is consistent with the median annualized rate of inpatient hospitalizations for severe VOCs of 2.5 at baseline. Therefore, it is not expected that patients will have more VOCs during the post-mobilization period as compared to the pre-mobilization period, and both are comparable to baseline median monthly rate of severe and serious VOCs recorded prior to entry into Study 121. In summary, the rate of VOCs was generally comparable between pre- and post- mobilization and apheresis time periods among subjects with SCD in Study 121 and was consistent to the rates at baseline. VOCs occurring during the mobilization and apheresis period in Study 121 were managed similarly to VOCs occurring throughout the rest of the study, with hydration, narcotics, and non-steroidal anti-inflammatory therapies according to investigator's medical judgement. Of the 12 subjects who had VOC within 7 days of mobilization and apheresis, 7 subjects completed mobilization and apheresis and were subsequently dosed with exa-cel, 1 subject is awaiting exa-cel dosing, and 4 subjects discontinued the study prior to exa-cel infusion (due to no longer meeting eligibility criteria for renal function, CD34+ dose not reached, reasons provided in the dossier, and patient decision, respectively). Accordingly, the applicant estimates, that no subject discontinued the study due to VOCs during mobilization and apheresis. The applicant also emphasizes that there is no established causal association between plerixafor and occurrence of VOCs. The proposed SmPC already includes measures aimed to minimize and reduce the likelihood of and risk from VOCs during mobilization and apheresis, with the recommendation that patients receive RBC exchange or simple transfusions prior to mobilization and apheresis, with the goal of maintaining HbS levels <30% of total Hb while keeping total Hb $\leq$ 11 g/dL prior to mobilization and apheresis. These are standard measures to minimize and reduce the likelihood of VOCs occurring during mobilization and apheresis that are well described in the literature and were also implemented in Study 121. Other types of measures to minimize the risk of the occurrence of VOCs, including hydration, are not standard measures, are not supported by the literature and were not included in Study 121 protocol and are therefore not considered suitable for inclusion in the SmPC.

At data cutoff date of 16 April 2023, the mobilization regimens were well-tolerated among the 117 subjects who initiated mobilization (TDT: N = 59; SCD: N = 58).

In total, 3 subjects with TDT and 11 subjects with SCD discontinued the respective studies after initiating mobilization and were not subsequently dosed with exa-cel. The reasons for discontinuations included the following:

• For subjects with TDT: withdrawal of consent for undisclosed reasons (1 subject); did not want to undergo a second apheresis procedure (1 subject); concerns with continued study participation (1 subject).

• For subjects with SCD: inadequate cell collection (6 subjects); no longer meeting eligibility criteria (1 subject); non-compliance (1 subject); withdrawal of consent (2 subjects); reasons provided in the dossier (1 subject [not reported as an AE]).

2.6.8.4. Laboratory findings

Standard laboratory evaluations, including hematology, chemistry, coagulation, immunology, and urinalysis, were performed in Studies 111 and 121. Laboratory testing was performed at screening, baseline, before the start of mobilization, before conditioning, and before exa-cel infusion, and at regular intervals (at least monthly through Month 6 visit and then every 3 months) after exa-cel infusion through the Month 24 visit.

Overall, there was no association between infection AEs or SAEs in subjects with TDT or SCD with longer times to neutrophil engraftment compared to subjects with shorter times to neutrophil engraftment.

Neutropenia is associated with increased risk of serious infection following myeloablative conditioning and HSCT, particularly during the period before neutrophil engraftment. Prophylactic anti-infective agents were used in the study according to individual site practices.

Overall, there was no association between bleeding AEs or SAEs in subjects with TDT or SCD with longer times to platelet engraftment compared to subjects with shorter times to platelet engraftment.

Following any HSCT, there is an increased risk of bleeding events following myeloablative conditioning, with the highest risk of bleeding being after HSCT before platelet engraftment. In a literature report, the peak incidence was the second week after transplant, with decreasing incidence of events thereafter.

In subjects with TDT and subjects with SCD, there were no clinically relevant trends in coagulation test results.

Overall, the incidence and timing of elevated transaminase (alanine transaminase [ALT] and aspartate transaminase [AST]) values were consistent with that seen with busulfan myeloablative conditioning and HSCT and elevations improved over time, with no additional exa-cel specific concerns identified.

Other hematology parameters over time were generally consistent for the TDT or SCD patient population and for subjects undergoing busulfan myeloablation and autologous HSCT.

There were no trends in median values of other non-LFT chemistry parameters. Chemistry results were generally consistent for subjects undergoing busulfan myeloablation and autologous HSCT.

In subjects with TDT, after busulfan myeloablative conditioning and exa-cel infusion, decreases in calcium and potassium were observed, primarily at Month 1, and improved over the subsequent months.

In subjects with SCD, after myeloablative busulfan conditioning and exa-cel infusion, decreases in calcium and potassium, as well as increases in magnesium were observed, primarily at Month 1, and improving over the subsequent months.

Across studies, all methemoglobin values were <10% over time, without increases over time, demonstrating the absence hemoglobin oxidation and methemoglobin formation.

In subjects with TDT, methemoglobin levels ranged from 0.3% to 7.7% in the subset of subjects within the Safety Analysis Set with reported methemoglobin.

In subjects with SCD, methemoglobin levels ranged from 0.1% to 2.3% in the subset of subjects within the Safety Analysis Set with reported methemoglobin.

2.6.8.5. In vitro biomarker test for patient selection for safety

Not applicable.

2.6.8.6. Safety in special populations

Age:

The observed safety profile was generally similar between subjects ≥12 and <18 years of age and ≥18 and ≤35 years of age and, across age groups, the AEs were considered consistent with myeloablative busulfan conditioning, HSCT, or underlying disease (SCD). No differences attributed to exa-cel were identified. The incidence of AEs and SAEs after exa-cel infusion to Month 24 for the 2 age groups (≥12 and <18 years [N = 24] and ≥18 and ≤35 years of age [N = 59]) was generally similar. There were no clinically relevant differences in achievement of engraftment (neutrophil or platelet), time to neutrophil engraftment, or time to platelet engraftment based on age.

Sex and race:

There were no clinically relevant differences attributable to exa-cel were identified based on sex or race.

Genotype:

No clinically relevant differences attributable to exa-cel were identified between TDT genotypes in Study 111 or SCD genotypes in Study 121.

Hepatic or renal impairment:

Exa-cel has not been studied in patients with hepatic impairment. Subjects with advanced liver disease were not eligible for Study 111 (TDT) or Study 121 (SCD).

Exa-cel has not been studied in patients with renal impairment. Subjects who had a baseline estimated glomerular filtration rate <60 mL/min/1.73 m2 were excluded from Study 111 (TDT) and Study 121 (SCD).

Pregnancy, birth, lactation:

Pregnancy and breastfeeding were exclusion criteria implemented during all clinical studies with exa-cel. No pregnancies after start of myeloablation or exa-cel infusion have been reported.

It is unknown whether exa-cel is excreted in human milk or transferred to the breast-feeding child. Because of the potential risks associated with busulfan conditioning, breast feeding should be discontinued during myeloablative conditioning.

There are no data on the effects of exa-cel on human fertility. Effects on male and female fertility have not been evaluated in animal studies. Infertility has been observed with myeloablative conditioning therefore fertility preservation options should be considered.

2.6.8.7. Immunological events

No immunological events were reported.

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

No drug-drug interaction studies were performed with exa-cel. Drug-drug interactions are not anticipated with exa-cel as it does not affect human physiological processes that can in turn alter the PK profiles of co-administered medications.

2.6.8.9. Discontinuation due to adverse events

No subject with TDT or subject with SCD had the single exa-cel infusion and discontinued exa-cel due to an AE.

No subject with TDT or subject with SCD discontinued busulfan due to an AE.

One subject (TDT) discontinued G-CSF during mobilization/apheresis (Grade 1 AE of splenomegaly) and subsequently completed mobilization/apheresis and continued in the study. No subjects with TDT or SCD discontinued plerixafor.

2.6.8.10. Post marketing experience

Not applicable.

2.6.9. Discussion on clinical safety

Overall, the short-term safety profile of exa-cel seems to be in line with the inherent safety aspects of myeloablation and HSCT. No additional exa-cel specific safety aspects were identified. In order to encompass the full extent of the safety aspects related to the procedure, the risks of mobilization and myeloablative conditioning must also be considered when assessing the benefit/risk of exa-cel. Such risks include secondary malignancies, bleeding or VOD. However, the provided dataset is considered not comprehensive regarding middle- and long-term safety of this gene editing therapy.

There were no age-related safety differences for the two age groups compared ($\geqslant$12 to <18 years vs. $\geqslant$18 to $\leqslant$35 years). No data were obtained in adults older than 35 yrs in any of the studies. This aspect may influence the proposed indication, because the initial intended indication is for "patients 12 years of age and older". Especially for TDT, in general it is considered that the mortality and significant morbidities associated with HSCT are lowest if the HSCT is administered during the mid-first decade of life, prior to the development of significant iron overload and/or other comorbidities such as liver fibrosis or hypersplenism. The indication was reworded to include phrasing on treating patients meeting transplant eligibility criteria.

No immunological events were reported, immunogenicity of the product was not assessed. Moreover, according to the CSP no analysis were performed to assess the presence of ADA before administration of the drug, and no analysis were performed to investigate potential immunogenicity/ immunological reactions following the administration of the drug. There have been no events reported that suggest any subject has experienced acute antibody-mediated or T-cell mediated response following exa-cel infusion. However, Cas9 nuclease delivered in protein form is of Streptococcus pyogenes origin. High prevalence of Streptococcus pyogenes Cas9 (SpCas9)-reactive T cells (PMID: 30374197) and antibodies against SpCas9 (PMID: 30692695) were described within the adult human population. It is speculated that a peptide fragments of any protein delivered into cells ex vivo are typically only expressed transiently on MHC class I molecules. At the end of manufacturing at least 99% of residual Cas9 is cleared in the process. The time- dependent expression of Cas9 during ex vivo gene editing is not discussed.

During manufacturing, cells are kept in culture following electroporation. At the time of infusion, Cas9 protein is still associated with the cells. This aspect poses an unaddressed safety and efficacy concern. Adaptive immune responses due to pre-existing Cas9 antibodies could potentially result in immune-mediated reactions or immune responses that result in the clearance of the cells that express residual Cas9. It is not discussed whether a recent Streptococcus pyogenes infection would pose additional risks for the patients. The applicant included a relevant statement to Section 4.4 Special warnings and precautions for use in the SmPC regarding the immunogenicity potential of Cas9.

During the phase of mobilization and apheresis, 15.3% of patients with TDT had AEs that were related to plerixafor and 47.5% had AEs related to G-CSF. 24.1% of patients with SCD had AEs related to plerixafor. 13.6% of TDT subjects and 60.3% of SCD subjects had SAEs. Especially SCD subjects had many SAEs and most of the SAE-experienced SCD subjects had SAE of sickle cell anemia with crisis (VOC). As the treatment entirety with exa-cel requires mobilization and apheresis, also AEs and SAEs of this phase are an essential part of the treatment. Therefore, the applicant has amended the SmPC by listing also the adverse reactions belonging to the phase of mobilization and apheresis in Section 4.8 Undesirable effects.

For one subject in clinical trial 111, as of month 12 a mutation was detected by NGS in the bone marrow aspirate. The applicant classified the finding on the gene mutation as of "unknown, unclear" clinical significance.

Mutations in this gene are a recurrent finding in myeloid malignancies. Mutated status has been associated with an inferior overall survival in acute myeloid leukaemia (AML), myelodysplastic syndromes (MDS), chronic myelomonocytic leukaemia (CMML), myelofibrosis, aplastic anaemia and age-related clonal haematopoiesis.

Development of clonal haematopoiesis may be considered a suspected pre-condition for the development of haemato-oncologic malignancies (PMID: 28068180). Mutations in this gene are frequently found in clonal hematopoiesis (CH) [also called clonal hematopoiesis of indeterminate potential (CHIP)], precursor states for hematologic neoplasms with somatic mutations in the absence of diagnostic criteria for hematologic malignancies.

Mutations in this gene are common in MDS patients and are associated with progression to leukemia, and are well-known independent predictors of poor overall survival in these patients (PMID: 21714648, PMID: 24220272).

According to the safety update for this patient no evidence of MDS or malignancy was found. The applicant carried out the analysis of potential off-targeting around the mutation site. The description of findings indicates that the analysis was carried out based on the reference assembly and included common genetic variation down to 1% allele frequency. No potential off-targeting was identified. GUIDE-seq also did not identify any potential off-target editing in a 200kb region across both healthy donor and patient material. The off-target analysis was carried out on the individual patient genome and on the reference assembly as well. Data presented indicate that the mutation was most likely present in the patient's cells before the gene-editing step.

WGS or NGS on a limited number of genes were only performed for two patients. Both analysis revealed patient specific mutations beyond the reference assembly. It cannot be excluded that other patient specific mutations were/will be present in the genomes of individual patients, and these hold the uncertainty of unlocking off targeting potential.

The applicant will need to set up a close monitoring in PSUR of cases, where genetic abnormalities, myelodysplasia or other pre-malignant findings are evidenced.

At the data cut-off of 06 September 2022, two further subjects did not achieve TI12, while remaining "clinically well". It is unknown if there is any mutation background contributing to these observations. While there seems to be no evidence of malignancy, the presence of clonal hematopoiesis or clonal cytopenia of undetermined significance (CCUS) was not assessed. At data cut-off of 16 April 2023, all 3 subjects have achieved substantial clinical benefit with significant reductions in annualized transfusion volume and have remained clinically well, including no serious infection or bleeding events, throughout the period after myeloablative conditioning and exa-cel infusion. For each subject, neutrophil and platelet engraftment was followed by normalizing neutrophil counts, increase in platelet counts, and improvement in bone marrow cellularity and M:E ratio after myeloablation. In all subjects, multiple bone marrow evaluations performed after exa-cel infusion demonstrated no dysplasia or blast count abnormalities. There was no evidence of myelodysplastic syndrome (MDS) or other hematological malignancies. As of 16 April 2023, 2 of the subjects had been followed for >26 months and 1 subject had been followed for >21 months after exa-cel infusion.

There are still several unknowns related to the consequences of CRISPR-Cas9 gene editing. By using the same guide RNA as in the product, it was shown that CRISPR–Cas9 editing generates structural defects of the nucleus, micronuclei and chromosome bridges, which initiate a mutational process called chromothripsis (PMID: 33846636). Chromothripsis is extensive chromosome rearrangement restricted

to one or a few chromosomes that can cause human congenital disease and cancer. Of course, should any patient develop cancer at a later stage, genetic analysis to identify potential causes may shed light on the frequency of such events, however, no data were generated in patients which could be used to address this aspect. The applicant only cited non clinical findings where such events were not observed.

There are further unknowns related to the human genetic diversity and variability, as there is only one assembly used to design gRNAs. Recent literature (PMID: 36522432) indicates that the same gRNA has a potential off-target produced by an allele common in African-ancestry populations that introduces a protospacer adjacent motif (PAM) sequence. In the case of BCL11A enhancer editing, up to ~10% of SCD patients with African ancestry would be expected to carry at least one rs114518452-C allele, leading to ~10% cleavage at an off-target site.

Currently there is insufficient evidence to evaluate the influence of human genetic diversity on the off targeting potential of the guide RNA. It would be desirable to implement strategies to solve this uncertainty regarding patient specific off targeting. In an ideal setup, all patient genomes should be sequenced before being treated with the gene editing product to identify potential off-targets relevant for CD34+ cell functions and oncogenesis. The applicant presented analysis of potential benefits and risks when employing patient specific WGS and off targeting analysis, as requested. The applicant concluded that using WGS and off-targeting analysis to investigate patient specific off-target potential does not have utility. The applicant indicated that in case off-target editing derived malignancies were to occur or new information should emerge that changes the utility of WGS and/or of off-targeting analysis, then the safety specification and SmPC will be updated.

Based on the data collected so far, the position of the applicant could be accepted. However, while no off-targeting was detected during the extensive preclinical assessments and in clinical setting yet, a theoretical risk of off-targeting due to patient-specific genetic variants cannot be excluded at this time. In order to ensure a swift and straightforward analysis of potential off-targeting occurring during future treatments with Casgevy, the CAT formulated a recommendation. Currently, a back-up collection of unmodified cells is established for each patient to cover the need for rescue treatment under certain conditions (compromise of Casgevy after initiation of myeloablative conditioning and before Casgevy infusion; neutrophil engraftment failure; or loss of engraftment after infusion with Casgevy). Following a successful Casgevy administration, the fate of these back-up cells is unknown, but their further storage seems superfluous. These cells, however, could potentially be used for any subsequent analysis (such as off-targeting and WGS). The applicant accepted the CAT recommendation and will store back-up cells or equivalent patient material (cells or DNA) for 15 years to ensure any subsequent off-targeting related analysis (CAT/CHMP Recommendation, REC). This aspect, combined with the close monitoring of patients seem to be sufficient measures to ensure a swift identification of potential, clinically relevant, off targeting effects.

In TDT patients, a dual-agent including granulocyte colony-stimulating factor [G-CSF] therapy and plerixafor was used for mobilization of stem cells. A total of 8 (13.6%) TDT subjects had SAEs that occurred during mobilization and before start of myeloablative busulfan conditioning. SAEs included bacteremia (2 subjects), nausea, vomiting, abdominal pain lower, acute kidney injury, alanine transaminase (ALT) increased, aspartate transaminase (AST) increased, blood bilirubin increased, dehydration, drug hypersensitivity, dysmenorrhea, gastroenteritis viral, hepatic siderosis, hypokalemia, hyponatremia, nephrolithiasis, ovarian cyst torsion, and paranasal cyst (1 subject each). It is acknowledged that G-CSF (filgrastim) has a safety profile, which might be causing many AEs (and SAEs) mentioned above, but also, it must be accomplished that all these AEs and SAEs belong inevitably to the treatment procedure with exa-cel. The applicant states, that the majority of AEs were consistent with the known safety profile of G-CSF or plerixafor and/or apheresis procedure in the context of underlying disease. To further assess whether mobilization and apheresis was affected by underlying disease of TDT, the applicant reviewed the occurrence of hemolysis during the mobilization and apheresis

procedure. Based on this analysis, no AEs of hemolysis or similar terms occurred in any subject around the time of mobilization and apheresis procedures. The underlying disease of TDT did not seem to affect the safety profile during mobilization and apheresis, which was similar to approved labels and well tolerated.

Fifty-five (94.8%) subjects with SCD had at least 1 AE during mobilization, and 14 (24.1%) subjects had AEs that were considered related, and 19 (32.8%) subjects had AEs that were considered possibly related to plerixafor. A total of 22 (37.9%) SCD subjects had an AE of sickle cell anemia with crisis (VOC), of which a total of 20 (34.5%) subjects had an SAE of sickle cell anemia with crisis (VOC), including 3 (5.2%) subjects who also had ACS (acute chest syndrome). The most common AEs during mobilization (>10% of subjects) were sickle cell anemia with crisis (VOC), nausea, vomiting, abdominal pain, constipation, diarrhea, paresthesia oral, vascular access site pain, hypomagnesemia, hypocalcemia, hypokalemia, headache, paresthesia, pruritus, pain in extremity, back pain, and arthralgia. According to the applicant, the majority of these AEs were consistent with the known safety profile of plerixafor or apheresis procedure and underlying disease. However, VOC is unique to the SCD population, and the incidence in Study 121 was comparable to that of other published studies in SCD during mobilization and apheresis and will be included in the ADR section of the exa-cel SmPC. Overall, mobilization and apheresis was well tolerated.

The rate of VOCs was generally comparable between pre- and post- mobilization and apheresis time periods among subjects with SCD in Study 121 and was consistent to the rates at baseline. VOCs occurring during the mobilization and apheresis period in Study 121 were managed similarly to VOCs occurring throughout the rest of the study, with hydration, narcotics, and non-steroidal anti-inflammatory therapies according to investigator's medical judgement. The proposed SmPC includes measures aimed to minimize and reduce the likelihood of and risk from VOCs during mobilization and apheresis, with the recommendation that patients receive RBC exchange or simple transfusions prior to mobilization and apheresis, with the goal of maintaining HbS levels <30% of total Hb while keeping total Hb $\leq$ 11 g/dL prior to mobilization and apheresis

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

Additional safety data needed in the context of a conditional MA

With respect to (middle and long-term) safety data on Casgevy further data are needed and will be provided according to SOBs, the long term follow up studies and the registry studies.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) aged 12 years and older, the MAH should submit the final results from the Study 111.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with severe sickle cell disease (SCD) aged 12 years and older, the MAH should submit the final results from the study 121.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with severe sickle cell disease (SCD) aged 12 years and older, the MAH should submit the final results from the study 151.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) and severe sickle cell disease (SCD) aged 12 years and older, the MAH should submit the interim results from the study 161.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with severe sickle cell disease (SCD) aged 12 years and older, the MAH should submit the final results from the Study 171.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) and severe sickle cell disease (SCD) aged 12 years and older, the MAH should conduct and submit the interim results of a study based on data from a registry according to an agreed protocol. Yearly progress reports shall also be submitted.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) and severe sickle cell disease (SCD) in patients aged 12 years and older, the MAH should submit the interim results from study 131.

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

- In order to further characterise the long-term safety and efficacy of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) and severe sickle cell disease (SCD) aged 12 years and older, the MAH should conduct and submit the results of a study based on data from a registry, according to an agreed protocol.

2.6.10. Conclusions on the clinical safety

Exa-cel was generally safe and well tolerated. Across indications, the AE profile of exa-cel was generally consistent with that expected from myeloablative busulfan conditioning and HSCT, with no additional exa-cel specific concerns identified. As treatment with Casgevy encompasses an HSCT procedure, the risks associated with mobilisation and conditioning are also part of the B/R of Casgevy.

Veno-occlusive liver disease, pneumonia (including fatal and life-threatening cases), respiratory failure, capillary leak syndrome, cerebral hemorrhage, seizure, drug-induced hepatitis, and infertility are also recognized toxicities for busulfan. Rarely, secondary malignancies have been reported after busulfan treatment.

Specific risks are longer platelet engraftment time (identified risk) and neutrophil engraftment failure (potential risk) for TDT and SCD.

The oncogenic risks of guide RNA off targeting is difficult to evaluate and a risk remains, despite the fact that no such findings were observed so far in the development of the product. The impact and risk of generating chromosomal aberrations (such as chromothripsis) at the on-target site remains unaddressed. Similarly, the impact of human genetic diversity and variability is insufficiently characterized and remains an uncertainty.

While short-term safety of exa-cel seems to be in line with the inherent safety aspects of myeloablation and HSCT, and no additional exa-cel specific safety aspects were identified, the provided dataset is considered incomprehensive regarding middle- and long-term safety of this gene editing therapy, with the proposed SOBs being expected to generate respective data.

The applicant accepted the CAT recommendation to store back-up cells or equivalent patient material (cells or DNA) for 15 years to ensure any subsequent off-targeting related analysis (REC).

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

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with

transfusion-dependent β-thalassemia (TDT) aged 12 years and older, the MAH should submit the final results from the Study 111.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with severe sickle cell disease (SCD) aged 12 years and older, the MAH should submit the final results from the study 121.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with severe sickle cell disease (SCD) aged 12 years and older, the MAH should submit the final results from the study 151.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) and severe sickle cell disease (SCD) aged 12 years and older, the MAH should submit the interim results from the study 161.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with severe sickle cell disease (SCD) aged 12 years and older, the MAH should submit the final results from the Study 171.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) and severe sickle cell disease (SCD) aged 12 years and older, the MAH should conduct and submit the interim results of a study based on data from a registry according to an agreed protocol. Yearly progress reports shall also be submitted.

- In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) and severe sickle cell disease (SCD) in patients aged 12 years and older, the MAH should submit the interim results from study 131.

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

- In order to further characterise the long-term safety and efficacy of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) and severe sickle cell disease (SCD) aged 12 years and older, the MAH should conduct and submit the results of a study based on data from a registry, according to an agreed protocol.

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

2.7. Risk Management Plan

2.7.1. Safety concerns

Important identified risks	• Delayed platelet engraftment
Important potential risks	• Neutrophil engraftment failure • Gene editing-related oncogenesis
Missing information	• Long-term effects • Pregnancy and lactation • Use in patients >35 years of age

2.7.2. Pharmacovigilance plan

Study/Status	Summary of Objectives	Safety Concerns Addressed	Milestones	Due Dates
Category 1 – Imposed mandatory additional PV activities which are Conditions of the MA (key to benefit risk)				
Study 101 (PASS) Planned	**Primary Objective** • Evaluate long-term safety outcomes in patients who received exa-cel for treatment of TDT or SCD • Evaluate long-term safety outcomes in patients who received exa-cel for treatment of TDT or SCD in comparison to patients receiving allo-HSCT **Secondary Objectives** • Evaluate long-term effectiveness outcomes in patients who received exa-cel for treatment of TDT or SCD • Evaluate long-term effectiveness outcomes in patients who received exa-cel for treatment of TDT or SCD in comparison to patients receiving allo-HSCT	• Delayed platelet engraftment • Neutrophil engraftment failure • Gene editing-related oncogenesis • Long-term effects • Pregnancy • Use in patients >35 years of age	Progress Reports Interim Reports Final Report	31 December 2028/2029 31 December 2033/2038 31 December 2043
Category 2 – Imposed mandatory additional PV activities which are Specific Obligations in the context of a conditional MA under exceptional circumstances (key to benefit risk)				
Not applicable				
Category 3 – Required additional PV activities (by the competent authority)				
HCP Survey (PASS) Planned	**Study Objective** • Assess the HCPs' understanding of the important safety information detailed in the Guide for HCPs • Assess the HCPs' awareness of the aRMM tools • Assess the HCPs' utilisation of aRMM tools (behaviour)	• Delayed platelet engraftment • Neutrophil engraftment failure • Gene editing-related oncogenesis • Long-term effects	Final Report	30 months after first market launch in the EU

allo: allogeneic; exa-cel: exagamglogene autotemcel; CRISPR-Cas9: clustered regularly interspaced short palindromic repeats-associated 9 nuclease; HbF: foetal haemoglobin; HCP: healthcare professional; hHSPCs: human haematopoietic stem and progenitor cells; HSCT: haematopoietic stem cell transplant; MA: marketing authorisation; PASS: post-authorisation safety study; PSUR: periodic safety update report; PV: pharmacovigilance; SCD: sickle cell disease; TDT: transfusion-dependent beta-thalassemia

Note: Study 101 (PASS) progress and interim reports will also be summarised and included within the PSUR.

In addition, the following studies imposed as specific obligations in the context of a conditional MA will provide (long-term) safety results:

Study/Status	Summary of Objectives	Efficacy Uncertainties Addressed	Milestones	Due Dates
Efficacy studies which are conditions of the marketing authorisation				
Not applicable				
Efficacy studies which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
Study 101 (PASS) Planned	**Primary Objective** • Evaluate long-term safety outcomes in patients who received exa-cel for treatment of TDT or SCD	• Efficacy in additional subjects	Progress Reports	31 August 2024/2025/2026

Study/Status	Summary of Objectives	Efficacy Uncertainties Addressed	Milestones	Due Dates
	• Evaluate long-term safety outcomes in patients who received exa-cel for treatment of TDT or SCD in comparison to patients receiving allo-HSCT **Secondary Objectives** • Evaluate long-term effectiveness outcomes in patients who received exa-cel for treatment of TDT or SCD • Evaluate long-term effectiveness outcomes in patients who received exa-cel for treatment of TDT or SCD in comparison to patients receiving allo-HSCT		Interim Report 1	31 December 2027
Study 111 in subjects with TDT ages 12 to 35 years Ongoing	**Primary Objective** • To evaluate the safety and efficacy of a single dose of autologous CRISPR-Cas9 modified $CD34^+$ hHSPCs (exa-cel) **Secondary Objectives** • To quantify percentage of edited alleles in peripheral blood leukocytes and $CD34^+$ cells of the bone marrow • To assess the production of HbF after exa-cel infusion • To assess the effects of infusion of exa-cel on disease-specific events and clinical status **Exploratory Objective** • To assess the ability of biomarkers to characterise exa-cel effect and predict treatment outcomes	• Efficacy in additional subjects	Final Report	31 August 2026
Study 121 in subjects with SCD ages 12 to 35 years Ongoing	**Primary Objective** • To evaluate the safety and efficacy of a single dose of autologous CRISPR-Cas9 modified $CD34^+$ hHSPCs (exa-cel) **Secondary Objectives** • Assess the effects of infusion of exa-cel on disease-specific events and clinical status • Quantify gene editing efficiency **Exploratory Objective** • Assess the ability of biomarkers to characterise exa-cel effect and predict treatment outcomes	• Efficacy in additional subjects	Final Report	31 August 2026
Study 131 Long-term follow-up study in subjects with TDT and SCD Ongoing	In subjects who received exa-cel for treatment of TDT or SCD **Primary Objective** • To evaluate long-term safety for 15 years after exa-cel infusion **Secondary Objective** • To evaluate efficacy of exa-cel for 15 years after exa-cel infusion	• Efficacy in additional subjects	Interim Reports	31 August 2026/2029
Study 151 in subjects with SCD ages 2 to 11 years Ongoing	**Primary Objective** • To evaluate efficacy of a single dose of exa-cel in paediatric subjects with severe SCD **Secondary Objective** • To evaluate safety and tolerability of a single dose of exa-cel • Assess the effects of infusion of exa-cel on disease-specific events and clinical status Quantify gene editing efficiency	• Efficacy in additional subjects	Final Report	31 December 2027

Study/Status	Summary of Objectives	Efficacy Uncertainties Addressed	Milestones	Due Dates
Study 161 in subjects with TDT or SCD ages 12 to 35 years Ongoing	**Primary Objective** • To assess HbF levels over time, after a single dose of exa-cel in adolescent and adult subjects with either TDT or severe SCD **Secondary Objective** • To evaluate efficacy and safety of a single dose of exa-cel • Assess the effects of infusion of exa-cel on disease-specific events and clinical status • Quantify gene editing efficiency	• Efficacy in additional subjects	Interim Report	31 December 2027
Study 171 in subjects with SCD ages 12 to 35 years Planned	**Primary Objective** • To evaluate efficacy of a single dose of exa-cel in adolescent and adult subjects with severe SCD, HbSC genotype **Secondary Objectives** • To evaluate safety of a single dose of exa-cel in adolescent and adult subjects with SCD, HbSC genotype • Assess the effects of infusion of exa-cel on disease-specific events and clinical status • Quantify gene editing efficiency	• Efficacy in additional subjects	Final Report	30 June 2032

allo: allogeneic; exa-cel: exagamglogene autotemcel; HbF: foetal haemoglobin; HbSC: βS/βC; HSCT: haematopoietic stem cell transplant; PASS: post-authorisation safety study; PSUR: periodic safety update report; SCD: sickle cell disease; TDT: transfusion-dependent b-thalassemia

Note: Study 101 (PASS) progress and interim reports and Study 131 interim reports will also be summarised and included within the Periodic Safety Update Report.

2.7.3. Risk minimisation measures

Safety Concern	Risk Minimisation Measures	Pharmacovigilance Activities
Delayed platelet engraftment	**Routine risk minimisation measures:** <u>SmPC Sections 4.1, 4.2, and 4.4</u> • Indication for treatment of patients with β-hemoglobinopathies for whom HSCT is appropriate, as stated in SmPC Section 4.1. • Administration of exa-cel must be performed in a treatment centre by physician(s) with experience in HSCT and in the treatment of patients with β-hemoglobinopathies, as stated in SmPC Section 4.2. • Recommendations for monitoring platelet counts and managing symptoms of bleeding are provided in SmPC Section 4.4. <u>PL Sections 2 and 4</u> • Advice on how to identify symptoms of bleeding and when to contact the doctor is given in PL Sections 2 and 4. Restricted prescription medicine **Additional risk minimisation measures:** • Guide for HCPs • Patient Card • Guide for Patients/Carers	**Routine pharmacovigilance activities beyond adverse reaction reporting and signal detection** Platelet Engraftment Safety Information Collection Questionnaire **Additional PV activities:** • Study 101 (PASS) Progress reports: 31 December 2028/2029; Interim reports: 31 December 2033/2038; Final report: 31 December 2043 • HCP Survey (PASS) Final Report: 30 months after first market launch in the EU <u>*Efficacy studies that will provide relevant safety results:*</u> • Study 101 (PASS) Progress reports: 31 August 2024/2025/2026 Interim report: 31 December 2027 • Study 111 in subjects with TDT ages 12 to 35 years; Final Report: 31 August 2026

		• Study 121 in subjects with SCD ages 12 to 35 years; Final Report: 31 August 2026 • Study 151 in subjects with SCD ages 2 to 11 years; Final Report: 31 December 2027 • Study 161 in subjects with TDT or SCD ages 12 to 35 years; Interim Report: 31 December 2027 • Study 171 in subjects with SCD ages 12 to 35 years; Final Report: 30 June 2032
Neutrophil engraftment failure	**Routine risk minimisation measures:** <u>SmPC Sections 4.1, 4.2, and 4.4</u> • Indication for treatment of patients with β-hemoglobinopathies for whom HSCT is appropriate, as stated in SmPC Section 4.1. • Administration of exa-cel must be performed in a treatment centre by physician(s) with experience in HSCT and in the treatment of patients with β-hemoglobinopathies, as stated in SmPC Section 4.2. • Collection of unmodified rescue CD34+ stem cells is required prior to myeloablative conditioning and infusion with exa-cel, as outlined in SmPC Section 4.2. • Guidance for administering unmodified rescue cells in the event of neutrophil engraftment failure is provided in SmPC Sections 4.2 and 4.4. • Recommendations for monitoring neutrophil counts and managing infections are provided in SmPC Section 4.4. <u>PL Sections 2 and 4</u> • Information on what to expect if engraftment fails is provided in PL Section 2. • Advice on how to identify symptoms of infection and when to contact the doctor is given in PL Sections 2 and 4. Restricted prescription medicine **Additional risk minimisation measures:** • Guide for HCPs • Patient Card • Guide for Patients/Carers	**Routine pharmacovigilance activities beyond adverse reaction reporting and signal detection** Neutrophil Engraftment Failure Safety Information Collection Questionnaire **Additional PV activities:** • Study 101(PASS) Progress reports: 31 December 2028/2029; Interim reports: 31 December 2033/2038; Final report: 31 December 2043 • HCP Survey (PASS) Final Report: 30 months after first market launch in the EU <u>*Efficacy studies that will provide relevant safety results:*</u> • Study 101 (PASS) Progress reports: 31 August 2024/2025/2026; Interim report: 31 December 2027 • Study 111 in subjects with TDT ages 12 to 35 years; Final Report: 31 August 2026 • Study 121 in subjects with SCD ages 12 to 35 years; Final Report: 31 August 2026 • Study 151 in subjects with SCD ages 2 to 11 years; Final Report: 31 December 2027 • Study 161 in subjects with TDT or SCD ages 12 to 35 years; Interim Report: 31 December 2027 • Study 171 in subjects with SCD ages 12 to 35 years; Final Report: 30 June 2032
Gene editing-related oncogenesis	**Routine risk minimisation measures:** <u>SmPC Section 4.4</u> • Description of the lack of myelodysplasia, leukaemia, or lymphoma from the clinical studies and a recommendation to monitor at least annually (including complete blood count) for 15 years after treatment is provided in SmPC Section 4.4 Restricted prescription medicine	**Routine pharmacovigilance activities beyond adverse reaction reporting and signal detection** Haematologic Malignancy Safety Information Collection Questionnaire **Additional PV activities:**

	Additional risk minimisation measures: • Guide for HCPs • Patient Card • Guide for Patients/Carers	• Study 101 (PASS) Progress reports: 31 December 2028/2029; Interim reports: 31 December 2033/2038; Final report: 31 December 2043 • HCP Survey (PASS) Final Report: 30 months after first market launch in the EU *Efficacy studies that will provide relevant safety results:* • Study 101 (PASS) Progress reports: 31 August 2024/2025/2026; Interim report: 31 December 2027 • Study 131 Long-term follow-up study in subjects with TDT or SCD; Interim Reports: 31 August 2026/2029
Long-term effects	**Routine risk minimisation measures:** SmPC Section 4.4 • Recommendation for long-term follow up is provided in SmPC Section 4.4. PL Section 2 • Expectations for long-term monitoring are described in PL Section 2. Restricted prescription medicine **Additional risk minimisation measures:** • Guide for HCPs • Guide for Patients/Carers	**Routine pharmacovigilance activities beyond adverse reaction reporting and signal detection** None **Additional PV activities:** • Study 101 (PASS) Progress reports: 31 December 2028/2029; Interim reports: 31 December 2033/2038; Final report: 31 December 2043 • HCP Survey (PASS) Final Report: 30 months after first market launch in the EU *Efficacy studies that will provide relevant safety results:* • Study 101 (PASS) Progress reports: 31 August 2024/2025/2026; Interim report: 31 December 2027 • Study 131 Long-term follow-up study in subjects with TDT or SCD; Interim Reports: 31 August 2026/2029
Pregnancy and lactation	**Routine risk minimisation measures:** SmPC Section 4.6 • Recommendations for contraception use, breastfeeding, and pregnancy, including a negative pregnancy test prior to the start of any treatment, are provided in SmPC Section 4.6. • Exa-cel must not be administered during pregnancy or breastfeeding due to risks associated with myeloablative conditioning, as stated in SmPC Section 4.6 PL Section 2 • Expectations for use of contraception, pregnancy testing, and breastfeeding are described in PL Section 2.	**Routine pharmacovigilance activities beyond adverse reaction reporting and signal detection** Pregnancy and Lactation Safety Information Collection Questionnaire **Additional PV activities:** • Study 101 (PASS) Progress reports: 31 December 2028/2029; Interim reports: 31 December 2033/2038; Final report: 31 December 2043 *Efficacy studies that will provide relevant safety results:* • Study 101 (PASS) Progress reports: 31 August

	• Advice for talking to the doctor prior to starting treatment is given in PL Section 2. Restricted prescription medicine **Additional risk minimisation measures:** None	2024/2025/2026; Interim report: 31 December 2027 • Study 131 Long-term follow-up study in subjects with TDT or SCD; Interim Reports: 31 August 2026/2029
Use in patients >35 years of age	**Routine risk minimisation measures:** SmPC Section 4.2 • Recommendation to consider the benefits of treatment against the risks of HSCT Restricted prescription medicine **Additional risk minimisation measures:** None	**Routine pharmacovigilance activities beyond adverse reaction reporting and signal detection** None **Additional PV activities:** • Study 101 (PASS) Progress reports: 31 December 2028/2029; Interim reports: 31 December 2033/2038; Final report: 31 December 2043 *Efficacy studies that will provide relevant safety results:* • Study 101 (PASS) Progress reports: 31 August 2024/2025/2026; Interim report: 31 December 2027
EU: European Union; HSCT: haematopoietic stem cell transplantation; PASS: Post-authorisation safety study; PL: Package Leaflet; PV: pharmacovigilance; Q4: Quarter 4; SCD: sickle cell disease; SmPC: Summary of Product Characteristics; TDT: transfusion-dependent β-thalassemia Note: Study 101 (PASS) progress and interim reports and Study 131 interim reports will also be summarised and included within the Periodic Safety Update Report.		

2.7.4. Conclusion

The CAT considers that the risk management plan version 1.1 is acceptable.

The CHMP endorses the CAT conclusion on the RMP as described above.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

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

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 15.11.2023. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. 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.*

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Casgevy (exagamglogene autotemcel) is included in the additional monitoring list as

- It contains a new active substance
- It is a biological product
- It is approved under a conditional marketing authorisation

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

Transfusion-dependent thalassemia

3.1. Therapeutic Context

3.1.1. Disease or condition

Treatment of transfusion-dependent β-thalassemia (TDT) in patients 12 years of age and older for whom haemopoietic stem cell (HSC) transplantation is appropriate and a human leukocyte antigen (HLA)-matched related haematopoietic stem cell (HSC) donor is not available.

3.1.2. Available therapies and unmet medical need

The **symptomatic** and recommended **treatment** for TDT is lifelong regular blood transfusions, usually administered every two to five weeks. Blood transfusions expose the patient to a variety of risks and adverse events, including but not limited to transfusion reactions, alloimmunisation, transfusion-related acute lung injuries, and transfusion transmitted infections (2021 Guidelines for the management of TDT, TFI). In addition, though chronic blood transfusion regimens are effective at preventing the hallmark symptoms and physical manifestations of disease, they introduce a large iron overload (Cao et al., 1996) that may lead to mortality through iron associated heart and liver toxicity (Vichinsky et al., 2005) To prevent this, iron overload must be managed with iron chelation regimens that are usually initiated at an early age (Saliba et al., 2015). Poor compliance with chelation regimens remains a key challenge. Despite the improvements with current therapies, there is poor quality of life and overall survival until the age of 30 years is only 55% (Modell et al., 2000; Delea et al., 2007).

Additionally, Reblozyl (luspatercept) was approved in the EU for treatment of adult patients with transfusion-dependent anaemia associated with beta-thalassaemia in June 2020.

Currently, the only **curative treatment option** for TDT is allogeneic haematopoietic stem cell transplant (allo-HSCT). There are significant risks associated with allo-HSCT such as serious infections, graft failure and graft-versus-host disease (GvHD), some of which can be fatal. As such, transplants are offered primarily to subjects who have available human leukocyte antigen (HLA)-matched sibling donors, who are young (<16 years of age), and who do not have significant iron overload. Because of the need of an HLA-matched sibling donor, allo-HSCT is available to only <25% of eligible patients with remainder of the patients requiring lifelong transfusions and chelation. Transplants using alternative donor sources such as unrelated cord blood and haploidentical donors remain experimental due to higher risk of engraftment failure and GvHD.

TDT is a seriously debilitating disease. The absence of suitable donors, the significant risks associated with transplantation, and the requirement for post-transplant immunosuppression therapy to prevent GvHD indicate an unmet medical need for novel therapies with transformative potential for subjects with TDT.

Regarding Zynteglo, lentiviral gene therapy for non-β0/β0-TDT approved in 2019, the EU marketing authorization had been withdrawn upon request of the marketing authorization holder.

3.1.3. Main clinical studies

The main body of evidence for efficacy is derived from the single pivotal phase 1/2/3, single-arm study 111 in TDT patients aged 12-35 years, inclusive at time of informed consent, for whom a human leukocyte antigen (HLA) matched related haematopoietic stem cell (HSC) donor was not available. TDT was defined as:

- Documented homozygous β-thalassemia or compound heterozygous β-thalassemia including β-thalassemia/hemoglobin E (HbE).
- A history of at least 100 mL/kg/year or 10 units/year of packed RBC transfusions in the prior 2 years before signing the consent or the last rescreening for patients going through re-screening.

Patients are to be followed for 24 months after exa-cel in study 111. Upon completion, they are asked to enrol into a dedicated long-term FU study 131 to cover 15 years after exa-cel.

Both studies are **ongoing,** enrolment into the pivotal trial is completed. For MAA, interim analysis data (data cutoff date 06-Sep-2022) and an updated analysis (data cut-off of 16 April 2023) of the pivotal study was submitted. Of n=59 enrolled patients, 54 subjects had received exa-cel infusion and are included in the full analysis set (FAS); 42 patients were included in the primary efficacy set (PES), including 13 adolescents.

3.2. Favourable effects

39/42 evaluable patients achieved transfusion independence lasting at least 12 months after exa-cel infusion. All patients, who met the primary endpoint, remained transfusion independent for the duration of follow-up.

Mean (SD) HbF levels were 7.8 (2.9) g/dL at Month 3, increased and were maintained with mean ≥10.9 g/dL from Month 6 through the duration of follow-up.

Mean (SD) total Hb levels were 11.4 (2.2) g/dL at Month 3, increased and were maintained with mean ≥ 12.2 g/dL from Month 6 through the duration of follow-up.

3.3. Uncertainties and limitations about favourable effects

The study design and complex study evolution have methodological deficiencies: It is a single pivotal phase 1/2/3 trial evolving from a first-in-human phase 1/2 study, with protocol amendments including changes to the primary efficacy endpoint and eligibility criteria that have raised concerns related to the internal validity of the trial and the potentially compromised data integrity. The type 1 error control strategy was uncommon and not fully comprehensible, of the studies was not controlled and effect estimates do not adequately reflect all enrolled subjects. The PES includes patients treated earlier when eligibility criteria were different.

The clinical study population was restricted to patients aged 12 to 35 years, inclusive at time of informed consent.

Given that transfusion thresholds are lower than (sex-specific) reference ranges for haemoglobin, transfusion independence does not automatically indicate normal haemoglobin levels, thus patients may continue to be anaemic.

Last, only a subset of patients was evaluable for the primary endpoint in this interim analysis, and long-term persistence of the effect is yet to be demonstrated.

3.4. Unfavourable effects

Treatment with exa-cel is preceded by mobilization/ CD34+ stem cell collection, and by myeloablative conditioning using busulfan. Exa-cel was generally safe and well tolerated. The safety profile of exa-cel was consistent with the risks of myeloablative busulfan conditioning and HSCT, with no additional exa-cel specific safety aspects identified. The type of AEs and timing of onset were generally consistent with that anticipated due to myeloablative conditioning and were expected findings in the peritransplant period. The majority of AEs and SAEs occurred within 6 months following exa-cel infusion, with most occurring within the first 3 months.

The most common AEs (occurring in >40% of subjects) were events described in the busulfan product information and included stomatitis, nausea, febrile neutropenia, headache, anemia, thrombocytopenia, mucosal inflammation, and vomiting in subjects with TDT.

In subjects with TDT, after myeloablation with busulfan and exa-cel infusion, 7 (13.0%) subjects had an AE of VOD, including 2 subject with a Grade 3 AE and 5 subjects with SAEs (Grade 2 or Grade 3). None of the VOD events were Grade 4. All events were considered possibly related or related to busulfan. All subjects received VOD prophylaxis starting from the time of busulfan conditioning and continuing after exa-cel infusion. The overall incidence and pattern of VOD events is consistent with the literature for subjects undergoing busulfan-based myeloablative conditioning and autologous HSCT with no additional exa-cel specific concerns identified.

Overall, of the subjects who completed busulfan conditioning and received exa-cel, 19 (35.2%) subjects with TDT had SAEs. Two subjects with TDT had SAEs that were considered possibly related or related to exa-cel (some of which were also related to busulfan). The SAEs in both subjects resolved. These events are recognized complications following myeloablative conditioning and autologous HSCT. A total of 8 (13.6%) TDT subjects had SAEs that occurred during mobilization and before start of myeloablative busulfan conditioning.

Specific risks are longer platelet engraftment time (identified risk) and neutrophil engraftment failure (potential risk) for TDT.

3.5. Uncertainties and limitations about unfavourable effects

The relatively low number of patients treated in both indications represent limitations.

While short-term safety profile of exa-cel seems to be in line with the inherent safety aspects of myeloablation and HSCT, little is known about mid-to-long term safety due to the limited sample size and limited follow-up time. The risk of malignancies, in particular, is currently unknown, which might be increased due to both the myeloablative conditioning and off-target gene editing.

Safety was characterized in patients aged 12-35 years. No data were obtained in adults older than 35 years. In general, it is considered that the mortality and significant morbidities associated with HSCT are lowest if the HSCT is administered during the mid-first decade of life, prior to the development of significant iron overload and/or other comorbidities such as liver fibrosis or hypersplenism. This aspect was taken into consideration for the indication wording.

For one subject a mutation was detected by NGS in the bone marrow aspirate after treatment. The applicant classified the finding on the gene mutation as of "unknown, unclear" clinical significance. The applicant provided further analysis investigating the origin of the mutation. Data presented indicate that the mutation was most likely present in the patient's cells before the gene-editing step. No potential for off-target editing around the variant site was detected, an analysis carried out both on the reference and patient sequence.

NGS was not performed in the trial populations before or after the interventional therapy. The extent of off targeting under clinical conditions is therefore unknown. However, it is currently not known whether such an approach adds to the detection and understanding of relevant off-target editing and its clinical consequences.

The (oncogenic) risks of guide RNA off targeting and of generating chromosomal aberrations (such as chromothripsis) at the on-target site is unknown. The significance of the *in vivo* model used for addressing the tumorigenic risk associated with potential off-target editing and/or chromosomal translocations induced during the editing process of the $CD34^+$ hHSPCs is limited, as the validity of the model remains questionable. Human genetic diversity and variability could have an impact on the off targeting potential and remains an uncertainty. However, the tumorigenic risk of exa-cel was addressed by the evaluation of the potential of SPY101-RNP to induce off-target editing and chromosomal aberrations, which has been sufficiently addressed using in silico and *in vitro* approaches. For a detailed discussion of this topic see 3.2.6

No immunological events were reported, the immunogenicity of the product was not assessed. Moreover, according to the CSP no analysis was performed to assess the presence of ADA before administration of the drug, and no analyses were performed to investigate potential immunogenicity/immunological reactions following the administration of the drug. At the time of infusion, Cas9 protein is still associated with the cells. This aspect is addressed in the SmPC Section 4.4, Special warnings and precautions for use.

3.6. Effects Table

Table 17. Effects Table for *Casgevy for treatment of TDT (data cut-off: 16-April-2023)*

Effect	Short Description	Unit	Treatment	Control (baseline)	Uncertainties/ Strength of evidence	References
Favourable Effects						
Transfusion independence (TI_{12})	TI ≥12m and Hb ≥9g/dl	N (%)	39/42 (92.9)	0	final analysis pending; methodological uncertainties regarding study design and conduct	
Total Hb	Mean from month 6 in absence of transfusions	g/dl	≥12.2	n.a.	final analysis pending, Hb not necessarily within reference ranges	
Unfavourable Effects						
SAEs	Patients with at least 1 SAE	N (%)	19 (35.2)			
Febrile neutropenia	Grade ≥ 3	N (%)	29 (53.7)		Related to Busulfan	
Venoocclusive liver disease	Grade ≥ 3	N (%)	6 (11.1)		Related to Busulfan	
Pneumonia	Any grade	N (%)	4 (7.4)		Related to Busulfan	
De novo gene mutation	Patients with gene mutation detected by NGS	N (%)	1 (1.9)		Unknown origin, could be related to exa-cel	

Abbreviations: FU (Follow-up); IA (Interim analysis); TI (transfusion independence); Hb (haemoglobin); HbF (foetal haemoglobin), m (months); NGS (next generation sequencing); SAE (serious adverse event)

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Regarding the favourable effects, durable transfusion independence with continuous Hb values above commonly accepted transfusion thresholds accompanied by an increase of HbF that can only be attributed to exa-cel is compelling; this is self-standing evidence of efficacy in a transfusion-dependent patient population, as it is not compatible with the natural course of disease, thereby outweighing methodological concerns derived from the single pivotal FIH phase 1/2/3 study. These concerns related to numerous changes to the study design during the course of this open label pivotal study.

Demonstration of durability, however, requires a longer follow-up in a sufficient number of patients, hence the final analysis of the pivotal study generating 24 months of follow-up in 59 patients could still

be considered incomprehensive. Thus a conditional marketing authorisation is considered the appropriate regulatory approach.

While total haemoglobin levels above transfusion-thresholds are relevant for contextualisation of transfusion independence, total haemoglobin levels below the lower age- and/or sex-specific reference range per definition indicate anaemia, which may warrant medical attention in specific outer conditions, e.g. pregnancies. The SmPC informs on the proportion of patients with Hb levels below the respective WHO age and sex dependent reference threshold, and the package leaflet informs patients of this risk.

On the unfavourable effects, overall, the short-term safety profile of exa-cel seems to be in line with the inherent safety aspects of myeloablation and HSCT. No additional exa-cel specific safety aspects were identified.

No data were obtained in adults older than 35 years at time of informed consent, which is reflected in the SmPC. In general, it is considered that the mortality and significant morbidities associated with HSCT are lowest if the HSCT is administered during the mid-first decade of life, prior to the development of significant iron overload and/or other comorbidities such as liver fibrosis or hypersplenism. The indication clearly refers to patients meeting transplant eligibility criteria.

There are still several unknowns related to the consequences of CRISPR-Cas9 gene editing. The same guide RNA as in the product may generate chromothripsis as consequence of specific on-target editing. There are further unknowns related to the human genetic diversity and variability and the potential of off-target editing.

3.7.2. Balance of benefits and risks

Exa-cel is considered the gene therapy equivalent to standard-of-care curative HSCT in patients for whom allogeneic HSCT is not an option due to the lack of a suitable donor. The efficacy, although based on an interim analysis, has been demonstrated based on a pre-specified analysis and could be considered sufficient as such. Updated efficacy results will be provided post-authorisation to show that these estimates are stable and exclude the possibility of overly favourable estimates describing efficacy. No exa-cel specific safety aspects were identified, and the short-term safety aspects are acceptable. Long-term effects, especially possible risks related to genome editing will be followed up in the post-marketing setting.

The nitrosamine NDMA has been detected in most batches of the excipient Cryostor CS5 that is used for freezing the cells (see Quality section). The applicant is committed to use Cryostor CS5 with a defined maximum amount of NDMA. Furthermore, the applicant commits to provide the outcome of the initiated investigation with respect to root cause analysis of detection of NDMA in the CryoStor CS5 buffer, implemented CAPA and potential revisions to the commercial control strategy post-authorisation.

The amount of NDMA that could possibly be administered to a patient treated with Casgevy, under worst case calculations, corresponds to about five-fold of the intake that is considered acceptable for lifetime daily exposure (96 ng/day). The Non Clinical Working Party confirmed that the risk associated with a one-time exposure of 5 times the acceptable intake of NDMA is many times lower than the risk from chronic exposure to 96 ng/day (acceptable intake) and therefore this added risk is considered acceptable from a safety point of view when balanced to the benefits of the product. The demonstrated clinical benefit of exa-cel in a situation of clear unmet medical need outweighs the low risk of accidental exposure to NDMA by the presence of this contamination. Considering the toxicology assessment provided and

since Casgevy is given only once in a lifetime, the proposed NMDA limit for all incoming batches of Cryostor CS5 is considered acceptable by CAT and CHMP.

Thus, for a restricted TDT patient population, i.e. transfusion-dependent beta-thalassemia patients who are eligible for HSCT but for whom a human leukocyte antigen (HLA)-matched related hematopoietic stem cell (HSC) donor is not available, the favourable effects outweigh the unfavourable effects and uncertainties.

3.7.3. Additional considerations on the benefit-risk balance

Quality of evidence

The main body of evidence for efficacy is derived from an ongoing, single pivotal trial with a limited number of patients enrolled and shortcomings in the design and conduct as discussed in detail previously. The SOBs include the ongoing trial 161, enrolling both TDT and SCD patients. This trial is expected to provide supportive data on safety and efficacy of exa-cel in TDT patients.

Precision of effect size

Early withdrawals or subjects for whom the product could not be manufactured exist; they negatively impact the effect size. The limited number of patients enrolled and the fact the trial is still ongoing and results are based on interim analyses only further impact the precision of the estimated effect size. With the SOBs, determination of the effect size is expected to be more precise.

Clinical meaningfulness of the endpoint

The key endpoints are clinically relevant, and the biomarker endpoint Hb-level can be directly linked to the mechanism of action.

Duration of efficacy

The overall follow-up is limited, precluding final estimation of durability of the effect. Clonal haematopoiesis is known to be age-related and may result in a loss of efficacy. No data is available yet to confirm or reject occurrence of clonal haematopoiesis in patients having been treated with exa-cel; longer term data is expected to provide respective information. The proposed SOBs include an interim study report for the ongoing LTFU study at the time of completion of the respective pivotal study. A second interim analysis is planned after five years of follow-up post exa-cel.

Safety exposure and length of follow-up

While short-term safety of exa-cel seems to be in line with the inherent safety aspects of myeloablation and HSCT, and no additional exa-cel specific safety aspects were identified, the provided dataset is considered incomprehensive regarding middle- and long-term safety of this gene editing therapy. Both more patients, representing the population defined by the proposed indication, and additional follow-up data are needed to address this aspect, which is reflected in the SOBs.

Pharmacologic rationale

The pharmacologic rationale of exa-cel, i.e. a targeted and permanent genetic modification to hHSPCs that leads to an increase in fetal hemoglobin (HbF) protein levels, is strong.

In principle, treatment of TDT with exa-cel is an autologous HSCT of genetically modified autologous CD34+. Ex vivo genome editing of autologous CD34+ aims at reduction of BCL11A gene transcription and subsequent decrease in BCL11A protein level with concomitant increases in γ-globin expression,

and, upon erythroid differentiation, increased levels of HbF *in vivo*. Increased levels of HbF are expected to result in increased endogenous total Hb in TDT, thus alleviating transfusion burden in TDT.

Natural history/course of the disease

The demonstrated transfusion independence is per definition not achievable in transfusion dependent thalassemia patients.

Based on the issues listed above, the clinical data provided in the MAA are not considered comprehensive.

Sickle-cell disease

3.7.4. Therapeutic Context

3.7.4.1 Disease or condition

Treatment of severe sickle cell disease (SCD) in patients 12 years of age and older with recurrent vaso-occlusive crises for whom haemopoietic stem cell (HSC) transplantation is appropriate and a human leukocyte antigen (HLA)-matched related haematopoietic stem cell (HSC) donor is not available.

3.7.4.2 Available therapies and unmet medical need

Approved therapies to prevent complications of SCD include hydroxyurea (HU), and voxelotor. These therapies reduce complications of SCD; however, patients can still have breakthrough VOCs. However, HU is not effective in all patients, is not well tolerated, nor is it curative, and has carcinogenic and teratogenic risks.

The treatment of clinical manifestations consists mainly of the management of pain episodes (hydration, anti-inflammatory agents, pain medication, massage etc.). Fever and suspected infection are treated with appropriate antibiotics. Life-threatening or severe complications (e.g., severe acute chest syndrome or stroke) often require red blood cell transfusions. Blood transfusions are usually combined with iron chelators, which are necessary in patients with long-term anaemias such as sickle cell disease. Splenectomy may be necessary for splenic sequestration.

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only known cure for SCD, but HSCT is only available to about 20% of patients who have a matched donor, and graft-versus-host disease (GvHD) is a known risk. Therefore, there is unmet medical need for the treatment of SCD.

3.7.4.3 Main clinical studies

The main body of evidence for efficacy is derived from a single pivotal phase 1/2/3, single-arm study 121 in patients aged 12-35 years (inclusive at time of informed consent) suffering from severe SCD, inclusive, for whom a human leukocyte antigen (HLA) matched related haematopoietic stem cell (HSC) donor was not available. Severe SCD was defined by the occurrence of at least 2 severe VOCs per year during the 2-year period before screening, while receiving appropriate supportive care (e.g., pain management plan, hydroxyurea), while severe VOC was defined as any 1 of the following events:

- Acute **pain** event that requires a visit to a medical facility and administration of pain medications (opioids or IV NSAIDs) or RBC transfusions
- Acute chest syndrome, as indicated by the presence of a new pulmonary infiltrate associated with pneumonia-like symptoms, **pain**, or fever
- Priapism lasting >2 hours and requiring a visit to a medical facility
- Splenic sequestration, as defined by an enlarged spleen, left upper quadrant pain, and an acute decrease in hemoglobin concentration of ≥2 g/dL.

Patients are to be followed for 24 months after exa-cel in study 121. Upon completion, they are asked to enrol into a dedicated long-term FU study 131 to cover 15 years after exa-cel.

Both studies are **ongoing,** enrolment into the pivotal trial is completed. In total, n=63 patients were enrolled, of which 58/63 patients had βS/βS, two (2) βS/β+, and three (3) βS/β0 genotype. For MAA, interim analysis data (data cutoff date 16-Sep-2022) and an updated analysis (data cut-off of 16 April 2023) of the pivotal study was submitted. 43 subjects had received exa-cel infusion and are included in the full analysis set (FAS); 29 patients were included in the primary efficacy set (PES), including six (6) adolescents. One treated patient discontinued study 121 from a death due to reasons unrelated to the study treatment.

3.7.5. Favourable effects

Primary Efficacy Endpoint: Following infusion with exa-cel, 28 of 29 (96.6%) subjects in the PES in Study 121 achieved VOC free for a continuous 12-month period (VF12; 95% CI: 82.2%, 99.9%; P < 0.0001).

Key Secondary Efficacy Endpoint: Following infusion with exa-cel, 29 of 29 (100%) subjects in the PES in Study 121 achieved inpatient hospitalization free for severe VOC for a continuous 12-month period (HF12; 95% CI: 88.1%, 100.0%).

In the FAS, mean (SD) proportion of total Hb comprised by HbF (HbF%) was 37.3% (9.0%) at Month 3, increased and was generally maintained with mean ≥40% from Month 6 through the duration of follow-up.

3.7.6. Uncertainties and limitations about favourable effects

The study design and complex study evolution, i.e. a single pivotal phase 1/2/3 trial evolving from a first-in-human phase 1/2 study, with numerous protocol amendments including changes to the primary efficacy endpoint and eligibility criteria raised concerns related to the internal validity of the trial and potentially compromise the integrity of the data. The type 1 error control strategy was uncommon and not fully comprehensible, and effect estimates do not adequately reflect all enrolled subjects. A high number of subjects discontinued the study early (e.g. due to inability to manufacture drug product). The clinical study population was restricted to patients aged 12 to 35 years resulting in an age gap compared to the intended patient population.

The primary endpoint addresses VOCs, some of which can be characterized objectively, like pulmonary infiltrate or splenic sequestration, or is based on subjectively experienced pain. Post-HSCT pain in patients with SCD can be related to factors that may not represent VOC events due to active disease. Pain events can be due to consequences of pre-existing disease such as hypersensitivity to pain (e.g., triggered by infections or trauma), exacerbation of chronic pain, and behavioural patterns.

Clonal haematopoiesis is a phenomenon recognized as being age-related. In cases of clonal haematopoiesis, a "sufficient" HbF expression is not predictable based on allelic editing data. Available clinical data does not indicate such a case has occurred.

Last, only a subset of patients was evaluable for the primary endpoint in this interim analysis, and long-term persistence of the effect is yet to be demonstrated.

3.7.7. Unfavourable effects

Treatment with exa-cel is preceded by mobilization/ CD34+ stem cell collection, and by myeloablative conditioning using busulfan. Exa-cel was generally safe and well tolerated. Across both indications, the safety profile of exa-cel was consistent with the risks of myeloablative busulfan conditioning and HSCT, with no additional exa-cel specific safety aspects identified. The type of AEs and timing of onset were generally consistent with that anticipated due to myeloablative conditioning and were expected findings in the peritransplant period. The majority of AEs and SAEs occurred within 6 months following exa-cel infusion, with most occurring within the first 3 months.

The most common AEs (occurring in >40% of subjects) were events described in the busulfan product information and included stomatitis, nausea, febrile neutropenia, headache, vomiting, decreased appetite, abdominal pain, constipation, arthralgia, pruritus, pain in extremity, and laboratory related events (platelet count decreased) in subjects with SCD.

In subjects with SCD, after myeloablation with busulfan and exa-cel infusion, 1 (2.3%) subject had a non-serious, Grade 3 AE of VOD. The event was considered related to busulfan and not related to exa-cel. All subjects received VOD prophylaxis starting from the time of busulfan conditioning and continuing after exa-cel infusion. The overall incidence and pattern of VOD events is consistent with the literature for subjects undergoing busulfan-based myeloablative conditioning and autologous HSCT with no additional exa-cel specific concerns identified. There was 1 death which occurred in the SCD study. The event was considered not related to exa-cel and possibly related to busulfan.

Overall, of the subjects who completed busulfan conditioning and received exa-cel, 16 (37.2%) subjects with SCD subjects had SAEs. There were no SAEs that were considered possibly related or related to exa-cel in subjects with SCD. Sickle cell anemia with crisis (3 [5.2%] subjects), followed by bone pain and abdominal pain (2 [3.4%] subjects, each), were the most common SAEs considered related or possibly related to plerixafor. A total of 22 (37.9%) subjects had an AE of sickle cell anemia with crisis (VOC), including 4 (6.9%) subjects who also had ACS (acute chest syndrome).

A specific risk is neutrophil engraftment failure which is captured as a potential risk in the RMP for SCD.

3.7.8. Uncertainties and limitations about unfavourable effects

The relatively low number of patients treated in both indications represent limitations.

While short-term safety profile of exa-cel seems to be in line with the inherent safety aspects of myeloablation and HSCT, little is known about mid-to-long term safety due to the limited follow-up time. For example, the risk of malignancies which may occur because of both myeloablative conditioning and off-target gene editing remains uncertain.

Safety was characterized in patients aged 12-34 years. No data were obtained in adults older than 34 yrs. This aspect was taken into consideration and reflected in the indication wording.

NGS was not performed in the trial populations before or after the interventional therapy. The extent of off targeting under clinical conditions is therefore unknown. However, it is also currently not known whether such an approach adds to the detection and understanding of relevant off-target editing and its clinical consequences.

The (oncogenic) risks of guide RNA off targeting and of generating chromosomal aberrations (such as chromothripsis) at the on-target site is unknown. The significance of the *in vivo* model used for addressing the tumorigenic risk associated with potential off-target editing and/or chromosomal translocations induced during the editing process of the $CD34^+$ hHSPCs is limited, as the validity of the model remains questionable. Human genetic diversity and variability could have an impact on the off targeting potential and remains an uncertainty. However, the tumorigenic risk of exa-cel was addressed by the evaluation of the potential of SPY101-RNP to induce off-target editing and chromosomal aberrations, which has been sufficiently addressed using in silico and *in vitro* approaches.

No immunological events were reported, the immunogenicity of the product was not assessed. Moreover, according to the CSP no analysis was performed to assess the presence of ADA before administration of the drug, and no analyses were performed to investigate potential immunogenicity/immunological reactions following the administration of the drug. At the time of infusion, Cas9 protein is still associated with the cells. This aspect is addressed in the SmPC Section 4.4, Special warnings and precautions for use.

3.7.9. Effects Table

Table 14. Effects Table for Casgevy for treatment of SCD (data cut-off: 16-April-2023)

Effect	Short Description	Unit	Treatment	Control (baseline)	Uncertainties/ Strength of evidence	Ref.
Favourable Effects						
VF12	absence of severe VOCs for at least 12 consecutive months	N (%)	28 (96.6)	0 (0)	final analysis pending; methodological uncertainties regarding study design and conduct; VOC (pain) is a subjective experience	
HF12	free from inpatient hospitalization for severe VOCs for at least 12 months	N (%)	29 (100)	0 (0)	final analysis pending; methodological uncertainties regarding study design and conduct;	
Unfavourable Effects						
SAEs	Patients with at least 1 SAE	N (%)	16 (37.2)			
Febrile neutropenia	Grade ≥ 3	N (%)	20 (46.5)		Related to Busulfan	
Pneumonia	Any grade	N (%)	5 (11.6)		Related to Busulfan	
Deaths		N (%)	1 (2.3)		Unrelated to study treatment	

Abbreviations: IA (Interim analysis); Hb (haemoglobin); HbF (foetal haemoglobin); HF (hospitalisation free); SAE (serious adverse event); VF (VOC free); VOC (vaso-occlusive crisis)

3.7.10. Benefit-risk assessment and discussion

3.7.11. Importance of favourable and unfavourable effects

Regarding the favourable effects, absence of VOCs in a patient population that had severe SCD as defined by at least two events of severe VOCs annually in the two prior years to treatment and absence of hospitalizations accompanied by an increase of HbF that can only be attributed to exa-cel is compelling and self-standing evidence of efficacy, which outweigh methodological concerns derived from the single pivotal FIH phase 1/2/3 study. These concerns related to numerous changes to the study design during the course of this open label pivotal study.

Proof of durability, however, requires a longer follow-up in a sufficient number of patients, and the final analysis of the pivotal study generating 24 months of follow-up in 63 patients is still not considered comprehensive.

No data were obtained in adults older than 35 years at time of informed consent, which is reflected in the SmPC. Only a limited number of paediatric patients (six) was evaluable for the primary endpoint, in line with respective findings in adults. The MoA is considered age-independent.

Available clinical data for non-βS/βS-genotype is limited, but the MoA of exa-cel is considered independent on genotype.

On the unfavourable effects, overall, the **short-term** safety profile of exa-cel seems to be in line with the inherent safety aspects of myeloablation and HSCT. No additional exa-cel specific safety aspects were identified.

There are still several unknowns related to the consequences of CRISPR-Cas9 gene editing. The same guide RNA as in the product may generate chromothripsis as consequence of specific on-target editing. There are further unknowns related to the human genetic diversity and variability and the potential of off-target editing.

3.7.12. Balance of benefits and risks

Exa-cel is considered the gene therapy equivalent to standard-of-care curative HSCT in patients for whom allogeneic HSCT is not an option due to the lack of a suitable donor. The efficacy, although based on an interim analysis, has been demonstrated based on a pre-specified analysis and could be considered sufficient as such. Updated efficacy results will be provided post-authorisation to show that these estimates are stable and exclude the possibility of overly favourable estimates describing efficacy.

No exa-cel specific safety aspects were identified, and the short-term safety aspects are acceptable. Long-term effects, especially possible risks related to genome editing will have to be followed up in the post-marketing setting.

The nitrosamine NDMA has been detected in most batches of the excipient Cryostor CS5 that is used for freezing the cells (see Quality section). The applicant is committed to use Cryostor CS5 with a defined maximum amount of NDMA. Furthermore, the applicant commits to provide the outcome of the initiated investigation with respect to root cause analysis of detection of NDMA in the CryoStor CS5 buffer, implemented CAPA and potential revisions to the commercial control strategy post-authorisation. The amount of NDMA that could possibly be administered to a patient treated with Casgevy, under worst case calculations, corresponds to about five-fold of the intake that is considered acceptable for lifetime daily exposure (96 ng/day). The Non Clinical Working Party confirmed that the risk associated with a one-time exposure of 5 times the acceptable intake of NDMA is many times lower than the risk from

chronic exposure to 96 ng/day (acceptable intake) and therefore this added risk is considered acceptable from a safety point of view when balanced to the benefits of the product. The demonstrated clinical benefit of exa-cel in a situation of clear unmet medical need outweighs the low risk of accidental exposure to NDMA by the presence of this contamination. Considering the toxicology assessment provided and since Casgevy is given only once in a lifetime, the proposed NMDA limit for all incoming batches of Cryostor CS5 is considered acceptable by CAT and CHMP.

Thus, for a restricted SCD patient population, i.e. adults with severe SCD who are eligible for HSCT, but for whom a human leukocyte antigen (HLA)-matched related hematopoietic stem cell (HSC) donor is not available, the favourable effects outweigh the unfavourable effects and uncertainties.

3.7.13. Additional considerations on the benefit-risk balance

Quality of evidence

The main body of evidence for efficacy is derived from an ongoing, single pivotal trial with a limited number of patients enrolled and shortcomings in the design and conduct as discussed in detail previously. With the proposed SOB, the body of evidence will be extended by data of three additional clinical trials, increasing the number of patients from n=46 to a minimum of 84.

Precision of effect size

A significant proportion of patients were unable or unwilling to complete the treatment procedure. The proportion of patients who were enrolled but never received the treatment was high even in this highly selected study population, and may be even higher in clinical practice. These patients negatively impact the effect size and are not yet adequately reflected in the estimated effect. The limited number of patients enrolled and the fact the trial is still ongoing and results are based on interim analyses only further impact the precision of the estimated effect size. The proposed SOBs cover three additional interventional trials, resulting in a substantially higher number of patients for the generation of comprehensive data regarding efficacy in the target population. The ongoing clinical trial in both paediatric and adult patients may also generate supportive or refuting data to the current observation that the majority, i.e. n=5/6 patients with insufficient CD34+ cells for manufacturing were adults. Insufficient mobilization/apheresis might be a consequence of (longer) hydroxyurea use.

The proposed PASS currently would not generate data on the proportion of patients intended to be treated, but that would never receive exa-cel. However, due to the autologous nature of the product and the circumstances of manufacturing, the MAH should be able to provide information on the fraction of patients for which the mobilization/apheresis process was started, but who will not be treated.

Clinical meaningfulness of the endpoint

The key endpoints are clinically relevant, and the biomarker endpoint Hb-level can be directly linked to the mechanism of action.

Duration of efficacy

The overall follow-up is limited, precluding final estimation of durability of the effect. With the proposed SOBs, a longer overall follow-up will be available.

Safety exposure and length of follow-up

While short-term safety of exa-cel seems to be in line with the inherent safety aspects of myeloablation and HSCT, and no additional exa-cel specific safety aspects were identified, the provided dataset is considered incomprehensive regarding middle- and long-term safety of this gene editing therapy. Both

more patients, representing the population defined by the proposed indication, and additional follow-up data are needed to address this aspect, which is reflected in the proposed SOBs.

Pharmacologic rationale

The pharmacologic rationale of exa-cel, i.e. a targeted and permanent genetic modification to hHSPCs that leads to an increase in fetal hemoglobin (HbF) protein levels, is strong.

In principle, treatment of SCD with exa-cel is an autologous HSCT of genetically modified autologous CD34+. Ex vivo genome editing of autologous CD34+ aims at reduction of BCL11A gene transcription and subsequent decrease in BCL11A protein level with concomitant increases in γ-globin expression, and, upon erythroid differentiation, increased levels of HbF *in vivo*. HbF itself is considered to have antickling properties by inhibiting deoxy sickle hemoglobin (HbS) polymerization (doi.org/10.1182/blood-2013-09-528067). In addition, increased levels of HbF are expected to result in accordingly decreased HbS in SCD, and ultimately reduce VOCs in SCD.

The mechanism of action is considered to be genotype-agnostic; a confirmation regarding genotypes not being included in the pivotal trial is expected from the proposed SOBs.

Natural history/course of the disease

The natural history renders the demonstrated freedom of vaso-occlusive crises or hospitalizations due to vaso-occlusive crises most likely to be due to exa-cel treatment.

Based on the issues listed above, the clinical data presented in the MA are not considered comprehensive.

Conditional marketing authorisation

As comprehensive data on the product are not available, a conditional marketing authorisation was proposed by the CAT during the assessment, after having consulted the applicant.

The product falls within the scope of Article 14-a of Regulation (EC) No 726/2004 concerning conditional marketing authorisations, as it aims at the treatment of a seriously debilitating disease.

Furthermore, the CAT considers that the product fulfils the requirements for a conditional marketing authorisation:

- The benefit-risk balance is positive, as discussed.
- It is likely that the applicant will be able to provide comprehensive data coming from the ongoing or planned studies 111, 121, 131, 151, 161 and 171 and from a registry study that will be established post-authorisation. The present limitations of the dataset will be addressed with seven specific obligations intended to provide additional data on the durability of the effect and long-term safety. By 31 August 2029, the data expected from these specific obligations will increase the available follow-up to at least five years in >100 exa-cel treated patients, with a maximum of >10 years; at present >16 months follow-up data is available for 42 exa-cel treated TDT patients, and for 29 exa-cel treated SCD patients, respectively. The current maximum reported follow-up is 51.1 months of a TDT patient and 46.2 months of a SCD patient.
- Unmet medical needs will be addressed, as a one-time treatment with Casgevy eliminates or markedly reduces transfusion requirements, with improvements in iron overload in patients with TDT and reduces the number of VOC, particularly hospitalizations due to VOC, reduces blood transfusions and the need for other medications that come with side effects or have to be taken frequently, in patients with in severe SCD. Casgevy therefore has benefits over available

treatment options in TDT and SCD. This is fully supported by the feedback received from patients' organisations summarised below for each indication:

TDT

Treatment with regular blood transfusions is exhausting and prevents patients from leading a normal social and professional life because schedules must be arranged around hospital appointments. From the patient perspective, medicines that reduce or eliminate transfusions are the most meaningful, particularly if this leads to a reduction in iron overload.

Existing therapies for TDT include: RBC transfusions supported by iron chelation therapy, allogeneic stem cell transplantation, and Reblozyl (luspatercept).

Exa-cel provides a clinically relevant advantage by eliminating or profoundly reducing the need for RBC transfusions. A reduction in RBC transfusions and ineffective erythropoiesis is expected to reduce iron overload and eventually the need for iron chelation therapies. Reduction in iron overload and ineffective erythropoiesis are known to occur slowly, following successful allo-HSCT, because the body's homeostatic processes for iron metabolism and removal are inefficient and require months to years to process. As a one-time treatment with exa-cel eliminates or markedly reduces transfusion requirements, with improvements in iron overload. Exa-cel therefore has benefits over chronic transfusion therapy and no issues with compliance.

Exa-cel offers a clinically relevant and marked advantage to the subset of patients with TDT who are eligible for HSCT but do not have a matched-related allogeneic HSCT donor. Exa-cel infusion improved biomarkers related to tissue iron overload and ineffective erythropoiesis that were clinically meaningful and sustained. PROs indicated clinically meaningful improved quality of life. Furthermore, there were no instances of graft versus host disease, primary or secondary graft failure.

Exa-cel is superior to luspatercept in terms of achieving transfusion independence, reducing anemia and is a once-only treatment, not a chronic treatment. Exa-cel has also demonstrated efficacy and safety in adolescent patients, unlike luspatercept.

SCD

The most clinically meaningful treatments would be one that reduce the number of VOC, particularly hospitalizations due to VOC, reduce blood transfusions which are time consuming and are associated with many complications, and reduce the need for other medications that come with side effects or have to be taken frequently.

Existing therapies for SCD include: allogeneic stem cell transplantation, RBC transfusion therapy, hydroxycarbamide, and Oxbryta (voxelotor).

Exa-cel provides a major therapeutic advantage for the subset of the severe SCD population who would be considered for curative therapy but do not have a suitable HLA-matched related donor. As an autologous therapy exa-cel does not carry risks of acute and chronic GVHD, and other severe complications related to the need for immunosuppressive therapies.

Exa-cel provides a clinically relevant advantage by eliminating the need for RBC transfusions for SCD-related indications. In addition, exa-cel is a one-time treatment which markedly reduces or eliminates severe VOCs and hospitalizations for severe VOCs.

Compared to Hydroxycarbamide, exa-cel provides improved efficacy in terms of VOC elimination or marked reduction, increases in total Hb and elimination of the need of transfusions. Exa-cel also increases total Hb to a greater extent than Hydroxycarbamide. Lack of compliance due to side effects is a noted issue with Hydroxycarbamide; exa-cel as a one-

time therapy does not have compliance concerns. Unlike Hydroxycarbamide, Exa-cel eliminates the needs for SCD related transfusions.

Exa-cel offers greatly improved efficacy in terms of elimination of or reduction in VOCs, improvements in total Hb, and elimination of the need for RBC transfusions for SCD compared to voxelotor.

- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required. As benefit-risk balance on basis of the current data is regarded positive, an additional therapy option for transfusion-dependent β-thalassemia and severe sickle cell disease patients for whom haemopoietic stem cell (HSC) transplantation is appropriate and a human leukocyte antigen (HLA) matched related haematopoietic stem cell (HSC) donor is not available is considered beneficial.

The CHMP endorses the CAT conclusion on conditional marketing authorisation as described above.

3.8. Conclusions

The overall benefit/risk balance of Casgevy is positive, subject to the conditions stated in section 'Recommendations'.

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

4. Recommendations

Similarity with authorised orphan medicinal products

The CAT by consensus is of the opinion that Casgevy is not similar to Oxbryta and Reblozyl within the meaning of Article 3 of Commission Regulation (EC) No. 847/2000.

The CHMP endorses the CAT conclusion on similarity as described above.

Outcome

Based on the CAT review of data on quality, safety and efficacy, the CAT considers by consensus that the benefit- risk balance of Casgevy is favourable in the following indication(s):

<u>β-thalassemia (TDT)</u>

Casgevy is indicated for the treatment of transfusion-dependent β-thalassemia (TDT) in patients 12 years of age and older for whom haematopoietic stem cell (HSC) transplantation is appropriate and a human leukocyte antigen (HLA)-matched related HSC donor is not available.

<u>Sickle cell disease (SCD)</u>

Casgevy is indicated for the treatment of severe sickle cell disease (SCD) in patients 12 years of age and older with recurrent vaso-occlusive crises (VOCs) for whom haematopoietic stem cell (HSC) transplantation is appropriate and a human leukocyte antigen (HLA)-matched related HSC donor is not available.

The CAT therefore recommends the granting of the conditional 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).

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

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

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 marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

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

- **Additional risk minimisation measures**

Prior to the use of Casgevy (exagamglogene autotemcel) in each Member State, the Marketing Authorisation Holder (MAH) must agree about the content and format of the educational programme with the National Competent Authority.

The MAH shall ensure that in each Member State where Casgevy is marketed, all healthcare professionals (HCPs) and patients/carers who are expected to prescribe, use, or oversee the administration of Casgevy have access to/are provided with the following 2 educational packages aimed at highlighting the important identified and potential risks of Casgevy. These packages will be translated in the local language to ensure understanding of proposed mitigation measures by physicians and patients:

- **The Physician Educational Material consists of**
  - Guide for Healthcare Professionals;
  - Summary of Product Characteristics;
  - Guide for Patients/Carers;
  - Patient Card.
- **The Patient Information Pack consists of**
  - Guide for Patients/Carers;
  - Patient Card;
  - Patient Information Leaflet

- **Guide for healthcare professionals** shall contain the following key elements:
    - The HCP should inform patients treated with Casgevy that there is an important identified risk of delayed platelet engraftment and important potential risks of neutrophil engraftment failure and gene editing-related oncogenesis; and details on how these risks can be minimised.

  When presenting Casgevy as a treatment option and before a treatment decision is made, the HCP should discuss the risk-benefit of Casgevy, including the following:

    - Delayed platelet engraftment
        - Platelet counts should be monitored and managed according to standard guidelines and medical judgement. Blood cell count determination and other appropriate testing should be promptly considered whenever clinical symptoms suggestive of bleeding arise.
        - Patients should be counselled regarding the risk of delayed platelet engraftment, what symptoms and signs to be aware which could indicate bleeding, and the need to seek medical assistance if they experience any signs or symptoms suggestive of bleeding.
    - Neutrophil engraftment failure
        - Patients should be monitored for absolute neutrophil counts and infections and should be managed according to standard guidelines and medical judgement. In the event of neutrophil engraftment failure, patients should be infused with unmodified rescue $CD34^+$ cells.
        - Patients should be counselled regarding the fact that if they were to experience neutrophil engraftment failure after treatment with Casgevy, they would require an infusion of back-up $CD34^+$ cells and would not obtain the benefit of Casgevy treatment and still be exposed to possible long-term risks.
    - Gene editing-related oncogenesis
        - Gene editing-related oncogenesis is a theoretical risk. After treatment with Casgevy, patients should be monitored annually (including complete blood count) according to standard guidelines and medical judgement. If blood and bone marrow samples are taken for the diagnosis of haematologic malignancy, HCPs should take additional samples for analysis by the MAH to evaluate the association of malignancy with Casgevy treatment, should a malignancy be confirmed.
        - Patient should be counselled regarding the theoretical risk of gene editing-related oncogenesis and to seek medical attention if these signs and symptoms of myelodysplasia, leukaemia, and lymphoma are present.
    - The HCP should provide the Patient Card and Guide for Patients/Carers to patients/carers.
    - There is limited information regarding long-term effects. Therefore, participation in the long-term, registry-based study evaluating the long-term safety and effectiveness outcomes in patients who received Casgevy for treatment of TDT or SCD is encouraged. The HCP should remind patients about the importance to enrol in the 15-year, registry-based study of the long-term effects and how to obtain further information.

- **Patient alert card** shall contain the following key elements**:**
    - This card is to inform HCPs that the patient has received Casgevy infusion.
    - The patient should show the Patient Card to a doctor or nurse whenever they have medical appointments.
    - The patient should have blood tests as directed by the doctor.
    - The patient should seek medical advice for any signs of low platelet cell or white blood cell levels: severe headache, abnormal bruising, prolonged bleeding, or bleeding without injury (such as nosebleeds, bleeding from gums, blood in the urine, stool, or vomit, or coughing up blood), fever, chills, or infections.
    - Blood cancers are a theoretical risk. The patient should seek medical advice for any signs of fatigue, unexplained fever, night sweats, unexplained weight loss, frequent infections, shortness of breath, or swelling of lymph glands.

- **Guide for patients/carers** shall contain the following key elements**:**

  The guide explains the importance to fully understand the risk-benefit of Casgevy treatment and that there is limited information about the long-term effects

  Therefore, before a decision is made about starting the therapy, the doctor will discuss the following with the patient/carer:
    - How the important identified risk of delayed platelet engraftment and important potential risk of neutrophil engraftment failure can be recognised and minimised, including the need for monitoring of platelet and neutrophils regularly with regular blood tests until they have returned to a safe level.
    - Explain that there is a theoretical risk of gene editing-related oncogenesis and the need to monitor annually.
    - Explain that, in the event of neutrophil engraftment failure after treatment with Casgevy, unmodified rescue cells will be infused and the patient will not obtain benefit from Casgevy whilst still being exposed to the possible long-term risks.
    - Advise to seek medical advice for any signs of low platelets: severe headache, abnormal bruising, prolonged bleeding, or bleeding without injury (such as nosebleeds, bleeding from gums, blood in the urine, stool, or vomit, or coughing up blood).
    - Advise patient to seek medical advice for any signs of low white blood cell levels: fever, chills, or infections.
    - As blood cancers are a theoretical risk, advise to seek medical advice for any signs of blood cancers such as fatigue, unexplained fever, night sweats, unexplained weight loss, frequent infections, shortness of breath, or swelling of lymph glands.
    - The patient will receive a Patient Card that should be shown to any doctor or nurse at any medical appointments.
    - Inform that there is limited information regarding the long-term effects of Casgevy and the importance to participate in the registry-based study for long-term surveillance of 15 years.

The CHMP does endorse the CAT conclusion on the additional risk minimisation measures.

- **Obligation to conduct post-authorisation measures**

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

Description	Due date
In order to further characterise the long-term safety and efficacy of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) and severe sickle cell disease (SCD) aged 12 years and older, the MAH should conduct and submit the results of a study based on data from a registry, according to an agreed protocol.	31 December 2043

The CHMP endorses the CAT conclusions on the obligation to conduct post-authorisation measures as described above.

Specific Obligation to complete post-authorisation measures for the conditional marketing authorisation

This being a conditional marketing authorisation and pursuant to Article 14-a of Regulation (EC) No 726/2004, the MAH shall complete, within the stated timeframe, the following measures:

Description	Due date
In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) aged 12 years and older, the MAH should submit the final results from the study 111, a phase 1/2/3 study evaluating the safety and efficacy of a single dose of exagamglogene autotemcel in subjects with transfusion-dependent β-thalassemia.	31 August 2026
In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with severe sickle cell disease (SCD) aged 12 years and older, the MAH should submit the final results from the study 121, a Phase 1/2/3 Study to evaluate the safety and efficacy of a single dose of exagamglogene autotemcel in subjects with severe SCD.	31 August 2026
In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with severe sickle cell disease (SCD) aged 12 years and older, the MAH should submit the final results from the study 151, a Phase 3 Study to evaluate the safety and efficacy of a single dose of exagamglogene autotemcel in paediatric patients with severe SCD aged between 2 to 11 years.	31 December 2027
In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) and severe sickle cell disease (SCD) aged 12 years and older, the MAH should submit the interim results from the study 161, a phase 3b study to evaluate the efficacy and safety of a single dose of exagamglogene autotemcel in subjects with TDT or severe SCD.	31 December 2027
In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with severe sickle cell disease (SCD) aged 12 years and older, the MAH should submit the final results from the study 171, a phase 3 study to evaluate the safety and efficacy of a single dose of exagamglogene autotemcel in subjects with severe SCD, β^S/β^C genotype.	30 June 2032
In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) and severe sickle cell disease (SCD) aged 12 years and older, the MAH should conduct and submit the interim results of a study based on data from a registry, according to an agreed protocol. Yearly progress reports shall also be submitted.	31 December 2027

Description	Due date
In order to confirm the efficacy and safety of exagamglogene autotemcel in patients with transfusion-dependent β-thalassemia (TDT) and severe sickle cell disease (SCD) in patients aged 12 years and older, the MAH should submit the interim results from study 131, a long term follow-up open-label trial evaluating the safety and efficacy of exagamglogene autotemcel for 15 years in subjects with TDT and severe SCD who received treatment with exagamglogene autotemcel in previous clinical trials.	Interim reports: 31 August 2026 and 31 August 2029

The CHMP endorses the CAT conclusion on the specific obligation to complete post-authorisation measures for the conditional marketing authorisation as described above.

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

Not applicable.

New Active Substance Status

Based on the review of available data on the active substance, the CAT considers that exagamglogene autotemcel is to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union.

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