26 April 2019
EMA/56140/2020/Corr.[1]
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Zynteglo

International non-proprietary name: betibeglogene autotemcel

Procedure No. EMEA/H/C/003691/0000

Note

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

[1] Changes in this updated version consist in the redaction of personal data, in compliance with Regulation (EU) 2018/1725, updated INN and assigned ATC code.

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Administrative information

Name of the medicinal product:	Zynteglo
Applicant:	bluebird bio (Netherlands) B.V. Stadsplateau 7 WTC Utrecht 3521AZ Utrecht The Netherlands
Active substance:	Autologous CD34+ cell-enriched population that contains haematopoietic stem cells transduced with lentiviral vector encoding the $\beta^{A\text{-}T87Q}$-globin gene
International Nonproprietary Name/Common Name:	betibeglogene autotemcel
Pharmaco-therapeutic group (ATC Code):	B06AX02: Other haematological agents
Therapeutic indication(s):	Zynteglo is indicated for the treatment of patients 12 years and older with transfusion-dependent β-thalassaemia (TDT) who do not have a β^0/β^0 genotype, for whom haematopoietic stem cell (HSC) transplantation is appropriate but a human leukocyte antigen (HLA)-matched related HSC donor is not available (see sections 4.4 and 5.1).
Pharmaceutical form(s):	Dispersion for infusion
Strength(s):	1.2-20 x 10^6 cells/ml
Route(s) of administration:	Intravenous use
Packaging:	bag (Fluorinated Ethylene Propylene)
Package size(s):	1 bag (or more)

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List of abbreviations

aa	amino acid
Ab	antibody
AE	adverse event
allogeneic HSCT	allogeneic haematopoietic stem cell transplantation
ALT	alanine aminotransferase
AS	active substance
AST	aspartate aminotransferase
ATMP	advanced therapy medicinal product
AUC	area under the curve
BFU	burst-forming units - erythroid
BM	bone marrow
BME	bone marrow engraftment
c/dg	copies per diploid genome
CAT	Committee for Advanced Therapies
CFC	colony-forming cell
CI	confidence interval
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CPPs	critical process parameters
CQAs	critical quality attributes
CSR	clinical study report
DLco	diffusing capacity of the lungs for carbon monoxide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DP	drug product
DP VCN	drug product vector copy number
EC	European Commission
ELISA	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EOI	events of interest
EoP	End-of-production
EU	European Union
EURD	European Union reference date
FEP	fluorinated ethylene propylene
FP	finished product
GCP	Good Clinical Practice
G-CSF	granulocyte-colony stimulating factor
GFR	glomerular filtration rate
GLP	Good Laboratory Practice
GVHD	graft-vs-host disease
Hb	haemoglobin
HbA	haemoglobin A
HbA^{T87Q}	haemoglobin containing $\beta^{A\text{-}T87Q}$-globin
HbE	haemoglobin E

HbF	fetal haemoglobin
HBV	hepatitis B virus
HCV	hepatitis C virus
HGVS	Human Genome Variation Society
HIV	human immunodeficiency virus
HIV-1	human immunodeficiency virus type-1
HLA	human leukocyte antigen
HPC-A	hematopoietic progenitor cells obtained by apheresis
HPLC	high-performance liquid chromatography
HRQoL	health-related quality of life
HSC	haematopoietic stem cell
HSCT	haematopoietic stem cell transplant(ation)
HTA	health technology assessment
HTLV-1	human T-lymphotrophic virus-1
IL	interleukin
IL-2Rγ	interleukin-2 receptor gamma
INN	international nonproprietary name
IPC	in-process control
IS	integration site(s)
ISA	integration site analysis/analyses
ITS	interrupted time series
ITT	Intent-to-Treat
IV	intravenous
IVIM	in vitro immortalisation
LAM-PCR	linear amplification-mediated polymerase chain reaction
LCR	locus control region
LIC	liver iron content
Lin-	lineage-depleted
LTC-IC	long-term culture-initiating cell
LT-HSCs	long-term hematopoietic stem cells
LVV	lentiviral vector
%LVV+ cells	percentage of cells containing lentiviral vector sequences
MAA	Marketing Authorisation Application
MAH	Marketing Authorisation Holder
MCB	master cell bank
MDS	myelodysplastic syndrome
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
NC	not calculated
NE	neutrophil engraftment
NOR	normal operating range
nr	non-restrictive
NSG	NOD scid gamma
PAGE	polyacrylamide gel electrophoresis
PAR	proven acceptable range

PB VCN	peripheral blood vector copy number
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PCS	potentially clinically significant
PD	pharmacodynamics
PE	platelet engraftment
PIP	paediatric investigation plan
PK	pharmacokinetics
POC	proof-of-concept
PPQ	process performance qualification
pRBC	packed red blood cell
QoL	quality of life
qPCR	quantitative polymerase chain reaction
QRD	Quality Review of Documents
RBC	red blood cell
RCL	replication competent lentivirus
RMP	Risk Management Plan
RP-HPLC	reversed-phase high-performance liquid chromatography
RPR	rapid plasma reagin
SAE	serious adverse event
SAP	Statistical Analysis Plan
SCD	sickle cell disease
SEP	Successful Engraftment Population
SIN	self-inactivating
SmPC	Summary of Product Characteristics
SOC	system organ class
SOE	schedule of events
SOP	standard operating procedure
TDT	transfusion-dependent β-thalassaemia
TI	transfusion independence
TP	Transplant Population
TR	transfusion reduction
ULN	upper limit of normal
VCN	vector copy number
VOD	veno-occlusive liver disease
VSV-G	vesicular stomatitis virus glycoprotein G
WBC	white blood cell
WCB	working cell bank

1. Background information on the procedure

1.1. Submission of the dossier

The applicant bluebird bio (Netherlands) B.V. submitted on 21 August 2018 an application for marketing authorisation to the European Medicines Agency (EMA) for Zynteglo, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No. 726/2004.

Zynteglo was designated as an orphan medicinal product EU/3/12/1091 on 24 January 2013 in the following condition: treatment of ß-thalassaemia intermedia and major.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Zynteglo 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: ema.europa.eu/en/medicines/human/EPAR/zynteglo

Zynteglo was granted eligibility to PRIME on 15 September 2016 in the following indication: treatment of transfusion-dependent ß-thalassaemia.

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

- The therapeutic armamentarium for the treatment of ß-thalassaemia can be considered insufficient. The symptomatic approach of transfusions combined with iron chelation therapy may result in a certain improvement of life expectancy. The challenges with regard to side effects, insufficiency of a non-curative approach as well as non-adherence as a result of the burden of treatment remain. The only curative treatment option, allogeneic haematopoietic stem cell transplant (HSCT), is associated with severe side-effects and reactions. The treatment of transfusion-dependent ß-thalassaemia (TDT), therefore, represents an unmet medical need.
- Clinical data from 22 patients at that time suggested persistency of lentiviral vector (LVV) sequences in patients' peripheral blood cells with all patients producing therapeutic haemoglobin containing β^{A-T87Q}-globin (HbA^{T87Q}), associated with clinically relevant improvements. The majority of patients producing varying amounts of endogenous HbF, HbE, and/or HbA, no longer required transfusion support by 6 months after drug product infusion in Phase 1/2 studies. Patients with at least 6 months follow-up who had β^0/β^0 genotype and therefore no endogenous production of β-globin, showed a reduction in volume and/or frequency of transfusions requirements in the post-engraftment period compared to the 24 months prior to enrolment.
- If confirmed by further clinical investigations, the proposed product, Zynteglo, has the potential to exhibit an advantageous safety profile compared to patients treated with allogeneic haematopoietic stem cell transplantation (allogeneic HSCT) with a reduced risk of graft-versus-host disease (GVHD), opportunity not to administer immunosuppressive agents, and a lower risk of immune-mediated graft rejection with an autologous transplantation is argued to be lower. As compared to treatment using chronic pRBC transfusions, there is no risk of sensitisation to alloantigens and reduced risk of increased iron burden.
- The potential to significantly address the unmet medical need was therefore supported by the proof-of-concept (POC) both in non-clinical and clinical investigations as well as its correlation to clinical outcome parameters linked to clinical improvement and potential long-term well-being in patients.

The applicant applied for the following indication: "Adolescents and adults with transfusion-dependent β-thalassaemia (TDT) who do not have a β^0 mutation at both alleles of the β-globin (*HBB*) gene (i.e., patients with a non-β^0/β^0 genotype), for whom haematopoietic stem cell (HSC) transplantation is appropriate but a human leukocyte antigen (HLA)-matched related HSC donor is not available."

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC as amended - complete and independent application

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 studies.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No. 1901/2006, the application included an EMA Decision(s) P/0067/2018 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0067/2018 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

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

Applicant's request(s) for consideration

Conditional marketing authorisation and Accelerated assessment

The applicant requested consideration of its application for a conditional marketing authorisation in accordance with Article 14-a of the above-mentioned Regulation.

The applicant requested accelerated assessment in accordance to Article 14(9) of Regulation (EC) No. 726/2004.

New active Substance status

The applicant requested the active substance "autologous CD34+ cell-enriched population that contains haematopoietic stem cells transduced with lentiviral vector encoding the $\beta^{A\text{-}T87Q}$-globin gene" 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.

Scientific recommendation on Classification

The applicant bluebird bio (Netherlands) B.V. submitted on 21 August 2018 an application for Scientific recommendation on Classification to the EMA for Zynteglo, which was designated as an Advanced Therapy Medicinal Product EMA/989166/2011 on 19 December 2011.

PRIME support

Upon granting of eligibility to PRIME, the Rapporteur was appointed by the CHMP. A kick-off meeting was subsequently organised with the EMA, Rapporteur, assessors' team and experts from relevant scientific committees. 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: change in the manufacturing process and increase in potency attributes, environmental risk assessment, characterisation of transfusion requirements change over time for paediatric patients and pharmacodynamic model, post-authorisation registry, proposals for post-authorisation specific obligations, and maintenance of the orphan designation.

Protocol Assistance

The applicant received Protocol Assistance from the CHMP on the development for the indication from the CHMP on:

- 25 April 2015 (EMEA/H/SAH/038/1/2015/PA/SME/ADT/II),
- 23 July 2015 (EMEA/H/SA/3150/1/2015/PA/SME/ADT/II),
- 16 September 2016 (EMEA/H/SA/3150/1/FU/1/2016/PA/SME/ADT/I),
- 21 April 2017 (EMEA/H/SA/038/2/2017/PA/SME/ADT/PR/III), and
- 9 November 2017 (EMEA/H/SA/3150/1/FU6/2017/PA/SME/ADT/PR/I).

The Protocol Assistance pertained to the following quality aspects:

- Advice on manufacturing aspects and their impact on clinical trial material, particularly following changes to manufacturing site and improvement of the manufacturing process: robustness of characterisation and analytical methods, release and stability, specifications for clinical trials material, technology transfer, number of lots for Marketing Authorisation Application (MAA) submission, timing of data submission, qualification of the apheresis collection centres and traceability.

The Protocol Assistance pertained to the following clinical aspects in the context of parallel EMA/health technology assessment (HTA) advice:

- The suitability of data acquired with clinical trial material from the refined manufacturing process to support an MAA, including number of treated patients and their genotype.
- Adequacy of the proposed endpoints.
- The earliest time point for efficacy and safety data to support submission of a conditional MAA.
- Statistical analysis plans.
- Extrapolation models for prediction of both long-term efficacy and for inclusion of children in the trials.
- Design of confirmatory trials and open label real-world monitoring to investigate long-term efficacy and safety.
- Proposed pharmacovigilance and risk minimisation plans.

1.2. Steps taken for the assessment of the product

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

CAT Rapporteur: Johannes Hendrikus Ovelgonne CAT Co-Rapporteur: Violaine Closson Carella

CHMP Coordinator (Rapporteur): Paula Boudewina van Hennik

CHMP Coordinator (Co-Rapporteur): Alexandre Moreau

Accelerated Assessment procedure was agreed-upon by CAT and CHMP on	26 July 2018
The procedure started on	4 October 2018
The Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	21 December 2018
The Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	21 December 2018
In accordance with Article 6(3) of Regulation (EC) No. 726/2004, the Rapporteur and Co-Rapporteur declared that they had completed their assessment report in less than 80 days	21 December 2018
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	7 January 2019
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	17 January 2019
The following GCP inspection(s) were requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	
– A GCP inspection of the Clinical Trial HGB-207 at 3 sites, an investigator site in the USA, an investigator site in Thailand and the sponsor in the USA, between 4 December 2018 and 4 January 2019. The outcome of the inspections carried out was issued on	23 January 2019
The CAT agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	25 January 2019
The applicant submitted the responses to the CAT consolidated List of Questions on	20 February 2019
The Rapporteurs circulated the Joint Assessment Report on the responses to the List of Questions to all CAT and CHMP members on	8 March 2019
The Rapporteurs circulated the updated Joint Assessment Report on the responses to the List of Questions to all CAT and CHMP members on	15 March 2019
The CAT, 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 Zynteglo on	22 March 2019
EC requested clarifications on the SmPC to the CAT/CHMP	3 April 2019
The CAT, in the light of the overall data submitted and the scientific discussion within the Committee, readopted a positive opinion for granting a marketing authorisation to Zynteglo on	17 April 2019
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, readopted a positive opinion for granting a marketing authorisation to Zynteglo on	26 April 2019

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Zynteglo is indicated for the treatment of patients 12 years and older with transfusion-dependent β-thalassaemia (TDT) who do not have a β^0/β^0 genotype, for whom HSC transplantation is appropriate but a human leukocyte antigen (HLA)-matched related HSC donor is not available.

Per the 2014 Thalassaemia International Federation (TIF) Guidelines (Cappellini *et al.* 2014), the severity of disease for symptomatic patients is evaluated based on transfusion requirements. As such, patients with β-thalassaemia are classified either as transfusion-dependent or as non-transfusion dependent. Another classification of disease severity, which was used exclusively until recently, divided most patients with symptomatic disease into two groups: β-thalassaemia major and β-thalassaemia intermedia, with the former group being considered more severe, including patients who were started on regular transfusions soon after birth, and the latter being considered less severe, including patients started on regular transfusions later in childhood or those only requiring intermittent transfusions throughout life. Transfusion-dependent patients include those who have been historically diagnosed with β-thalassaemia major and some patients who are considered to have β-thalassaemia intermedia.

2.1.2. Epidemiology

The incidence and prevalence of β-thalassaemia are geographically variable, with endemic populations primarily found in South Asia, the Middle East, North Africa, and Southern Europe (Colah *et al.* 2010; Galanello and Origa 2010; Cappellini *et al.* 2014). The β^E mutation is very common in people of Southeast Asian descent (Williams and Weatherall 2012). While migration is changing the global distribution of the disease, β-thalassaemia is still a rare disease in most of Europe. The total annual incidence of symptomatic individuals is estimated at 1 in 100,000 throughout the world (Galanello and Origa 2010). Reliable sources of multinational epidemiology data in the EU for TDT are not widely available, which is compounded by differing terminologies used to describe transfusion dependence. The prevalence of TDT is higher in endemic Southern European countries such as Italy, where it is estimated that there are approximately 6000 cases of TDT (Angelucci *et al.* 2016). Northern European, non-endemic countries typically have a prevalence of <1000 (Angelucci *et al.* 2016). Based on England National Health Service (NHS) Hospital Episode Statistics (HES) database data, 1,007 β-thalassaemia patients in England were identified with at least 10 years of medical records (April 2005-March 2016). Of these, 524 were diagnosed with TDT. These numbers suggest a conservative estimate of prevalence in the EU of at least 10,000 patients.

Although survival with β-thalassaemia has dramatically improved over the past 20 years due to improvements in transfusion and iron chelation management and monitoring, iron overload and associated morbidities remain a major challenge in the management of TDT and treatment-related complications are the primary source of mortality (Cappellini *et al.* 2014). Mortality for patients with TDT remains significantly increased compared to that of the general population, as exemplified by a historical prospective study in Greece of patients with TDT in which overall survival until the age of 50 was 65%

and the standardised mortality rate (standardised for sex and ages 20 to 40 years) compared to the general population was 13.5 from 2002 to 2008 (Ladis *et al.* 2011).

2.1.3. Biologic features

Hundreds of β-thalassaemia mutations have been described that affect transcription, RNA processing, or translation of the β-globin gene and could result in clinical presentation of β-thalassaemia (Cao and Galanello 2010). Null mutations that eliminate the production of functional β-globin are referred to as β^0 mutations. Mutations that reduce expression of functional β-globin are referred to as β^+ and produce varying quantities of functional β-globin. Genotypes of patients with β-thalassaemia are usually denoted according to these classes of mutation (i.e., β^0/β^0, β^0/β^+, β^+/β^+). Certain common mutations may have their own designation, such as β^E, that results in reduced production of β-globin because of alternative splicing, as well as a single amino acid change (glutamic acid to lysine at codon 26) that alters its electrophoretic mobility. All mutations that are not null mutations are classified as non-β^0 mutations, including β^E. β-thalassaemia is an autosomal recessive disorder.

2.1.4. Clinical presentation, diagnosis and stage/prognosis

Absence or reduction in β-globin production results in an accumulation of excess uncomplexed α-globin chains in erythroblasts. The clinical implications of the α-globin/β-globin imbalance are two-fold: 1) patients lack sufficient red blood cells (RBCs) and haemoglobin (Hb) to effectively transport oxygen throughout the body, resulting in severe anaemia; and 2) ineffective erythropoiesis can lead to morbidities via splenomegaly, marrow expansion, concomitant bone deformities, and iron overload.

Individuals homozygous or compound heterozygous for mutant alleles are clinically heterogeneous and present with disease that ranges in severity from asymptomatic to transfusion-dependent and life-shortening. The severity of β-thalassaemia is also a function of the degree of imbalance in the production of α- and non-α-globins. In its most severe form, TDT causes patients to have life-threatening anaemia requiring frequent and lifelong pRBC transfusions for survival, resulting in unavoidable iron overload which in turn, in the absence of appropriate and maintained iron chelation, can cause serious cardiac, liver, and endocrine comorbidities, and shortened lifespan compared to the general population. Patients who have been inadequately transfused and/or chelated can present with serious complications including cardiomyopathy, pulmonary hypertension, osteoporosis, skeletal deformities, arthropathy, hepato-splenomegaly, delayed puberty and gonadal failure (Rund and Rachmilewitz 2005). Even with the correct application of blood transfusions and chelation treatments, patients with β-thalassaemia have many complications (Bonifazi *et al.* 2017).

2.1.5. Management

About the product

Zynteglo is an Advanced Therapeutic Medicinal Product (ATMP) and was classified by the EMA Committee for Advanced Therapies (CAT) as a gene therapy medicinal product (EMA/505476/2012). The active substance of Zynteglo is an autologous CD34+ cell-enriched population that contains hematopoietic stem cells transduced with lentiviral vector encoding the $\beta^{A\text{-}T87Q}$-globin gene.

Zynteglo is a gene therapy medicinal product designed to provide functional β-globin to patients with TDT, thus circumventing their need for chronic pRBC transfusions.

The applicant applied for the following indication: "Adolescents and adults with transfusion-dependent β-thalassaemia (TDT) who do not have a β^0 mutation at both alleles of the β-globin (*HBB*) gene (i.e., patients with a non-β^0/β^0 genotype), for whom haematopoietic stem cell (HSC) transplantation is

appropriate but a human leukocyte antigen (HLA)-matched related HSC donor is not available."

The CAT and CHMP agreed to the following indication: "Zynteglo is indicated for the treatment of patients 12 years and older with transfusion-dependent β-thalassaemia (TDT) who do not have a β^0/β^0 genotype, for whom haematopoietic stem cell (HSC) transplantation is appropriate but a human leukocyte antigen (HLA)-matched related HSC donor is not available."

It is intended for autologous use and should only be administered once. It is for intravenous infusion. The minimum recommended dose of Zynteglo is 5×10^6 CD34+ cells/kg. In clinical studies, doses up to 20×10^6 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 a qualified treatment centre by a physician(s) with experience in HSC transplantation and in the treatment of patients with TDT (see section 4.2 of the SmPC).

Manufacture of Zynteglo involves the isolation of CD34+ HSCs from the patient, followed by transduction of these cells *ex vivo* with BB305 lentiviral vector to introduce the $\beta^{A\text{-}T87Q}$-globin gene. Successful transduction results in the integration of the $\beta^{A\text{-}T87Q}$-globin gene into the genomic DNA of the patient's HSCs. After the patient has undergone myeloablation, the patient receives Zynteglo via a single intravenous infusion, with the aim of reconstituting their HSC repertoire with cells stably carrying integrated copies of the transgene. After engraftment and differentiation of the transduced HSCs in the patient, expression of $\beta^{A\text{-}T87Q}$-globin is driven by the globin locus control region, which determines that genes under its control are expressed specifically in cells of the erythroid lineage. This treatment therefore has the potential to correct the α-globin/β-globin imbalance in erythrocytes and allow for the production of sufficient endogenous Hb to correct anaemia and enable transfusion independence.

Type of Application and aspects on development

The CHMP and CAT agreed to the applicant's request for an accelerated assessment as the product was considered to be of major public health interest. This was based on the preliminary results, the novel mode of action and the unmet medical need.

The applicant requested consideration of its application for a Conditional Marketing Authorisation in accordance with Article 14-a of the above-mentioned Regulation, based on the following criteria:

- The benefit-risk balance is positive.
- It is likely that the applicant will be able to provide comprehensive data. The applicant will be able to provide comprehensive data relevant to the initial indication including long-term safety and efficacy data for adult and adolescent patients with TDT who have a non-β^0/β^0 genotype, post initial approval from the following sources: Phase 3 Study HGB-207, Phase 3 Study HGB-212 and long-term follow-up Study LTF-303.
- The unmet medical need has been addressed. The therapeutic armamentarium for the treatment of ß-thalassaemia can be considered insufficient. The symptomatic approach of transfusions combined with iron chelation therapy may result in a certain improvement of life expectancy. The challenges with regard to side effects, insufficiency of a non-curative approach as well as non-adherence as a result of the burden of treatment remain. The only curative treatment option, allogeneic HSCT, is associated with severe side-effects and reactions. The treatment of TDT, therefore, represents an unmet medical need.
- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required. The majority of patients with TDT who have a non-β^0/β^0 genotype treated with Zynteglo in Studies HGB-204 and HGB-205 have achieved TI (11 out of 14;

78.6%). For Study HGB-207, 4 out of 5 TI-evaluable patients achieved TI (data cut: 13 December 2018), and 6 additional patients from the ongoing Study HGB-207 with available data at Month 6 produced close to 8 g/dL or more HbA^{T87Q} at Month 6 and are predicted to achieve TI with ≥98% probability. Achievement of TI is clinically meaningful, eliminating the need for regular blood transfusions and associated comorbidities due to iron overload.

2.2. Quality aspects

2.2.1. Introduction

The finished product (FP) Zynteglo (formerly referred to as LentiGlobin for TDT) is presented as a dispersion for infusion containing 1.2 to 20 x 10^6 cells/mL of an autologous CD34+ cell-enriched population that contains hematopoietic stem cells (HSCs) transduced with lentiviral vector (LVV) encoding the β^{A-T87Q}-globin gene as active substance (AS). The only other ingredient is CryoStor CS5. The product is available in a 20 mL cryopreservation bag (or multiple bags), made of fluorinated ethylene propylene (FEP).

2.2.2. Active Substance

The active substance (AS) consists of an autologous CD34+ cell-enriched population that contains hematopoietic stem cells (HSCs) transduced with lentiviral vector (LVV) encoding the β^{A-T87Q}-globin gene and is produced from two starting materials, the BB305 lentiviral vector and autologous hematopoietic progenitor cells obtained by apheresis (HPC-A).

The section on the active substance is separated into two parts; part 1 for the LVV and part 2 for the transduced autologous cells. The active substance (transduced autologous cells) is formulated into the FP by formulation in the cryopreservation solution and filling into cryopreservation bags.

General information (lentiviral vector)

BB305 LVV is a replication-defective, self-inactivating (SIN), third-generation human immunodeficiency virus type-1 (HIV-1)-based LVV pseudotyped with envelope protein of the vesicular stomatitis virus (i.e., vesicular stomatitis virus glycoprotein G; VSV-G), carrying the human β-globin gene with a single modification at codon 87 under the transcriptional control of the erythroid specific human β-globin promoter and erythroid specific enhancer elements (DNase I hypersensitive sites HS2, HS3, and HS4) of the β-globin locus control region (see Figure 1). The β^{A-T87Q}-globin variant can be distinguished from the wild-type β^{A}-globin by HPLC.

Figure 1: BB305 LVV Provirus Sequence Map

Figure definitions: dU3, promoter deleted unique 3'; R, repeat; U5 unique 5'; Ψ+, packaging signal; cPPT, central polypurine tract; RRE, rev responsive element; UTR, untranslated region; E3, Exon3; I2, Intron2; E2, Exon2 with threonine87glutamine codon; I1, intron1; E1, Exon1; Prom, promoter; HS2-HS3-HS4, hypersensitive site 2,3,4; ppt, polypurine tract*.

BB305 LVV contains proteins responsible for forming the LVV particle that are encoded by the helper plasmids that are used to transfect human embryonic kidney (HEK293T) cells during LVV production.

Manufacture, characterisation and process controls (lentiviral vector)

LVV Manufacturing process

BB305 LVV is produced by transient transfection. BB305 LVV buds from the production cells, is harvested, purified via chromatography, and formulated prior to frozen storage.

The LVV manufacturing process begins with thawing HEK293T cells from the working cell bank (WCB). These cells are transfected with plasmid BB305 and packaging plasmids and incubated at a specified temperature and duration.

Subsequently, the supernatants containing the LVV are collected. This crude harvest is clarified by filtration, purified by chromatography, concentrated and formulated. The sterile filtered BB305 LVV is filled in vials.

For each process step, critical process parameters (CPPs), non-CPPs, and in-process controls (IPCs) are provided. The parameters are controlled within specified ranges.

LVV Control of materials

Sufficient detail was provided on the raw and starting materials used and their quality control is appropriately described.

The testing of the master cell bank (MCB) and WCB and end-of-production (EoP) cells is in agreement with Ph. Eur. 5.2.3 and Ph. Eur. 5.14.

Plasmids

An overview of the sites involved in plasmid manufacturing and a detailed description of the manufacturing process and its development, control and validation is provided. Each plasmid is manufactured from a bacterial WCB and further purified. The generation and testing of the cell banks (MCB and WCB), including testing EoP cells is described in detail.

The controls of the plasmids are in agreement with Ph. Eur. 5.14 and the section on bacterial cells used for manufacture of plasmid vectors for human use. Release specifications for the plasmids include tests for purity), strength, identity and safety. Testing of the MCB, WCB and EoP cells is in agreement with the requirements of Ph. Eur. 5.14. Method descriptions and validation summaries are provided.

Generally stability of the plasmids over a shelf life of 36 months at ≤ -65°C has sufficiently been demonstrated.

LVV Control of critical steps and intermediates

Process characterisation studies were deployed to identify the impact of the process parameters on critical quality attributes (CQAs) and establish proven acceptable range (PAR) for the process parameters.

The choice and ranges of the various process parameters studied are considered appropriate.

LVV Process validation

The BB305 LVV commercial manufacturing process was validated. BB305 LVV batches were manufactured under a prospectively established validation protocol. All measured parameters and attributes met the pre-defined protocol acceptance criteria. All process parameters were within the normal operating range (NOR). All process parameter and performance attribute results met the protocol acceptance criteria.

A media-fill program is implemented to confirm the ability to conduct aseptic processing. The sterilizing filter was validated for its intended use.

Qualification of shipping of LVV to long-term storage facilities was demonstrated.

LVV Manufacturing process development

The manufacturing process history has been adequately described. Comparability data, including extensive characterisation of transduced cells, were provided.

A risk assessment on the potential extractables of single-use product-contact materials was provided and demonstrated that an extractable study was not necessary.

LVV Characterisation

Characterisation testing of BB305 LVV was performed. This elucidation includes the analysis of particle size distribution, potential aggregation, particle buoyant density, particle morphology, protein composition, total nanoparticle count, and activity as assessed by the transduction of CD34+ target cells. These revealed some minor differences between the LVV of the clinical and commercial process.

Relevant process-related impurities have been identified. Their removal is addressed in process validation and characterisation studies.

Specifications (lentiviral vector)

The release and stability specifications for BB305 LVV cover potency, identity, safety, purity, appearance, and quality.

Replication competent lentivirus (RCL) test is in place and appropriately validated. The main consideration with respect to RCL safety of the product is the design of the LVV, based on which the chance of formation of RCL is considered very remote (also see ERA assessment) and the clinical data that have not given any indication of RCL.

The applicant has provided further justifications for the limits proposed for each of the CQA. Quantitative acceptance criteria are based on data from manufacturing history. The Applicant committed to review the specifications again when additional batches have been manufactured.

LVV Batch analyses

Release testing results are provided for BB305 LVV batches. Three consecutive BB305 LVV batches were manufactured under a prospectively established validation protocol.

LVV Reference standard

The reference standard was manufactured using the proposed commercial LVV manufacturing process. The qualification and characterisation tests and results, and the stability testing strategy of the reference standard are considered to be appropriate.

LVV Container closure

BB305 LVV is filled into break-resistant, transparent, plastic vials that are stoppered with chlorobutyl, elastomeric stoppers and capped with flip-off, aluminium seals. Tests on extractables and leachables have been performed using appropriate worst-case conditions. The results of these tests have been provided during the procedure and gave no reason for concern; therefore, the container closure system can be accepted and considered to be appropriately qualified.

Stability (lentiviral vector)

The proposed shelf life for the lentiviral vector is 24 months frozen.

Stability batches have been placed on stability at the intended long-term storage condition to demonstrate the biological and physical stability through the proposed 2 years shelf-life. Data from primary batches and supportive batches remain within specification.

The primary batches were also placed on stability at an accelerated storage condition. The results of these studies were completed and provided during assessment.

General information (transduced autologous cells)

The AS consists of an autologous CD34+ cell-enriched population that contains hematopoietic stem cells transduced with BB305 lentiviral vector (LVV) encoding the $\beta^{A\text{-}T87Q}$-globin gene (international nonproprietary name [INN] request pending). The $\beta^{A\text{-}T87Q}$-globin gene is the human, adult, β^{A}-globin gene with a glutamine amino acid residue substituted for a threonine at position 87.

The mechanism of action is described as engraftment in the bone marrow (BM) of the transduced CD34+ HSCs and differentiation, to produce RBC containing biologically active $\beta^{A\text{-}T87Q}$-globin that will combine with α-globin to produce functional Hb.

Manufacture, characterisation and process controls (transduced autologous cells)

Manufacturer

The AS is manufactured and controlled at Apceth BioPharma GmbH, Ottobrunn, Germany. MIA and GMP certificate references are provided. The latest inspection was conducted on 2018-06-19. A QP declaration is also provided.

Manufacturing process

The manufacturing process starts from the starting material, the haematopoietic progenitor cells obtained by apheresis (HPC-A) and follows a continuous process up to formulation and filling of the FP. HPC-A is subject to a platelet wash and CD34+ cell enrichment. The purpose of the CD34+ cell enrichment step is to separate CD34+ cells from other cells.

After enrichment, a pre-transduction stimulation is performed. The purpose of the Pre-Transduction Stimulation Culture process step is to make cells receptive to transduction. The cells are then transduced with BB305 LVV. Cells are then washed and re-suspended.
These washed cells constitute the active substance. The process is continuous until formulation of the FP.

No reprocessing is described.

Information on buffers, media and objectives of the individual process steps are included.

Tabular overviews of CPPs and IPCs have been adequately described.

The description of the manufacturing process is considered to be adequate and sufficient information and detail in relation to process parameters targets and ranges for each step has been provided.

Control of Materials

Raw materials

An adequate overview of the compendial and non-compendial raw materials used in the active substance manufacturing process is provided.

Compendial raw materials of human origin have a marketing authorisation in the EU.

For all raw materials, representative certificates of analysis CoAs or CoCs are provided. In general, the provided information demonstrates appropriate quality of the raw materials.

Representative certificates of analysis for the non-equipment product-contact materials have been provided.

Two components of the active substance manufacturing process were identified for extractables testing through a risk assessment. From both materials some compounds were above the Analytical Evaluation Threshold (AET). For all extractable compounds the Maximum Acceptable Concentration (MAC) calculated on justified Permissive Daily Exposure (PDE) was greater than the estimated concentration within the extraction solution.

Apheresis material

HPC-A consists of autologous peripheral blood mononuclear cells (PBMCs) collected by apheresis from each patient following mobilisation of leukocytes. Apheresis is performed by apheresis collection centres (ACC) that are certified as either Tissue or Blood Establishments in accordance with national legislation implementing relevant EU Directives. Qualification of the apheresis centres by the Applicant involves apheresis assessment, quality audits, quality agreement, on-site training and a follow-up and maintenance program.

A brief description of the HPC-A collection process is provided. Collection of PBMCs is performed following standard operating procedures.

The apheresis collection devices and kits are specified and CE-declarations of conformity are provided.

Control of critical steps and intermediates

The IPCs in the AS manufacturing process are defined.

The actions taken in case an action limit is not met are sufficiently described and acceptable. The limits of the currently proposed IPCs are justified by in-process data from FP.

Specified process steps were identified as critical.

Process validation and/or evaluation

As the manufacturing process of Zynteglo is continuous, process validation is discussed in the FP section.

Manufacturing Process Development

Development

Several manufacturing process versions have been described during development. The nomenclature corresponds to FP manufactured for the initial clinical trial (Study HGB-205), and FP manufactured before (Study HGB-204) and after optimization of the manufacturing process (Study HGB-207 and 212).

The proposed manufacturing changes, and the steps to ensure that the clinical batches from earlier processes were representative of the clinical profile of refined process product, were discussed in 4 Protocol Assistance procedures prior to submission of the Marketing Authorisation application.

The results of studies sufficiently demonstrate that FP manufactured according to base and refined processes is comparable. The observed differences could be acceptable from a safety and efficacy point of view. Uncertainties were adequately addressed by the Applicant in response to the LoQ.

It is sufficiently demonstrated that FP batches manufactured according to the refined process at different sites are comparable.

Changes made to the manufacturing process at the commercial manufacturing site were supported by data that demonstrate that consistent potency can be obtained. Comparability between the refined manufacturing process and the commercial manufacturing process was not sufficiently addressed. In response to questions, the Applicant indicates that the change was aimed to target VCNs to retain product efficacy while reducing theoretical risk of oncogenesis. The available data were, however, initially too limited to conclude on comparable product efficacy as it was insufficiently demonstrated that clinical data from base manufacturing process batches, can be considered representative of the commercial process. Tight control of potency attributes (i.e. within the range of refined process batches) was therefore considered necessary but was not provided by the proposed specifications. This issue was raised as a Major Objection because sub-potent batches are a considerable risk to the patient in case of sub-optimal efficacy because, based on the SmPC, the treatment cannot be repeated. In response, the Applicant agreed to revise the acceptance limits or provided further justification to maintain the proposed criteria for potency attributes. The Applicant provided a commitment to re-evaluate the acceptance criteria for potency attributes when batch release data from an additional 20 commercial FP batches are available. The re-evaluation will also consider the available clinical data and will aim to demonstrate that the release specification acceptance criteria are sufficient to ensure consistent clinical efficacy and safety. This commitment will be included as an Annex II condition.

Control strategy

A summary of the FP manufacturing process design and development, including classification of quality attributes and justification for IPCs is provided. Assessment of criticality of process parameters and definition of their operational range was based on a risk estimate and process characterisation studies (DOE). Further detail was provided on the risk assessment and the characterisation studies during the procedure. The provided information sufficiently justifies the proposed AS process parameter classification and PARs.

Characterisation

Functional characterisation

Functional characterisation results demonstrate an increased in some potency attribute levels in batches manufactured according to the refined process compared to batches manufactured according to the base process.

Differences in characterisation results were observed in VCN distribution across cell populations for refined and commercial process finished product lots as compared to base process lots. As cells with high VCN could be at higher risk of insertional mutagenesis, an acceptance criterion was introduced to ensure the distribution of VCN was adequately controlled within the range of clinical batches as requested. In this context, the Applicant has committed to provide the finalised data of the test method once available.

Impurities

With regard to product-derived impurities, extensive phenotypic characterization was performed, demonstrating that the percentage of cellular impurities is low.

The CD34+ cell and transduced cell levels are controlled at FP release.

Specification (transduced cells)

No specification for the active substance (AS) is provided as the AS is manufactured and processed into FP without interruption. An AS reference standard is not required.

As the manufacturing process of Zynteglo is continuous, no container closure system is described for the AS.

Stability (transduced cells)

Zynteglo AS is manufactured and processed into the FP without interruption. Stability testing of the AS is not applicable.

Comparability Exercise for Active Substance

See under manufacturing process development.

2.2.3. Finished Medicinal Product

Description of the product and pharmaceutical development

Description and composition of the finished product:

The FP is a dispersion for infusion, filled in one or two bags, each containing 1.2 to 20 x 10^6 cells/mL in 20 mL Cryostor CS5, a commercially available cryopreservation solution.

Zynteglo is an autologous, advanced therapy medicinal product (ATMP) suitable for infusion and conveys functional copies of the $\beta^{A\text{-}T87Q}$-globin gene in HSCs to myeloablated patients who have TDT. The applicant has provided key attributes and their control is included in the section on physicochemical and biological properties.

The posology as described in SmPC is a minimum of 5 × 10^6 CD34+ cells/kg body weight. The patient population is limited to non-β^0/β^0 genotype, transfusion-dependent adolescents and adults.

If the initial lot of FP for any patient does not contain the minimum cell dose, the patient may undergo additional cycles of mobilization and apheresis in order to obtain more cells. The autologous cells obtained from each additional cycle of mobilization and apheresis may be manufactured to produce an additional lot of FP. In this case, more than one lot would be used to make up the dose. Up to four bags of FP may be used per dose.

Pharmaceutical development

The Applicant has adequately justified the selection of CryoStor CS5 cryopreservation media as excipient for the formulation of the FP.

An overview of the manufacturing process development has been provided (see also section on active substance above).

Container closure system, microbiological attributes, and compatibility

The primary container closures selected for the FP are sterile, individually packaged, single-use FEP bags. Sufficient information on the selection of the container closure system has been provided. The selected container closure system supports product stability and is expected to be suitable for its intended use.

The suitability of the primary container closure was determined by evaluating the biocompatibility, physiochemical, and extractables data provided by the manufacturer. Extractables testing was performed on the primary container closure in a solution simulating a worst-case organic extraction.

The Applicant has provided a brief discussion of relevant aspects for microbiological safety, including testing of the FP and the steam sterilized container, which is considered acceptable.

Manufacture of the product and process controls

Manufacturer

The FP is manufactured at apceth BioPharma GmbH Ottobrunn, in Germany, directly in continuity of the AS manufacturing process.

Description of the manufacturing process and process controls

To manufacture Zynteglo FP, the AS cells are resuspended in CryoStor CS5 at a concentration of 1.2 x 10^6 to 20 x 10^6 cells/mL and filled into cryopreservation bags. Each bag is placed in a secondary container and a chilled metal cassette, and frozen before being placed in the vapour phase of liquid nitrogen for storage. Upon request, the Applicant provided additional information on process parameters and their classification and the justification of the proposed ranges. The provided information is considered adequate and sufficiently justifies the proposed FP process parameters, process parameter classification and PARs.

Process validation and/or evaluation

The PPQ study was performed using healthy donor cells. This approach has been accepted. Three consecutive FP batches were manufactured from apheresis material.

The PPQ study results suggest that the intended commercial manufacturing process is capable to consistently yield a product meeting the acceptance criteria.

The available batch analysis data support consistency of the FP manufactured at the commercial site.

Aseptic process simulation studies

Aseptic process simulation (media fill simulation) was successfully completed for three consecutive process simulation runs. It was confirmed that the media fill simulation covers all the aseptic processing steps.

Shipping validation

The shipping system used for transporting Zynteglo FP from the manufacturing facility to FP infusion centres within the European Union Regions has been qualified, taking a risk-based approach through Operational Qualification and Performance Qualification under worst case conditions.

Control of excipients

Information on the composition and specification of the excipient is provided. Ph. Eur. or equivalent quality of the ingredients is sufficiently demonstrated.

Container closure system

The primary container closure for Zynteglo is a 20-mL fluorinated ethylene propylene (FEP) cryopreservation bag.

Sufficient information on the container closure system has been provided. The choice of this container is appropriate for the type of product. Information on extractables/leachables and microbiological attributes has also been provided.

Product specification

Specification

The proposed commercial FP release specification includes potency, identity, purity, strength, safety (sterility, endotoxin, mycoplasma), and quality. Upon request, the Applicant has indicated at what stage the FP release tests are performed.

The set of specifications is in line with regulatory requirements.

Analytical procedures and reference standards

An overview of the analytical methods and their validation has been provided. In general, is has been demonstrated that methods are appropriately validated and give no reason for concern.

A reference standard is not used in the testing and release of the FP.

Batch analysis

Batch analysis data include batches manufactured according to clinical studies. The data support consistency of the FP manufactured at the commercial site. Batch analysis data of (additional) batches manufactured according to the commercial process were provided upon request. All the provided results conform to the FP specification.

Stability of the product

The proposed shelf life for Zynteglo FP is 1 year frozen at ≤-140° C. The product should be stored in the vapour phase of liquid nitrogen at ≤-140° C until ready for thaw and administration. Once thawed, it can be kept at room temperature (20°C to 25°C) for a maximum of 4 hours.

The product should be kept in the infusion bag(s) in the metal cassette(s) and should not be re-frozen after thawing.

Stability studies have been performed in accordance with current ICH/CHMP guidelines.

Long term stability data support the proposed shelf life of 1 year at ≤ -140°C. All results reported comply with the specifications and no trends are observed.

Stability at 25°C was studied. Results complied with the specifications after 4 hours. In the SmPC it is stated that the product has a maximum shelf life of 4 hours. This is considered to be acceptable.

Comparability exercise for finished medicinal product

See pharmaceutical development.

Adventitious agents

Non-viral adventitious agents

The information provided on the control of non-viral adventitious agents is considered adequate. Control of contamination of the manufacturing facility is briefly described by referring to cGMP compliance, personnel, cleaning/sanitization, single-use materials, facility design and procedural controls. Media fill simulation studies have been successfully performed.

Animal derived materials are discussed with regards to the risk for BSE/TSE (Bovine Spongiform Encephalopathy/Transmissible Spongiform Encephalopathy). Relevant BSE/TSE certificates of suitability have been provided. The risk for BSE/TSE is considered negligible.

Viral adventitious agents

Virus safety relies on the adequate control of starting materials and raw materials. The manufacturing process of LVV, AS and FP contains no steps capable of inactivating or removing viruses.

An overview table is provided of the testing for potential adventitious agents performed at various stages of the process (starting materials, in process LVV, final LVV, FP).

For all materials of human or animal origin assessments of the risk for adventitious virus transmission are performed. In general, the information on human- and animal-derived materials is considered to be sufficient and gives no reason for concern.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

The dossier content submitted has been of adequate quality; however, several issues have been raised during the procedure. Most issues could be adequately addressed by the Applicant during the procedure. The initial concerns with regard to the presence of cells with higher VCN in the finished product were adequately addressed by the Applicant. An acceptance criterion is implemented that will also limit cells with high VCN and data are provided that demonstrate that potential overestimation of potency has no clinical relevance. In addition, the Applicant indicates that the changes used in the commercial process were aimed not only at improving process consistency but also to target VCN values to retain product efficacy while reducing theoretical risk of oncogenesis. This is supported by the provided data for the commercial batches.

The intention is acknowledged from a safety point of view. The available data were initially, however, too limited to conclude on comparable product efficacy. Tight control of potency attributes (i.e. within the range of refined process batches) was therefore considered necessary but was not provided by the proposed specifications. Therefore, during the procedure this issue was raised as a Major Objection because sub-potent batches are a considerable risk to the patient due to the risks associated with of sub-optimal efficacy because the treatment cannot be repeated. In response to this major concern, the Applicant agreed to increase the specification or provided further justification to maintain the specification for potency attributes. In addition, the Applicant provided a commitment to re-evaluation of the justified initial specification when data from the release testing of an additional 20 commercial finished product batches are available. This is acceptable. However, it was considered that the re-evaluation of the specification should be extended to other potency tests and the Applicant should also take into account the available clinical data. The Applicant has agreed to the following Annex II condition: in order to further confirm the appropriateness of the acceptance criteria, the Marketing Authorisation Holder (MAH) should re-evaluate the acceptance criteria for attributes related to potency tests using batch release data and clinical results after 6 months follow-up of 20 patients treated with commercial batches.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of Zynteglo active substance and FP 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.

The CAT has identified the following measures necessary to address the identified quality developments issues that may have a potential impact on the safe and effective use of the medicinal product:

- In order to further confirm the appropriateness of the acceptance criteria, the applicant should re-evaluate the acceptance criteria for attributes related to potency tests using batch release data and clinical results after 6 months follow-up of 20 patients treated with commercial batches.

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

2.2.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CAT recommends the following points for investigation:

14 recommendations aimed at improving control of raw materials, providing additional stability data for starting materials, installing in process controls and/or limits for LVV and active substance manufacture, completing characterisation of LVV particles, providing further data on LVV and finished product test methods, revising acceptance criteria as warranted, re-evaluation of specifications after manufacture of additional batches of LVV and finished product, implementing additional test methods and acceptance criteria, respectively.

The CHMP endorse the CAT assessment regarding the recommendations for future quality development.

2.3. Non-clinical aspects

2.3.1. Introduction

The non-clinical data package included a series of *in vitro* experiments as well as *in vivo* animal studies, either using a test article manufactured according to a base manufacturing process or a refined manufacturing process.

In vivo pivotal studies were combined to assess therapeutic proof-of-concept (POC), pharmacology, biodistribution, general safety, single-dose toxicity and genotoxicity of BB3ept05 LVV-transduced cells.

Considering the nature of the final drug product (human autologous thalassaemic CD34+ haematopoietic stem cells transduced with the BB305 lentiviral vector), the test articles used during non-clinical studies were surrogates, and consisted of either thalassaemic mouse bone marrow cells transduced with BB305 LVV, or human healthy donor HSCs transduced with BB305 LVV, administered to β-thalassaemic ($Hbb^{th1}/^{th1}$) mice and immunodeficient NOD *scid* gamma (NSG) mice, respectively.

2.3.2. Pharmacology

Primary pharmacodynamic studies

Zynteglo contains autologous HSCs modified to express the transgenic protein $\beta^{A\text{-}T87Q}$-globin, the primary pharmacodynamic effect after engraftment and differentiation of the HSCs is the production of $\beta^{A\text{-}T87Q}$-globin. The $\beta^{A\text{-}T87Q}$-globin expressed by the integrated transgene can be distinguished from the wild type β^{A}-globin and other globins by RP-HPLC, using standards containing $\beta^{A\text{-}T87Q}$-globin and other globins with known retention times. The relative amount of $\beta^{A\text{-}T87Q}$-globin produced is reported as the $\%\beta^{A\text{-}T87Q}$-globin.

Primary pharmacodynamics was assessed in all *in vitro* and *in vivo* non-clinical studies.

***In vitro studies*:**

Table 1: *In vitro* primary pharmacodynamic studies

Study number	Species/Strain	Test or Control Articles	Method of Admin.	Doses cells/ kg	Gender and No. per Group	Noteworthy Findings
NC-11-001	Human SCD (β^S/β^E, lacking β^A) BM CD34+ cells (98.3% CD34+); MS-5 stromal cell line	HPV569 LVV or BB305 LVV	*In vitro*	NA	NA	As compared to HPV569 LVV, BB305 LVV was a more efficient transducer of human SCD BM CD34+ cells. After transduction with BB305 LVV, $\beta^{A\text{-}T87Q}$-globin represented **22%** of the total β-like-globin produced by SCD BM CD34+ cells.
NC-11-004	Human SCD (β^S/β^{+thal}, low levels of β^A) BM CD34+ cells (99.7% CD34+); MS-5 stromal cell line	HPV524 LVV, HPV569 LVV or BB305 LVV	*In vitro*	NA	NA	As compared to HPV569 LVV and HPV524 LVV, BB305 LVV was a more efficient transducer of human SCD BM CD34+ cells. After transduction with BB305 LVV, $\beta^{A\text{-}T87Q}$ globin represented **32%** of the total β-like-globin produced by SCD BM CD34+ cells.
B2-16-276	Human healthy donor mPB and BM CD34+ cells	BlaM LVV, BB313 (GFP) LVV or BB305 LVV	*In vitro*	NA	NA	As compared to the base manufacturing process, manufacturing BB305 LVV-transduced HSCs with a refined manufacturing process resulted in a greater proportion of transduced (%LVV+) cells and higher VCN values, without affecting cellular or colony differentiation

In two different studies (NC-11-001 and 004) the effect of transduction with earlier versions of the BB305 LVV (HPV569 LVV and HPV524 LVV) and BB305 LVV on vector copy number (VCN) values and $\beta^{A\text{-}T87Q}$-globin production in SCD (β^S/β^E, lacking β^A) BM CD34+ cells in colony-forming cell (CFC, reflecting late progenitors) and long-term culture initiating cell (LTC-IC, reflecting early progenitors) culture assays was determined. BB305 LVV largely outcompeted HPV569 LVV and performed slightly better than HPV524 LVV in transduction efficiency (VCN) of both early and late progenitors, erythroid and non-erythroid cell precursors and in percentage of LVV+ cells. After transduction with BB305 LVV, $\beta^{A\text{-}T87Q}$-globin represented 22% of the total β-like-globin produced by SCD BM CD34+ cells. Study B2-16-276 showed that, compared to the base manufacturing process, there was an increase in the proportion of colonies with a VCN greater than 4 copies per diploid genome (c/dg) in the refined manufacturing process. Overall, most colonies have VCN values between 1 and 4 c/dg with the refined manufacturing process. Several individual colonies of cell lot 518 bb305_13913 and _20113 have a VCN upper limit close to or above 10 c/dg. In contrast, the VCN of cultured long-term repopulating cells was below 2.5 c/dg. Each DP lot contains both long-term hematopoietic stem cells (LT-HSCs) and short-term repopulating cells. It is assumed that 1% of CD34+ cells are LT-HSCs according to the Applicant.

***In vivo studies*:**

BB305 LVV-transduced cells were evaluated in the β-thalassaemic ($Hbb^{th1}/^{th1}$) mouse model and in the NSG mice.

Table 2: Overview of *in vivo* primary pharmacodynamic studies

Study number	Species/Strain	Test or Control Articles	Method of Admin.	Doses cells/kg	Gender and No. per Group	Noteworthy Findings
NC-11-002	CD45.2+ C57BL/6 $Hbb^{th1/th1}$ β-thalass-aemic mice	Mock-, HPV569 LVV- or BB305 LVV-transduced CD45.2+ C57BL/6 $Hbb^{th1/th1}$ β-thalassaemic mouse syngeneic Lin- BMCs	IV (primary trans-plantation)	11×10^6	8 to 11 M or 7 to 11 F	Primary transplantation of HPV569 LVV- or BB305 LVV-transduced BMCs from primary donor β-thalassaemic mice into primary recipient β-thalassaemic mice resulted in successful and equivalent BM engraftment, correction of dyserythropoiesis, productive expression of $\beta^{A\text{-}T87Q}$-globin, and effective treatment of β-thalassaemia, thereby showing gene therapy POC correction of the β-thalassaemic phenotype.
NC-12-019	CD45.1+ C57BL/6 mice	Month 4 BMCs from CD45.2+ C57BL/6 $Hbb^{th1/th1}$ β-thalassaemic mice that received primary transplantation of mock-, HPV569 LVV- or BB305 LVV-transduced CD45.2+ C57BL/6 $Hbb^{th1/th1}$ β-thalassaemic mouse syngeneic Lin- BMCs in Study No. NC-11-002.	IV (secondary trans-plantation)	240×10^6	17 to 23 M or 9 to 21 F	Secondary transplantation of HPV569 LVV- and BB305 LVV-transduced BMCs from primary recipient/secondary donor β-thalassaemic mice into wildtype mice resulted in successful and equivalent BM engraftment, and maintained improvement of the gene therapy-corrected β-thalassaemic phenotype.
B2-15-161	NSG mice	Mock- or BB305 LVV-transduced human CD34+ HSCs	IV (trans-plantation)	50×10^6	10 to 15 F	Transduction of human heathy donor CD34+ HSCs with BB305 LVV using the refined manufacturing process had no adverse effects on long-term BME in immunodeficient, myeloablated NSG

						mice. As compared to the base manufacturing process, the refined manufacturing process resulted in comparable long-term BME, with no differences in BM cellular differentiation, but with higher %LVV+ cells, VCN values and $\beta^{A\text{-}T87Q}$-globin production in LTE BMCs.
B2-16-200	NSG mice	BB305 LVV-transduced human CD34+ HSCs	IV (trans-plantation)	42×106	15 F	Transduction of human CD34+ HSCs with BB305 LVV with the base manufacturing process or the refined manufacturing process had no adverse effects on long-term BME in immunodeficient, myeloablated NSG mice. As compared to Process 1, Process 2 resulted in slightly lower long-term BM engraftment, with no differences in BM cellular differentiation, but with higher %LVV+ cells, VCN values and $\beta^{A\text{-}T87Q}$-globin production in LTE BMCs. Highly-transduced HSCs did not appear to give rise to highly-transduced LTE BMCs.

Short-term repopulating cells contained in the CD34+ drug product have a restricted lifespan *in vivo* and primarily contribute to short-term hematopoietic reconstitution, whereas long-term repopulating cells contribute to long-term repopulating into both myeloid and lymphoid cell lineages.

Moreover, it is highlighted that transduction of CD34+ cells is stochastic within a given drug product and committed progenitors are presumably more easily transduced than primitive cells.

It is hypothesised that cells with a higher VCN were mainly committed progenitors of short-term lifespan (i.e., do not participate in long term engraftment) mitigating the risk of insertional mutagenesis. As data on VCN in LT-HSCs is lacking, this hypothesis could not be verified.

Transduction of CD34+ HSCs of healthy donors with the refined manufacturing process resulted in VCN between 2 and 6 c/dg depending on cell lot, LVV lot and operator. However, drug product VCN variability can also be attributed to (uncontrollable) intrinsic inter-patient differences of CD34+ HSCs and not the manufacturing process or LVV potency.

Experiments comparing peripheral blood CD34+ HSC with those from bone marrow showed comparable VCN. These studies confirm that the refined manufacturing process yields increased LVV+ CD34+ cells with VCN in range of approximately 4 c/dg.

In 92% of the transplanted animals, donor cell engraftment was above 80% with an average 0.8-3.6 c/dg. There was no difference between male and female engraftment rate. In literature, differences have been attributed to male specific expression of a MHC antigen. The differences observed in study NC-11-002 are not due to engraftment but most likely due to experimental variance and were not toxicologically relevant.

Engraftment of transduced cells resulted in increased haemoglobin and haematocrit levels, increased RBC counts and decreased reticulocyte% compared to mock control animals after 2 and 3 months. After 4 months, for animals given BB305 LVV-transduced BMC, the ratio of $\beta^{A\text{-}T87Q}$-globin in peripheral blood was above 30%. These improvements were largely comparable between HPV569 LVV- and BB305 LVV-transduced groups. There were no differences on WBC count, peripheral blood or bone marrow cell subsets compared to the sham group. Platelet counts appeared to be deceased in female transduced mice and slightly reduced in males at 2 months. Slight reductions in platelet counts were still apparent after 3 months in males and females. This is due to an overestimation of platelets in mock animals due to abnormal erythrocytes being counted by the analyser, which is a known phenomenon. Ter119+ CD71 cells, which are erythroid progenitors, were significantly increased in bone marrow of mice transplanted with BB305 LVV-transduced BMC compared to sham mice. This corresponded with improved dyserythropoiesis. In BB305 LVV-transduced BMC transplanted mice, differentiated erythroid cell counts were 40% higher than mock controls. Haemoglobin and haematocrit were increased and reticulocytes were decreased in animals expressing VCN of 1-2.6 c/dg. Homeostasis was not investigated (also see Study NC-12-019). Total bilirubin concentration was decreased in animals given HPV569 LVV- or BB305 LVV-transduced BMC compared to mock controls. This was related to reduced destruction of RBC and haemoglobin degradation in mice treated with transduced BM cells. BB305 LVV-transduced BMC transplanted mice had reduced spleen weight and corresponded to a reduced spleen size compared to sham mice. This variability stems from (uncontrollable) intrinsic inter patient differences of CD34+ HSCs and not the manufacturing process or LVV potency.

Subsequently, bone marrow harvested from β-thalassaemic ($Hbb^{th1}/^{th1}$) mice transduced with either HPV569 LVV or BB305 LVV was transplanted in lethally irradiated (11 Gy) CD45.1 C57BL/6. $Hbb^{th1}/^{th1}$ mice were transduced with Lin depleted $Hbb^{th1}/^{th1}$ CD45.2 four months earlier. Mice were followed for 6 months post-transplant. Engraftment was obtained with more than 90% of donor cells in bone marrow of secondary recipients after 6 months. High engraftment of CD45.2 cells was reached in both males and females in all groups after 4 months. Average percentage of CD45.2 cells was in excess of 85%. After 6

months, engraftment of CD45.2 cells remained high. There appeared to be an increase in variance in BB305 males. Nevertheless, average CD45.2 reached 90% or more in all groups.

The average vector copy number per cell in the bone marrow (BM) 4 months post-Bone Marrow Transplant (BMT) was 1.61 ± 0.95 and 2.33 ± 1.36 copies per cell for HPV569 and BB305 lentiviral vectors respectively. Five months post-BMT, the average VCN in blood cells was 1.63 ± 1.02 and 2.31 ± 1.40 copy per cell for HPV569 LVV and BB305 LVV respectively. At the end of the study, the average VCN per cell in the bone marrow of mice prematurely dead or sacrificed at the end of the study was 1.39± 0.82 and 2.02 ± 1.52 copies per cell for HPV569 LVV and BB305 LVV groups respectively, suggesting that stable reconstitution had been achieved. The maximum (individual highest) VCN measured in bone marrow in this study was 3.7 c/dg.

Red blood cell count, haemoglobin and mean corpuscular haemoglobin concentration, mean corpuscular and packed cell volume were increased in animals given HPV569 LVV- and BB305 LVV-transduced BMC compared to mock control animals. Reticulocyte counts were decreased. From the haematology data, it is clear that thalassaemic phenotype is improved, although mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration remain somewhat lower than wildtype historical controls. Furthermore, anisochromia and anisocytosis were observed in transplanted animals, consistent with the phenotype of β-thalassaemia. Minimal changes in platelet count were observed in HPV569 LVV and BB305 LVV transduced animals compared to mock controls. Nevertheless, the changes in platelets are minimal and are not considered to be toxicologically relevant. Monocytes and neutrophils were increased in females but not males treated with BB305 LVV-transduced BMC. However, the data are mostly within control values of historical data, and therefore not considered to be related to treatment. There is minimal variability in BMC chimerism (%CD45.2+ cells) and moderate variability in %Gr1/Mac1+ myeloid cells, %CD3+ T cells and %B220+ B cells after 4, 5 and 6 months suggesting that there are no drug product related effects on homeostasis.

These transduced BMCs, containing β-globin, abrogate the typical thalassaemic phenotype. Furthermore, re-transplantation of transduced bone marrow results in successful engraftment of CD45.2 cells with a stable VCN and a maintained correction of the β-thalassaemic phenotype for the duration of the study.

Two studies with the product to be used in the clinic were undertaken in which BB305 LVV-transduced human CD34+ cells were administered to immunodeficient, myeloablated NSG ((non-obese diabetic (NOD) severe combined immune deficiency (scid) interleukin-2 receptor gamma (IL-2Rγ) knockout (gamma)) mice. Transduction of human healthy donor CD34+ HSCs with BB305 LVV occurred in the presence of the base manufacturing process or the refined manufacturing process). These NSG mice are immunodeficient and characterised by the absence of mature T or B cells, lack of functional NK cells and deficiencies in cytokine signalling; they are therefore unable to reject the human CD34+ HSCs. Of note, NSG (and wildtype) mice do not support the differentiation of human HSCs into erythrocytes, and therefore, $\beta^{A\text{-}T87Q}$-globin production (which relies upon an erythroid promoter and is therefore restricted to cells of erythroid lineage) cannot be directly measured in NSG mice receiving BB305 LVV-transduced HSCs. Rather, BME is assessed by evaluating individual BM samples for relative numbers (%) of human-origin haematopoiesis-derived cells, composite VCN values and Integration Site Analysis (ISA) profiles, and by placing pooled BM samples into ex vivo colony cultures to determine the colony composite VCN values, proportion (%) of colonies transduced and the relative amounts (%) of β-globin chains, including $\%\beta^{A\text{-}T87Q}$-globin, produced by the colonies.

In a first *in vivo* experiment in NSG mice (B2-15-161), elements of the manufacturing process were refined. As a result, the applicant switched their production process to the refined manufacturing process The products resulting from these two processes were once more compared in an *in vivo* experiment in NSG mice (B2-16-200). For each of the studies, CD34+ cells from one healthy human donor were used. In both studies, colony or composite (culture) VCN value, the % of LVV positive cells were determined

on test article (before transplantation) and BM-derived materials 2 and 4 months post-transplantation. BME analyses were performed 2 and 4 months post-transplantation. In study 15-161 the test article material was cultured in several ways to discriminate between the early (LTC-IC) and the late progenitors (colony-forming cell, CFC) and provide information on transduction efficiency in these two cell populations. In study 16-200, the %$\beta^{A\text{-}T87Q}$-globin was determined for burst-forming units - erythroid (BFU) and cultured erythrocytes.

From both studies it becomes clear that the switch to the refined manufacturing process yields higher VCN values and a higher percentage of LVV positive cells, resulting in higher $\beta^{A\text{-}T87Q}$-globin expression levels. It does not seem to influence BM engraftment as this is quite similar between mock-treated and product-treated animals, where the product is generated by either process.

The proportion of early and late progenitors present in transduced HSCs were estimated from LTC-IC and CFC cultures. CFC cultures on post-transplant bone marrow or peripheral blood of TDT patients were not performed. However, data on patients with sickle cell disease (from Study HGB-206) are available. These data suggest that the proportion of PB and BM cells above 5 c/dg decline with longer term engraftment, supporting the notion that hematopoietic colony-forming cells with high VCN values are less able to establish long-term BM engraftment in humans.

Related to the switch to the refined manufacturing process the VCN values and the percentage of LVV+ cells increased, but these two parameters also showed a larger variability. It is not clear whether this phenomenon is also observed in the clinical results, for instance as a larger variety in efficiency. The level of LVV positive cells before and 4 months after transplantation is higher in study B2-16-200 (85% pre-transplantation -> 67% 4 months post-transplantation) as compared to B2-15-161 (53% pre-transplantation -> 43% 4 months post-transplantation). Also, the maximal colony VCN values for both studies differ. The colony VCN of pre transplantation material prepared under the refined manufacturing process reached a maximum of ~8 c/dg in study B2-15-161, whereas the maximal colony VCN pre transplantation was ~16 c/dg in study B2 15-200. In the latter study, the variability of colony VCN numbers is also higher for the refined manufacturing process, in both pre- and post-transplantation samples. Based on these data obtained with cell material from 2 healthy donors it appears that there is a batch to batch variability, which is attributable in large part to inherent differences in the transducibility of individual donor's HSCs for which the reason is not yet understood.

As becomes apparent from the analysis with material from the refined manufacturing process, VCN values in pre-transplantation material, representing early and late progenitors, are higher as compared to VCN values obtained from 2 or 4 months post transplantation material, which may be representative for engrafted cells. Also, the colony VCN values obtained from LTC-IC cultured test material (reflecting early progenitors) are lower compared to colony VCN values from CFC cultured test material (reflecting late progenitors). $\beta^{A\text{-}T87Q}$-globin expression from BFU or cultured erythrocytes seems to be higher from the pre-transplantation material, compared to $\beta^{A\text{-}T87Q}$-globin expression from BFU or cultured erythrocytes using BM material 2 or 4 months post-transplantation. Higher $\beta^{A\text{-}T87Q}$-globin expression from pre-transplantation material as compared to post-transplantation material may indicate higher insertional activity in myeloid lineage late progenitor CD34+ cells that will not engraft and these cells are likely to disappear shortly after transplantation. Cells obtained from the BM 2 or 4 months post-transplantation rather reflect the early progenitors that have engrafted. Apparently, for these cells lower VCN levels are observed. Data thus seem to indicate that the high VCN values present in the pre-transplantation material are mainly present in late progenitors that are not likely to engraft, which is regarded as a positive signal with regards to the mutagenic risk related to insertion. However, these suggestions are only based on data generated with cells retrieved from two healthy donors (one donor for study B2-15-161 and one donor for study B2-16-200), which is regarded quite limited.

Although it was not the purpose of these studies, the product could be considered generally safe according to a safety evaluation on the basis of animal observations, body weights and haematology parameters. However, in these studies (B2-15-161 and B2-16-200) no data is presented on persistence of BB305 LVV-transduced cells in other tissues/organs (other than peripheral blood and bone marrow), such as spleen, thymus, and lymph nodes. As studies NC-11-002 and NC-12-019 showed persistence of BB305 LVV-transduced β-thalassaemic mouse BM-derived cells in bone marrow, peripheral blood and/or lymphoid tissues (thymus and lymph nodes) of mice through Month 4 or Month 6, it is thus assumed that transduced cells were furthermore also distributed to a non-exhaustive list of tissues/organs.

Secondary pharmacodynamic studies

No stand-alone secondary pharmacodynamics studies were performed with BB305 LVV-transduced BMCs and/or HSCs (see discussion on non-clinical aspects).

Safety pharmacology programme

No stand-alone safety pharmacology studies were performed with BB305 LVV-transduced BMCs and/or HSCs (see discussion on non-clinical aspects).

Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies were conducted (see discussion on non-clinical aspects).

2.3.3. Pharmacokinetics

As Zynteglo is an *ex vivo* genetically modified autologous HSC-based product, traditional absorption, distribution, metabolism and excretion (ADME) studies are not relevant for Zynteglo.

The pharmacokinetic and biodistribution properties of BB305 LVV-transduced β-thalassaemic mouse BMCs were evaluated *in vivo* in a pivotal combined therapeutic POC, pharmacology, single-dose toxicity and genotoxicity study in the β-thalassaemic mice. The pharmacokinetics and biodistribution of BB305 LVV-transduced human healthy donor HSCs were also evaluated *in vivo* in the NSG mice. To assess biodistribution and *in vivo* persistence of BB305 LVV-transduced cells in animals, different markers/parameters were used in non-clinical studies: $\beta^{A\text{-}T87Q}$-globin production, percent transduced (%LVV+) cells or colonies, vector copy number (VCN) values.

2.3.4. Toxicology

The four pivotal single-dose toxicity studies included studies evaluating BB305 LVV-transduced β-thalassaemic mouse BMCs and administered by primary transplantation to Hbbth1/th1 β-thalassaemic mice initially and then by secondary (serial) transplantation to wildtype C57BL/6 mice. Pivotal single-dose toxicity studies also evaluated BB305 LVV-transduced healthy human donor HSCs manufactured by using the refined manufacturing process and administered by primary transplantation to immunodeficient (NSG) mice. Although not fully Good Laboratory Practice (GLP) compliant, these *in vivo* studies (NC-11-002, NC-12-019, B2-15-161 and B2-16-200) were pivotal and combined pharmacology, biodistribution, single-dose toxicity and genotoxicity studies, conducted in the murine animal model of β-thalassaemia (C57BL/6 Hbbth1/th1 β-thalassaemic mice) and in the immunodeficient NSG mice.

Single dose toxicity

Four pivotal single dose toxicity studies were conducted in immunocompetent and immunodeficient mice with a surrogate of Zynteglo FP, via the IV route.

Table 3: Overview of single dose toxicity studies and findings

Study number	Species/ Strain	Test or Control Articles	Method of Admin.	Doses cells/kg	Gender and No. per Group	Observed Maximum Nonlethal Dose (cells/kg)	Toxicity Noteworthy Findings
NC-11-002	CD45.2+ (Ly5.2+) C57BL/6 $Hbb^{th1/th1}$ β-thalassaemic mice	Mock-, HPV569 LVV- or BB305 LVV-transduced CD45.2+ C57BL/6 $Hbb^{th1/th1}$ β-thalassaemic mouse syngeneic Lin- BMCs	IV (primary trans-plantation)	11×10^6	8 to 11 M or 7 to 11 F	11×10^6	No toxicity associated with primary transplantation of HPV569 LVV- or BB305 LVV-transduced BMCs from primary donor β-thalassaemic mice into primary recipient β-thalassaemic mice. No evidence of oncogenesis related to insertional mutagenesis.
NC-12-019	CD45.1+ (Ly5.1+) C57BL/6 mice	Month 4 BMCs from CD45.2+ C57BL/6 β-thalassaemic $Hbb^{th1}/^{th1}$ mice that received primary transplantation of mock-, HPV569 LVV- or BB305 LVV-transduced CD45.2+ C57BL/6 $Hbb^{th1}/^{th1}$ β-thalassaemic mouse syngeneic Lin- BMCs	IV (secondary trans-plantation)	240×10^6	17 to 23 M or 9 to 21 F	240×10^6	No toxicity associated with secondary transplantation of HPV569 LVV- and BB305 LVV-transduced BMCs from primary recipient/secondary donor β-thalassaemic mice into secondary recipient wildtype mice. No evidence of clonal expansion or oncogenesis related to insertional mutagenesis.
B2-15-161	NSG mice	Mock- or BB305 LVV-transduced human CD34+ HSCs	IV (BM trans-plantation)	50×10^6	10 to 15 F	50×10^6	No toxicity associated with BB305 LVV-transduced human HSCs manufactured by the base manufacturing process or the refined manufacturing process. No evidence of clonal expansion or oncogenesis related to insertional mutagenesis.
B2-16-200	NSG mice	BB305 LVV-transduced human CD34+ HSCs	IV (BM trans-plantation)	42×10^6	15 F	42×10^6	No toxicity associated with BB305 LVV-transduced human HSCs manufactured by the base manufacturing process or the refined manufacturing process. No evidence of clonal expansion or oncogenesis related to insertional mutagenesis.

Repeat dose toxicity

No repeated dose toxicity studies were performed as the treatment foresees only one unique injection (see discussion on non-clinical aspects).

Genotoxicity

The BB305 LVV genotoxic potential has been studied during *in vitro* and *in vivo* studies.

Carcinogenicity

No carcinogenicity studies were submitted with Zynteglo.

Reproduction toxicity

No developmental and/or reproductive toxicity (DART) studies were submitted to support the use of Zynteglo for the treatment of TDT.

Toxicokinetic data

Persistent exposure to BB305 LVV-transduced mouse BMCs and/or to mouse HSC-derived BMCs was shown through 4 months or 6 months in all animals of both sexes.

Local tolerance

Local tolerance was assessed in the course of *in vivo* studies.

Other toxicity studies

No immunotoxicity studies have been performed.

2.3.5. Ecotoxicity/environmental risk assessment

As this product contains a genetically modified organism, an environmental risk assessment has been conducted in accordance with Directive 2001/18/CE on the deliberate release into the environment.

Transduced cells of Zynteglo FP are not able to survive in the environment; they are not infectious and are eliminated in non-target people by their immune system. Immunodeficient people, other than the patient for whom the product is intended, will not come into contact with Zynteglo as they will not be involved in administration of the product. Zynteglo FP cells would be recognised as foreign and eliminated by the immune system of an exposed person other than the cell donor (DePolo *et al.* 2000). It cannot be excluded that in immunocompromised people other than the specific patients, Zynteglo FP would persist and assume the function of normal CD34+ cells with expression of $\beta^{A\text{-}T87Q}$-globin. Due to the low immunogenicity of $\beta^{A\text{-}T87Q}$-globin observed in clinical studies, it is not anticipated that this would cause an adverse effect. Additionally, administration to the correct patient is assured by traceability procedures.

Residual amounts of free particles of BB305 LVV are below the level of quantification in Zynteglo FP. In the event a free particle would be present, lentiviral vectors pseudotyped with VSV-G have been reported to be inactivated by human serum complement (98.8% after 1 hour) if the LVV particles have not entered cells. Besides, BB305 LVV is inactivated rapidly under environmental conditions.

In conclusion, neither Zynteglo FP nor BB305 LVV are expected to be released into the environment at large. Zynteglo contains transduced, autologous, human cells which are re-infused into the same patient. It is the goal of the product to proliferate within the patient and the cells cannot survive outside of the patient and do not compete with other species in the natural environment.

2.3.6. Discussion on the non-clinical aspects

The non-clinical program was well conducted and consisted in four pivotal and combined *in vivo* studies conducted to assess proof of concept, pharmacology, biodistribution, single dose toxicity and genotoxicity of Zynteglo FP cells. Even if not fully compliant to GLP principles, these four *in vivo* combined studies allowed the assessment of both primary pharmacodynamics and toxicity.

No secondary pharmacodynamics studies were performed which is considered acceptable since the secondary pharmacodynamic effects of BB305 LVV-transduced BMCs (i.e. normalisation of total β-globin and Hb levels, normalisation of the hematologic parameters and correction of the β-thalassaemic phenotype) were evaluated in of pivotal pharmacology studies in the β-thalassaemic murine animal model. Results showed that treatment of thalassaemic mice resulted in the correction of the β-thalassaemic phenotype.

No safety pharmacology studies were performed which is considered acceptable as there was no evidence of adverse effects on the circulatory, respiratory, neurologic or digestive systems observed in any of the *in vivo* studies conducted. Considering the nature and origin of the cells (autologous human cells) and the absence of undesirable effects observed during *in vivo* studies, the lack of stand-alone safety pharmacology studies is considered acceptable.

Transduction efficiency in vitro

Several individual colonies of cell lot 518 bb305_13913 and _20113 had a VCN upper limit close to or above 10 c/dg. In contrast, the VCN of cultured long-term repopulating cells was below 2.5 c/dg. Therefore, it is understood that cells containing high VCN are likely early progenitors and are not likely to reconstitute in bone marrow. Transduction of CD34+ HSCs of healthy donors with the refined manufacturing process resulted in VCN between 2 and 6 c/dg depending on cell lot, LVV lot and operator. This variability stems from (uncontrollable) intrinsic inter patient differences of CD34+ HSCs and not the manufacturing process or LVV potency.

Effects observed in the in vivo study in the homologous animal model

In 92% of the transplanted animals, donor cell engraftment was above 80% with an average 0.8-3.6 c/dg. However, the submitted data representing successful engraftment did not differentiate between HPV569 LVV- or BB305 LVV-transduced cells. Furthermore, females transplanted with male BMCs appear to have identical engraftment rates. Gender differences are noted in the haematological, pathological and histopathological assessment. This is in line with literature where it has been reported that reciprocal transplantation can result in gender differences. In addition, because the drug product is an autologous application, such differences are not expected. Platelet counts appeared to be deceased in female transduced mice and slightly reduced in males at 2 months. Slight reductions in platelet counts are still apparent after 3 months in males and females. This is due to an overestimation of platelets in mock animals due to abnormal erythrocytes counted by the analyser, which is a known phenomenon.

Four animals died during the study but the cause of death could not be attributed to a treatment related effect. Microscopic changes in mock-transduced mice showed marked to severe increases in extramedullary haematopoiesis in spleen. This also corresponded to a minimal to moderate increase in erythroid cells in bone marrow and is typical for thalassaemic mice. These changes were still present in HPV569 and BB305 transduced mice, albeit at much lower severity, representing correction of the

thalassaemic phenotype. No other microscopic changes were noted. The presence of tumours or tumour cells (e.g. lymphoma) was not recorded in study NC-11-002 (primary transplantation). In study NC-12-019 (secondary transplantation) leukaemia/lymphoma was observed which was attributed to the long time since donor cells irradiation (10 months prior to the end of this study, vs 4 months in study NC-11-002). As irradiation to support grafting of BB305 LVV-transduced CD34+ cells is not part of the clinical treatment, this observation is not regarded of relevance for human.

Effects observed in the in vivo study in the non-homologous animal model

Related to the switch from to the refined manufacturing process, the VCN values and the percentage of LVV+ cells increased, but the results of these two parameters showed a larger variability. It is not clear whether this phenomenon is also revealed within the clinical results, for instance in a larger variability in efficacy. The in level of LVV positive cells before and 4 months after transplantation is higher in study B2-16-200 (85% pre transplantation, sample vs 67% in BM sampled 4 months post transplantation) as compared to B2-15-161 (53% pre transplantation, 43% 4 months post transplantation). Also the maximal colony VCN for both studies differed. The colony VCN of pre-transplantation material prepared under the refined manufacturing process reached a maximum of ~8 c/dg in study B2-15-161, whereas the maximal colony VCN pre transplantation was ~16 c/dg in study B2 15-200. In the latter study, the variability of colony VCN numbers is also higher for the refined manufacturing process as compared to Process 1, in both pre- and post-transplantation samples.

As becomes apparent from the analysis with material from the refined manufacturing process, VCN values in pre-transplantation material, representing early and late progenitors, are higher as compared to VCN values obtained from 2 or 4 months post transplantation material, which may be representative for engrafted cells. Also the colony VCN values obtained from LTC-IC cultured test material (reflecting early progenitors) are lower compared to colony VCN values from CFC cultured test material (reflecting late progenitors). $\beta^{A\ T87Q}$-globin expression from BFU or cultured erythrocytes seems to be higher from the pre-transplantation material, compared to $\beta^{A\text{-}T87Q}$-globin expression from BFU or cultured erythrocytes using BM material 2 or 4 months post-transplantation. Higher $\beta^{A\text{-}T87Q}$-globin expression from pre-transplantation material as compared to post-transplantation material, may indicate higher insertional activity in myeloid lineage late progenitor CD34+ cells that will not engraft and these cells are likely to disappear shortly after transplantation. Cells obtained from the BM 2 or 4 months post-transplantation rather reflect the early progenitors that have engrafted. Apparently, for these cells lower VCN levels are observed. Data thus seem to indicate that the high VCN values present in the pre-transplantation material are mainly present in late progenitors that are not likely to engraft, which is regarded as a positive signal with regards to the mutagenic risk related to insertion. However, these suggestions are only based on data generated with cells retrieved from two healthy donors (one donor for study B2-15-161 and one donor for study B2-16-200) and on data present in literature (Charrier *et al.,* 2010). VCN data from LTC-IC cultured from BB305 LVV-transduced human CD34+ cells would have provided further support for this explanation.

In these studies (B2-15-161 and B2-16-200) no data is presented on persistence of BB305 LVV-transduced cells in other tissues/organs (other than peripheral blood and bone marrow), such as spleen, thymus, and lymph nodes. Since studies NC-11-002 and NC-12-019 showed persistence of BB305 LVV-transduced β thalassaemic mouse BM-derived cells in bone marrow, peripheral blood and/or lymphoid tissues (thymus and lymph nodes) of mice through Month 4 or Month 6, it is thus assumed that in the studies B2-15-161 and B2-16-200 the transduced cells also distribute also distribute tissues/organs similar to what observed in studies NC-11-002 and NC-12-019.

Mutagenic or tumorigenic risk

An in vitro *in vitro* immortalisation (IVIM) assay (NC-12-016) was conducted to analyse the potential of the LVV to induce an immortalized or a transformed phenotype in the transduced mouse lineage depleted

(Lin-) BMCs cells. Evidence of in vitro genotoxicity was observed in the GRV (RFS91) and LVV (l.v-SF) positive controls in an IVIM assay, confirming the sensitivity of the assay. The mutagenic potential was strongly reduced for HPV524 LVV, HPV569 LVV and BB305 LVV. Of these, BB505 LVV showed the least genotoxic potential. In this in vitro assay, insertions were found in or near the following genes; inside Cdk17, Rad21, Cdk14, or Cadps2, near Pth2r and an insertion upstream of Pdha 2. None of these genes are related to haematopoietic malignancies. Neither is for any of the six gene products an apparent relationship with oncogenicity established. In human, IS in all of the six genes were identified, but none of them were among the top 10 clones or resulted in a malignancy. The issue of the integration and the potential effect upon treatment of patients with BB305 LVV-transduced CD34+ cells will also be further followed in the long-term registry for this GTMP with integrative LVV.

Based on available data it is not possible to conclude that the refined manufacturing process resulted in 1.3-fold more unique mappable IS, and 1.2-fold higher proportion of unique mappable IS within CIS. These observations are rather indicative for the variability between the different batches generated under the refined manufacturing process. However, the variability around this mean is very large. For instance, for cell batch 1 transduced via the base manufacturing process, the unique mappable IS and unique mappable CIS in IS are high, whereas these are low for the same cell batch, transduced with the refined manufacturing process. Remarkably, for cell batch 3 the exact opposite observation is made. For cell batch 2, the transduction method only results in a small difference of mappable IS or CIS in IS.

Variability in percentage of LVV+ cells and colony and composite VCN in early and late progenitors as well as uncertainties on level of unique mappable IS in CIS (see above) hamper the estimation whether the tumorigenic risk is purely theoretical or whether this might be a small but real risk for the patient. The applicant provided a discussion on the extent of the potential tumorigenic risk including (DP) characterisation data on 10 patients treated with BB305-LVV transduced CD34+ cells. The highly polyclonal semi-random integration profile that is expected for SIN LVVs is observed in mouse and confirmed in human. In the > 550,000 unique, mappable ISs identified no contribution of one individual IS reached >30% at any single time point. Clonal outgrowth or haematologic malignancies in any of the patients receiving Zynteglo was not observed yet. Together this indicates that the risk for tumorigenesis upon transduction of CD34+cells with BB305 LVV is rather limited and is likely theoretical, also for DP manufactured with the refined manufacturing process. Patients benefit more from treatment / product transduced with BB305 LVV using the refined manufacturing process (increased $\beta^{A\text{-}T87Q}$-globin and total haemoglobin concentrations) as compared to the base manufacturing process.

The requested (DP) characterisation data on 10 patients treated with BB305 LVV-transduced CD34+ cells lacked VCN data on LTC-IC cultured cells. This was regarded essential to support the proposed hypothesis that HSCs with high VCN values (and greater numbers of IS) fail to establish long-term engraftment. Currently, this hypothesis is supported by results from mouse studies and by literature data (Charrier, 2010) but not by clinical data with the current product for the proposed indication. It would have been of interest to observe a relationship between VCN of early progenitors and MOI, viral batch used and clinical parameters as β-globin production, Hb and transfusion independence. As VCN data on the early progenitors or long-term repopulating cells is missing, such a relationship cannot be identified.

Genotoxicity

The BB305 LVV genotoxic potential has been studied during *in vitro* and *in vivo* studies. *In* an IVIM assay, BB305 LVV-transduced mouse bone marrow cells showed reduced mutagenic potential as compared to positive control LVVs. Insertion site analysis of pre-transplantation transduced mouse BMCs and human CD34+ HSCs displayed the expected SIN LVV integration profile, with no enrichment for insertion in or near cancer-related genes.

There was no evidence that higher VCN values are associated with enrichment for integration within cancer-related genes or increased risk of insertional mutagenesis leading to oncogenesis. In the nonclinical studies conducted, ISA profiles of BB305 LVV-transduced β-thalassaemic mouse BMCs at Months 4 or 6 post-transplantation suggested that there was no preferred integration in the MDS1-EVI1 (MECOM), LMO2 or HMGA2 genes. Comparison of the Top 10 IS has not revealed any evidence of clonal dominance. Integration occurred across the genome, with no enrichment for integration near cancer-related genes.

Carcinogenicity

No carcinogenicity studies were conducted with Zynteglo. The final product is intended for a single IV administration, and conventional long-term carcinogenicity studies in rodents do not apply to ATMPs. However, from a risk-based perspective, as the final product consists of (immature) CD34+ HSCs transduced by a lentiviral vector, insertional mutagenesis resulting in oncogenesis (e.g., leukaemia/lymphoma) was the primary theoretical safety concern identified by the Applicant.

As the refined manufacturing process has been shown to induce a greater proportion of transduced (%LVV+) cells, higher composite VCN values and greater $\beta^{A\text{-}T87Q}$-globin production, a huge number of in vitro/ and in vivo genotoxicity studies were consequently conducted with the aim to assess the risk related with SIN LVV-mediated insertional mutagenesis, when using Zynteglo. No tumorigenicity and no evidence of an oncogenesis event related to insertional mutagenesis were observed in the nonclinical studies using BB305 LVV-transduced β-thalassaemic mouse BMCs or human healthy donor HSCs.

Reproduction toxicity

Zynteglo in contraindicated in pregnant women as reflected in section 4.3 of the SmPC, this is because of myeloablative conditioning.

Toxicokinetic data

Persistent exposure to BB305 LVV-transduced mouse BMCs and/or to mouse HSC-derived BMCs was demonstrated through 4 months or 6 months in all animals of both sexes.

Local tolerance

Local tolerance was assessed in the course of *in vivo* studies. No adverse effects were noted at the site of injection. The absence of studies is considered acceptable.

Other toxicity studies

No immunotoxicity studies have been performed since immunogenicity with $\beta^{A\text{-}T87Q}$-globin is not anticipated. Patients will be immunosuppressed at the time of treatment (busulfan conditioning) and Zynteglo is given on a single unique occasion.

Environmental Risk Assessment

Considering the data provided, Zynteglo is not expected to pose a risk to the environment, providing handling precautions are carried out as stated in the SmPC in order to minimise any potential risks to the environment. Patients treated with Zynteglo are informed not to donate blood, bone marrow or organs in order to prevent transplantation of Zynteglo FP cells to immunodeficient persons as indicated in section 4.4 of the SmPC.

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

2.3.7. Conclusion on the non-clinical aspects

Overall, the nonclinical program has been well conducted and supports the approval of Zynteglo. The

risk of insertional mutagenesis has also been adequately addressed in the non-clinical studies.

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

2.4. Clinical aspects

2.4.1. Introduction

GCP

The Clinical trials were performed in accordance with Good Clinical Practice (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

Table 4: Overview of Clinical Studies Evaluating the Drug Product in Patients with TDT

Study Identifier (Status); Location of CSR or Protocol (as applicable)	Study Title	Number of Patients with TDT[1] and Genotype	Drug Product Characteristics and Recommended Cell Dose	Recommended Busulfan Average Daily AUC	Primary Efficacy Endpoint(s) from Study Protocol	Data Cut Dates
HGB-205 (ongoing) Module 5.3.5.2 Interim CSR HGB-205 (patients with TDT only[2])	A Phase 1/2 Open Label Study Evaluating the Safety and Efficacy of Gene Therapy of the β-Hemoglobinopathies (Sickle Cell Anemia and β-Thalassemia Major) by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral $\beta^{A\text{-}T87Q}$-globin Globin Vector (LentiGlobin BB305 Drug Product)	7 planned (TDT or SCD[2]) 4 TDT treated 4 TDT completed (all non-β^0/β^0)	Original manufacturing process ≥3.0 × 10^6 CD34+ cells/kg	4000 to 5200 μM*min[3]	RBC transfusion requirements (measured in milliliters [mL] per kilogram [kg]) per month and per year post-transplant. Number of total in-patient hospitalization days (post-transplant discharge) at 6, 12, and 24 months.	Interim CSR Data Cut: 11 Oct 2017 Module 2.7.2/2.7.3 Data Cut: 07 Mar 2018 Additional Data Cut: 13 Dec 2018
HGB-204 (completed: 21 February 2018) Module 5.3.5.2 CSR HGB-204	A Phase 1/2 Open Label Study Evaluating the Safety and Efficacy of Gene Therapy in Patients with β-thalassemia Major by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral $\beta^{A\text{-}T87Q}$-globin Vector (LentiGlobin BB305 Drug Product)	18 planned 18 treated 18 completed (10 non-β^0/β^0; 8 β^0/β^0) 1 discontinued before conditioning	Base manufacturing process ≥3.0 × 10^6 CD34+ cells/kg	3600 to 5000 μM*min[3]	The sustained production of ≥2.0 g/dL of haemoglobin A (HbA) containing $\beta^{A\text{-}T87Q}$-globin for the 6 months between Month 18 and Month 24 post-transplant.	CSR Data Lock Point: 07 Mar 2018 Module 2.7.2/2.7.3 Data Cut: 07 Mar 2018 Additional Data Cut: 13 Dec 2018
HGB-207 (ongoing) Module 5.3.5.2 Interim CSR HGB-207	A Phase 3, Single Arm Study Evaluating the Efficacy and Safety of Gene Therapy in Patients with Transfusion-dependent β-Thalassemia, who do not have the β^0/β^0 Genotype, by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral $\beta^{A\text{-}T87Q}$-Globin Vector in Patients ≤50 Years of Age	23 planned (15 for ≥12 and ≤50 years of age; 8 for <12 years of age) 15 treated 0 completed 1 discontinued before conditioning	Refined or commercial manufacturing process ≥5.0 × 10^6 CD34+ cells/kg	3800 to 4500 μM*min[4]	The proportion of patients who meet the definition of "transfusion independence" (TI). TI is defined as a weighted average Hb ≥9 g/dL without any RBC transfusions for a continuous period of ≥12 months at any time during the study after drug product infusion. (Calculation of time period of TI will start when patients achieve an Hb ≥9 g/dL with no transfusions in the preceding 60 days).	Interim CSR Data Cut: 22 Feb 2018 Module 2.7.2/2.7.3 Data Cut: 22 Feb 2018 Additional Module 2.7.2/2.7.3 Late-Breaking Data Cut: 15 May 2018 Additional Data Cut: 13 Dec 2018

Study Identifier (Status); Location of CSR or Protocol (as applicable)	Study Title	Number of Patients with TDT[1] and Genotype	Drug Product Characteristics and Recommended Cell Dose	Recommended Busulfan Average Daily AUC	Primary Efficacy Endpoint(s) from Study Protocol	Data Cut Dates
HGB-212 (ongoing) Module 5.3.5.2 Protocol HGB-212	A Phase 3 Single Arm Study Evaluating the Efficacy and Safety of Gene Therapy in Patients with Transfusion-dependent β-Thalassemia, who have a β^0/β^0 Genotype, by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral $\beta^{A\text{-}T87Q}$-Globin Vector in Patients ≤50 Years of Age	15 planned (5 for ≥18 and ≤50 years of age; 10 for <18 years of age) 5 treated 0 completed	Refined or commercial manufacturing process $\geq 5.0 \times 10^6$ CD34+ cells/kg	3800 to 4500 μM*min[4]	The proportion of patients who meet the definition of "transfusion reduction" (TR), defined as demonstration of a ≥60% reduction in volume of packed red blood cell (pRBC) transfusion requirements (in mL/kg) in the post-treatment time period of Months 12 to 24, compared to the average annual transfusion requirements in the 24 months prior to study enrollment	CSR Data Cut: NA Module 2.7.2/2.7.3 Data Cut: 07 Mar 2018 Additional Data Cut: 13 Dec 2018
LTF-303 (ongoing) Module 5.3.5.2 Interim CSR LTF-303 (patients with TDT only[5])	A Longterm Follow-up of Patients with Hemoglobinopathies Treated with Ex Vivo Gene Therapy Using Autologous Hematopoietic Stem Cells Transduced with Lentiviral Vector	Long-term follow-up for all patients with a haemoglobinopathy who received drug product during bluebird bio-sponsored studies[5] 22 treated patients with TDT enrolled (18 from Study HGB-204; 4 from Study HGB-205)	Not applicable	Not applicable	For patients with TDT, assessments include, but are not limited to interval transfusions required (pRBC mL/kg), haemoglobin levels, therapeutic phlebotomies, iron chelator use, and measures of iron overload.	CSR Data Cut: 21 Nov 2017 Module 2.7.2/2.7.3 Data Cut: 07 Mar 2018 Additional Data Cut: 13 Dec 2018

[1] Number of patients as of 13 December 2018
[2] Note: patients with SCD (N=3 treated) were not included in the efficacy analysis for this module or in the interim CSR HGB 205. A final CSR for HGB-205 with data from all patients will be written after completion of the study.
[3] Changed from 3200 to 4400 μM*min in Protocol HGB-205 Version 7.0 (19 May 2016) and in Protocol HGB-204 Version 3.1 (17 March 2014).
[4] Changed from 4000 to 5000 μM*min in Protocol HGB-207 Version 3.0 (19 June 2018) and in Protocol HGB-212 Version 2.0 (19 June 2018).
[5] Note: patients with SCD were not included in the efficacy analysis for this module or the interim CSR LTF-303. A final CSR for LTF-303 with data from all patients will be written after completion of the study

Methods of pooling data:

Pools are described as used in the SAP submitted with the MAA. Data for patients in long-term follow-up LTF-303 were integrated with their data from parent studies and presented by parent studies within the pools described below.

- For pooled PD analysis, the All Studies non-β^0/β^0 Pooling (HGB-204, HGB-205 TDT patients, HGB-207) was the primary analysis set. The PD analysis was also performed on the All Studies Pooling (HGB-204, HGB-205 TDT patients, HGB-207 and HGB-212 that includes all genotypes).
- For pooled efficacy analysis, the Phase 1/2 Studies non-β^0/β^0 Pooling (HGB-204, HGB-205 TDT patients) was the primary analysis set. The efficacy analysis was also performed on the Phase 1/2 Studies Pooling (HGB-204, HGB-205 TDT patients). Studies HGB-207 and HGB-212 were not included in the pooled efficacy analysis, because these studies were recently initiated and are currently ongoing, and therefore few patients were evaluable for efficacy endpoints.
- The All Studies non-β^0/β^0 Pooling (HGB-204, HGB-205 TDT patients, HGB-207) was the primary analysis set for correlation analysis. The correlation analysis was also performed on the All Studies Pooling (HGB-204, HGB-205 TDT patients, HGB-207 and HGB-212 that includes all genotypes).
- The Phase 1/2 Studies non-β^0/β^0 Pooling (HGB-204, HGB-205 TDT patients) were used to fit the linear regression model, the repeated measures mixed model, and the exact logistic regression model to predict relevant outcomes in HGB-207.

2.4.2. Pharmacokinetics

No pharmacokinetics studies were submitted (see discussion on clinical pharmacology).

Because of the nature of Zynteglo, conventional methods cannot be used for pharmacokinetic monitoring. No dedicated PK-PD studies have been conducted because the standard clinical pharmacology studies such as dose escalation/dose range finding, human absorption, metabolism and excretion, drug-drug interaction and special population studies are not considered feasible for gene therapy products.

2.4.3. Pharmacodynamics

Mechanism of action

After collection of mobilised peripheral blood samples, the patient's autologous cells, enriched for CD34+ HSCs, undergo *ex vivo* transduction with BB305 lentiviral vector (LVV) (one cycle of transduction) to produce Zynteglo finished product, which is then infused intravenously (IV) into the patient after myeloablative busulfan conditioning to prepare bone marrow (BM) "niches" for engraftment of the HSCs. Zynteglo finished product is intended for autologous use only, as a single IV administration (transplantation), to treat non-β^0/β^0 TDT.

The BB305 lentiviral vector (LVV), used for the transduction of autologous HSCs, is a replication-defective, self-inactivating (SIN), third-generation, human immunodeficiency virus type 1 (HIV-1)-based LVV, pseudotyped with the vesicular stomatitis virus glycoprotein G (VSV-G). The BB305 LVV encodes a single amino acid (aa)-modified human β^A-globin protein (β^A threonine [Thr; T] to glutamine [Gln; Q] at aa position 87 [$\beta^{A\text{-}T87Q}$-globin]) under the transcriptional control of the erythroid-specific human β-globin promoter and erythroid-specific enhancer elements (DNase I hypersensitive sites HS2, HS3, and HS4) of the human β-globin locus control region (LCR).

Zynteglo finished product addresses the underlying genetic cause of TDT by adding functional copies of the $\beta^{A\text{-}T87Q}$-globin gene. It provides a one-time treatment for patients with TDT, potentially eliminating the need for life-long supportive care with regular transfusions and iron chelation therapy. For TDT, production of the transgenic protein, $\beta^{A\text{-}T87Q}$-globin, provides/restores the synthesis of functional β-globin chains.

After engraftment and differentiation of the HSCs from Zynteglo finished product, the *in vivo* expression of the integrated $\beta^{A\text{-}T87Q}$-globin transgene in cells of the erythroid lineage allows the formation of HbA^{T87Q} (i.e., $\alpha_2\beta^{A\text{-}T87Q}{}_2$). Restoration of functional β-globin production should reduce the accumulation of excess uncomplexed α-globin in erythroblasts, thus preventing premature death of the cells, enhancing erythropoiesis, preventing haemolysis, increasing total Hb levels and greatly reducing or eliminating the need for transfusions.

Primary and Secondary pharmacology

Pharmacodynamic parameters were evaluated in both Phase 1/2 (HGB-204 and HGB-205) and Phase 3 (HGB-207 and HGB-212) studies, as well as in long-term follow-up Study LTF-303.

Analytical methods

The measurement of pharmacodynamics (PD) parameters that detect the presence of integrated proviral sequences and the expression of the transgene in differentiated cells can be used to determine successful delivery and persistence of the drug product. If the patient's HSCs are successfully transduced, they incorporate the $\beta^{A\text{-}T87Q}$-globin gene into their genome, and therefore their progeny contain the transgene.

Therefore, the presence of LVV sequences in differentiated nucleated peripheral blood cells indicates the presence of transduced cells amongst their HSC precursors. The presence of LVV sequences in the genomic DNA of differentiated cells is detected using quantitative polymerase chain reaction (qPCR) and is expressed as vector copy number (VCN) (VCN, i.e., vector copies per diploid genome, c/dg). Because VCN is determined on a heterogeneous population of cells, VCN is a composite value, dependent both on % cells transduced as well as number of copies per transduced cell. Although all nucleated cells derived from a successfully transduced HSCs will have LVV sequences in their genome and contribute to the VCN in peripheral blood, only cells within the erythroid lineage will produce the transcription factors required to drive expression of the $\beta^{A\text{-}T87Q}$-globin, because the transgene is under the control of the erythroid lineage specific globin LCR. Only cells of the erythroid lineage will express the transgenic $\beta^{A\text{-}T87Q}$-globin and subsequently contain HbA^{T87Q} that results from the combination of endogenous α-globin with the transgenic $\beta^{A\text{-}T87Q}$-globin. The presence of $\beta^{A\text{-}T87Q}$-globin is detected using RP-HPLC. The levels of $\beta^{A\text{-}T87Q}$-globin in blood were evaluated.

In clinical studies using Zynteglo, both VCN in drug product (DP VCN) and VCN in peripheral blood (PB VCN) of treated patients are reported. DP VCN is measured in genomic DNA from pooled colony-forming cells after 14 days of in vitro culture. PB VCN is measured in genomic DNA from peripheral blood cells (i.e., VCN of nucleated cells derived from HSCs after differentiation in vivo, within the treated patient).

RP-HPLC methods were developed and validated for relative quantification of globin chains; the methods were able to separate wildtype β^{A}-globin (β^{A}) from the transgene product $\beta^{A\text{-}T87Q}$-globin ($\beta^{A\text{-}T87Q}$). The relative levels of α-globin and of the β-like globins (% β-like globin chains), in conjunction with the determination of total Hb in another sample in the same visit window, allowed for the calculation of the proportion and quantity of the following Hb fractions that comprise total Hb (with associated β-like-globins involved): HbA^{T87Q} ($\beta^{A\text{-}T87Q}$), HbA (β^{A}), HbE (β^{E}; only for patients with the β^{E} mutation), HbA_2 (δ), and HbF (γA and γG).

Primary pharmacology

For pooled PD analysis, the All Studies non-β^0/β^0 Pooling (HGB-204, HGB-205 TDT patients, HGB-207) was the primary analysis set. The PD analysis was also performed on the All Studies Pooling (HGB-204, HGB-205 TDT patients, HGB-207 and HGB-212 that includes all genotypes).

All 4 studies had a similar study design. All studies were single arm studies enrolling patients with genetic defects in their β-globin gene (thalassaemia or sickle cell disease). All patients underwent haematopoietic stem cell collection (mobilisation with granulocyte colony-stimulating factor [G-CSF] and plerixafor followed by apheresis for patients with TDT), and myeloablative conditioning using busulfan before the administration of Zynteglo. During each parent study, busulfan concentrations were monitored and busulfan dosing adjusted to target recommended AUC to effectively remove endogenous untransduced HSCs.

After drug product administration via IV infusion, PB VCN was monitored at all scheduled visits to monitor engraftment of transduced HSCs. Total Hb also was measured, and HbA^{T87Q}, HbA, HbA_2, HbF, and HbE (as relevant) were calculated at all scheduled visits after drug product administration to monitor both transgene expression and expression of endogenous globins.

Study population

As of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies, a total of 33 patients ≥ 12 years of age with TDT have been treated, of these 24 patients having the non-β^0/β^0 genotype.

Across Studies HGB-204, HGB-205 and HGB-207, similar median cell doses were administered with an overall median (min, max) total cell dose administered to patients with TDT of all genotypes (all studies)

of 8.100 (5.00, 19.40)×10^6 CD34+ cells/kg, and to patients with TDT of non-β^0/β^0 genotype (all studies) was 7.950 (5.00, 19.40)×10^6 CD34+ cells/kg.

Myeloablation of the patients before drug product infusion is required to deplete endogenous HSCs, thus allowing repopulation of the patient with HSCs containing the transgene without dilution due to the presence of untransduced cells. Busulfan was used as a single agent. Busulfan's effectiveness in myeloablation is dependent on pharmacologic exposure, as measured by the AUC, and dosing was adjusted based on PK monitoring to meet the targeted AUC levels as defined in each protocol. Busulfan average daily AUC used during these studies: median (min, max) of 4337.0 (3030, 5789) uM*min in patients of non-β^0/β^0 genotype (in Studies HGB-204, HGB-205, and HGB-207; N=24).

Vector Copy Number (VCN) within Cell Samples

DP VCN data from Studies HGB-204, HGB-205, HGB-207 and (the long-term follow up study) LTF-303 provided the following values for patients of non-β^0/β^0 genotype: median (min, max) DP VCN was highest in Study HGB-207 (3.350 [2.40, 5.40] c/dg; N=10), followed by that in Study HGB-205 (1.300 [0.80, 2.10] c/dg; N=4) and lowest in Study HGB-204 (0.750 [0.30, 1.05] c/dg); N=10).

Study HGB-204 included both non-β^0/β^0 genotype and β^0/β^0 genotype patients. The DP VCN values were as follows: median (min, max) of 0.750 (0.30, 1.05) c/dg for patients of non-β^0/β^0 genotype (N=10) vs 0.650 (0.30, 1.50) c/dg for patients of β^0/β^0 genotype (N=8) for patients treated in Study HGB-204.

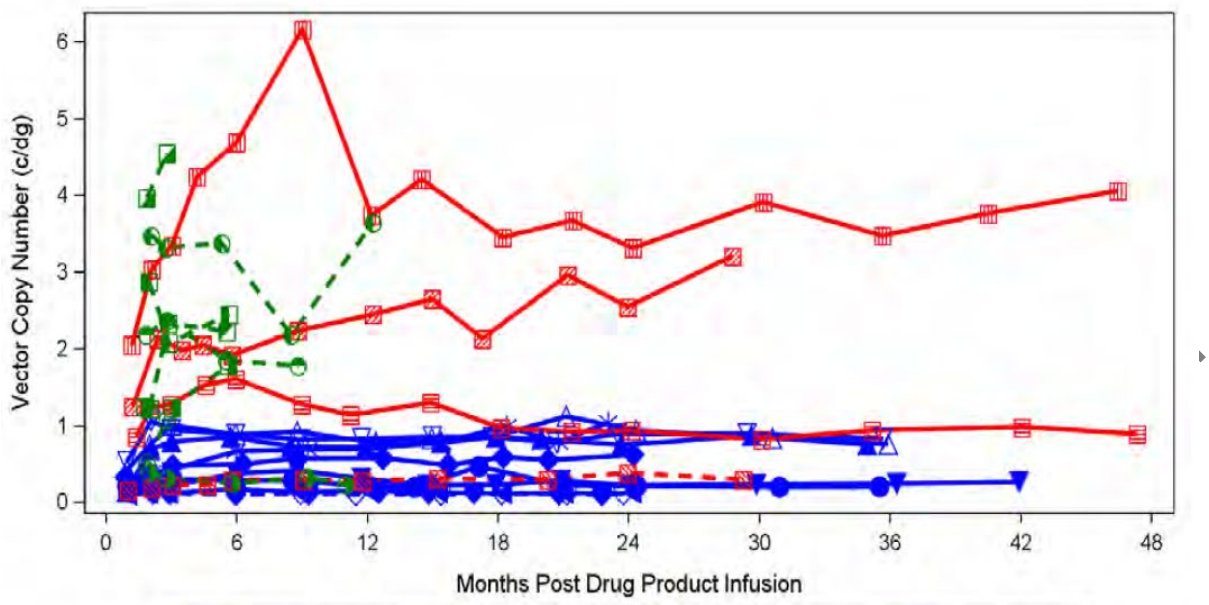
PB VCN was determined in peripheral blood of patients from Month 1 through Month 48 (Table 5 and Figure 2).

Table 5: PB VCN Summary (TP)

		Phase 1/2 Studies						Phase 3 Studies		All Studies	
Parameter	Statistic	HGB-204 Non-β^0/β^0	HGB-204 β^0/β^0	HGB-204 Overall	HGB-205 Non-β^0/β^0	Pooled Non-β^0/β^0	Pooled overall	HGB-207 Non-β^0/β^0	HGB-212 β^0/β^0	Overall Non-β^0/β^0	Overall
DP VCN (average/subject, c/dg)	N	10	8	18	4	14	22	10	1	24	33
	Mean (SD)	0.715 (0.2310)	0.756 (0.3755)	0.733 (0.2946)	1.375 (0.5620)	0.904 (0.4534)	0.850 (0.4237)	3.540 (0.8972)	3.100 (-)	2.002 (1.4812)	1.733 (1.3996)
	Median	0.750	0.650	0.700	1.300	0.800	0.800	3.350	3.100	1.300	1.000
	(Min, max)	0.30, 1.05	0.30, 1.50	0.30, 1.50	0.80, 2.10	0.30, 2.10	0.30, 2.10	2.40, 5.40	3.10, 3.10	0.30, 5.40	0.30, 5.40
PB VCN at Month 6 (c/dg)	N	10	8	18	4	14	22	6	0	20	28
	Mean (SD)	0.483 (0.3199)	0.396 (0.2584)	0.444 (0.2892)	2.127 (1.8488)	0.953 (1.2058)	0.750 (0.9988)	1.993 (1.0289)	-	1.265 (1.2298)	1.016 (1.1142)
	Median	0.440	0.333	0.356	1.765	0.583	0.391	2.043	-	0.867	0.518
	(Min, max)	0.10, 0.88	0.08, 0.95	0.08, 0.95	0.29, 4.69	0.10, 4.69	0.08, 4.69	0.25, 3.38	-	0.10, 4.69	0.08, 4.69
PB VCN at Month 24 (c/dg)	N	10	8	18	4	14	22	0	0	14	22
	Mean (SD)	0.501 (0.3852)	0.379 (0.2502)	0.447 (0.3289)	1.796 (1.3665)	0.871 (0.9498)	0.692 (0.7986)	-	-	0.871 (0.9498)	0.692 (0.7986)
	Median	0.422	0.312	0.312	1.743	0.685	0.405	-	-	0.685	0.405
	(Min, max)	0.10, 1.04	0.06 0.87	0.06, 1.04	0.38, 3.31	0.10, 3.31	0.06, 3.31	-	-	0.10, 3.31	0.06, 3.31

Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies

Figure 2: VCN Over Time in Peripheral Blood for Non-β⁰/β⁰, by Patient

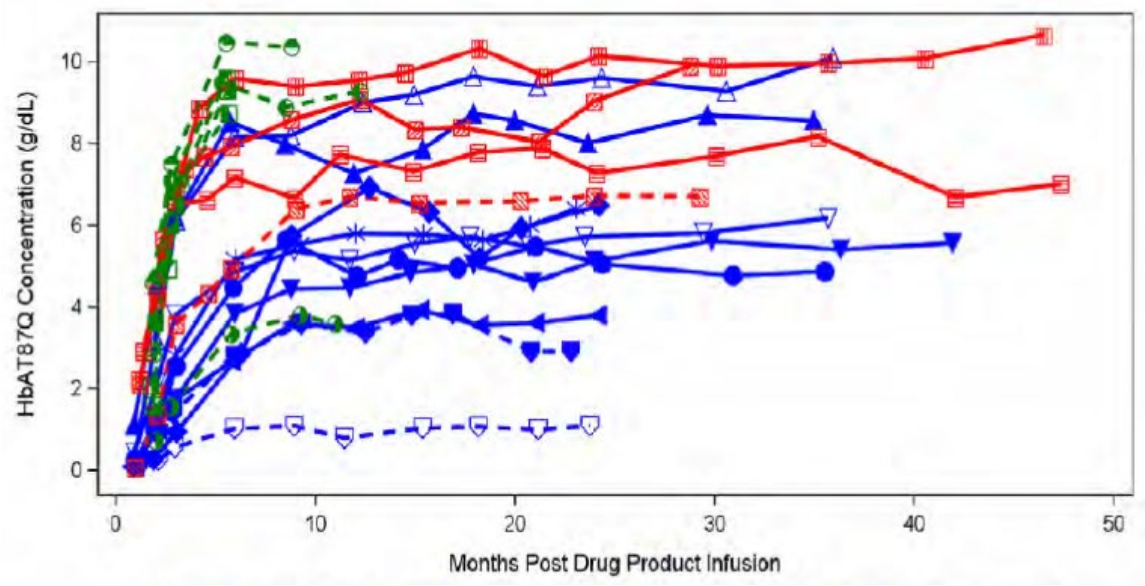


Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies
Note: Symbols indicate individual patients. Blue = patients from Study HGB-204, red = patients from Study HGB-205, and green = patients from Study HGB-207. Patients who achieved transfusion independence (TI) at any time are represented with solid lines, and patients who have not yet achieved TI are represented with dashed lines.

HbA^{T87Q} in Peripheral Blood

Median HbA^{T87Q} levels in the non-β⁰/β⁰ patients are shown in Figure 3 with parent study indicated by colour: red (HGB-205), blue (HGB-204), and green (HGB-207). The median (min, max) for HbA^{T87Q} at Month 6 for patients with non-β⁰/β⁰ genotype for Study HGB-204 was 4.153 (1.03, 8.52) g/dL, for Study HGB-205 was 7.543 (4.94, 9.59) g/dL, and for Study HGB-207 was 9.409 (3.35, 10.47) g/dL. Data indicate that HbA^{T87Q} levels generally increase from Month 1 through approximately Month 6 to Month 9, after which time they stabilise.

Figure 3: HbA^{T87Q} Over Time in Peripheral Blood for non-β⁰/β⁰, by Patient

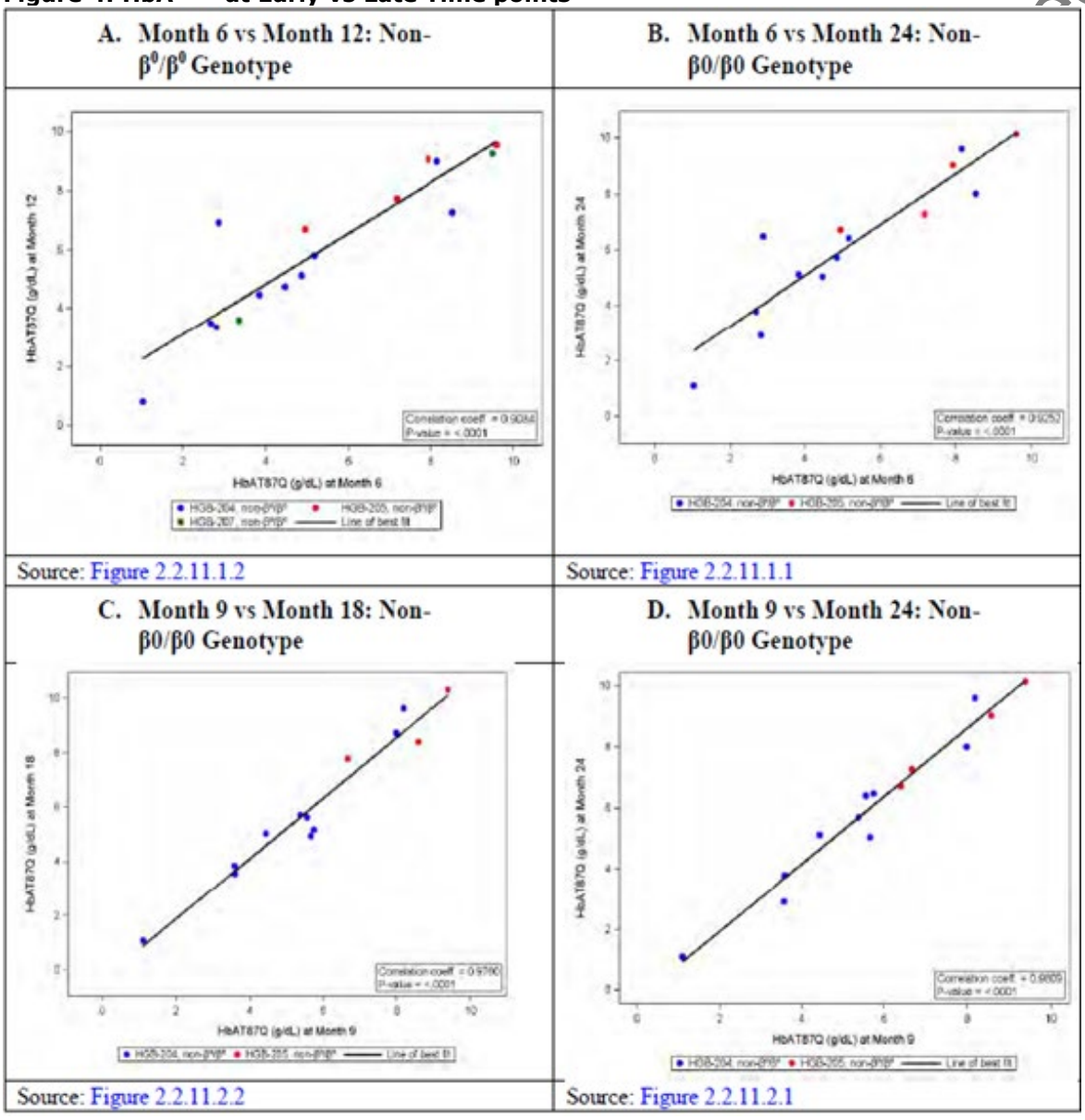


Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies
Note: Symbols indicate individual patients. Blue = patients from Study HGB-204, red = patients from Study HGB-205, and green = patients from Study HGB-207. Patients who achieved transfusion independence (TI) at any time are represented with solid lines, and patients who have not yet achieved TI are represented with dashed lines.

Relationship Between HbA^{T87Q} Levels at Early Time points (Months 6 or 9) vs HbA^{T87Q} Levels at Later Time points (Month 18 or 24)

Due to similarity of HbA^{T87Q} kinetics over time between patients, HbA^{T87Q} values at early time points tend to correlate with HbA^{T87Q} values at later time points. Using data on the non-β^0/β^0 genotype patients from the Phase 1/2 studies, strong correlations were observed between the HbA^{T87Q} value at Month 6 (r = 0.9252) or Month 9 (r = 0.9809) and HbA^{T87Q} measured at Month 24. This suggests that HbA^{T87Q} values measured at these two time points, particularly at Month 9, could be used to predict HbA^{T87Q} levels at Month 24 (see Figure 4).

Figure 4: HbA^{T87Q} at Early vs Late Time points



Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies

Hb Fractions Over Time

Hb fractions over time are presented in Figure 5 for transfusion-free patients of non-β^0/β^0 genotype, where transfusion-free is defined as not receiving a pRBC transfusion for a continuous period of at least 12 months. Endogenous HbE or HbA make important contributions to total Hb for patients of non-β^0/β^0

genotype who were able to be maintained transfusion-free in Studies HGB-204 and HGB-205. In these Phase 1/ 2 studies, at Month 24 for transfusion-free patients of non-β^0/β^0 genotype, median (min, max) HbA^{T87Q} was 6.600 (3.80, 10.13; N=12) g/dL, HbE was 2.900 (2.08, 4.78; N=8) g/dL, HbA was 2.551 (1.00, 6.44; N=4) g/dL, HbF was 0.384 (0.08, 1.37; N=12) g/dL, and HbA_2 was 0.328 (0.19, 0.56; N=12) g/dL.

Panel A shows that HbA^{T87Q} levels increase initially as patients begin to express the transgene and reach plateau levels around Month 6 to Month 9. In Phase 1/2 studies, median (min, max) HbA^{T87Q} was 6.600 (3.80, 10.13; N=12) g/dL at Month 24 for transfusion-free patients of non-β^0/β^0 genotype.

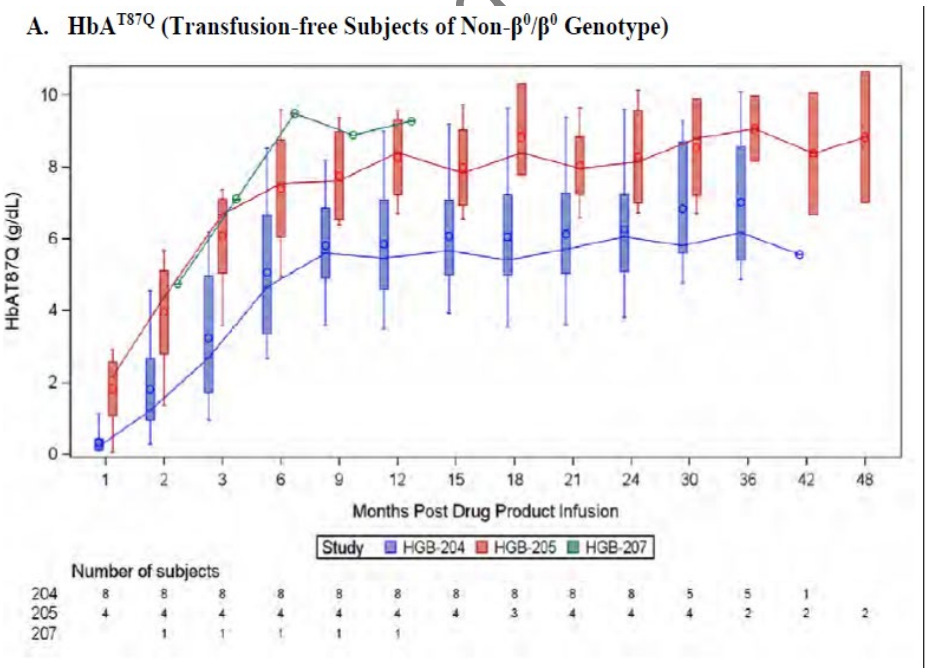
Panel B shows the kinetics of HbE in patients with at least one β^E allele. After drug product infusion, HbE kinetics paralleled that of HbA^{T87Q}, with endogenous levels of HbE, reaching plateau levels by Months 6 to 9. In Phase 1/2 studies, median (min, max) HbE was 2.900 (2.08, 4.78; N=8) g/dL at Month 24 for transfusion-free patients of non-β^0/β^0 genotype.

Panel C shows kinetics of HbA in patients with at least one β^+ allele. After drug product infusion, HbA levels initially decreased as patients stopped pRBC transfusions and exogenous red blood cells (RBC) were metabolized, and then plateau by Months 6 to 9, reflecting endogenous HbA production. In Phase 1/2 studies, median (min, max) HbA was 2.551 (1.00, 6.44; N=4) g/dL at Month 24 for transfusion-free patients of non-β^0/β^0 genotype.

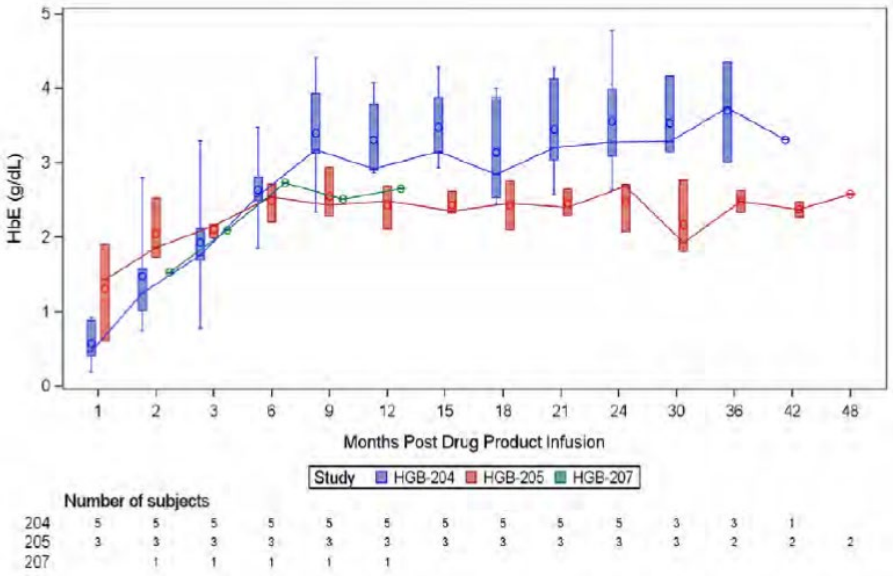
Panel D shows kinetics of HbF in all transfusion-free patients of non-β^0/β^0 genotype. HbF levels tended to show a peak around 2 to 3 months after drug product infusion. The variability of HbF (in Phase 1/2 studies, median (min, max) HbF was 0.384 (0.08, 1.37; N=12) g/dL at Month 24 for transfusion-free patients of non-β^0/β^0 genotype) reflected variation in the levels of HbF that patients stably expressed.

Panel E shows that in general, HbA_2 contributed relatively low amounts to the total Hb levels of transfusion-free patients. In Phase 1/2 studies, median (min, max) HbA_2 was 0.328 (0.19, 0.56; N=12) g/dL at Month 24 for transfusion-free patients of non-β^0/β^0 genotype.

Figure 5: Boxplot of Hb fractions over time among transfusion-free patients of Non-β^0/β^0 Genotype



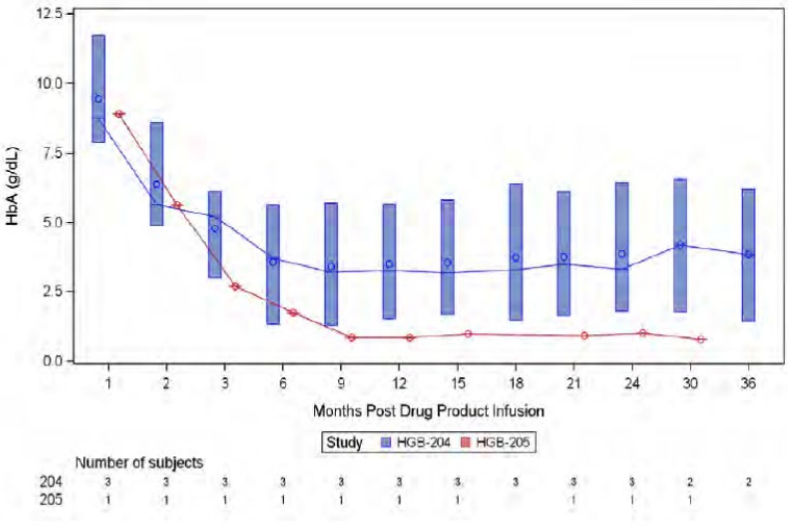
B. HbE (Transfusion-free Subjects with a β^E Mutation)



Number of subjects

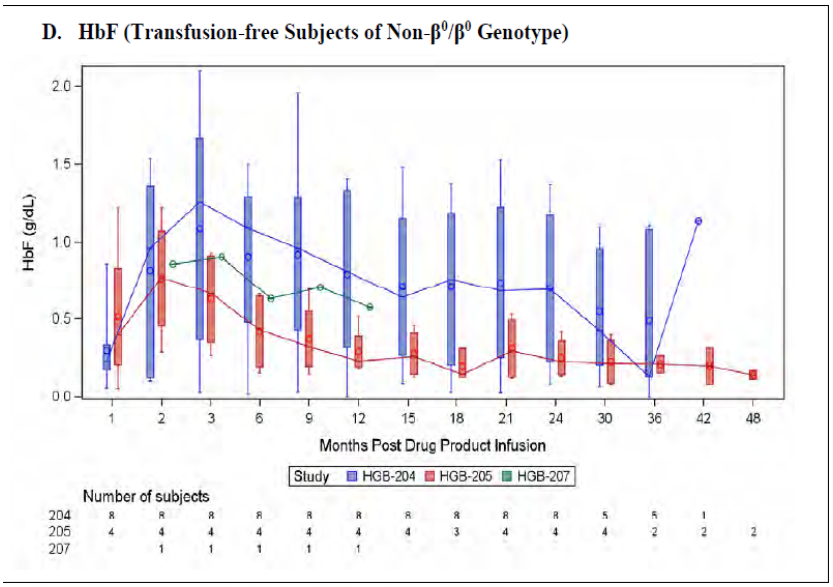
204	5	5	5	5	5	5	5	5	5	5	3	3	1	
205	3	3	3	3	3	3	3	3	3	3	3	2	2	2
207		1	1	1	1	1								

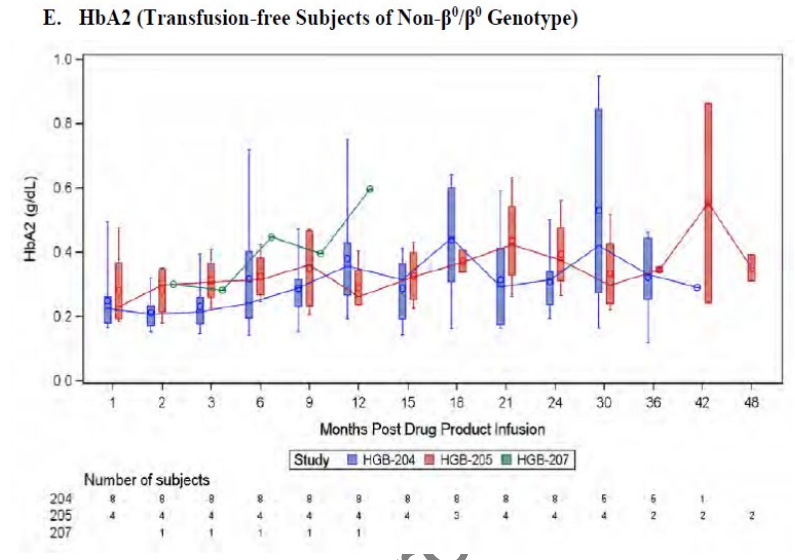
C. HbA (Transfusion-free Subjects With a β^+ Mutation])



Number of subjects

204	3	3	3	3	3	3	3	3	3	3	2	2
205	1	1	1	1	1	1	1		1	1	1	





Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies

Ratio of α-globin to β-like-globins

In study HGB-205: After becoming transfusion independent, all patients were able to maintain a stable α/β-like ratio close to 1, ranging from 0.86 in one patient through 1.26 in another patient at Month 24.

In Study HGB-204: After becoming transfusion independent, the patients were able to maintain a stable α/β-like ratio close to 1 with ratios ranging from 1.07 through 1.34 at Month 24.

Relationship between Dosing Characteristics and PD Parameters

In general, the highest HbA^{T87Q} after drug product infusion was reported for the patients with the highest DP VCN. Despite the existence of one patient in Study HGB-207 that had a much lower HbA^{T87Q} than DP VCN, results showed a correlation for patients of non-β^0/β^0 genotypes (p=0.0001; Table 6). Only limited data for Phase 3 studies conducted with the refined or commercial manufacturing process are available.

Table 6: Relationships between dosing characteristics and PD parameters (Pearson correlations)

X-axis (Independent)	Y-axis (Dependent)	Population	R value	p-value
DP VCN	PB VCN at M6	TP Non-β^0/β^0	R=0.6916 R=0.6424	p=<0.0001 p=0.0023
	HbA^{T87Q} at M6	TP Non-β^0/β^0	R=0.7520 R=0.7561	p=<0.0001 p=0.0001
	HbA^{T87Q} at M24	TP Non-β^0/β^0	R=0.6910 R=0.8182	p=0.0004 p=0.0003
Total cell dose	HbA^{T87Q} at M6	TP Non-β^0/β^0	R=-0.0692 R=-0.0435	p=0.7263 p=0.8557
	HbA^{T87Q} at M24	TP Non-β^0/β^0	R=0.2364 R=0.2385	p=0.2895 p=0.4116

Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies

Predictive Modelling

Development of Models

Given the strong correlation between HbA^{T87Q} at early and later time points, linear regression models can be used to predict HbA^{T87Q} at Month 12/24 or Month 18/24 for Study HGB-207 patients using their observed HbA^{T87Q} data at Month 6 or Month 9. To establish the linear relationship, data from the non-β^0/β^0 patients of Studies HGB-204 and HGB-205 were used (N=14) (Table 7).

Table 7: Fitted Univariate Linear Regression Models

Model	X	Y	Estimated Slope	95% CI of Slope	p-value
Model 1	HbA^{T87Q} at M6	HbA^{T87Q} at M12	0.86	(0.59, 1.14)	<0.0001
Model 2	HbA^{T87Q} at M6	HbA^{T87Q} at M24	0.91	(0.68, 1.15)	<0.0001
Model 3	HbA^{T87Q} at M9	HbA^{T87Q} at M18	1.11	(0.95, 1.28)	<0.0001
Model 4	HbA^{T87Q} at M9	HbA^{T87Q} at M24	1.11	(0.97, 1.25)	<0.0001

Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies

As a sensitivity analysis, the repeated measures mixed model was applied to assess HbA^{T87Q} expression post Month 6 and up to Month 24 (inclusive). Model 5 used HbA^{T87Q} at Month 6 as a predictor while including visits (Months 9, 12, 15, 18, 21, and 24) as a categorical covariate. The results of Model 5 were very similar to those of Model 1 and Model 2.

Use of Models to Predict HGB-207 Results

The fitted models were applied to predict HbA^{T87Q} at either Months 12, 18, or 24, based on Study HGB-207 patients with observed HbA^{T87Q} at Months 6 or 9 (Table 8). These models predict that 5/6 of these patients will be producing approximately 9 g/dL or greater of HbA^{T87Q} at Months 12, 18 and 24 in Study

HGB-207. Therefore, total Hb is expected to consistently reach levels such that chronic pRBC transfusions are no longer required (>9 g/dL) (Cappellini *et al.* 2014).

Table 8: Predicted HbA^{T87Q} Expression at Later Timepoints of Study HGB-207 Patients

Subject ID	Using observed values at Month 6				Using observed value at Month 9	
	Model 1: Predicted HbA^{T87Q} Expression (g/dL) at Month 12	Model 5: Predicted HbA^{T87Q} Expression (g/dL) at Month 12	Model 2: Predicted HbA^{T87Q} Expression (g/dL) at Month 24	Model 5: Predicted HbA^{T87Q} Expression (g/dL) at Month 24	Model 3: Predicted HbA^{T87Q} Expression (g/dL) at Month 18	Model 4: Predicted HbA^{T87Q} Expression (g/dL) at Month 24
	9.6326	9.6655	10.0715	9.9039	9.5516	9.5989
	4.3362	4.3139	4.4779	4.5523	3.8832	3.9344
	10.4762	10.5179	10.9626	10.7564	11.1859	11.232
	8.9738	8.9999	9.3758	9.2383	NA	NA
	9.4864	9.5178	9.9171	9.7562	NA	NA
	9.7357	9.7697	10.1805	10.0082	NA	NA

Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies

Despite the similar ranges of HbA^{T87Q} produced by patients with non-β^0/β^0 and β^0/β^0 genotypes, the observed clinical outcomes were different in these 2 groups in Phase 1/2 studies. Within Study HGB-204, only 1/8 (12.5%) patients of β^0/β^0 genotype was able to achieve TI by the end of the study, presumably because patients of β^0/β^0 genotype produce no endogenous β-globin. In contrast, the majority (8/10, 80%) of patients of non-β^0/β^0 genotype in Study HGB-204 were able to meet the criteria to establish TI, presumably because of the contributions of endogenous globins. Based on results presented Month 6/9 HbA^{T87Q} values are associated with HbA^{T87Q} values at Month 12/18/24. Thus Month 6/9 HbA^{T87Q} values were also used to predict the binary outcome of whether or not patients would achieve TI at Month 24, or at any time during the study.

Relationship of HbA^{T87Q} Production to Clinical Outcome Model Development

Exact logistic regression models were fitted to 14 non-β^0/β^0 patients from Studies HGB-204 and HGB-205 to evaluate the relationship between the probability of achieving TI at Month 24/any time and HbA^{T87Q} at Month 6/9. The HGB-204 and HGB-205 non-β^0/β^0 patients were pooled to increase sample size for a more accurate fit. Among the 14 patients, 11 patients achieved TI at any time/Month 24 and 3 patients did not achieve TI at any time/Month 24.

Model 6 established the relationship between probability of achieving TI at any time and the continuous HbA^{T87Q} (g/dL) value at Month 6. This suggested that the probability of achieving TI at any time and the HbA^{T87Q} (g/dL) at Month 6 were highly correlated. Model 7 showed a similar relationship between probability of achieving TI at any time and the HbA^{T87Q} (g/dL) at Month 9.

Table 9: Univariate Exact Logistic Regression Models: Achieving TI at Any Time and HbA^{T87Q} at Months 6 and 9

Model:	X	Y	Exact odds ratio	95% CI of Exact odds ratio	p-value
Model 6	HbA^{T87Q} at M6	Probability of TI at Any Time	2.01	(0.94, 6.92)	0.0824
Model 7	HbA^{T87Q} at M9	Probability of TI at Any Time	1.99	(0.96, 6.47)	0.0714

Subgroup analysis

Subgroup Analysis of PB VCN

Subgroup analyses for PB VCN were performed by age (12 to <18 vs ≥18 years of age), gender (male vs female), and race (Asian vs White; data shown in summary of Clin. Pharmacology). All subgroups showed similar kinetics, with median PB VCN values stabilizing by approximately Month 6.

Subgroup Analysis of HbA^{T87Q}

Subgroup analyses for HbA^{T87Q} were performed by age (12 to <18 vs ≥18 years of age;), gender (male vs female) and race (Asian vs White). All subgroups show similar kinetics, with HbA^{T87Q} values stabilizing by approximately 6 to 9 months. No meaningful differences were observed (data shown in summary of Clin. Pharmacology).

2.4.4. Updated data on PD parameters: 13 December 2018

An additional data cut was performed of the HGB-207, HGB-212, and LTF-303 clinical databases on 13 December 2018. As of 13 December 2018, a total of 42 patients ≥ 12 years of age with TDT have been treated, of these 32 patients having the non-β^0/β^0 genotype.

All patients were administered Zynteglo with a median (min, max) dose of 7.80×10^6 (5.0, 19.4) CD34+ cells/kg (N=32). Patients remained in the hospital for a median (min, max) of 44.0 (27, 92) days from conditioning through discharge (N=32).

Median (min, max) DP VCN was 3.400 (2.40, 5.60) c/dg (N = 15). Once established, PB VCN levels generally stabilise by Month 6; PB VCN at Month 6 was 2.451 (0.25, 5.41) c/dg for patients of non-β^0/β^0 genotype in Study HGB-207, (N = 11). For patients in all studies, both PB VCN and HbA^{T87Q} levels have been persistent through last follow-up (up to Month 60 as of 13 December 2018).

Table 10: VCN for studies HGB-204, HGB-205 and HGB-207

Parameter	Statistic	HGB-204 (N =10)	HGB-205 (N = 4)	HGB-207
DP VCN (c/dg)	Median (min, max)	0.750 (0.30, 1.05)	1.300 (0.800, 2.10)	3.400 (2.40, 5.60) N = 15
M6 PB VCN (c/dg)	Median (min, max)	0.440 (0.10, 0.88)	1.765 (0.29, 4.69)	2.451 (0.25, 5.41) N = 11
M6 HbA^{T87Q} (g/dL)	Median (min, max)	4.153 (1.03, 8.52)	7.543 (4.94, 9.59)	9.494 (3.35, 10.60) N = 11
M24 HbA^{T87Q} (g/dL)	Median (min, max)	5.418 (1.10, 9.60)	8.147 (6.72, 10.13)	Predicted range (4.4779, 11.0829)[1] N = 11

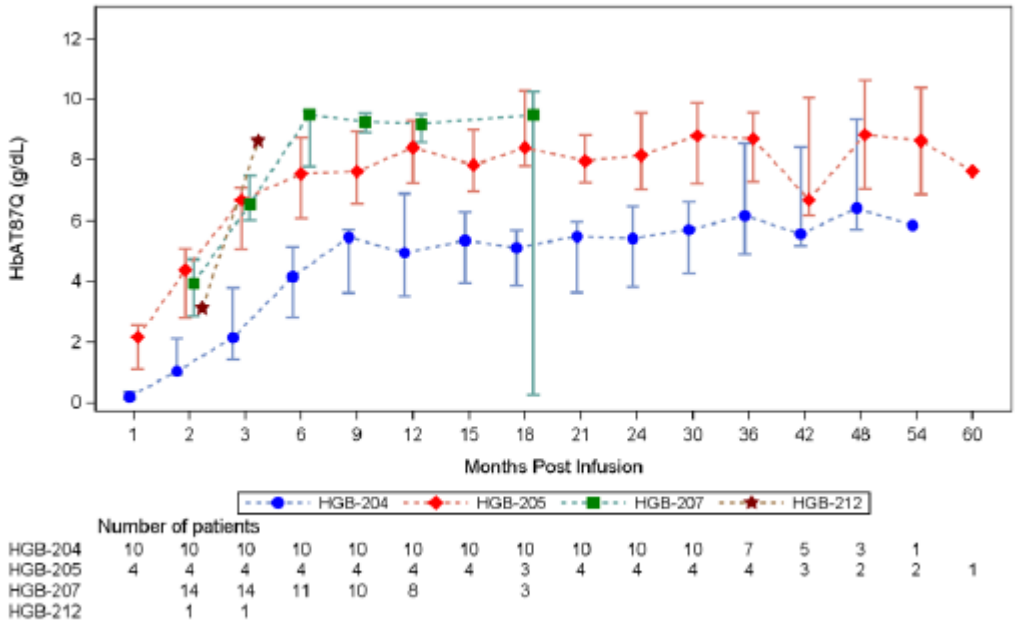
Data as of 13 December 2018

All patients with TDT with a non-β^0/β^0 genotype who received Zynteglo with at least 3 months of follow-up produced HbA^{T87Q} (N=10, HGB-204; N=4, HGB-205; N=14, HGB-207; N=1, HGB-212). For patients with at least 6 months of follow-up, HbA^{T87Q} generally increased steadily after Zynteglo infusion and stabilised by approximately Month 6 to 9 post infusion. Patients had a Month 6 median (min, max) HbA^{T87Q} of 4.90 (1.0, 9.6) g/dL in the Phase 1/2 studies (N=14, HGB-204 and HGB-205) and 9.49 (3.4, 10.6) g/dL in the ongoing Phase 3 study (N=11, HGB-207). Phase 3 studies are conducted with the refined or commercial manufacturing process, resulting in increased drug product vector copy number (DP VCN), PB VCN, and HbA^{T87Q} as compared to Phase 1/2 studies. With the increased HbA^{T87Q} levels in Phase 3 studies, endogenous haemoglobin (Hb) may not be as important a determinant of total Hb post

treatment, and patients may be able to increase total Hb to levels sufficient to achieve and maintain transfusion independence (TI) regardless of genotype and endogenous β-globin production.

HbA^{T87Q} remained generally stable through Month 24 with a median (min, max) of 6.44 (1.1, 10.1) g/dL (N=14, HGB-204 and HGB-205), and through long-term follow-up in LTF-303, demonstrating stable integration of the $\beta^{A\text{-}T87Q}$-globin gene into long-term HSCs and stable expression of the $\beta^{A\text{-}T87Q}$-globin gene in cells of the erythroid lineage (Figure 6).

Figure 6: Median HbA^{T87Q} over time for non-β^0/β^0 patients



Data as of 13 December 2018

Ratio of α-globin to β-like-globins: In patients in study HGB 207, with at least 6 months of follow-up, the median α-globin to β-like-globin chain ratio, as observed at last visit, was 1.07, range of 1.02 to 1.14, (N=11).

2.4.5. Discussion on clinical pharmacology

Because of the nature of Zynteglo, conventional methods cannot be used for pharmacokinetic monitoring. No dedicated PK-PD studies have been conducted because the standard clinical pharmacology studies such as dose escalation/dose range finding, human absorption, metabolism and excretion, drug-drug interaction and special population studies are not considered feasible for gene therapy products.

The presence of integrated viral sequences and the subsequent expression of the transgene are presented as pharmacodynamic (PD) parameters to determine the incorporation and persistence of the drug product. The presence of LVV sequences and vector copy numbers can be established in the drug product, bone marrow cells and peripheral blood cells (PB VCN). When HSCs are transduced with BB305 LVV, they incorporate $\beta^{A\text{-}T87Q}$-globin gene in their genome. The HbA^{T87Q} will be produced by all their progenitor cells that have differentiated into the erythroid lineage.

PD parameters have been presented from two finalised phase 1/2 clinical trials (HGB-204 and HGB-205) and 2 ongoing phase 3 clinical trials (HGB-207 and HGB-212). After drug product administration via IV infusion, PB VCN was monitored at all scheduled visits to monitor engraftment of transduced HSCs. For optimization of drug product, transduction, and transplantation procedures, PB VCN was a useful marker,

even though PB VCN is considered to be of limited relevance as clinical outcome. The ultimate goal of the therapy is to achieve adequate amounts of total haemoglobin (Hb) to obtain transfusion independence (primary efficacy endpoint), and for this purpose HbA^{T87Q} levels can be used as surrogate. HbA^{T87Q} production alone or supplemented with endogenous Hb (e.g. HbE, HbF, etc) is required to achieve levels of 9 g/dl or higher to meet the definition of transfusion independence.

In all studies, successful engraftment was achieved in all patients, and busulfan doses and total cell doses were sufficient to achieve effective haematopoietic reconstitution. A dose effect could not be investigated for Zynteglo due to the individual nature of the product. In Phase 1/2 clinical studies HGB-205 and HGB-204, the minimum recommended dose of Zynteglo was 3.0×10^6 CD34+ cells/kg; this was based on practice for autologous transplantation. The recommended cell dose is a single dose of drug product with $\geq 5.0 \times 10^6$ CD34+ cells/kg body weight. The doses administered in clinical studies to date are 5.0 to 19.4×10^6 CD34+ cells/kg. The minimum recommended dose is the same for adults and adolescents 12 years of age and older (see Summary of Product Characteristics [SmPC]). An upper dose limit has not been determined. As a matter of fact, the number of CD34+ cells administered to a patient is limited by the number of cells that could be collected from the patient. There is no maximum dose of CD34+ cells for infusion of autologous cell products prescribed in the literature.

All patients with TDT who received Zynteglo had LVV sequences in peripheral blood cells at their last visit. In all studies the VCN in peripheral blood was generally lower than in the DP, this is likely due to a dilution effect as busulfan only ablates HSCs and not mature white blood cells. PB VCN kinetics over time are similar between patients, showing a plateau at approximately 6 months after Zynteglo infusion followed by stable expression over time (a correlation coefficient of ≥0.9 between PB VCN at Month 24 versus PB VCN at Month 6). Stable values were maintained in the patient with the longest follow up of approximately 60 months after drug product infusion.

All patients who received Zynteglo produced Hb containing $\beta^{A\text{-}T87Q}$-globin (HbA^{T87Q}). Median HbA^{T87Q} levels increase from Month 1 through approximately Month 6 to Month 9, after which time they stabilize. Significant correlations ($p<0.0001$) were observed between HbA^{T87Q} at Month 6 or Month 9 and that at Month 24 (HGB-204 and HGB-205). The amount of HbA^{T87Q} produced was variable between patients. For β^0/β^0 patients in Phase 1/2 Study HGB-204, the within patient variability can be explained by dilution after periodic pRBC transfusions, on which the majority were still dependent. For non-β^0/β^0 patients, the variation in HbA^{T87Q} production between patients could be due to a variety of factors associated with drug product manufacturing and administration as well as between-patient variability.

Additional analyses indicate that there is a relationship between PB VCN and HbA^{T87Q} which appears to be linear at PB VCN levels below approximately 1.0 c/dg, but that above that, the curve flattens out, appearing to plateau at >2.0 c/dg ($R^2 = 0.8$, $p<0.0001$, $N = 34$). A similar relation is noted between DP VCN and HbA^{T87Q} at Month 6 ($R^2 = 0.6$, $p<0.0001$, $N = 34$). In general, patients with the highest DP %LVV+ cells had the highest PB VCN after drug product infusion and, as a consequence, a similar relationship with HbA^{T87Q} at Month 6 ($R^2 = 0.7$, $p<0.0001$).

There appears to be an inverse correlation between HbA^{T87Q} production and endogenous β-globin at Month 6 in patients not receiving pRBC transfusions.

Non-β^0/β^0 patients produce low amounts of endogenous HbA or HbE (depending on type of β-globin mutation), as well as HbA_2 and HbF. All endogenous Hb fractions (i.e., HbF, HbA_2, HbA or HbE as relevant) reached stable levels by approximately the same time as the transgenic HbA^{T87Q} (i.e., by approximately 6 to 9 months) in patients no longer receiving pRBC transfusions and were maintained at stable levels out to at least 24 months.

In HGB-204 and HGB-205, patients were reported with an α/β chain ratio of above 1 at Month 24, which in most cases was an increase from the ratio before transplantation. The synthesis of these chains is

very tightly coordinated so that the ratio of alpha globin to ß globin is normally 1 +/- 0.05. The clinical relevance of this observed imbalance in α/β chains and the increase from baseline is not known. There might be a ceiling of β-globin or total Hb within a cell at which mechanisms of metabolism or down-regulation exist that prevent β-globin accumulation. However, it is unclear whether dysregulation of these mechanisms of degradation of excess β-globin could occur.

Study HGB-207 is still ongoing with a median follow-up time of 5.59 (0.8, 13.2) months. In line with the results from the phase 1/2 studies, the preliminary data from study HGB-207 showed production of HbA^{T87Q}. A linear regression model was built based on studies HGB-204 and HGB-205 in order to predict levels of HbA^{T87Q} at Month 12/24 with data from Month 6. The Applicant performed multiple correlations providing a strong correlation on the kinetics of PB VCN and HbA^{T87Q} at different time points. The correlations and regression models suggested that Month 9 had a stronger relationship with Months 18 and 24 (r = 0.9760 and 0.9809, respectively) than Month 6 data have with Months 12 and 24 (r = 0.8929 and 0.9252, respectively); thus the models predicting outcomes based on Month 9 data are preferred. Logistic regression models were also fitted using data from the 14 non-β^0/β^0 patients from HGB-204 and HGB-205, in order to evaluate the relationship between the probability of achieving TI at Month 24/any time and HbA^{T87Q} at Month 6/9. However, the models have not been validated given the small patient sample. The prediction intervals were wide, which reflects the uncertain prediction in the models. Thus, no conclusion can be drawn in light of the model uncertainties.

During the clinical studies, the drug manufacturing process was changed for optimization of transgene expression. This change in manufacturing process in studies HGB-205, HGB-204, and HGB-207 had a clear influence on the median DP VCN achieved with the highest median DP VCN in HGB-207, consistent with improved transduction efficiency of the refined and commercial manufacturing processes. A relation between the DP VCN and PD parameters has been shown and thus this process change also influenced PB VCN and HbA^{T87Q} levels. In line with this, higher HbA^{T87Q} levels were obtained in patients treated in Studies HGB-207 and HGB-212, as compared to patients treated in Study HGB-204. Therefore, even though clinical experience with the commercial manufacturing process in Phase 3 studies is more limited, high rate of transfusion independence could be expected.

2.4.6. Conclusions on clinical pharmacology

The presented data show that Zynteglo administration at all tested doses resulted in successful engraftment in all patients with LVV sequences integrated into HSCs, resulting in transgene expression and subsequent $\beta^{A\text{-}T87Q}$-globin in treated non-β^0/β^0 patients. Transgene expression and $\beta^{A\text{-}T87Q}$-globin production plateaued at approximately 6 to 9 months after Zynteglo infusion, although long-term follow-up is limited. Despite some uncertainties identified with the regression model used to predict HbA^{T87Q} at Months 12/24 in study HGB-207, it confirmed that the results were in line with previous phase 1/2 studies conducted with the product.

The CHMP endorse the Committee for Advanced Therapies (CAT) assessment regarding the conclusions on the Clinical pharmacology as described above.

2.5. Clinical efficacy

2.5.1. Dose response studies

Phase 1/2 study HGB-205

Study HGB-205 was an "Open Label Study Evaluating the Safety and Efficacy of Gene Therapy of the β-Hemoglobinopathies (Sickle Cell Anemia and β-Thalassemia Major) by Transplantation of Autologous CD34+ Stem Cells Transduced *ex vivo* with a Lentiviral $\beta^{A\text{-}T87Q}$-Globin Vector" (Zynteglo finished product).

Drug product in this study was manufactured using the original manufacturing process.

Study Participants

Inclusion Criteria:

1. Between 5 and 35 years of age, inclusive.
    - Adult patients (between 18 and 35 years of age, inclusive, at the time of consent) must be able to provide written consent.
    - For paediatric patients (between 5 and 17 years of age, inclusive, at the time of consent), a competent parent or legal guardian must be able to provide written informed consent. When possible, involvement of the child >7 years of age in the decision is highly recommended, and written assent will be obtained and should be clearly documented.
    - Patients aged 5 to 14 years require the approval from the Comité de Surveillance prior to enrollment.
2. Have severe SCD or TDT, regardless of the genotype (e.g., β^0/β^0, β^+/β^+, β^E/β^0, β^S/β^S, β^S/β^0, β^S/β^+), with the diagnosis confirmed by Hb studies. Patients with TDT must be stable and maintained on an appropriate iron chelation regimen. Transfusion dependence is defined as requiring at least 100 mL/kg/year of packed RBCs.
3. Be eligible for allogeneic haematopoietic stem cell transplantation (allogeneic HSCT) based on institutional medical guidelines, but without a matched, related donor.
4. Be willing and able, in the Investigator's opinion, to comply with the study procedures outlined in the study protocol. If a paediatric patient, the patient's parent/legal guardian also must be willing and able to comply with the study procedures outlined in the study protocol.
5. Have been treated and followed for at least the past 2 years in a specialised centre that maintained detailed medical records, including transfusion history.

Exclusion Criteria

1. Availability of a willing, 10/10-matched HLA-identical sibling haematopoietic cell donor, unless recommendation for enrolment is provided by the Comité de Surveillance following a review of the case.
2. Positive for presence of human immunodeficiency virus type 1 or 2 (HIV-1 or HIV-2), human T-lymphotrophic virus-1 or -2 (HTLV-1 or HTLV-2), vesicular stomatitis virus glycoprotein (VSV-G) antibody (Ab).
3. Clinically significant, active bacterial, viral, fungal, or parasitic infection.
4. Contraindication to anaesthesia for bone marrow harvesting.

5. Any prior or current malignancy, myeloproliferative or immunodeficiency disorder.
6. A white blood cell (WBC) count $<3 \times 10^9$/L and/or platelet count $<120 \times 10^9$/L.
7. Receipt of an allogeneic transplant.
8. Receipt of erythropoietin within 3 months before hematopoietic stem cell transplantation (HSCT) harvest.
9. Immediate family member with a known or suspected Familial Cancer Syndrome (including but not limited to breast, colorectal, ovarian, prostate, and pancreatic cancers).
10. Diagnosis of significant psychiatric disorder of the patient that could seriously impede the ability to participate in the study.
11. Active relapsing malaria.
12. Pregnancy or breastfeeding in a postpartum female or absence of adequate contraception for fertile patients. Females of childbearing potential must agree to use a medically acceptable method of birth control such as oral contraceptive, intrauterine device, barrier and spermicide, or contraceptive implant/injection throughout the 27-month study period.
13. History of major organ damage including:
    - Liver disease, with transaminase levels >3× upper limit of normal. (This observation will not be exclusionary if a liver biopsy shows no evidence of extensive bridging fibrosis, cirrhosis, or acute hepatitis).
    - Histopathological evidence of extensive bridging fibrosis, cirrhosis, or acute hepatitis on liver biopsy.
    - Heart disease, with a left ventricular ejection fraction <25%.
    - Kidney disease with a calculated creatinine clearance <30% normal value.
    - Severe iron overload, which in the opinion of the physician is grounds for exclusion.
    - A cardiac T2* <10 ms by magnetic resonance imaging (MRI).
    - Evidence of clinically significant pulmonary hypertension requiring medical intervention.
14. Any other condition that would render the patient ineligible for HSCT, as determined by the attending transplant physician.
15. Participation in another clinical study with an investigational drug within 30 days of Screening.
16. Patients who have the desire to become a parent within the 27-month study period.
17. Prior receipt of gene therapy.
18. An assessment by the Investigator that the patient or parents of the patient will not comply with the study procedures outlined in the study protocol.
19. Hydroxyurea therapy within 3 months before haematopoietic stem cell collection.

Baseline data

Seven patients satisfied the eligibility criteria for this study: 4 adolescents with TDT and 3 adolescents/young adults with SCD were enrolled and treated.

All 4 patients with TDT enrolled in this study were of a non-β^0/β^0 genotypes. Three patients were compound heterozygous for the β^0 and β^E alleles (β^E/β^0 genotype). The remaining patient had a β^+/β^+

genotype (homozygous for the IVS I-110 G→A mutation, a β^+ mutation that consistently results in dramatically reduced β-globin production and is responsible for severe disease; Human Genome Variation Society [HGVS] nomenclature *HBB*:c.93-21G>A). Patients received a single intravenous dose of Zynteglo ranging from 8.79 × 10^6 to 13.6 × 10^6 CD34+ cells/kg. Drug product vector copy number (DP VCN) ranged from 0.8 to 2.1 c/dg.

Outcomes and estimation

Table 11: Summary of Key Efficacy Results

	Subjects who Achieved TI			Subject who did not achieve TI
Parameter	**1201**	**1202**	**1206**	**1203**
Achieved TI	Y	Y	Y	N
Reduction from baseline in pRBC transfusions between M6 to M24	100%	100%	100%	100%
Weighted average Hb (g/dL)	10.6 (during TI)	13.1 (during TI)	11.3 (during TI)	8.5 (during 6 months after DPI through end of study)
Decrease in Liver Iron Content (LIC) (Baseline vs M24)	Y	Y	Y	N
Myocardial T2*>20ms throughout study	Y	Y	Y	Y
Decrease in ferritin (Baseline vs M24)	Y	Y	N	Y
Stopped iron chelation/started phlebotomy	N	Y	Y	N
Normal or near normal serum transferrin receptor at M24	Y	Y	Y	N
Normal reticulocytes/ erythrocytes (%) at M24	Y	Y	N	N
Normal % nucleated RBCs (erythroblasts) at M24	N	Y	N	N

Abbrev.: DPI, drug product infusion; LIC, liver iron content; M, month; pRBC, packed red blood cell; TI, transfusion independence

Data as of 11 October 2017

Table 12: Change in pRBC Transfusion Requirements (TP)

	Baseline[1]				6 Months (183 days post-infusion) through Month 24 Visit						
					Annualized pRBC Volume			Annualized Number of Transfusions			
Subject	**Annualized Volume (mL/kg/year)**	**Annualized Number of Transfusions**	**Weighted Average Nadir[2] (g/dL)**	**Trigger Hb[3] (g/dL)**	**Volume (mL/kg/ year)**	**Change from BL (mL/kg/ year)**	**Percent Change from BL**	**Number**	**Change from BL**	**Percent Change from BL**	**Weighted Average (g/dL)**
	138.8	10.5	8.2	8.2	0.0	-138.8	-100.0	0.0	-10.5	-100.0	10.6
	187.7	12.0	10.6	9.5	0.0	-187.7	-100.0	0.0	-12.0	-100.0	13.2
	176.0	13.0	8.1	8.7	0.0	-176.0	-100.0	0.0	-13.0	-100.0	8.5
	197.3	13.0	10.8	9.5	0.0	-197.3	-100.0	0.0	-13.0	-100.0	11.4

Source: Table 14.2.4

Abbrev.: BL, baseline; Hb, hemoglobin; pRBC, packed red blood cell

[1] 2-year period prior to enrollment

[2] Weighted average Hb nadir is defined as an average area under the curve where the Hb closest but within 3 days prior to a transfusion is used as the Hb nadir. Hb values on the day of the transfusion will be considered for nadir calculations.

[3] Hb value that triggered a pRBC transfusion pre-enrollment, as reported by treating physician

Data as of 11 October 2017

Three of the 4 patients with TDT in this study achieved TI. Of the 4 TDT patients, 3 patients received their last pRBC transfusion within 13 days after Zynteglo infusion and all 3 patients remained transfusion-free through Month 24. These 3 patients maintained a total Hb ≥9.0 g/dL from Month 1 through end of the study at Month 24.

One patient who was homozygous for IVS-I-110 mutation was not able to maintain a total Hb ≥9.0 g/dL in the absence of pRBC transfusions and received last pRBC transfusion on Day 94 when total Hb dropped to 7.4 g/dL. The weighted average Hb between 6 months after drug product infusion and last study visit was 8.5 g/dL, which was above his average pre-enrolment pre-transfusion nadir of 8.1 g/dL. His HbA^{T87Q} levels increased slowly and stabilized by Month 9 at 6.40 g/dL with a total Hb of 8.2 g/dL, and he remained transfusion free through Month 24.

HbA^{T87Q} concentration at Month 24 for the 3 patients who achieved TI ranged from 7.27 g/dL to 10.13 g/dL. These 3 patients all had the genotype β^E/β^0 and therefore also were producing ≥2.0 g/dL of HbE.

TI status was maintained stably through the end of the study, up to a duration of approximately 22 months, with a weighted average Hb during the period of TI for 3 patients ranging from 10.6 to 13.1 g/dL

The 3 patients who achieved TI had a decrease in liver iron content (LIC) during the study, and 2 of the 3 patients discontinued iron chelation therapy and started phlebotomy. The patient who did not achieve TI did not show an improvement in LIC and remained on iron chelation therapy.

All patients had normal cardiac iron content (MRI results: myocardial T2* >20 ms) at baseline and throughout the study.

The 3 patients who achieved TI had near normal levels of serum transferrin receptor, and 2 of these 3 patients had normal levels of reticulocytes, whereas the patient who did not achieve TI had higher than normal levels of both reticulocytes and serum transferrin receptor. One patient who achieved TI had no nucleated RBCs (erythroblasts) in peripheral blood, whereas erythroblasts were still detected in the peripheral blood of the other 3 patients.

Phase 1/2 study HGB 204

This was a single-arm, multi-site, single dose, Phase 1/2 study in which patients with TDT were treated with Zynteglo finished product. TDT was defined as patients who received at least 100 mL/kg/year of pRBCs or ≥8 transfusions of pRBCs per year in each of the 2 years prior to enrolment. Treatment was staggered with a second patient beginning myeloablative conditioning only after the first patient 1) engrafted (defined as 3 consecutive absolute neutrophil count [ANC] laboratory values $\geq 0.5 \times 10^9$/L obtained on different days); and 2) had no Zynteglo finished product treatment-related serious adverse event (SAE) unexpected to occur with HSCT.

Eighteen patients (15 adults and 3 adolescents) were treated with a single dose of Zynteglo finished product; 10 patients had a non-β^0/β^0 genotype and 8 patients had a β^0/β^0 genotype. The base manufacturing process used in Study HGB-204 was similar to that used in Study HGB-205 but was optimized for multi-site clinical use.

Inclusion Criteria:

1. Patients between 12 and 35 years of age, inclusive, at the time of consent or assent (as applicable), and able to provide written consent (adults, or legal guardians, as applicable) or assent (adolescents).
2. Diagnosis of β-thalassemia major and a history of at least 100 mL/kg/year of pRBCs or ≥8 transfusions of pRBCs per year for the prior 2 years.
3. Documented baseline, or pretransfusion, Hb level of ≤7 g/dL.
4. Clinically stable, have a Karnofsky performance status of ≥60, and eligible to undergo HSCT.
5. Treated and followed for at least the past 2 years in a specialized centre that maintained detailed medical records, including transfusion history.

Exclusion Criteria:

1. Positive for presence of human immunodeficiency virus type 1 or 2 (HIV-1 or HIV-2), hepatitis B virus (HBV), or hepatitis C virus (HCV). (Note that patients who are positive for anti-HBV antibody [to either core or envelope proteins] or for anti-HCV antibody are eligible as long as they have a negative HBV or HCV viral load by qPCR. Where clinically and/or regionally indicated, one or more of the following tests may be performed, in which case positive results would exclude the patient from participating: human T-lymphotrophic virus-1 (HTLV-1) or HTLV-2, syphilis (rapid plasma reagin [RPR]), toxoplasmosis, Trypanosoma cruzi, or West Nile Virus).
2. Active bacterial, viral, fungal, or parasitic infection.
3. A white blood cell (WBC) count $< 3 \times 10^9$/L, and / or platelet count $< 100 \times 10^9$/L not related to hypersplenism.
4. Uncorrected bleeding disorder.
5. Any prior or current malignancy or myeloproliferative or immunodeficiency disorder.
6. Immediate family member with a known or suspected Familial Cancer Syndrome (including but not limited to hereditary breast and ovarian cancer syndrome, hereditary non-polyposis colorectal cancer syndrome and familial adenomatous polyposis).
7. Prior HSCT.
8. Advanced liver disease, defined as:

a. Baseline alanine aminotransferase (ALT) or direct bilirubin value >3 × the upper limit of normal (ULN), or

b. Liver biopsy demonstrating cirrhosis, any evidence of bridging fibrosis, or active hepatitis.

9. Baseline estimated glomerular filtration rate (GFR) <70 mL/min/1.73 m^2, as determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation for ≥ 18 years of age, and Bedside Schwartz equation calculator <18 years of age (see http://www.kidney.org/professionals/kdoqi/gfr_calculator.cfm).
10. Uncontrolled seizure disorder.
11. Diffusion capacity of carbon monoxide (DLco) <50% of predicted (corrected for Hb and/or alveolar volume, as clinically indicated).
12. A cardiac T2* <10 ms by MRI
13. Any other evidence of severe iron overload that, in the investigator' s opinion, warrants exclusion.
14. Clinically significant pulmonary hypertension, as defined by the requirement for ongoing pharmacologic treatment or the consistent or intermittent use of supplemental home oxygen.
15. Participation in another clinical study with an investigational drug within 30 days of Screening.
16. Failure to obtain appropriate informed consent.
17. Any other condition that would render the patient ineligible for HSCT, as determined by the attending transplant physician or investigator.
18. Contraindications to the conditioning regimen.
19. Prior receipt of gene therapy.
20. Diagnosis of significant psychiatric disorder of the patient that could seriously impede the ability to participate in the study.
21. Pregnancy or breastfeeding in a postpartum female or absence of adequate contraception for fertile patients. Females of child-bearing potential are required to use effective contraception from Screening through at least 6 months after Zynteglo finished product infusion. Male patients are required to use effective contraception (including condoms) from Screening through at least 6 months after Zynteglo finished product infusion.
22. An assessment by the investigator that the patient would not comply with the study procedures outlined in the protocol.

Patient Withdrawal

Patients had the right to withdraw from the study at any time for any reason. After giving informed consent or assent (if applicable), patients could withdraw or be withdrawn from study-related procedures and treatments (e.g., mobilization/apheresis, busulfan conditioning) under the following conditions:

- withdrawal of consent or assent (if applicable),
- the patient was unable to comply with protocol-defined visits or other requirements of the protocol,
- any medical condition which, in the opinion of the investigators, put the patient at risk for continuing treatment or follow-up studies, or
- adequate cells were not collected during harvests, or failure of transduced cells to be

dispositioned for clinical use.

In addition, after patients were treated with Zynteglo finished product, they could have been withdrawn from the study under the following additional condition: the patient had undetectable VCN (<0.0003 c/dg) in peripheral blood cells for 2 consecutive measurements at least 1 month apart and at least 12 months after Zynteglo finished product infusion.

Blood Transfusions

Within the period of 30 days prior to and during mobilization and apheresis, the patient's transfusion regimen was adjusted to maintain a minimum of 10 g/dL of Hb to suppress dyserythropoiesis, which can impede the isolation of CD34+ cells-enriched for undifferentiated HSC.

During follow-up, the goal was to maintain a target Hb ≥9 g/dL. In general, transfusions were to be avoided for Hb ≥9 g/dL, unless the need was medically justified (e.g., as a pre-requirement for surgery). It was recommended that patients should receive pRBC transfusions for any Hb <7.0 g/dL, and for clinically symptomatic anaemia, irrespective of Hb level.

Efficacy Variables

The following assessments were performed to determine the efficacy of Zynteglo finished product:

- pRBC transfusion requirements (mL/kg/year and number of transfusions/year)
- Hb nadirs, total Hb (and Hb fractions)

In addition, exploratory analyses of the following parameters were performed, as available data allowed:

- Assessment of stress erythropoiesis/dyserythropoiesis (reticulocytes and nucleated RBCs in blood, soluble transferrin receptor in blood, and qualitative assessment of bone marrow, as available)
- Iron burden (LIC by MRI or SQUID; cardiac T2* on MRI; clinical chemistry laboratory iron studies)
- Iron chelator use and phlebotomy
- Health Related Quality of Life (HRQoL) questionnaires (these questionnaires were introduced late in the study, such that most patients lacked baseline responses)
- Days and number of in-patient hospitalisations (annualised, post-discharge)

Table 13: Disposition by Genotype (ITT)

Parameter	Statistic	Non-β^0/β^0 (N=11)	β^0/β^0 (N=8)	Overall (N=19)
Subjects Mobilized	n (%)	11 (100.0)	8 (100.0)	19 (100.0)
Subjects Infused with Drug Product	n (%)	10 (90.9)	8 (100.0)	18 (94.7)
Subjects with Successful Engraftment [a]	n (%)	10 (90.9)	8 (100.0)	18 (94.7)
Subjects Completing the Study through their Month 24 Visit	n (%)	10 (90.9)	8 (100.0)	18 (94.7)
Subjects who Discontinued from Study	n (%)	1 (9.1)	0	1 (5.3)
Reasons for Study Discontinuation:				
Adverse Event	n (%)	0	0	0
Investigator Decision	n (%)	1 (9.1) [b]	0	1 (5.3) [b]

Source: Table 14.1.1.2; Listing 16.2.1.1

[a] Defined as ANC ≥0.5 × 10^9/L for 3 consecutive days post-LentiGlobin BB305 Drug Product infusion or ANC ≥0.5 × 10^9/L for 3 consecutive measurements done on separate days.

[b] Discontinued from the study due to inadequate stem cell mobilization (Listing 16.2.1.1)

Data as of 07 March 2018

Table 14: Summary of key demographic and medical history parameters, including transfusion history for the 2 years prior to study enrolment (TP, by Genotype)

Parameter	Statistic	Non-β^0/β^0 (N=10)	β^0/β^0 (N=8)	Overall (N=18)
Spleen Present				
Yes	n (%)	7 (70.0)	5 (62.5)	12 (66.7)
No	n (%)	3 (30.0)	3 (37.5)	6 (33.3)
Medical History in 2 Years Prior to Study Enrollment				
pRBC (mL/kg/year)	Median (min, max)	151.28 (140.0, 234.5)	182.59 (124.4, 273.2)	169.05 (124.4, 273.2)
pRBC (number transfusions/year)	Median (min, max)	13.75 (10.0, 16.5)	13.75 (12.5, 17.5)	13.75 (10.0, 17.5)
Weighted mean Hb nadir (g/dL)	Median (min, max)	9.1 (7.0, 9.8)	9.4 (8.7, 10.1)	9.31 (7.0, 10.1)
Hb trigger (g/dL)	Median (min, max)	7.0 (7.0, 9.0) N=5 [b]	9.0 (7.0, 9.0) N=3 [b]	8.0 (7.0, 9.0) N=8 [b]
Hospitalization, 2 years prior to enrollment (days/year)	n (%)	0	0	0
Hospitalization, 2 years prior to enrollment (visits/year)	n (%)	0	0	0
β-Thalassemia Major Diagnosis (months)	Median (min, max)	69.5 (0, 315)	4.5 (0, 92)	7.5 (0, 315)
1st pRBC transfusion (months)	Median (min, max)	30.0 (0, 132)	6.0 (2, 12)	10.0 (0, 132)
Estab. regular pRBC transfusion regimen (months)	Median (min, max)	72.0 (8, 312)	7.0 (2, 84)	42.0 (2, 312)
Start iron chelation (years)	Median (min, max)	7.5 (2, 26)	3.5 (2, 18)	6.5 (2, 26)

Source: Tables 14.1.2.2, 14.1.3.2, 14.1.4.2, Listing 16.2.6.14
[a] Age of consent applicable for adults; age of assent applicable for subjects <18 years old
[b] When data for all subjects is not available, N is given

Data as of 07 March 2018.

Outcomes and estimation

Eighteen patients with TDT were treated with Zynteglo finished product and all completed the study. All patients had successful neutrophil and platelet engraftment (PE).

Primary Efficacy Endpoint (Per Protocol): Proportion of Patients with Sustained Production of ≥2.0 g/dL of HbA^{T87Q} Between Month 18 and Month 24.

16/18 patients (88.9%; lower 1-sided 95% confidence interval of 69.0%) treated with Zynteglo finished product were producing ≥2.0 g/dL of HbA^{T87Q} at their Month 18 Visit, and all of these patients maintained this production through Month 24. The 2 patients producing less than 2.0 g/dL HbA^{T87Q} at their Month 18

and 24 Visit were both treated with Zynteglo finished product with low DP VCN (0.3 c/dg and 0.45 c/dg, respectively), and their subsequent low PB VCN resulted in the low HbA^{T87Q} production.

Table 15: Proportion of patients with sustained production of ≥2.0 g/dL of HbA^{T87Q} between Month 18 and Month 24 (TP)

Parameter	Statistic	non-β^0/β^0 (N=10)	β^0/β^0 (N=8)	Overall (N=18)
Subjects with ≥2.0 g/dL of HbA^{T87Q} at Month 18 [a]	n (%) Lower 1-sided 95% CI [b]	9 (90.0%) 60.6	7 (87.5%) 52.9	16 (88.9%) 69.0
Subjects with ≥2.0 g/dL of HbA^{T87Q} at Month 24 [c]	n (%) Lower 1-sided 95% CI [b]	9 (90.0%) 60.6	7 (87.5%) 52.9	16 (88.9%) 69.0
Subjects with sustained production of ≥2.0 g/dL of HbA^{T87Q} for the 6 months between Month 18 and Month 24 [c]	n (%) Lower 1-sided 95% CI [b]	9 (90.0%) 60.6	7 (87.5%) 52.9	16 (88.9%) 69.0

Source: Table 14.2.3
[a] Denominator is based on N=18 subjects reaching Month 18 visit or discontinued
[b] The Clopper-Pearson Exact method was used to calculate the lower 1-sided 95% CI for the proportion of subjects meeting this criterion.
[c] Denominator is based on N=18 subjects

Data as of 07 March 2018

Proportion of Patients Who Achieved Transfusion Independence

Of the 8 patients of non-β^0/β^0 genotype who achieved TI after infusion with Zynteglo finished product, all 8 remained TI at Month 24. Their last pRBC transfusion before becoming TI was at median (min, max) of 2.00 (0.3, 5.8) months after Zynteglo finished product infusion, and their TI status was maintained with a median (min, max) weighted average Hb of 9.99 (9.3, 12.8) g/dL. The individual weighted average Hb for patients after they achieved TI were above their weighted nadirs before study entry when they were receiving pRBC transfusions, with one exception: 1 patient who had an average weighted nadir of 9.7 g/dL before treatment and 9.3 g/dL during TI. TI patients of non-β^0/β^0 genotype at Month 24 produced between 3.80 to 9.60 g/dL of HbA^{T87Q}, with the remainder of their total Hb made of up other endogenous Hb fractions, primarily by HbE (2.64 to 4.78 g/dL) in patients with a β^E allele or HbA (1.80 to 6.44 g/dL) in patients with a β^+ allele. Patients of non-β^0/β^0 genotype who did not achieve TI were those that produced the lowest HbA^{T87Q} among non-β^0/β^0 patients, 2.92 g/dL and 1.10 g/dL, respectively at Month 24.

Table 16: Transfusion Independence, by Genotype (TP)

Parameter	Statistic	Non-β^0/β^0 (N=10)	β^0/β^0 (N=8)	Overall (N=18)
TI at any time [a]	n (%)	8 (80.0)	1 (12.5)	9 (50.0)
	Lower 1-sided 95% CI	49.3	0.6	29.1
Subjects with TI at 24 Months	n (%)	8 (80.0)	0	8 (44.4)
Subjects with TI, sensitivity failure analysis [b]	n (%)	8 (80.0)	0	8 (44.4)
Duration of TI (months) *(TI at any time subjects only)*	N	8	1	9
	Mean (SD)	18.78 (2.587)	16.13 (--)	18.49 (2.576)
	Median	18.91	16.13	17.28
	Min, Max	15.2, 21.4	16.1, 16.1	15.2, 21.4
Time from Drug Product infusion to Last pRBC Transfusion before becoming TI (months) *(TI at any time subjects only)*	N	8	1	9
	Mean (SD)	2.33 (2.098)	1.81 (--)	2.27 (1.970)
	Median	2.00	1.81	1.81
	Min, Max	0.3, 5.8	1.8, 1.8	0.3, 5.8
Weighted average Hb during TI (g/dL) *(TI at any time subjects only)*	N	8	1	9
	Mean (SD)	10.44 (1.277)	10.11 (--)	10.41 (1.200)
	Median	9.99	10.11	10.11
	Min, Max	9.3, 12.8	10.1, 10.1	9.3, 12.8

Source: Table 14.2.5.1

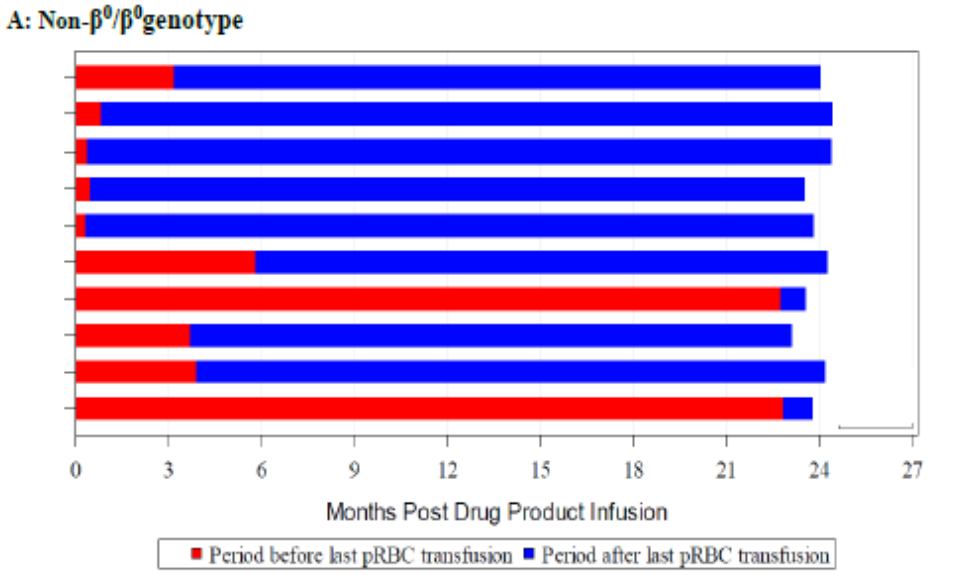
[a] Transfusion independence (TI) is defined as a weighted average Hb ≥9 g/dL without any pRBC transfusions for a continuous period of ≥12 months at any time during the study after LentiGlobin BB305 Drug Product infusion. Time period of TI will start when subjects achieve an Hb ≥9 g/dL with no transfusions in the preceding 60 days.

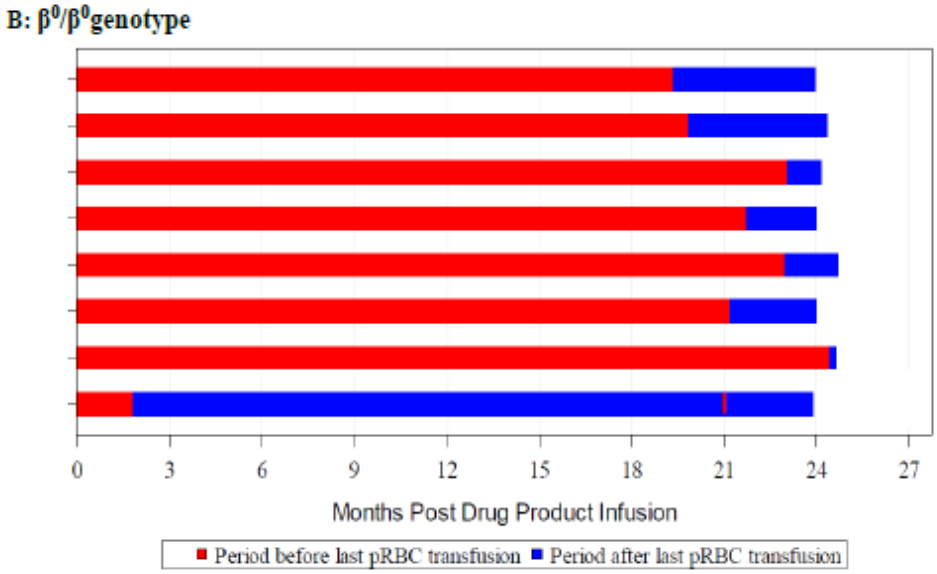
[b] Subjects who achieved TI and then discontinued early or received any pRBC transfusions are considered failures

Data as of 07 March 2018

The median (min, max) time between Zynteglo finished product infusion and last pRBC transfusion for all patients was 12.58 (0.3, 24.4) months, with a median (min, max) duration of not receiving pRBC transfusions at their last visit of 11.52 (0.2, 24.0) months. The wide range in these values reflect the fact that some patients no longer required pRBC transfusions after only a few weeks after drug product infusion, whereas other patients required regular transfusions throughout the study.

Figure 7: Duration of Transfusion Periods, by Genotype (TP)





Source: Figure 14.2.5.2
Note: , the red bar is the period before the last pRBC transfusion prior to achieving transfusion independence; the vertical line within the blue bar indicates a single pRBC transfusion for an acute event (Cat Scratch Disease) that occurred after achieving transfusion independence.

Data as of 07 March 2018

Transfusion Reduction

Transfusion requirements for all patients (β^0/β^0 and non-β^0/β^0) for the following time periods were also compared, using the 2-year period prior to study enrolment versus the period starting from 6 months after infusion with Zynteglo through to Month 24.

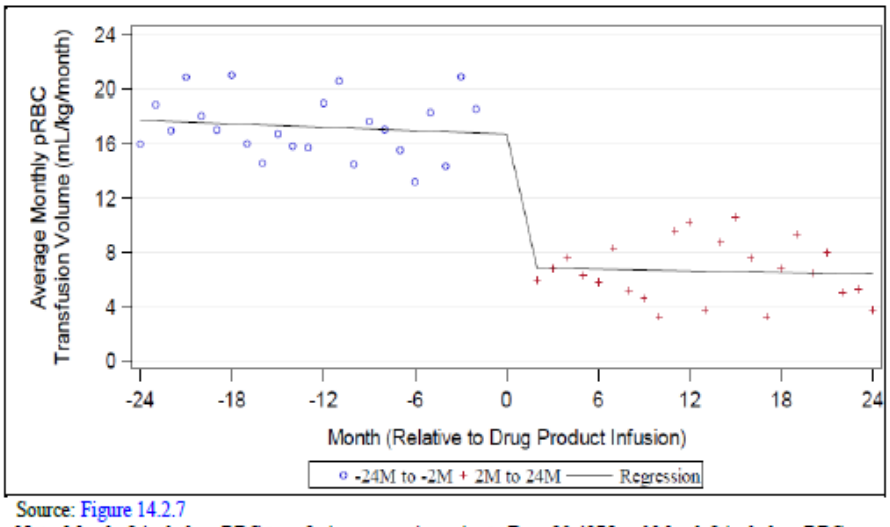
- 14/18 (77.8%, lower 1-sided 95% confidence interval of 56.1%) patients had at least a 60% reduction in transfusion requirements during the period from 6 months through approximately 24 months after Zynteglo finished product infusion, compared with their Baseline pre-treatment transfusion levels, while maintaining Hb nadirs within 1 g/dL of their pretreatment nadirs.

- In general, patients producing higher amounts of HbA^{T87Q} were more likely to achieve greater reductions in pRBC transfusion requirements (patients of β^0/β^0 genotype) and/or a greater likelihood of achieving TI (patients of non-β^0/β^0 genotype).

Interrupted Time Series Analyses

pRBC transfusion requirement over time was also analysed by interrupted time series (ITS, Kontopantelis et al., 2015) for β^0/β^0 patients. The monthly pRBC transfusion volume during each of the 48 months (24 months prior to drug product infusion and 24 months post-drug product infusion) was calculated for each patient, and then the mean pRBC transfusion volume over all β^0/β^0 patients was calculated for each of these 48 months. Due to the expected high volume of transfusion around drug product infusion, the month immediately prior to and after the drug product infusion were excluded from this analysis. The results show that monthly pRBC transfusion volume is significantly lower for β^0/β^0 patients after drug product infusion ($p<0.0001$). The lines before and after drug product infusion represent regression lines fitted by the ITS model. The drop represents the reduction in transfusion 1 month after drug product infusion as fitted by the model.

Figure 8: Interrupted Time Series Regression Model: Monthly pRBC Transfusion Volume Pre- and Post-Drug Product Infusion (β^0/β^0 Patients)



Source: Figure 14.2.7

Note: Month -2 includes pRBC transfusions occurring prior to Day -30.4375 and Month 2 includes pRBC transfusions occurring after Day 30.4375. Due to the expected high volume of transfusion around drug product infusion, the month immediately prior to and after the drug product infusion are excluded from this analysis.

Protocol HGB-209

Table 14.2.7

Interrupted Time Series Regression Model: Monthly pRBC Transfusion Volume Pre and Post DP Infusion by Genotype b0/b0

Genotype	Parameter	Regression Coefficient	Standard Error	95% CI Lower	95% CI Upper	p-value
b0/b0	Slope before DP infusion	-0.04	0.0711	-0.18	0.10	0.5690
	Level Change	-9.85	1.4675	-12.81	-6.89	<0.0001
	Slope Change	0.02	0.1006	-0.18	0.22	0.8529

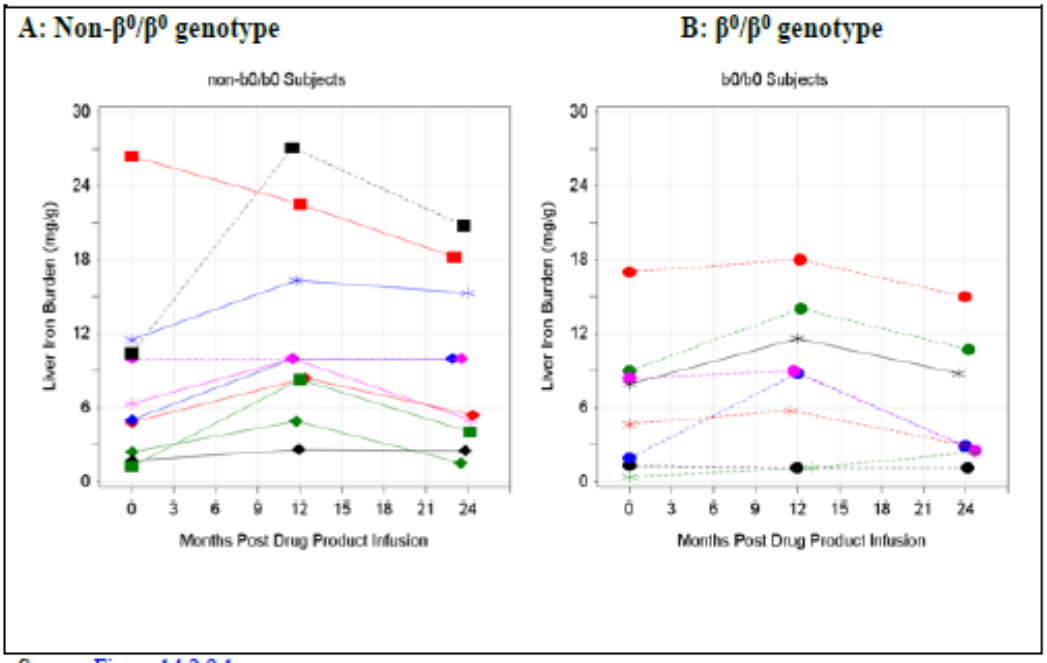
Data as of 07 March 2018

Iron burden

Iron overload during the study was evaluated by assessing LIC and cardiac T2* by MRI or SQUID, and serum ferritin, serum iron, transferrin, and transferrin saturation in peripheral blood. Patients who produced sufficient HbA^{T87Q} to become TI may be expected eventually to have a reduced iron burden compared to patients who continue to require regular pRBC transfusions, after undergoing iron chelation/removal regimens to remove excess iron previously accumulated during their disease. Therefore, comparisons within these exploratory analyses focus on TI versus non-TI patients, to determine if any differences were detectable during this study.

A transient increase in iron burden (as deduced from LIC and serum ferritin levels) was observed in several patients, presumably due to pretreatment hypertransfusion and/or delay in restarting chelation regimens after Zynteglo finished product infusion, and to the effect of myeloablation and transplantation.

Figure 9: Liver Iron Burden (MRI/SQUID Iron Assessment) Over Time, by Patient (TP)



Source: Figure 14.2.9.1
Note: X-axis "zero" = Baseline value defined as last value before initiation of conditioning
Note: Subjects achieving TI at any time are represented with solid lines and subjects not achieving TI at any time are represented with dashed lines.
Note: LIC results from Liver MRI and SQUID modalities are combined for analysis.
Note: For subjects with cardiac T2* >20 ms who no longer require pRBC transfusions, protocol recommended chelation for LIC>15 mg/g and for LIC>8 to 15 mg/g dependent on serum ferritin and hepatitis serology; chelation not recommended for LIC <8 mg/g.

Data as of 07 March 2018

16/18 patients had myocardial T2* >20 ms at Baseline and throughout the study; 2 patients had abnormal cardiac iron content throughout the study.

Measurement of myocardial iron is key to the clinical management of patients at risk of siderotic cardiomyopathy. The cardiovascular magnetic resonance relaxation parameter R2* (assessed clinically via its reciprocal T2*) measured in the ventricular septum is used to assess cardiac iron. Particulate intracellular iron causes shortening of the magnetic resonance relaxation parameter T2* (and hence increase in its reciprocal, R2*) due to microscopic magnetic field inhomogeneity.

Cardiac iron content was evaluated by MRI at Baseline (last value prior to conditioning), Month 12 and Month 24. No trend in cardiac iron content was observed: median % change from Baseline to Month 24

value was -5.26% for patients who achieved TI at any time during the study (N=9) versus 0% change for patients who did not achieve TI.

Serum Ferritin: The majority of patients who achieved TI and who were evaluable (having values at both Baseline and Month 24) showed improvements in ferritin (7/8 patients), serum transferrin (7/9 patients), serum iron (7/9 patients), and transferrin saturation (5/7 patients). The decrease in ferritin levels is generally greater for patients who achieved TI status versus patients who did not achieve TI status by Month 24: median change from Baseline at Month 24 was -26.1% for TI patients, versus -5.1% for non-TI patients, although results were variable between patients.

Table 17: Serum Ferritin at Month 24, by TI Status (TP)

	Statistic	Baseline Value (pmol/L)	Month 24 Value (pmol/L)	Month 24 Change from Baseline	Month 24 % Change from Baseline
Overall	N	17[a]	18	17[a]	17[a]
	Mean	4100.3	4330.0	168.4	10.0
	(SD)	(2227.91)	(3934.89)	(3554.25)	(98.53)
	Median	3146.8	2485.4	-471.9	-23.5
	Min, max	748, 8629	613, 13662	-4742, 10515	-82, 334
TI Subjects	N	8	9	8	8
	Mean	4221.3	3584.9	-859.6	-20.6
	(SD)	(2398.50)	(2159.21)	(1751.56)	(24.21)
	Median	3504.5	2973.0	-613.5	-26.1
	Min, max	1643, 8629	1171, 7499	-4742, 1481	-55, 25
Non-TI Subjects	N	9	9	9	9
	Mean	3992.8	5075.1	1082.2	37.2
	(SD)	(2205.86)	(5195.28)	(4537.04)	(130.91)
	Median	3146.8	2049.4	-134.8	-5.1
	Min, max	748, 7267	613, 13662	-4485, 10515	-82, 334

Source: Table 14.2.9.3
Note: Baseline is the last value before initiation of conditioning
[a]No Baseline ferritin for value reported

Data as of 07 March 2018

Serum transferrin: serum transferrin values for individual patients who achieved TI, at Baseline and at Month 24 shows that transferrin levels improved in 7/9 patients who achieved TI, generally in alignment with the patients who showed a ferritin decrease at Month 24.

Stress Erythropoiesis/Dyserythropoiesis

Although all patients who had achieved TI had some normal values in some exploratory assays for stress erythropoiesis, no patient had normal values in all exploratory analyses done. Specifically:

- 3/7 patients who achieved TI and had data available had stable normal levels of soluble transferrin receptor, 1 patient (Patient 1120) had levels that fluctuated between normal and slightly above-normal levels, and 3/7 patients had stable above-normal levels of soluble transferrin receptor, in the absence of pRBC transfusions.
- 5/9 patients who achieved TI at any time during the study had reticulocyte levels within 1% of the upper normal level of 2.36%, and 4 patients had reticulocyte levels ≥4%, in the absence of pRBC transfusions.

2.5.2. Main studies

The studies underlying the pooled analysis are presented in Section 2.4.1 and includes Phase 1/2 (HGB-204 and HGB-205) and Phase 3 (HGB-207 and HGB-212) studies, as well as in long-term follow-up Study LTF-303.

- Study HGB-205, "A Phase 1/2 Open Label Study Evaluating the Safety and Efficacy of Gene Therapy of the β-Hemoglobinopathies (Sickle Cell Anemia and β-Thalassemia Major) by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral βA-T87Q-Globin Vector (LentiGlobin BB305 Drug Product)"
- Study HGB-204, a single-arm, multi-site, single dose, "Phase 1/2, Open Label Study Evaluating the Safety and Efficacy of Gene Therapy in Patients with β-Thalassemia Major by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral β^{A-T87Q}-Globin Vector (LentiGlobin BB305 Drug Product)"
- Study HGB-207, "A Phase 3 Single Arm Study Evaluating the Efficacy and Safety of Gene Therapy in Patients with Transfusion-dependent β-Thalassaemia, who do not have the β^0/β^0 Genotype, by Transplantation of Autologous CD34+ Stem Cells Transduced *Ex Vivo* with a Lentiviral β^{A-T87Q}-Globin Vector in Patients ≤50 Years of Age"

Methods

Methods for Studies HGB-204 and HGB-205 are described above in Section 2.5.1.

Study HGB-207 is a single-arm, multisite, single-dose, Phase 3 study with 2 cohorts of patients with TDT who do not have a β^0 mutation at both alleles of the *β-globin (HBB)* gene (i.e., non-β^0/β^0):

Cohort 1 includes at least 15 patients ≥12 and ≤50 years of age

Cohort 2 includes at least 8 patients <12 years of age. (Note: as of 22 February 2018, no patients <12 years of age were in the Intent-to-Treat [ITT] population).

Study Participants

In general, the inclusion and exclusion criteria were comparable among studies HGB-204, HGB-205, HGB-207 and HGB-212. HGB-207 inclusion and exclusion criteria are depicted below, some discrepancies in the inclusion and exclusion criteria between studies are highlighted.

Inclusion Criteria

Patients must meet all of the following criteria to be considered eligible for enrolment in the study.

1. Patients ≤50 years of age at the time of consent or assent (as applicable), and able to provide written consent (adults, or legal guardians, as applicable) or assent (adolescents or children). Provided that the DMC has approved enrolling patients younger than 5 years of age, patients younger than 5 years of age may be enrolled if they weigh a minimum of 6 kg and are reasonably anticipated to be able to provide at least the minimum number of cells required to initiate the manufacturing process. Study HGB-205 included only patients with age between 5-35 and study HGB-204 included patients 12-35 years of age.

2. Diagnosis of TDT with a history of at least 100 mL/kg/year of pRBCs in the 2 years preceding enrolment (all patients), or be managed under standard thalassaemia guidelines (e.g., (Cappellini *et al.* 2014)) with ≥8 transfusions of pRBCs per year in the 2 years preceding enrolment (patients ≥12 years (Rachmilewitz

and Giardina 2011)). In study HGB-204, inclusion was for patients with TDT and in study HGB-205 for patients with TDT or severe SCD.

3. Clinically stable, have a Karnofsky performance status of ≥80 for adults (≥16 years of age) or a Lansky performance status of ≥80 for adolescents or children (<16 years of age), and eligible to undergo HSCT. Study HGB-204 included patients with Karnofsky performance status ≥ 60, and HGB-205 included patients eligible for allogeneic HSCT.

4. Treated and followed for at least the past 2 years in a specialized centre that maintained detailed medical records on RBC transfusions (including volume and units of RBCs and associated pre-transfusion Hb values, reticulocyte counts and relevant blood bank details as available), in-patient hospitalization, and iron chelation history.

Exclusion Criteria

Patients meeting any of the following criteria are being excluded from the study.

1. Presence of a mutation characterized as β^0 on both *HBB* alleles. For the purpose of this study, the *HBB* mutation IVS I-110 (G→A) [HGVS nomenclature: *HBB*:c.93-21G>A] will be considered equivalent to a β^0 mutation[2]

2. Positive for presence of human immunodeficiency virus type 1 (HIV-1) or 2 (HIV-2), HBV, or HCV. Syphilis (RPR) testing is also required and a positive test for syphilis is exclusionary where mandated by regional drug product manufacturing practices. Note that patients who have been vaccinated against hepatitis B [hepatitis B surface antibody-positive] who are negative for other markers of prior hepatitis B infection [e.g., negative for hepatitis B core antibody] are eligible. Patients with past exposure to HBV [HBc Ab positive and/or HBe Ab positive] are also eligible for the study provided they are negative by assessment for HBV DNA. Also note that patients who are positive for anti-hepatitis C antibody are eligible as long as they have a negative HCV viral load. Where clinically and/or regionally indicated, other tests may be performed, in which case positive results would exclude the patient from participating: for example, human T-lymphotropic virus-1 (HTLV-1) or -2 (HTLV-2), tuberculosis, toxoplasmosis, Trypanosoma cruzi, West Nile Virus, or Zika Virus.

3. Clinically significant and active bacterial, viral, fungal, or parasitic infection as determined by the clinical investigator.

4. A white blood cell (WBC) count <3×10^9/L, and/or platelet count <100×10^9/L not related to hypersplenism.

5. Uncorrected bleeding disorder.

6. Any prior or current malignancy (with the exception of adequately treated cone-biopsied in situ carcinoma of the cervix uteri and basal or squamous cell carcinoma of the skin) or myeloproliferative or significant immunodeficiency disorder.

7. Immediate family member (i.e., parent or siblings) with a known Familial Cancer Syndrome (including but not limited to hereditary breast and ovarian cancer syndrome, hereditary non-polyposis colorectal cancer syndrome and familial adenomatous polyposis).

8. Prior HSCT.

9. Advanced liver disease, defined as:

[2] Similar to β^0 alleles, the IVS-I-110 mutation is widely recognized as producing little to no β-globin (Borgna-Pignatti and Galanello, 2009), thus subjects with β^0/IVS-I-110 or IVS-I-110/IVS-I-110 genotypes were excluded from Study HGB-207 and grouped with the β^0/β^0 subjects in Study HGB-212.

a. Persistent aspartate aminotransferase (AST), alanine transaminase (ALT), or direct bilirubin value >3 × the upper limit of normal (ULN), or

b. Baseline prothrombin time or partial thromboplastin time >1.5× ULN, suspected of arising from liver disease, or

c. MRI of the liver demonstrating clear evidence of cirrhosis

d. MRI findings suggestive of active hepatitis, significant fibrosis, inconclusive evidence of cirrhosis, or liver iron concentration ≥15 mg/g require follow-up liver biopsy in patients ≥18 years of age. In patients <18 years of age, these MRI findings are exclusionary, unless in the opinion of the investigator, a liver biopsy could provide additional data to confirm eligibility and would be safe to perform. If a liver biopsy is performed based on MRI findings, any evidence of cirrhosis, bridging fibrosis, or significant active hepatitis will be exclusionary.

10. Baseline estimated GFR <70 mL/min/1.73 m^2, as determined using the CKD-EPI creatinine equation for ≥18 years of age, and Bedside Schwartz equation calculator for <18 years of age. (http://www.kidney.org/professionals/kdoqi/gfr_calculator.cfm)

11. Uncontrolled seizure disorder.

12. Diffusion capacity of carbon monoxide (DLco) <50% of predicted (corrected for Hb and/or alveolar volume, as clinically indicated).

13. A cardiac T2* <10 ms by MRI.

14. Any other evidence of severe iron overload that, in the investigator's opinion, warrants exclusion.

15. Participation in another clinical study with an investigational drug within 30 days of Screening.

16. Any other condition that would render the patient ineligible for HSCT, as determined by the attending transplant physician or investigator.

17. Prior receipt of gene therapy.

18. Diagnosis of significant psychiatric disorder of the patient that could seriously impede the ability to participate in the study.

19. Pregnancy or breastfeeding in a postpartum female or absence of adequate contraception for fertile patients. Females of childbearing potential and males are required to use two different effective methods of contraception from Screening through at least 6 months after drug product infusion. If patients are truly sexually abstinent (where true sexual abstinence is defined as being in line with the preferred and usual lifestyle of the patient), no second method is required.

20. An assessment by the investigator that the patient would not comply with the study procedures outlined in the protocol.

21. A known and available HLA-matched family donor. If required by regional regulatory authority, patients with a known and available matched unrelated donor will be excluded from the study.

22. Any contraindications to the use of G-CSF and plerixafor during the mobilization of HSCs and any contraindications to the use of busulfan and any other medicinal products required during the myeloablative conditioning, including hypersensitivity to the active substances or to any of the excipients.

Treatments

In general, study treatments were comparable among studies HGB-204, HGB-205, HGB-207 and HGB-212. HGB-207 study treatments are depicted below.

The study has 4 distinct stages, as follows.

Stage 1: Screening to determine eligibility for treatment. The *HBB* genotype for non-β^0/β^0 patients will be confirmed during Screening along with additional parameters of eligibility.

Stage 2: Autologous CD34+ cell collection, drug product manufacture and disposition.

Each patient will undergo HSC mobilization with a granulocyte colony-stimulating factor (G-CSF, e.g., filgrastim, lenograstim) and plerixafor. Peripheral blood mononuclear cells (PBMCs) will be collected by apheresis. Patients with a spleen were recommended to be dosed with G-CSF at 10 µg/kg/day and patients without a spleen dosed at 5 µg/kg/day, and to be dosed with 0.24 mg/kg/day plerixafor, with adjustments permitted based on white blood cell monitoring. A total of 2 mobilization cycles may be performed if needed and each mobilization cycle may include up to 3 apheresis days.

However, patients whose drug product fails to meet specifications for any reason may undergo repeat apheresis and manufacture of new drug product. Apheresis products can also be used for rescue cells; alternatively, a bone marrow harvest is also allowed to procure cells for rescue.

Stage 3: Myeloablative conditioning (4 days of conditioning followed by at least 48 hours of washout) and infusion of Zynteglo (Day 1)

After the transduced cells are dispositioned for clinical use, and the patient's eligibility has been re-confirmed (by clinical laboratory tests, physical examination, performance status, adverse event [AE] review and other tests based on institutional requirements), the patient will undergo myeloablative conditioning with busulfan.

Conditioning only begins after the following criteria are met:

- All the drug product to be used for a particular patient has been release tested, dispositioned for clinical use, and is stored at the clinical site;
- It is recommended that patients maintain a hypertransfusion regimen at least 30 days prior to conditioning to maintain a pre-transfusion Hb of ≥11 g/dL. Iron chelation will likely need to be adjusted to compensate for the increased iron load, until required discontinuation per protocol;
- The patient has undergone AE and concomitant medications assessments, physical examination, vital signs, and laboratory tests as per the SOE and continues to meet the eligibility criteria based on these results.

Busulfan will be administered at a starting dose of 3.2 mg/kg/day for 4 consecutive days via IV infusion on Days -6 through -3. Please refer to the busulfan prescribing information for details on appropriate method for determination of patient weight; e.g., (Busilvex SmPC).

A dose of 3.2 mg/kg daily, or 0.8 mg/kg every 6 hours, for 4 consecutive days, is recommended, with dose adjustment as needed based on pharmacokinetics monitoring to achieve a target daily busulfan of AUC of 4200 (range 3800 to 4500) µM*min. It is recommended that busulfan is administered at 0.8 mg/kg every 6 hours in children and adolescents with a target busulfan AUC of 1050 (range 950 to 1125)

μM*min for every 6 hour dosing to avoid higher peak concentrations while still providing equivalent daily exposure.

After completion of the 4-day course of busulfan, there must be a minimum of 48 hours of washout before drug product infusion. On study Day 1, thawed drug product will be administered via intravenous (IV) infusion at a dose of $\geq 5.0 \times 10^6$ CD34+ cells/kg.

Stage 4: Follow-up, through engraftment and up to 24 months after drug product infusion. Patients will be followed daily in the transplant unit for AEs, and laboratory parameters will be followed to monitor bone marrow engraftment. The patient will be discharged from the transplant unit once the patient is considered medically stable.

The goal during the follow-up period is to maintain Hb ≥9 g/dL. Transfusions should be avoided for Hb ≥9 g/dL unless the need is medically justified (e.g., as a pre-requirement for surgery). It is recommended that patients should receive pRBC transfusions for Hb <7 g/dL, and for clinically symptomatic anaemia, irrespective of Hb level.

Patients will be followed in this protocol for a period of approximately 24 months after Zynteglo infusion. Thereafter, patients will be asked to enroll in a separate long-term follow-up protocol (LTF-303) that will assess safety and efficacy beyond Month 24 for a total of 15 years after drug product infusion. The end of Study HGB-207 will be defined as the last visit for the last patient.

Concomitant Medications and Therapies

Permitted concomitant treatments during conditioning at the Investigator's discretion include but are not limited to:

- • Hydration beginning 12 hours before initiating conditioning and continuing through 24 hours thereafter
- Ondansetron and/or metoclopramide for prevention or treatment of nausea and vomiting.
- Anticonvulsants for seizure prophylaxis with the exception of phenytoin.
- Ursodeoxycholic acid or defibrotide for prevention of hepatic veno-occlusive disease/hepatic sinusoidal obstruction syndrome
- Anti-infectives for infection prophylaxis or treatment of febrile neutropenia per the study site standard of care

Iron Chelating Agents

To avoid potential toxic drug-to-drug interactions, chelation was required to be stopped 7 days prior to initiating conditioning with busulfan. Iron chelation was at the investigator's discretion and in accordance with institutional protocols. Briefly, for patients who continued to require transfusions, chelation was recommended to be restarted with deferasirox or deferoxamine at hospital discharge. It was recommended that deferiprone not be started for at least 6 months due to the potential risk of myelosuppression. For patients who no longer required pRBC transfusions within 3 months after Zynteglo infusion, the need for starting iron reduction methods at Day +90 post- Zynteglo infusion was recommended to be based on LIC and Cardiac T2* measurements (Table 18).

Table 18: Iron Reduction Guidelines Post-Zynteglo Infusion

Liver LIC (mg/g)	Cardiac T2* (ms)	
	>20 ms	20 to 10 ms
<8 mg/g	No chelation is required	Chelation is indicated
8-15 mg/g	Consider based on serum ferritin and hepatitis serology[a]	Chelation is indicated
>15 mg/g	Chelation is indicated	Chelation is indicated

[a] Chelation is recommended only if serum ferritin is >2000 ng/ml at Day +90 or hepatitis B/C serology was positive pre-transplant.

Objectives

The objectives of the pooled analyses are:

- To evaluate and summarise the associations between measures of myeloablation and pharmaco-dynamic (PD) parameters as well as associations between gene expression and key efficacy transfusion requirement parameters, transfusion independence (TI) and transfusion reduction (TR), among patients with TDT.
- To conduct a pooled efficacy analysis, evaluating TI and TR among pooled cohorts of patients with TDT.

Outcomes/endpoints

Primary Efficacy Endpoint:

The primary endpoint was similar for the pooled analyses with Studies HGB-204, HGB-205, and HGB-207, and for study HGB-207: the proportion of patients who meet the definition of transfusion independence (TI).

- TI is defined as a weighted average Hb ≥9 g/dL without any pRBC transfusions for a continuous period of ≥12 months at any time during the study after drug product infusion.

Secondary Efficacy Endpoints for Study HGB-207

- Characterisation of patients achieving TI:
  - Duration of TI
  - Time from drug product infusion to last pRBC transfusion prior to becoming TI
  - Time from drug product infusion to TI
  - Weighted average nadir Hb during TI
  - Proportion of patients who meet the definition of TI at End-of-Study Visit
  - Transfusion-free survival
- Characterisation of transfusion reduction (TR):
  - Proportion of patients with a reduction in the mL/kg/year pRBCs transfused from 12 months post-drug product infusion through the Month 24 Visit of at least 50%, 60%, 75%, 90% or 100% compared to the average mL/kg/year pRBC transfusion requirement during the 2 years prior to enrolment
  - Number and volume of pRBC transfusions from 12 months post-drug product infusion through the Month 24 Visit compared to the average number and volume of annual

transfusions during the 2 years prior to enrolment

- Weighted average nadir Hb during the 2 years prior to enrolment compared to weighted average nadir Hb from 12 months post-DP infusion through the Month 24 Visit
- Characterisation of use of iron chelation among all patients:
  - Proportion of patients who have discontinued iron chelation therapy for at least 6 months
  - Change in dose of iron chelation therapy from baseline for those patients not discontinuing chelation for at least 6 months
- Evaluation of the change in iron burden over time, as measured by:
  - Change in LIC by MRI at baseline to Month 12 and Month 24
  - Change in cardiac T2* on MRI at baseline to Month 12 and Month 24
  - Change in serum ferritin from baseline through Month 24 Visit

Exploratory Efficacy Endpoints for Study HGB-207

- Evaluation of health-related quality of life (HRQoL) over time using the following validated tools:
  - Paediatrics: Paediatric Quality of Life Inventory (PedsQL; parent general core and general core)
  - Adolescents: PedsQL (parent general core and general core) and EuroQol-5D (Youth version) (EQ-5D-Y)
  - Adults: EuroQol-5D (EQ-5D), Functional Assessment of Cancer Therapy-Bone Marrow Transplant (FACT-BMT), and Short Form-36 (SF-36) v2
- Assessment of growth and puberty parameters (age appropriate), bone density, diabetes, endocrine evaluations, and neurocognitive development (paediatric patients <18 years of age)
- Assessment of improvement in ineffective erythropoiesis
- Correlations of pre-treatment variables (e.g., drug product vector copy number [VCN]) with response
- Measures of health resource utilization (including comparing number of transfusions, number of hospitalizations, and iron chelation usage from 12 months post-drug product infusion through Month 24 Visit with the annual average of the corresponding parameters during the 2 years prior to enrolment.

Sample size for Study HGB-207

No formal sample size calculations were done.

Approximately 23 patients in total will be treated with drug product , at least 15 of whom must be ≥12 and ≤50 years of age (Cohort 1; at least 5 patients in Cohort 1 must be ≥12 and <18), and at least 8 of whom must be <12 years of age (Cohort 2). Replacement patients may be added if patients are screen failures or withdraw prior to Stage 3 (conditioning).

The sample size in Cohort 1 is based on the premise that excluding a treatment effect of <30% with a high probability is of value (demonstrating with 97.5% confidence that ≥30% of patients become TI). Moreover, a point estimate of the proportion of patients who become TI of at least 60% is considered clinically meaningful. This point estimate was selected based on the minimal criterion to be met in the

trials and was supported as the minimal target based on discussions with key opinion leaders with expertise in the treatment of β-thalassemia. Therefore, among the proposed sample size of 15 treated patients ≥12 and ≤50 years of age in Cohort 1, a point estimate for success of 60% is proposed (9 out of 15 patients), which would yield a lower 1-sided 97.5% exact confidence bound of 32.3%, exceeding the 30% minimal criterion. Among the proposed sample size of 8 treated patients <12 years of age in Cohort 2, a point estimate for success of 60% (5 out of 8 patients) would yield a lower 1-sided 97.5% exact confidence bound of 24.5%.

The following patient populations are evaluated and used for presentation and analysis of the data:

- Intent-to-Treat (ITT) population: All patients who initiate any study procedures, beginning with mobilization by G-CSF and/or plerixafor.
- Transplant Population (TP): All patients who receive Zynteglo finished product treatment.
- Successful Engraftment Population (SEP): All patients who have successful neutrophil engraftment (NE) after Zynteglo finished product infusion.

The ITT population is the primary population for the analysis of safety parameters. The TP is the primary population for the analysis of efficacy, pharmacodynamic and transplant parameter endpoints (i.e., success and kinetics of engraftment, and incidence of transplant-related mortality post-drug product infusion). The Successful Engraftment Population (SEP) was planned to provide supportive data for patients who engraft; however, given all patients with sufficient follow-up data for evaluation had successful engraftment, this population was not analysed.

Randomisation

Not applicable.

Blinding (masking)

Not applicable.

Statistical methods

The studies are primarily descriptive in nature. Data are presented by patient and summarized overall within each analysis population, and by age and sex, as applicable. Tabulations were produced for appropriate demographic, baseline, efficacy, pharmacodynamic, and safety parameters. For categorical variables, summary tabulations of the number and percentage of patients within each category of the parameter are presented. For continuous variables, the number of patients, mean, standard deviation (SD), median, minimum, and maximum values are presented. Descriptive summary statistics as well as 1-sided 97.5% Clopper-Pearson exact binomial confidence intervals are presented on selected parameters expressed as proportions.

Baseline Definitions

Two years of retrospective pre-study enrolment data is collected for each patient in the study, so that each patient may serve as his/her own control for the parameters of pRBC transfusion requirements, weighted average nadir Hb concentrations, in-patient hospitalizations (number and duration), and iron chelation use. For these parameters, baseline is annualized over the 2 years prior to study entry (date of informed consent). For pRBC transfusion requirements, there is 1 baseline parameter, the average per year. For the number of in-patient hospitalization days (defined as hospitalization duration of at least 24 hours), in addition to the total number of hospitalizations in the 2 years prior to study entry, the baseline average per year is calculated. For iron chelation use, the baseline average dose per year is

calculated. For other efficacy parameters as well as for pharmacodynamics parameters, baseline is defined as the most recent measurement prior to conditioning; the conditioning start date is defined as the first date of busulfan administration. For safety parameters, including shifts in key laboratory parameters, the most recent value prior to mobilisation is used as the baseline assessment**.**

Formal multiplicity adjustment will not be performed. It is expected that the majority of the secondary and exploratory endpoints will demonstrate a positive effect of Zynteglo. There are multiple secondary endpoints, which will enable a more complete understanding of the clinical impact of therapy with Zynteglo.

Subpopulations

Selected analyses are stratified by age at informed consent/assent (<12, ≥12 to <18, and ≥18 years), race and sex. Additionally, some endpoints are presented for subpopulations by genotype: non-β^0/β^0 patients (containing the β^E mutation, or the β^+ mutation), β^0/β^0 patients.

Withdrawals, Dropouts, Loss to Follow-up

Patients withdrawn from the study prior to conditioning (myeloablation) will be replaced. Patients who begin conditioning but are subsequently withdrawn will not be replaced.

Patients who enrol in the trial but discontinue prior to myeloablation should be followed for at least 1 month after completion of harvesting, or until resolution of any study procedure-related AEs, whichever is later. In the rare case a patient undergoes myeloablation but does not undergo Zynteglo infusion, follow-up should continue on trial for at least 3 months, or until resolution of any study procedure-related AEs, whichever is later.

If withdrawal is after drug product infusion, patients will be asked to complete the same assessments as specified in the SOE for Month 24 (Early Termination Visit assessments) and will be asked to enroll in the long-term follow-up Study LTF-303.

Results

Participant flow

HGB-204: 19 eligible patients were enrolled in Study HGB-204, and 18 patients were infused with Zynteglo, all of whom successfully engrafted. One patient was withdrawn from the study due to "inadequate stem cell mobilization" after a single mobilization/apheresis cycle. 18 patients successfully engrafted completed the Study through their Month 24 Visit.

HGB-205: 4 patients with TDT underwent screening, and all were found to be eligible for this study. All 4 were treated with Zynteglo, all 4 successfully engrafted and completed the study through approximately 2 years post-drug product infusion.

HGB-207: As of 22 February 2018, 23 patients underwent screening, and 4 were found to be ineligible for this study. Three patients were excluded due to advanced liver disease and 1 patient was excluded due to a cardiac T2* <10 ms measured by MRI. 14 patients were mobilized, 1 patient underwent 1 cycle of mobilization and apheresis with G-CSF and plerixafor but discontinued before conditioning because of pregnancy. 10 patients were conditioned with busulfan and were administered Zynteglo (TP); and 3 patients have not yet undergone conditioning/Zynteglo infusion. Of the 10 patients who were infused with drug product at the time of data cut for the interim clinical study report (CSR), all 9 patients with at least 43 days of follow-up had achieved neutrophil engraftment, 6 patients had completed at least their Month 6 Visit and 2 patients had completed at least their Month 12 Visit (Table 19).

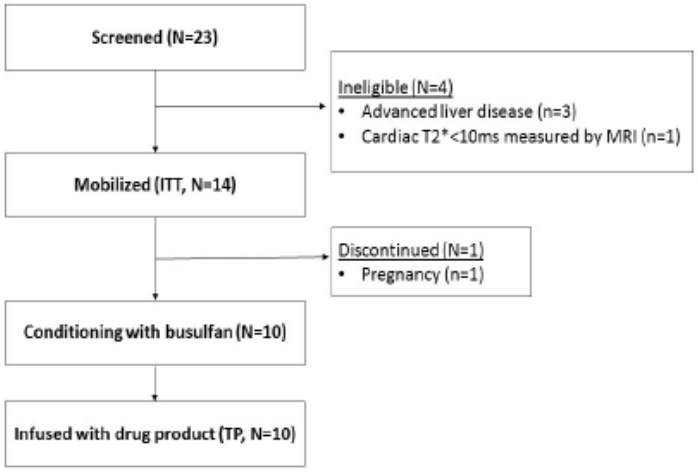
Table 19: HGB-207 disposition (ITT)

Parameter	Statistic	Overall (N=14)
Subjects Mobilized (ITT)[1]	n (%)	14 (100.0)
Subjects Infused with Drug Product (TP)[2]	n (%)	10 (71.4)
Subjects with Successful Engraftment[3]	n (%)	9 (64.3)
Subjects Completing Month 3 Visit Post-Drug Product Infusion	n (%)	8 (57.1)
Subjects Completing Month 6 Visit Post-Drug Product Infusion	n (%)	6 (42.9)
Subjects Completing Month 9 Visit Post-Drug Product Infusion	n (%)	3 (21.4)
Subjects Completing Month 12 Visit Post-Drug Product Infusion	n (%)	2 (14.3)
Subjects Completing Month 18 Visit Post-Drug Product Infusion	n (%)	0
Subjects who Discontinued from Study	n (%)	1 (7.1)
Reasons for Study Discontinuation Other[4]	n (%)	1 (7.1)

Source: Table 14.1.1.1, Listing 16.2.1.1
[1]Intent to treat population (ITT)
[2]Transplant Population (TP)
[3]Successful Engraftment Population (SEP)= all subjects who have successful neutrophil engraftment after LentiGlobin BB305 Drug Product infusion to date, defined as ANC $\geq 0.5 \times 10^9$/L for 3 consecutive days post drug product infusion or ANC $\geq 0.5 \times 10^9$/L for 3 consecutive measurements done on separate days. At the time of data lock, one subject had not yet reached successful engraftment due to insufficient follow-up time.
[4] Discontinued after a single cycle of mobilization/apheresis, but before conditioning because of "pregnancy on study".

Data as of 22 February 2018

Figure 10: **Consort diagram showing disposition for Study HGB-207**



Data as of 22 February 2018

Recruitment for Study HGB-207

4 patients were found ineligible for the study during screening. 3 patients were excluded due to advanced liver disease and 1 patient was excluded due to a cardiac T2* <10ms measured by MRI.

Of the 14 mobilised patients, 10 patients were infused with the drug product of which 9 engrafted successfully and one patient had insufficient follow up time for successful engraftment. As of 22 February 2018, follow-up time is limited and no patients had sufficient follow-up time that is needed for assessment of the primary endpoint.

Conduct of Study HGB-207

Enrolment in Study HGB-207 began under protocol version 1.3 (27 January 2016). The protocol was amended 3 times (Table 20).

Table 20: Substantive Changes in Protocol Amendments

Version, Date, Countries Effective	Key Substantive Changes
V1.3, 27JAN2016, Global	Original to enroll patients
V1.4, 06FEB2016, UK	Added new exclusion criteria to exclude any subjects with known sensitivities to drugs being used in the trial
V1.5, 14NOV2016, Germany	Added criteria to exclude patients with a known and available HLA-matched unrelated donor Added success criteria defined based on the proportion of subjects achieving transfusion independence Changed procedure to increase frequency of vital sign monitoring to every 2 hours post-drug product infusion Outlined assessments that will need to be repeated if mobilization is delayed for ≥3 months after screening Exclusion criteria added to require more stringent liver assessment before being eligible for treatment Exclusion criteria added to exclude subjects with known sensitivities to drugs being used in the trial
V2.0, 05JAN2017, Global	Added cohort of subjects <12 years of age
V3.0, 19 JUN2018, Global	Target average daily busulfan AUC reduced from 4500 (range 4000 to 5000) µM*min to 4200 (range 3800 to 4500) µM*min. Children and adolescents were recommended to follow a q6h dosing regimen, with a target AUC of 1050 (range 950 to 1125) µM*min. Prophylaxis with ursodeoxycholic acid (preferred) or defibrotide is required before initiation of conditioning to help prevent the occurrence of VOD/SOS. Secondary efficacy endpoints clarified to match SAP language which includes measuring weighted average Hb during TI and volume of pRBC transfusions from Month 12 through Month 24. Also added endpoint to clarify that total Hb levels are collected at designated time points.

As of 22 February 2018

Changes to the Planned Statistical Methods for Study HGB-207

The HGB-207 SAP outlines any differences in the planned analytical objectives relative to those planned in the study protocol. Changes to analyses outlined in the current SAP are as follows:

1. Due to limited follow-up at the time of the data-lock for this interim analysis:

   a. The primary efficacy endpoint TI and secondary efficacy endpoint characterising patients with TI were not performed.

   b. For transfusion reduction and weighted average nadir Hb analyses that pre-specify a Month 12 to Month 24 analysis period for comparison with baseline, a modified analysis period of hospital discharge after drug product infusion to the last visit for each patient was utilized to detect any emerging trends in the data. Data from the pre-enrollment period and the post-hospital discharge period were annualized for the purposes of comparison.

   c. Any data for the following endpoints are included only in data listings:

      i. Secondary efficacy endpoints of chelation therapy discontinuation and change in chelation therapy dose, and change in iron burden over time (LIC, cardiac T2*)

      ii. Exploratory endpoints of Quality of life (QoL), assessment of growth and puberty parameters, assessment of improvement in ineffective erythropoiesis, and measures of health resource utilization for the Month 12 through Month 24 pre-specified period

2. The characterisation of patients with TI endpoint, 'Time from drug product infusion to last pRBC transfusion prior to becoming TI' was evaluated as 'Time from drug product infusion to last pRBC transfusion' for all TP patients with available data.

3. The characterisation of patients with TI endpoint, 'Transfusion-free survival' was evaluated descriptively as 'Time from last pRBC transfusion to last visit' given there were no deaths, and was also analysed for all TP patients with available data.

4. Hb was planned to be included as a parameter for lab shift table safety analysis, however, then was removed since already analysed as part of efficacy analyses.

5. The pharmacodynamic endpoint for gene transfer efficiency and expression was quantified by measuring $\beta^{A\text{-}T87Q}$-globin expression; however, the protocol and SAP both state this endpoint is assessed by the ratio of $\beta^{A\text{-}T87Q}$-globin to α-globin, which is included in a data listing, but this is not how $\beta^{A\text{-}T87Q}$-globin expression is assessed.

6. The ratio of α-globin chains to all β-globin chains was planned but not analysed given the planned and analysed ratio of α-globin chains to all β-like globin chains was the more clinically relevant ratio for pharmacodynamic analysis.

7. The correlation of $\beta^{A\text{-}T87Q}$-globin expression at early time points post-drug product infusion (e.g., 3 and 6 months) to $\beta^{A\text{-}T87Q}$-globin expression at later time points, as well as clinical outcomes) was not assessed at this early stage of the trial.

8. Events of interest (EOI) analysis was not performed in the study as the definition of EOI continues to evolve with the development of the product.

Treatment procedure for Study HGB-207

Below a short review of the treatment procedure for Study HGB-207 is presented in Table 21 and Table 22.

Table 21: Mobilisation/Apheresis Details for Study HGB-207 (ITT Population)

Parameter	Statistic	Splenectomized (N=5)	Not Splenectomized (N=9)	Overall (N=14)
Number of Mobilization Cycles / Subject				
1	n (%)	3 (60.0)	9 (100.0)	12 (85.7)
2	n (%)	2 (40.0)	0	2 (14.3)
G-CSF Average Daily Dose (µg/kg/day) [1]	N	5	9	14
	Mean (SD)	6.46 (2.135)	10.23 (0.721)	8.89 (2.286)
	Median	5.44	10.00	9.86
	Min, Max	5.1, 10.2	9.3, 11.6	5.1, 11.6
Plerixafor Average Daily Dose (mg/kg/day) [1]	N	5	9	14
	Mean (SD)	0.246 (0.0069)	0.241 (0.0168)	0.243 (0.0139)
	Median	0.245	0.235	0.242
	Min, Max	0.24, 0.25	0.22, 0.28	0.22, 0.28
Number of Apheresis Procedures Performed per Mobilization Cycle [2]	N	5	9	14
	Mean (SD)	2.60 (0.418)	1.89 (0.333)	2.14 (0.497)
	Median	2.50	2.00	2.00
	Min, Max	2.0, 3.0	1.0, 2.0	1.0, 3.0
Total Blood Volume Processed during Apheresis (mL) [3]				
Cycle 1	N	5	9	14
	Mean (SD)	31001.2 (12423.72)	15253.8 (4929.98)	20877.9 (11124.87)
	Median	23918.0	15878.0	18925.0
	Min, Max	19608, 49087	8788, 22050	8788, 49087

Parameter	Statistic	Splenectomized (N=5)	Not Splenectomized (N=9)	Overall (N=14)
Cycle 2	N	2	0	2
	Mean (SD)	23869.0 (4174.76)		23869.0 (4174.76)
	Median	23869.0		23869.0
	Min, Max	20917, 26821		20917, 26821
Average Total Blood Volume Processed during Apheresis (mL) [2]	N	5	9	14
	Mean (SD)	15761.13 (7316.333)	8136.78 (2220.549)	10859.76 (5820.458)
	Median	16362.33	8769.50	9149.50
	Min, Max	7972.7, 26284.2	4394.0, 11025.0	4394.0, 26284.2
Number of CD34+ Cells Collected (Cells×10^6/kg)	N	5	9	14
	Mean (SD)	15.38 (4.061)	26.21 (7.602)	22.34 (8.344)
	Median	16.56	23.70	21.59
	Min, Max	8.9, 18.8	12.3, 35.1	8.9, 35.1
Number Of CD34+ Cells Sent for Transduction (Cells×10^6/kg)	N	5	9	14
	Mean (SD)	12.37 (3.585)	19.40 (7.068)	16.89 (6.850)
	Median	13.52	17.60	15.53
	Min, Max	6.4, 15.7	10.5, 30.9	6.4, 30.9
Number Of CD34+ Cells Sent for Rescue (Cells×10^6/kg)	N	5	9	14
	Mean (SD)	3.01 (1.003)	7.49 (4.352)	5.89 (4.112)
	Median	2.73	6.10	4.80
	Min, Max	2.1, 4.7	2.4, 15.7	2.1, 15.7

[1] Sum of total daily doses divided by weight (kg) (closest but prior to date of mobilization) and number of days dosed.
[2] For subjects with more than one mobilization cycle, data will be averaged across both cycles first.
[3] Total blood volume is summed within each cycle.
Source: Listings 16.2.3, 16.2.4.3, 16.2.4.7, 16.2.4.8.1, 16.2.4.8.2

Data as of 22 February 2018

Table 22: Drug Product Dosing and Infusion Details, by Age Group and Sex (TP)

Parameter	Statistic		Age			Sex		Overall (N=10)
		<12	12 to <18	>=18	M	F	
Duration of Hospitalization (days) [1]	N	0	2	6	3	5	8
	Mean (SD)		53.0 (5.66)	42.3 (5.61)	50.0 (6.56)	42.0 (6.20)	45.0 (7.17)
	Median		53.0	43.5	49.0	43.0	44.0
	Min, Max		49, 57	32, 49	44, 57	32, 49	32, 57
Number of Drug Product Lots Administered							
1	n (%)	0	4 (100.0)	5 (83.3)	4 (100.0)	5 (83.3)	9 (90.0)
2	n (%)	0	0	1 (16.7)	0	1 (16.7)	1 (10.0)
3	n (%)	0	0	0	0	0	0
4	n (%)	0	0	0	0	0	0
Total Cell Dose (CD34+ cells x 10^6/kg)	N	0	4	6	4	6	10
	Mean (SD)		9.70 (6.551)	8.77 (2.984)	6.88 (1.176)	10.65 (5.235)	9.14 (4.414)
	Median		7.20	8.00	7.20	9.45	7.65
	Min, Max		5.0, 19.4	5.2, 13.6	5.2, 7.9	5.0, 19.4	5.0, 19.4

Parameter	Statistic	Age			Sex		Overall (N=10)
		<12	12 to <18	>=18	M	F	
Vector Copy Number (VCN) in Drug Product (average per subject; c/dg) [2]	N	0	4	6	4	6	10
	Mean (SD)		4.150 (0.9950)	3.133 (0.5989)	3.350 (0.2887)	3.667 (1.1622)	3.540 (0.8972)
	Median		3.950	2.950	3.350	3.450	3.350
	Min, Max		3.30, 5.40	2.40, 4.00	3.00, 3.70	2.40, 5.40	2.40, 5.40
VCN in Drug Product (average per lot; c/dg) [3]	N	0	4	7	4	7	11
	Mean (SD)		4.150 (0.9950)	3.086 (0.6067)	3.350 (0.2887)	3.543 (1.1341)	3.473 (0.8979)
	Median		3.950	3.000	3.350	3.200	3.300
	Min, Max		3.30, 5.40	2.40, 4.00	3.00, 3.70	2.40, 5.40	2.40, 5.40
Time to Neutrophil Engraftment (days) [4]	N	0	3	6	4	5	9
	Mean (SD)		24.7 (5.77)	21.7 (3.78)	22.8 (4.99)	22.6 (4.51)	22.7 (4.42)
	Median		28.0	21.5	22.5	24.0	24.0
	Min, Max		18, 28	17, 26	18, 28	17, 28	17, 28

Parameter	Statistic	Age			Sex		Overall (N=10)
		<12	12 to <18	>=18	M	F	
Time to Platelet Engraftment (days) [5]	N	0	1	6	3	4	7
	Mean (SD)		51.0 (-)	43.0 (4.00)	46.3 (4.04)	42.5 (5.07)	44.1 (4.74)
	Median		51.0	44.0	44.0	44.5	44.0
	Min, Max		51, 51	35, 46	44, 51	35, 46	35, 51
Time to Platelet Engraftment [5]							
<=30 days	n (%)	0	0	0	0	0	0
>30 to <=60 days	n (%)	0	1 (25.0)	6 (100.0)	3 (75.0)	4 (66.7)	7 (70.0)
>60 to <=90 days	n (%)	0	0	0	0	0	0
>90 days	n (%)	0	0	0	0	0	0
Subjects with Neutrophil Engraftment Success [6]	n (%)	0	3 (75.0)	6 (100.0)	4 (100.0)	5 (83.3)	9 (90.0)
Subjects with Platelet Engraftment Success [7]	n (%)	0	1 (25.0)	6 (100.0)	3 (75.0)	4 (66.7)	7 (70.0)

[1] From initiation of hospitalisation for conditioning to post drug product infusion discharge; subjects that have not been discharged from the hospital are not included.
[2] VCN in Drug Product (average per subject) calculates an average drug product VCN for each subject prior to statistical calculations
[3] VCN in Drug Product (average per lot) counts each drug product VCN separately prior to statistical calculations.
[4] Defined as the first of 3 consecutive absolute neutrophil count (ANC) laboratory values >= 0.5 × 10^9/L obtained on different days after a post-transplant value of <0.5 × 10^9/L. At the time of data-cut, one subject has not yet reached successful engraftment due to insufficient follow-up.
[5] Defined as the first of 3 consecutive platelet count laboratory values >= 20 × 10^9/L obtained on different days after a post-transplant value of <20 × 10^9/L, while no platelet transfusions were administered for 7 days immediately preceding and during the evaluation period.
[6] Defined as achieving neutrophil engraftment by Day 43 and not receiving back-up cells at any time during the neutropenic phase. At the time of data-cut, one subject has not yet reached successful engraftment due to insufficient follow-up.
[7] Defined as achieving platelet engraftment at any time during the study.

Data as of 22 February 2018

Baseline data

Table 23: Key Demography of Patients with TDT by Parent Study, Genotype, and Overall for Pooled Analyses (TP)

		Phase 1/2 Studies						Phase 3 Studies		All Studies	
		HGB-204			HGB-205	Pooled		HGB-207	HGB-212		
Parameter	Statistic	Non-β⁰/β⁰ (N = 10)	β⁰/β⁰ (N = 8)	All Genotypes (N = 18)	Non-β⁰/β⁰ (N = 4)	Non-β⁰/β⁰ (N = 14)	All Genotypes (N = 22)	Non-β⁰/β⁰ (N = 10)	β⁰/β⁰ (N = 1)	Non-β⁰/β⁰ (N = 24)	All Genotypes (N = 33)
Age at informed consent or assent[1] (years)											
	N	10	8	18		14	22	10		24	33
	Mean	22.2	24.1	23.1		20.9	22.0	18.3		19.8	21.0
	(SD)	(6.60)	(7.62)	(6.92)		(5.95)	(6.62)	(4.30)		(5.37)	(6.15)
	Median	19.5	23.0	20.0		18.5	20.0	19.0		18.5	20.0
	Min, Max	16, 34	12, 35	12, 35		16, 34	12, 35	12, 24		12, 34	12, 35
Age at informed consent or assent[1] (category)											
≥18 years	n (%)									16 (66.7)	24 (72.7)
≥12 to <18 years	n (%)									8 (33.3)	9 (27.3)
Gender											
Male	n (%)									9 (37.5)	12 (36.4)
Female	n (%)									15 (62.5)	21 (63.6)
Race											
Asian	n (%)	8 (80.0)	6 (75.0)	14 (77.8)	2 (50.0)	10 (71.4)	16 (72.7)	6 (60.0)		16 (66.7)	23 (69.7)
White	n (%)	2 (20.0)	2 (25.0)	4 (22.2)	2 (50.0)	4 (28.6)	6 (27.3)	4 (40.0)		8 (33.3)	10 (30.3)

Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies.
Source: Table 2.1.2
[1] Assent is applicable to subjects <18 years old.

Thalassaemia-Related Medical History

Table 24: Thalassaemia-Related Medical History by Parent Study, Genotype, and Overall for Pooled Analyses (TP)

		Phase 1/2 Studies						Phase 3 Studies		All Studies	
		HGB-204			HGB-205	Pooled		HGB-207	HGB-212		
Parameter	Statistic	Non-β^0/β^0 (N = 10)	β^0/β^0 (N = 8)	All Genotypes (N = 18)	Non-β^0/β^0 (N = 4)	Non-β^0/β^0 (N = 14)	All Genotypes (N = 22)	Non-β^0/β^0 (N = 10)	β^0/β^0 (N = 1)	Non-β^0/β^0 (N = 24)	All Genotypes (N = 33)
HBB genotype											
β^0/β^0	n (%)	0	8 (100.0)	8 (44.4)	0	0	8 (36.4)	0	1 (100.0)	0	9 (27.3)
β^E/β^0	n (%)	6 (60.0)	0	6 (33.3)	3 (75.0)	9 (64.3)	9 (40.9)	3 (30.0)	0	12 (50.0)	12 (36.4)
β^0/β^+	n (%)	1 (10.0)	0	1 (5.6)	0	1 (7.1)	1 (4.5)	5 (50.0)	0	6 (25.0)	6 (18.2)
β^+/β^+	n (%)	2 (20.0)	0	2 (11.1)	1 (25.0)	3 (21.4)	3 (13.6)	2 (20.0)	0	5 (20.8)	5 (15.2)
Other[1]	n (%)	1 (10.0)	0	1 (5.6)	0	1 (7.1)	1 (4.5)	0	0	1 (4.2)	1 (3.0)
Splenectomy											
Yes	n (%)	3 (30.0)	3 (37.5)	6 (33.3)	3 (75.0)	6 (42.9)	9 (40.9)	2 (20.0)	1 (100.0)	8 (33.3)	12 (36.4)
No	n (%)	7 (70.0)	5 (62.5)	12 (66.7)	1 (25.0)	8 (57.1)	13 (59.1)	8 (80.0)	0	16 (66.7)	21 (63.6)
Age at β-thalassemia major diagnosis[2] (months)											
										24	33
										49.8 (74.81)	39.9 (67.07)
										13.5	9.0
										0, 315	0, 315
Age at first pRBC transfusion (months)											
										22	30
										32.6 (33.95)	25.7 (31.18)
										18.0	11.5
										0, 132	0, 132
Age at starting regular pRBC transfusions (months)											
										24	33
										88.4 (79.33)	69.8 (75.32)
										72.0	48.0
										1, 312	1, 312

		Phase 1/2 Studies						Phase 3 Studies		All Studies	
		HGB-204			HGB-205	Pooled		HGB-207	HGB-212		
Parameter	Statistic	Non-β^0/β^0 (N = 10)	β^0/β^0 (N = 8)	All Genotypes (N = 18)	Non-β^0/β^0 (N = 4)	Non-β^0/β^0 (N = 14)	All Genotypes (N = 22)	Non-β^0/β^0 (N = 10)	β^0/β^0 (N = 1)	Non-β^0/β^0 (N = 24)	All Genotypes (N = 33)
Age at starting iron chelation (years)											
	N	10	8	18	4	14	22	10	1	24	33
	Mean (SD)	9.7 (7.07)	5.8 (5.37)	7.9 (6.51)	5.0 (4.97)	8.4 (6.72)	7.4 (6.26)	6.0 (4.24)	4.0 (-)	7.4 (5.83)	6.9 (5.61)
	Median	7.5	3.5	6.5	3.5	7.0	6.0	4.5	4.0	6.5	5.0
	Min, Max	2, 26	2, 18	2, 26	1, 12	1, 26	1, 26	2, 16	4, 4	1, 26	1, 26
Pre-treatment baseline annualized pRBC transfusion volume (mL/kg/year)											
	N	10	8	18	4	14	22	10	1	24	33
	Mean (SD)	164.06 (30.419)	196.11 (50.022)	178.31 (42.294)	174.96 (25.606)	167.18 (28.602)	177.70 (39.287)	204.31 (38.583)	160.21 (-)	182.65 (37.346)	185.23 (40.050)
	Median	151.28	182.59	169.05	181.85	154.78	171.15	211.29	160.21	171.11	171.73
	Min, Max	140.0, 234.5	124.4, 273.2	124.4, 273.2	138.8, 197.3	138.8, 234.5	124.4, 273.2	158.7, 251.3	160.2, 160.2	138.8, 251.3	124.4, 273.2
Pre-treatment baseline annualized pRBC transfusion frequency (#/year)											
	N	10	8	18	4	14	22	10	1	24	33
	Mean (SD)	13.45 (1.817)	14.44 (1.935)	13.89 (1.883)	12.13 (1.181)	13.07 (1.730)	13.57 (1.885)	17.75 (3.981)	12.50 (-)	15.02 (3.667)	14.80 (3.274)
	Median	13.75	13.75	13.75	12.50	13.00	13.00	17.75	12.50	14.00	14.00
	Min, Max	10.0, 16.5	12.5, 17.5	10.0, 17.5	10.5, 13.0	10.0, 16.5	10.0, 17.5	11.5, 24.5	12.5, 12.5	10.0, 24.5	10.0, 24.5
Pre-treatment baseline weighted average nadir Hb that preceded pRBC transfusions (g/dL)											
	N	10	8	18	4	14	22	10	1	24	33
	Mean (SD)	8.73 (1.014)	9.38 (0.431)	9.02 (0.855)	9.46 (1.479)	8.94 (1.155)	9.10 (0.967)	9.42 (0.767)	9.72 (-)	9.14 (1.022)	9.21 (0.900)
	Median	9.11	9.43	9.31	9.44	9.11	9.31	9.63	9.72	9.43	9.52
	Min, Max	7.0, 9.8	8.7, 10.1	7.0, 10.1	8.1, 10.8	7.0, 10.8	7.0, 10.8	7.5, 10.2	9.7, 9.7	7.0, 10.8	7.0, 10.8

Parameter	Statistic	Phase 1/2 Studies						Phase 3 Studies		All Studies	
		HGB-204			HGB-205	Pooled		HGB-207	HGB-212		
		Non-β^0/β^0 (N = 10)	β^0/β^0 (N = 8)	All Genotypes (N = 18)	Non-β^0/β^0 (N = 4)	Non-β^0/β^0 (N = 14)	All Genotypes (N = 22)	Non-β^0/β^0 (N = 10)	β^0/β^0 (N = 1)	Non-β^0/β^0 (N = 24)	All Genotypes (N = 33)
Pre-treatment baseline iron burden[3]											
Liver iron content (mg/g)											
	N	10	8	18	4	14	22	10	0[4]	24	32
	Min, Max	1.2, 26.4	0.4, 17.0	0.4, 26.4	3.9, 14.0	1.2, 26.4	0.4, 26.4	1.0, 19.61		1.0,19.61	0.4, 19.61
Cardiac T2* measurement (msec)											
	N	10	8	18	4	14	22	10	0[4]	24	32
	Min, Max	27, 54	10, 37	10, 54	29, 46	27, 54	10, 54	35.30, 50.92		27, 54	10, 54
Serum ferritin (pmol/L)											
	N	10	7	18	4	14	21	10	0[4]	24	31
	Min, Max	1643, 8629	748, 7267	748, 8629	2139, 7097	1643, 8629	748, 8629	349, 10020		349, 10020	349, 10020

Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies.
Source: Table 2.1.3; Table 2.1.4, Listing 2.2.6.5, Listing 2.2.6.6, Listing 2.2.6.7, Interim CSR HGB-207 Listing 16.2.6.7.2, Interim CSR HGB-207 Listing 16.2.6.8, Interim CSR HGB-207 Listing 16.2.6.9
Note: Retrospective pre-treatment transfusion history (pre-treatment baseline annualized pRBC transfusion volume, pre-treatment baseline annualized pRBC transfusion frequency, and pre-treatment baseline weighted average nadir Hb that preceded pRBC transfusions) were collected from the 2 years prior to date of informed consent.
[1] Other genotype reported as: *HBB*:c.92+1G>T & Unknown. The unknown allele is an unidentified β^+ mutation of S since this subject is able to produce some endogenous HbA.
[2] Age at β-thalassemia major diagnosis is calculated as (date of diagnosis - date of birth + 1) / 30.4375
[3] Pre-treatment baseline iron burden values are defined as the last value prior to initiation of conditioning.
[4] Due to the limited follow-up time for the 1 treated subject in Study HGB-212, iron burden data was not included for this subject in this module.

Amongst the transplant population (TP), 72.7% of patients (24/33) were of non-β^0/β^0 genotype. Out of these 24 non-β^0/β^0 patients, 12 patients (50.0%) had the β^E mutation that occurs with high frequency in many Asian countries, correlating with the predominance of patients of Asian descent in the study populations. There were no meaningful differences in percent of splenectomised patients between genotypes or across studies. In Study HGB-204, 30.0% of non-β^0/β^0 patients (3/10) were splenectomised versus 37.5% of β^0/β^0 patients (3/8). The median ages at which the β^0/β^0 subgroup in Study HGB-204 was diagnosed, had their first transfusion, began regular transfusions, and began iron chelation, tended to be younger than for the non-β^0/β^0 subgroup.

All patients were transfusion-dependent in accordance with study entrance criteria for Studies HGB-204, HGB-205, HGB-207, and HGB-212, which included a requirement for patients to have a history of pRBC transfusions of at least 100 mL/kg/year in the 2 years preceding enrolment, or be managed under standard thalassaemia guidelines with ≥8 pRBC transfusions per year in the 2 years preceding enrolment. Overall, there were no apparent differences in pre-treatment transfusion requirements or pre-treatment weighted nadir Hb between β^0/β^0 and non-β^0/β^0 patients with TDT (either within Study HGB-204, or comparing β^0/β^0 from Study HGB-204 to non-β^0/β^0 pooled from Studies HGB-204 and HGB-205). Pre-treatment transfusion requirements and weighted nadir Hb also appeared to be similar, with overlapping ranges, across all studies for non-β^0/β^0 patients. Pre-treatment baseline value ranges for liver iron burden by liver MRI/SQUID, cardiac iron burden by cardiac T2* measurements, and serum ferritin (defined as the last value prior to conditioning). Overall, there was a wide range in iron burden levels at baseline amongst the patients, spanning across the normal range to iron overload, but ranges overlapped between genotypes and across studies.

Numbers analysed

Across the 4 treatment studies investigating the use of Zynteglo for the treatment of TDT (parent Studies HGB-204, HGB-205, HGB-207, and HGB-212), a total of 39 patients with TDT have undergone mobilisation and apheresis for drug product manufacture (i.e., the Intent-to-Treat [ITT] Population) as of the data cuts on 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies. Patient with TDT enrolled in Phase 1/2 Studies HGB-204 and HGB-205 have completed these studies and continue to be followed in Study LTF-303, whereas patients with TDT are still being followed in ongoing Phase 3 Studies HGB-207 and HGB-212.

As of the data cuts on 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies:

- the treated patients from Study HGB-204 (N = 18) have been followed for a median (min, max) of 32.11 (23.1, 41.9) months post-drug product infusion;
- the treated patients with TDT from Study HGB-205 (N = 4) have been followed for a median (min, max) of 38.29 (28.8, 47.7) months post-drug product infusion;
- the treated patients from Study HGB-207 (N = 10) have been followed for a median (min, max) of 5.59 (0.8, 13.2) months post-drug product infusion;
- the single treated patient in Study HGB-212 (who had a β^0/β^0 genotype) has been followed for a duration of 3.0 months post-drug product infusion.

There have been 2 discontinuations from these studies; both patients had non-β^0/β^0 genotypes, both before the initiation of conditioning.

In total as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies, 33 patients were treated with Zynteglo of which 24 had a non-β^0/β^0 genotype. The mean duration of follow up is 21.86 months post drug product infusion for non-β^0/β^0 patients, with 14 patients reaching the 14 months of follow up needed for the assessment of the primary endpoint of transfusion independence.

Table 25: Disposition for Patients with TDT by Parent Study, Genotype, and Overall (ITT)

		Phase 1/2 Studies						Phase 3 Studies		All Studies	
		HGB-204			HGB-205[1]	Pooled[2]		HGB-207[3]	HGB-212[4]		
Parameter	Statistic	Non-β^0/β^0 (N = 11)	β^0/β^0 (N = 8)	All Genotypes (N = 19)	Non-β^0/β^0 (N = 4)	Non-β^0/β^0 (N = 15)	All Genotypes (N = 23)	Non-β^0/β^0 (N = 14)	All Genotypes (N = 2)	Non-β^0/β^0 (N = 30)	All Genotypes (N = 39)
Subjects mobilized (ITT)											
	n (%)	11 (100.0)	8 (100.0)	19 (100.0)	4 (100.0)	15 (100.0)	23 (100.0)	14 (100.0)	2 (100.0)	30 (100.0)	39 (100.0)
Subjects treated with drug product (TP)											
	n (%)	10 (90.9)	8 (100.0)	18 (94.7)	4 (100.0)	14 (93.3)	22 (95.7)	10 (71.4)	1 (50.0)	24 (80.0)	33 (84.6)
Subjects with successful engraftment[5] (SEP)											
	n (%)	10 (90.9)	8 (100.0)	18 (94.7)	4 (100.0)	14 (93.3)	22 (95.7)	9 (64.3)	1 (50.0)	23 (76.7)	32 (82.1)
Subjects completing follow-up Visit (Months post-DPI):											
Month 3	n (%)	10 (90.9)	8 (100.0)	18 (94.7)	4 (100.0)	14 (93.3)	22 (95.7)	8 (57.1)	1 (50.0)	22 (73.3)	31 (79.5)
Month 6	n (%)	10 (90.9)	8 (100.0)	18 (94.7)	4 (100.0)	14 (93.3)	22 (95.7)	6 (42.9)	0	20 (66.7)	28 (71.8)
Month 9	n (%)	10 (90.9)	8 (100.0)	18 (94.7)	4 (100.0)	14 (93.3)	22 (95.7)	3 (21.4)	0	17 (56.7)	25 (64.1)
Month 12	n (%)	10 (90.9)	8 (100.0)	18 (94.7)	4 (100.0)	14 (93.3)	22 (95.7)	2 (14.3)	0	16 (53.3)	24 (61.5)
Month 15	n (%)	10 (90.9)	8 (100.0)	18 (94.7)	4 (100.0)	14 (93.3)	22 (95.7)	0	0	14 (46.7)	22 (56.4)
Month 24	n (%)	10 (90.9)	8 (100.0)	18 (94.7)	4 (100.0)	14 (93.3)	22 (95.7)	0	0	14 (46.7)	22 (56.4)
Month 30	n (%)	5 (45.5)	5 (62.5)	10 (52.6)	4 (100.0)	9 (60.0)	14 (60.9)	0	0	9 (30.0)	14 (35.9)
Month 36	n (%)	5 (45.5)	4 (50.0)	9 (47.4)	2 (50.0)	7 (46.7)	11 (47.8)	0	0	7 (23.3)	11 (28.2)
Month 42	n (%)	2 (18.2)	1 (12.5)	3 (15.8)	2 (50.0)	4 (26.7)	5 (21.7)	0	0	4 (13.3)	5 (12.8)
Month 48	n (%)	0	0	0	2 (50.0)	2 (13.3)	2 (8.7)	0	0	2 (6.7)	2 (5.1)
Total duration of follow-up post DPI (months)											
	N	10	8	18	4	14	22	10	1	24	33
	Mean	30.84	32.00	31.35	38.26	32.96	32.61	6.33	3.02	21.86	23.75
	(SD)	(7.778)	(7.368)	(7.399)	(10.682)	(8.962)	(8.249)	(4.311)	(-)	(15.247)	(14.563)
	Median	29.59	32.48	32.11	38.29	32.10	32.11	5.59	3.02	23.70	24.15
	Min, Max	23.1, 41.9	23.9, 41.5	23.1, 41.9	28.8, 47.7	23.1, 47.7	23.1, 47.7	0.8, 13.2	3.0, 3.0	0.8, 47.7	0.8, 47.7

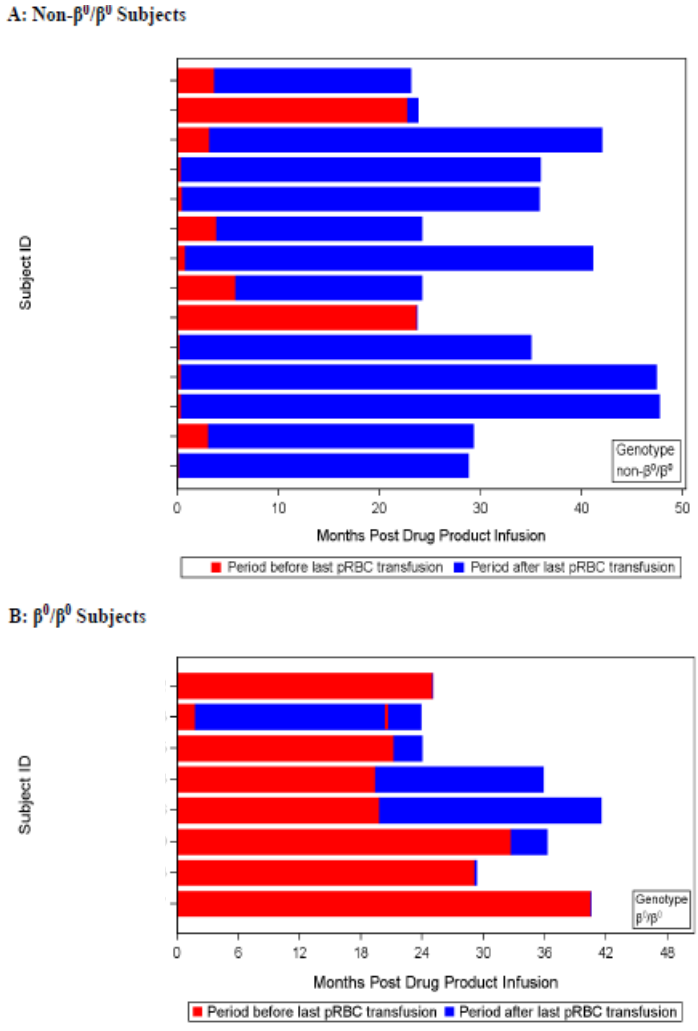
Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies

Outcomes and estimation

Transfusion Independence

The definition of transfusion independence (TI) requires 12 months without any pRBC transfusions while maintaining a weighted average Hb of ≥9 g/dL after a preceding 60 days without any pRBC transfusion. Therefore, no patients in Study HGB-207 were yet evaluable for TI at the 22 February 2018 data cut due to insufficient follow-up time in the parent study.

Figure 11: Duration of Transfusion Periods for Patients from Parent Studies HGB-204 and HGB-205 Through Last Available Visit in Study LTF-303, by Patient



Data as of 07 March 2018

Table 26: Proportion of Patients Who Achieved Transfusion Independence at Any Time, by Parent Study and Genotype (TP from Studies HGB-204 and HGB-205)

Parameter	Statistics	Subjects with TI at Any Time during Parent Study or Long-Term Follow-up					
		HGB-204			HGB-205	Overall	
		Non-β0/β0 (N = 10)	β0/β0 (N = 8)	All Genotypes (N = 18)	Non-β0/β0 (N = 4)	Non-β0/β0 (N = 14)	All Genotypes (N = 22)
Overall	N	10	8	18	4	14	22
	n (%)	8 (80.0)	2 (25.0)	10 (55.6)	3 (75.0)	11 (78.6)	13 (59.1)
	Lower 1-sided 95% CI	49.3	4.6	34.1	24.9	53.4	39.5
	2-sided 95% CI	44.4, 97.5	3.2, 65.1	30.8, 78.5	19.4, 99.4	49.2, 95.3	36.4, 79.3

Data as of 07 March 2018.
Source: Table 2.2.2.1
Abbrev.: CI, confidence interval; TI, transfusion independence; TP, Transplant Population.
Note: The Clopper-Pearson Exact method is used to calculate the lower 1-sided 95% CI and the 2-sided 95% CI for the proportion of subjects meeting this criterion.

For patients of non-β^0/β^0 genotype treated with Zynteglo in studies HGB-204 and HGB-205, 11 out of 14 patients (78.6%; 95% CI of 49.2% to 95.3%) met the definition of TI at any time, with similar proportion of non-β^0/β^0 patients meeting the definition of TI at any time across the 2 studies (8/10, 80%

for Study HGB-204; 3/4, 75% for Study HGB-205). Only 2 out of 8 β^0/β^0 patients (25.0%; 95% CI of 3.2% to 65.1%) met the definition of TI at any time.

Characterisation of Transfusion Independence (Secondary Efficacy Endpoints)

Table 27: Time from Drug Product Infusion to Last pRBC Transfusion, and Time to Reach Transfusion Independence (TP; TI Patients Only)

Parameter	Statistics	HGB-204			HGB-205	Overall	
		Non-β^0/β^0 (N = 10)	β^0/β^0 (N = 8)	All Genotypes (N = 18)	Non-β^0/β^0 (N = 4)	Non-β^0/β^0 (N = 14)	All Genotypes (N = 22)
Time from DPI to last pRBC transfusion prior to TI (months)	N	8	2	10	3	11	13
	Mean (SD)	2.33 (2.098)	10.58 (12.406)	3.98 (5.712)	0.32 (0.137)	1.78 (1.992)	3.13 (5.201)
	Median	2.00	10.58	2.50	0.36	0.46	0.82
	Min, Max	0.3, 5.8	1.8, 19.4	0.3, 19.4	0.2, 0.4	0.2, 5.8	0.2, 19.4
Time to Reach TI (months)	N	8	2	10	3	11	13
	Mean (SD)	17.60 (2.541)	26.60 (12.869)	19.40 (6.150)	15.13 (0.404)	16.93 (2.425)	18.42 (5.648)
	Median	17.10	26.60	17.95	14.90	15.60	15.80
	Min, Max	15.0, 20.9	17.5, 35.7	15.0, 35.7	14.9, 15.6	14.9, 20.9	14.9, 35.7

Data as of 07 March 2018.
Source: Table 2.2.3
Abbrev.: DPI, drug product infusion; pRBC, packed red blood cells; TI, transfusion independence; TP, Transplant Population.

For non-β0/β0 patients who achieved TI at any time (N = 11), median (min, max) duration of time from drug product infusion to last pRBC transfusion was relatively short for all patients, at 0.46 (0.2, 5.8) months. Median (min, max) time to reach TI was 15.60 (14.9, 20.9) months. The 3 non-β0/β0 TI patients from Study HGB-205 were on the lower end of this range for time to reach TI, reporting times of 14.9, 14.9, and 15.6 months. For the 2 β0/β0 patients from Study HGB-204 who achieved TI at any time, duration of time from drug product infusion to last pRBC transfusion was more variable at 1.8 and 19.4 months, with time to reach TI of 17.5 and 35.7 months.

Duration of Transfusion Independence

The duration of TI was analysed using Kaplan-Meier methods, which allows for the censoring of TI at the last Hb assessment if TI is maintained through all Hb assessments, as the true duration of TI cannot be determined when patients continue to maintain their TI status through latest study visit. By assuming TI ended at the last Hb assessment, the observed duration of TI can be calculated.

Table 28: Duration of Transfusion Independence, by Parent Study and Genotype (TP; TI Patients Only)

Parameter	Statistic	HGB-204			HGB-205	Overall	
		Non-β^0/β^0 (N = 10)	β^0/β^0 (N = 8)	All Genotypes (N = 18)	All Genotypes (N = 4)	Non-β^0/β^0 (N = 14)	All Genotypes (N = 22)
Duration of TI (months)[1]	# of events	0	1	1	0	0	1
	# of censored	8	1	9	3	11	12
	25th percentile	-	16.1	-	-	-	-
	(95% CI)	-	-	(16.1, -)	-	-	(16.1, -)
	Median	-	16.1	-	-	-	-
	(95% CI)	-	-	(16.1, -)	-	-	-
	75th percentile	-	16.1	-	-	-	-
	(95% CI)	-	-	-	-	-	-
	Min, Max	16.1+, 38.1+	14.4+, 16.1	14.4+, 38.1+	26.4+, 45.0+	16.1+, 45.0+	14.4+, 45.0+
Observed Duration of TI (months)[2]	N	8	2	10	3	11	13
	Mean (SD)	27.49 (9.010)	15.25 (1.202)	25.04 (9.483)	38.30 (10.333)	30.44 (10.182)	28.10 (10.911)
	Median	32.60	15.25	24.90	43.50	32.70	32.50
	Min, Max	16.1, 38.1	14.4, 16.1	14.4, 38.1	26.4, 45.0	16.1, 45.0	14.4, 45.0

Data as of 07 March 2018.
Source: Late Breaking Table 2.2.3
Note: Transfusion independence (TI) is defined as a weighted average Hb ≥9 g/dL without any RBC transfusions for a continuous period of ≥12 months at any time during the study after drug product infusion. Time period of TI will start when subjects achieve an Hb ≥9 g/dL with no transfusions in the preceding 60 days.
[1] If a TI were maintained through all Hb assessments, the duration of TI will be censored at the last Hb assessment date. The + sign indicates a value is censored. The duration of TI begins with t0 (the time when Hb is first >=9 g/dL with no transfusions in the preceding 60 days) and ends at the time point when subject receives a transfusion or the weighted average Hb falls below 9 g/dL, whichever is earlier. Number of events is the number of TIs that are confirmed ended at a Hb assessment. Number of censored is the number of TIs that are maintained through the last Hb assessment. When a TI is censored, the true duration of TI is unobserved and is longer than the duration from t0 to the last Hb assessment.
[2] Sensitivity analysis of TI assuming TI ended at the last Hb assessment date for censored TIs.

All non-β^0/β^0 patients who have achieved TI at any time (N = 11) have maintained their TI status through all Hb assessments; thus, the duration of TI was censored at the last Hb assessment and no events for loss of TI have yet been recorded. For these 11 non-β^0/β^0 patients who achieved TI at any time, the median duration of TI was not reached and the range for the duration of TI to date was 16.1+ to 45.0+ months. Median observed duration of TI was 32.70 months. The true duration of TI would be longer than these numbers reported.

No patients of β^0/β^0 genotype were TI at Month 24. One (1) β^0/β^0 patient had achieved TI during the parent study at 17.5 months post-drug product infusion. However, they received a single pRBC transfusion due to an acute event of Cat Scratch Disease prior to their Month 21 Visit and thus lost TI status. At Month 24 they had not yet been followed long enough to regain TI status. Another β^0/β^0 patient achieved TI at approximately Month 36, which was the last scheduled visit completed for this patient.

Weighted Average Haemoglobin during Transfusion Independence

For non-β^0/β^0 patients who achieved TI at any time (N = 11), median (min, max) weighted average Hb during TI was 10.64 (9.3, 13.1) g/dL. For the 2 β^0/β^0 patients who achieved TI at any time, their weighted average Hb during TI was 9.8 and 10.1 g/dL. For all patients who achieved TI at any time, during TI their weighted average Hb consists of Hb produced from transgenic and endogenous Hb fractions. This weighted average Hb was generally equal or greater than their baseline pre-treatment weighted average

nadir Hb that was primarily due to chronic pRBC transfusions. These baseline average nadirs ranged from 7.0 to 10.8 g/dL for non-β^0/β^0 patients and was 9.6 or 9.3 g/dL for the 2 β^0/β^0 patients, respectively.

The non-β^0/β^0 patients from Studies HGB-204 and HGB-205 who have achieved TI at any time reported median (min, max) total Hb levels of 10.20 (8.6, 13.4) g/dL at Month 6 (N = 11), 10.70 (9.1, 13.7) g/dL at Month 24 (N = 11), and 12.00 (9.4, 13.5) g/dL at Month 36 (N = 7). Four of these 11 TI patients reported total Hb levels within normal range (respective of their gender) at last visit.

Ancillary analyses

Predicting TI Status in Study HGB-207 From Observed HbA^{T87Q} at Month 6 and Month 9 in Non-β^0/β^0 Patients

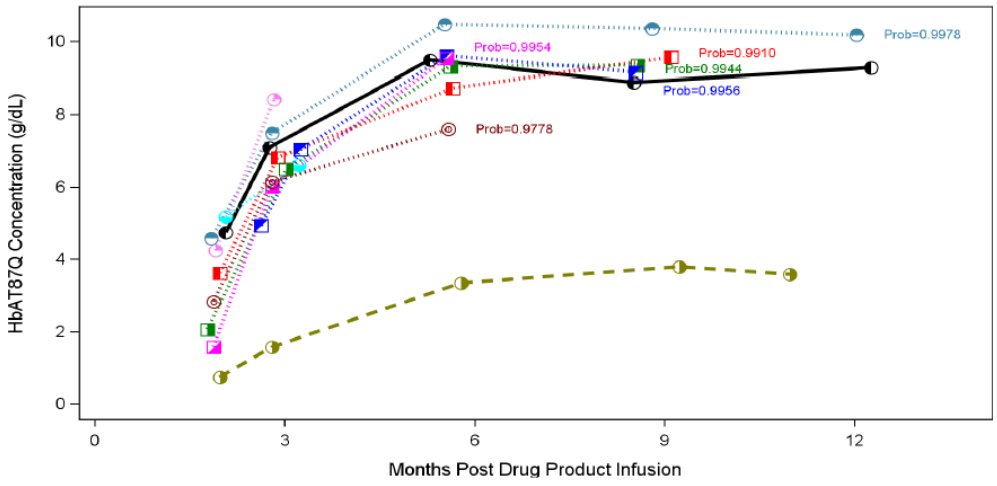
Maximum likelihood estimators from fitted logistic regression models (shown in PD section) were applied to predict the probability of achieving TI at any time or Month 24 for patients in Study HGB-207 with observed HbA^{T87Q} data at Months 6 and/or 9. The model based on month 6 data predicted that 5/6 patients in Study HGB-207 will have approximately 99% probability of achieving TI at any time or Month 24. Results for achieving TI at any time are summarised in Table 29, and observed HbA^{T87Q} values and predicted probabilities are combined and presented in Figure 12.

Table 29: Predicted Probability of Achieving TI of Study HGB-207 Patients

	Using Observed Values at Month 6	Using Observed Value at Month 9
Subject ID	Model 6: Predicted Probability of TI at Any Time	Model 7: Predicted Probability of TI at Any Time
	0.9955	0.9850
	0.6574	0.5737
	0.9979	0.9951
	0.9919	NA
	0.9949	NA
	0.9959	NA

Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies

Figure 12: HbA^{T87Q} over Time in Peripheral Blood with Predicted Probability of Achieving TI (TP; HGB-207)



Data as of 15 May 2018
Note: Individual patients are indicated by different colors. Patients achieving TI are represented with solid lines, patients not achieving TI are represented with dotted lines, and patients not having sufficient follow-up to be evaluable for TI are presented with dashed lines.

Transfusion Reduction (Phase 1/2 Studies HGB-204 and HGB-205)

The 3 non-β^0/β^0 patients from Studies HGB-204 and HGB-205 who have not achieved TI at any time (2 from Study HGB-204 and 1 from Study HGB-205), their percent change in annualized transfusion volume from the period from 6 months post-drug product infusion through Month 24 as compared to annualized baseline pre-treatment transfusion requirements was -26.8%, -86.9%, or -100%, respectively, and they had 3, 11.9, and 13 fewer transfusions per year for the period from 6 months post-drug product infusion through Month 24 as compared to baseline, respectively. All 3 patients maintained similar levels of transfusion reduction from end of the parent study at Month 24 through last study visit.

Transfusion Reduction (Phase 3 Study HGB-207)

For the interim analysis, data were not available to analyse the whole time period; therefore, annualized transfusion requirements for the following time periods were compared: 2 years prior to study enrolment versus hospital discharge after drug product infusion through the last visit. Eight patients in the TP had a "hospitalization for drug product infusion" discharge date, 7 out of 8 patients had 100% reduction in annualized transfusion volume and frequency from hospital discharge through last study visit as compared to pre-enrolment transfusion requirements, with weighted average nadir Hb of ≥11.1 g/dL, which is greater than their pre-enrolment weighted average nadir Hb, indicating that these are likely to be meaningful reductions in transfusion requirements.

Total Haemoglobin for Transfusion-free Patients

- All transfusion-free non-β^0/β^0 patients had their last pRBC transfusion by <178 days post-drug product infusion and remained transfusion-free at last study visit. (A patient is considered transfusion-free if they have a continuous period of ≥12 months without a pRBC transfusion.)
- Out of the 12 transfusion-free non-β^0/β^0 patients from Studies HGB-204 and HGB-205, 11 patients (92%) reported total Hb levels ≥9 g/dL during the majority of their transfusion-free period, with 4 patients reporting total Hb levels within normal range (respective of their gender) at last visit.
- One non-β^0/β^0 patient in Study HGB-207 is transfusion-free and this patient reported total Hb levels ranging from 10.6 to 13.3 g/dL post-drug product infusion; their Hb level has been within normal range since Month 6.

Change in Iron Burden

Iron overload during the study was evaluated by assessing LIC and cardiac T2* by MRI or SQUID, and serum ferritin, serum iron, transferrin, and transferrin saturation in peripheral blood.

In HGB-204: a transient increase in iron burden (as deduced from LIC and serum ferritin levels) was observed in several patients at app. 12 months post DPI, presumably due to pre-treatment hypertransfusion and/or delay in restarting chelation regimens after Zynteglo infusion, and to the effect of myeloablation and transplantation. Cardiac iron content was evaluated by MRI at Baseline (last value prior to conditioning), Month 12 and Month 24. No trend in cardiac iron content is observed. Median % change from Baseline to Month 24 value is -5.26% for patients who achieved TI at any time during the study (N=9) versus 0% change for patients who did not achieve TI.

The majority of patients who achieved TI and who were evaluable in study HGB-204 showed

improvements in ferritin (7/8 patients), serum transferrin (7/9 patients), serum iron (7/9 patients), and transferrin saturation (5/7 patients). The decrease in ferritin levels are generally greater for patients who achieved TI status versus patients who did not achieve TI status by Month 24: median change from Baseline at Month 24 was -26.1% for TI patients, versus -5.1% for non-TI patients, although results were variable between patients.

For study HGB-207, iron burden was not analysed in the interim CSR, due to the limited time of follow up. As of the data cut for the interim CSR, 2 out of 10 patients had re-started iron chelation after drug product infusion (follow-up of app. 7 and 12 months after drug product infusion).

Summary of main studies

The following tables summarise 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 30: Summary of Efficacy for the pooled efficacy analysis of patients treated in studies HGB-204 and HGB-205:

Title: Pooled Efficacy Analysis			
Study identifier	**Pooled Efficacy Analysis**		
Design	Phase 1/2 Studies non-β^0/β^0 Pooling (HGB-204, HGB-205 TDT patients) including long-term follow-up data for these patients in LTF-303 will be the primary analysis set, HGB-207 was not included due to the limited follow up.		
	Study start:	HGB-204: 05 September 2013 HGB-205:07 June 2013 LTF-303: 06 January 2014	
	Study Completion:	HGB-204: 21 February 2018 (Last patient/last visit), HGB-205: ongoing; 11 October 2017 (data cut date for pooled analyses) LTF-303: ongoing; 07 March 2018 (data cut date for pooled analyses)	
Hypothesis	None		
Treatments groups	N/A		Single arm
Endpoints and definitions	Primary endpoint	TI (transfusion independence)	defined as the proportion of patients with a weighted average haemoglobin (Hb) ≥9 g/dL without any pRBC transfusions for a continuous period of ≥12 months at any time during the study after Zynteglo infusion

	Secondary endpoint	TR	defined as the proportion of patients with at least 60% reduction in transfusion requirements compared to that in the 2 years prior to study enrolment
	Secondary endpoint	Characterization of TI	duration, time to achievement, time from infusion to last pRBC transfusion, weighted Hb during TI, proportion of patients with TI at Months 18 and 24
Database lock	Data cut date of 7 Mar 2018, only HGB-207 had data cut date of 22 Feb 2018		

Results and Analysis

Analysis description	**Primary Analysis**	
Analysis population and time point description	Transplant Population (TP): All patients in the ITT population who underwent Zynteglo infusion.	
Descriptive statistics and estimate variability	Treatment group	Non-β^0/β^0
	Number of patients	14
	TI 2-sided 95%CI	11 (78.6%) 49.2%, 95.3%
	TR in patients that did not achieve TI at any time (n=3) - % change in annualized transfusion volume from the period from 6 months post-DPI through Month 24 as compared to annualized baseline pre-treatment transfusion requirements: - fewer transfusions per year for the period from 6 months post-DPI through Month 24 as compared to baseline	-26.8%, -86.9%, -100% 3, 11.9, 13
	Observed duration of TI* * median duration of TI not reached	Median (min, max): 32.7 (16.1, 45.0)
	Time to reach TI (months)	Median (min, max): 15.6 (14.9, 20.9)
	weighted average Hb during TI median (min, max)	10.64 (9.3, 13.1) g/dL

	median Total Hb (g/dl) during TI (min, max)	*Month 6* (n=11): 10.20 (8.6, 13.4) *Month 24* (n=11): 10.70 (9.1, 13.7) *Month 36* (N = 7): 12.00 (9.4, 13.5)

Clinical studies in special populations

Study HGB-204 included 3 adolescents (out of 18 patients total), study HGB-205 included 2 adolescents (out of 4 patients total). Study HGB-207 is ongoing, as of the data cut on 22 February 2018, 4 out of 10 included patients were adolescents. It is noted that HGB-207 is designed to include 2 cohorts of patients with TDT of non-β^0/β^0 genotype: Cohort 1 should include at least 15 patients ≥12 and ≤50 years of age, and Cohort 2 should include at least 8 patients <12 years of age.

Moreover, it is noted that the oldest person included in All Studies and all genotypes was 35 years old. The oldest non-β^0/β^0 patients was 34 years old (included in HGB-204).

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

Supportive study

Long-term Follow up study LTF-303

This study is a multi-center, long-term safety and efficacy follow-up study. After monitoring of a patient in the parent study was completed (where the parent study includes approximately 2 years of follow-up after drug product infusion), patients were eligible to enrol in Study LTF-303. During Study LTF-303, patients were followed every 6 months from 2 years through 5 years post-drug product infusion and then annually from 5 years through 15 years post-drug production infusion for a total of 13 years of follow-up in Study LTF-303. The study started on 06 January 2014 (First patient signed informed consent). The interim CSR Data Cut Date: 21 November 2017 (After first patient with TDT treated with Zynteglo in parent study completed the Month 48 Visit). Study LTF-303 continues to be ongoing to date.

Seventeen patients with TDT treated with Zynteglo (13 from Study HGB-204 and 4 from Study HGB-205) consented to enrol in Study LTF-303, and all of these patients continue to participate in Study LTF-303 as of the data cut for this interim CSR, with a median (min, max) follow-up time of 29.93 (23.1, 47.3) months post-drug product infusion. Three patients with TDT treated with a related drug product manufactured from the HPV569 LVV encoding $\beta^{A\text{-}T87Q}$-globin (all from Study LG001) consented to enrol in Study LTF-303. One of these patients subsequently met the VCN discontinuation criterion and was discontinued from the study. The other 2 patients have been followed for approximately 5 or 10 years after drug product infusion.

Of the 17 enrolled Zynteglo-treated patients with TDT, 13 were female (76.5%) and 4 were male (23.5%). Median (min, max) age at consent or assent in parent study was 20.0 (16, 35) years; 14 patients were ≥18 years of age (82.4%) and 3 patients were adolescents (17.6%). Twelve patients identified as Asian (70.6%) and 5 patients identified as White (29.4%). Eleven patients were of non-β^0/β^0 genotype (64.7%) and 6 patients were of β^0/β^0 genotype (35.3%). The 2 eligible patients treated

with the related drug product manufactured from the HPV569 LVV enrolled in Study LTF-303 had the following demographic characteristics were young adults and of β^E/β^0 genotype.

Summary of Efficacy for LTF-303 as of 21 November 2017:

Transfusion Independence and Transfusion Reduction

- Out of the 17 treated patients with TDT, 10 patients (all with non-β^0/β^0 genotype) were TI upon entry into Study LTF-303 at Month 24. For patients who have additional follow-up in Study LTF-303 beyond entry at Month 24 (i.e., have completed at least their Month 30 Visit; N = 12 [7 TI and 5 non-TI]), the TI status achieved by the end of the parent study at Month 24 (either TI or non-TI) has been maintained for each patient throughout Study LTF-303.
- For patients who have achieved TI (N = 10), the median (min, max) duration of TI was 30.00 (17.1, 45.0) months as of the data cut, which includes the patient who has been followed for the longest time (with last scheduled visit completed of Month 48). For the 7 TI patients who have completed at least their Month 30 Visit, the weighted average Hb ranged from 9.7 to 13.2 g/dL for the period from 6 months through last visit, as compared their Hb levels during parent study with weighted average Hb ranging from 9.4 to 13.2 g/dL for the period from 6 months through end of parent study at Month 24.
- For the 7 patients who have not achieved TI (1 non-β^0/β^0 and 6 β^0/β^0), 6 patients have completed at least their Month 30 Visit and their annualized pRBC transfusion requirements (volume or frequency), and weighted average nadir Hb, were relatively stable from end of the parent study at Month 24 through long-term follow-up (up to Month 36). Two of these patients appear to show a sustained decrease in pRBC transfusion requirements during Study LTF-303 compared to requirements in the parent study but should be followed for a longer period of time to confirm this trend. Overall, for non-TI patients (N = 7), reductions in annualized pRBC transfusion volumes from 6 months through last visit as compared to pre-treatment requirements ranged from 40.1% to 100%, with 1 β^0/β^0 patient achieving <50% (40.1%), 1 β^0/β^0 patient achieving ≥50% to <60% (52.8%), 2 β^0/β^0 patients achieving ≥60% to <75%, 2 β^0/β^0 patients achieving ≥75% to <90%, and 1 non-β^0/β^0 patient achieving 100% reduction.

Iron Burden

- Only 4 patients had data for LIC beyond Month 24. For the 2 of these patients who had LIC values >7 mg/g (the threshold at which organ damage generally occurs) at baseline, both showed decreases in LIC over time, with the patient with the longest follow-up showing the biggest improvement in LIC.
- For the 3 patients with available data for cardiac iron content beyond Month 24, there was not a clear trend across patients for change in cardiac T2* values, with all patients maintaining normal values >20 ms. The patient with the longest follow-up showed improvement in cardiac iron burden.
- All 7 TI patients who have additional follow-up in Study LTF-303 beyond entry at Month 24 showed lower serum ferritin values at last visit as compared to Month 24. In particular, the TI patient with the longest follow-up showed a reduction in serum ferritin levels from 1393 pmol/L at Month 24 to 465 pmol/L a Month 48, reaching well below serum ferritin levels typically associated with clinically significant iron overload. For the 5 non-TI patients who have additional follow-up in Study LTF-303 beyond entry at Month 24 (N = 3 with last scheduled visit completed of Month 30 and N = 2 with Month 36), there was no clear trend across these patients for change in serum ferritin levels at last scheduled visit completed as compared to Month 24.

Dyserythropoiesis

- The 7 TI patients, all of whom were of non-β^0/β^0 genotype, who have additional follow-up in Study LTF-303 beyond entry at Month 24, maintained the pattern of reticulocyte/erythrocyte % established by the end of the parent study, with 4 patients having normal or near normal %, and 3 having higher than normal %.

For patients treated with the related drug product (N = 2):

- Neither of the 2 patients were able to achieve a stable total Hb ≥9 g/dL in the absence of pRBC transfusions during long-term follow-up.

2.5.3. Updated data on efficacy parameters: 13 December 2018

An additional data cut was performed of the HGB-207, HGB-212, and LTF-303 clinical database on 13 December 2018. A total of 48 patients with TDT have been treated with Zynteglo and followed for up to 60 months (up to 22 months in Phase 3 studies) as of 13 December 2018, including 42 patients ≥12 years of age with TDT (32 patients with a non-β^0/β^0 genotype and 10 patients with a β^0/β^0 genotype).

Below is a brief overview of the baseline characteristics and efficacy analysis as of 13 December 2018, based on 32 adult and adolescent patients with TDT and a non-β^0/β^0 genotype treated with Zynteglo (N=10, HGB-204; N=4, HGB-205; N=15, HGB-207; N=3, HGB-212).

Table 31: Baseline characteristics for non-β^0/β^0 patients with TDT ≥12 years of age treated with Zynteglo (Studies HGB-204, HGB-205, HGB-207, HGB-212, LTF-303)

Study	**Total number of patients** (young adults/ adolescents)	**Age** (years) median (min, max)	**Pre-enrolment transfusion volumes** (mL/kg/year) median (min, max)	**Pre-enrolment transfusion frequency** (number/year) median (min, max)
HGB-205	4 (2)	young adults/ adolescents	181.85 (138.8, 197.3)	12.50 (10.5, 13.0)
HGB-204	10 (2)	19.5 (16, 34)	151.28 (140.0, 234.5)	13.75 (10.0, 16.5)
HGB-207	15 (6)	20.0 (12, 34)	192.92 (152.3, 251.3)	17.50 (11.5, 37.0)
HGB-212	3 (1)	young adults/ adolescents	175.51 (170.7, 209.6)	21.50 (17.5, 39.5)

Adolescents were excluded from Phase 3 studies if they had a known and available HLA-matched related HSC donor. The median (min, max) age in the studies was 19.0 (12, 34) years, 56.3% were females, 59.4% were Asian, and 40.6% White/Caucasian. All patients had a Karnofsky performance score ≥80 and the majority had a performance score of 100 at baseline. Cardiac T2* at baseline was >20 msec. The median (min, max) serum ferritin at baseline was 3778.7 (784, 22517) pmol/L and median (min, max) liver iron concentration was 6.75 (1.0, 41.0) mg/g (N=10, HGB-204; N=4, HGB-205; N=15, HGB-207; N=3, HGB-212).

Transfusion Independence:

In total, 15 out of 19 non-β^0/β^0 patients have achieved TI (78.9%; 95% CI of 54.4% to 93.9%), suggesting that the great majority of patients with a non-β^0/β^0 genotype treated with Zynteglo will

achieve TI. All non-β^0/β^0 patients who achieved TI have maintained TI. The observed duration of TI ranged from 12.0 to 56.3 months post-drug product infusion.

Most non-β^0/β^0 patients treated in Studies HGB-204 and HGB-205 (11/14, 78.6%; 95% CI of 49.2% to 95.3%) have achieved TI (see Table 32 below). Among the 11 non-β^0/β^0 patients treated in Studies HGB-204 and HGB-205 who achieved TI, the median (min, max) weighted average Hb during TI was 10.51 (9.3, 13.2) g/dL. These patients have all maintained TI, with a min, max duration of TI of 21.2 to 56.3 months as of 13 December 2018 (see Table 33 below). Median (min, max) time to last RBC transfusion was 0.46 (0.2, 5.8) months following Zynteglo infusion.

The non-β^0/β^0 patients who did not achieve TI in Phase 1/2 studies were either producing relatively low amounts of HbA^{T87Q} (2 patients with 0.97 and 3.64 g/dL at last study visit, respectively) or low amounts of endogenous HbA (1 patient with 6.72 g/dL of HbA^{T87Q} at Month 24 but 1.0 g/dL of endogenous HbA at Month 24) compared to the other non-β^0/β^0 patients.

Table 32: Proportion of patients who achieved transfusion independence at any time, by parent study and genotype (TP from phase ½ studies HGB-204 and HGB-205

Statistics	Subjects with TI at Any Time during Parent Study or Long-Term Follow-up					
	HGB-204			HGB-205	Overall	
	Non-β^0/β^0 (N = 10)	β^0/β^0 (N = 8)	All Genotypes (N = 18)	Non-β^0/β^0 (N = 4)	Non-β^0/β^0 (N = 14)	All Genotypes (N = 22)
N	10	8	18	4	14	22
n (%)	8 (80.0)	3 (37.5)	11 (61.1)	3 (75.0)	11 (78.6)	14 (63.6)
Lower 1-sided 95% CI	49.3	11.1	39.2	24.9	53.4	43.9
2-sided 95% CI	44.4, 97.5	8.5, 75.5	35.7, 82.7	19.4, 99.4	49.2, 95.3	40.7, 82.8

Data as of 13 December 2018.
Source: Day 120 PD/SCE Table 2.2.3.1
Abbrev.: CI, confidence interval; TI, transfusion independence; TP, Transplant Population.
Transplant Population is defined as all subjects who received Zynteglo.
Note: The Clopper-Pearson Exact method is used to calculate the lower 1-sided 95% CI and the 2-sided 95% CI for the proportion of subjects meeting this criterion.

Table 33: Duration of Transfusion Independence, by Parent Study and Genotype (TP; TI Patients Only)

Parameter	Statistic	Phase 1/2				Phase 3	All Studies	
		HGB-204		HGB-205	Overall	HGB-207		
		Non-β^0/β^0 (N = 10)	β^0/β^0 (N = 8)	Non-β^0/β^0 (N = 4)	Non-β^0/β^0 (N = 14)	Non-β^0/β^0 (N = 15)	Non-β^0/β^0 (N = 32)	All Genotypes (N = 42)
Duration of TI (months)[1]	# of events	0	1	0	0	0	0	1
	# of censored	8	2	3	11	4	15	17
	25th percentile	-	16.1	-	-	-	-	-
	(95% CI)	-	(16.1, -)	-	-	-	-	(16.1, -)
	Median	-	-	-	-	-	-	-
	(95% CI)	-	(16.1, -)	-	-	-	-	-
	75th percentile	-	-	-	-	-	-	-
	(95% CI)	-	(16.1, -)	-	-	-	-	-
	Min, Max	21.2+, 45.3+	16.1, 20.8+	34.9+, 56.3+	21.2+, 56.3+	12.0+, 18.2+	12.0+, 56.3+	12.0+, 56.3+
Observed Duration of TI (months)[2]	N	8	3	3	11	4	15	18
	Mean (SD)	36.39 (8.659)	17.77 (2.631)	47.43 (11.161)	39.40 (10.199)	14.35 (2.909)	32.72 (14.408)	30.23 (14.306)
	Median	37.95	16.40	51.10	38.00	13.60	34.90	30.00
	Min, Max	21.2, 45.3	16.1, 20.8	34.9, 56.3	21.2, 56.3	12.0, 18.2	12.0, 56.3	12.0, 56.3

Data as of 13 December 2018.

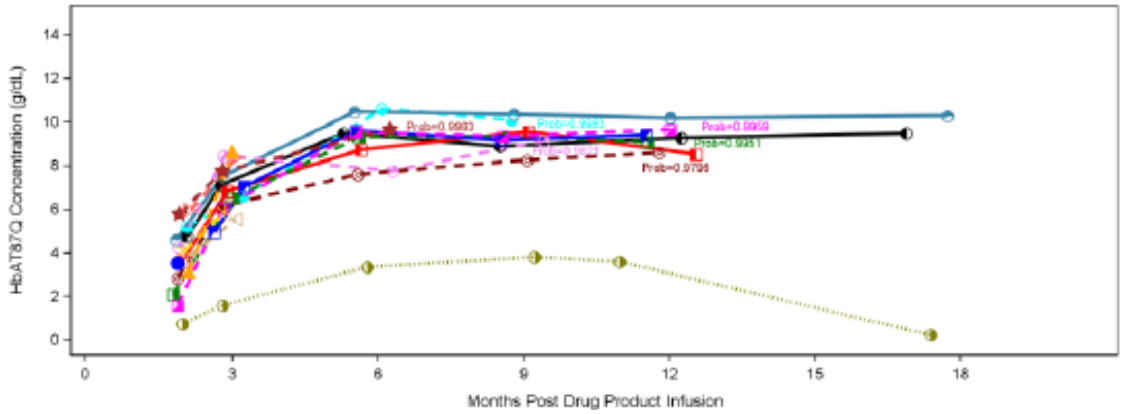
Study HGB-207:

A total of 4 out of the 5 TI-evaluable patients achieved TI (80.0%; 95% confidence interval [CI] of 28.4% to 99.5%). Median (min, max) time to reach TI for these 4 patients was 15.15 (15.0, 16.2) months post-drug product infusion with a min, max observed duration of TI to date of 12.0+, 18.2+ months and median (min, max) weighted average Hb during TI of 12.42 (11.5, 12.6) g/dL. Notably all patients who were predicted to achieve TI based on PD modelling that have become evaluable for TI (N = 3) have achieved TI, validating the predictive value of the logistic regression models (see figure below).

For the 6 additional non-β^0/β^0 patients ≥12 years of age (in Study HGB-207) with Month 6 HbA^{T87Q} data, the updated logistic regression model was applied to predict the probability of achieving TI by Month 24 and at any time. Results predict that they have an approximately 98% or higher probability of achieving TI by end of study.

Of the 11 non-β^0/β^0 patients ≥12 years of age who have completed at least their Month 6 Visit in Phase 3 studies, 10 patients (90.9%) have achieved TI (n = 4) or are predicted to achieve TI (n = 6), including 2 patients with the severe non-β^0 IVS-I-5 mutation (1 patient with IVS-I-5/IVS-I-5 and 1 patient with IVS-I-5/β^0) who produce approximately 0.3 g/dL or less of endogenous HbA in the absence of pRBC transfusions (1 patient who has achieved TI and the other predicted to achieve TI).

Table 34: HbA^{T87Q} over time in peripheral blood with predicted probability of achieving transfusion independence



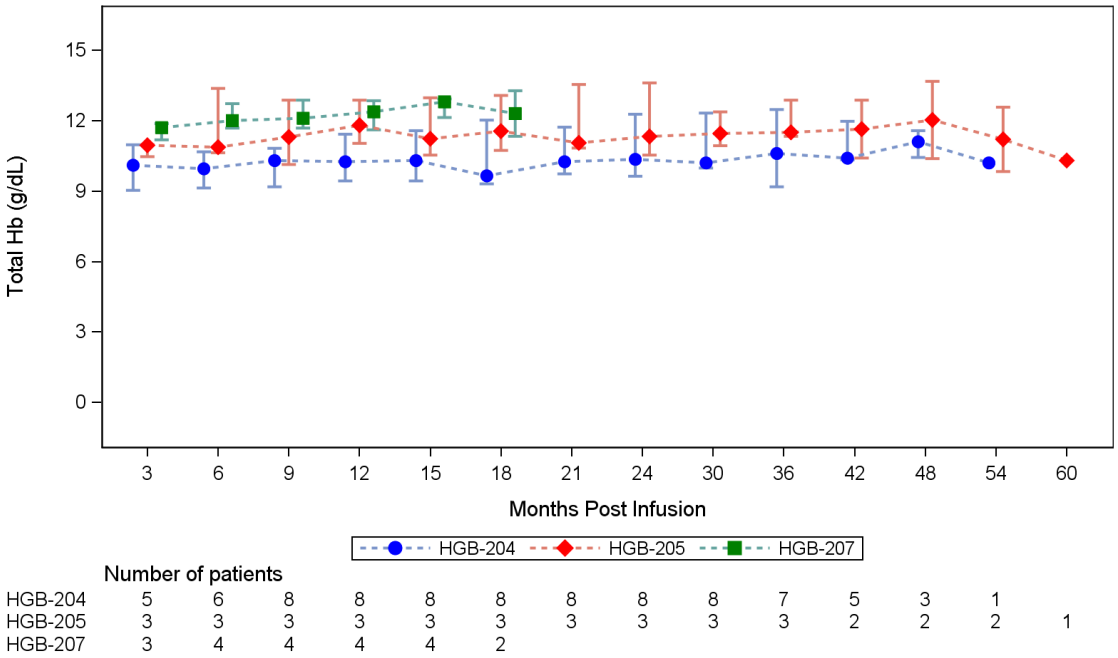
Data as of 13 December 2018
Source: Day 120 PD/SCE Figure 2.2.15
Abbrev.: Prob = probability of achieving transfusion independence
Note: Subjects achieving TI are represented with solid lines, subjects not achieving TI are represented with dotted lines. and subjects not having sufficient follow-up to be evaluable for TI are presented with dashed lines. Subject showed a decline in HbA^{T87Q} after resuming regular pRBC transfusions at approximately 15 months post-drug product infusion. All other subjects have not received any pRBC transfusions since less than 2.5 months post-drug product infusion.

Haemoglobin levels in TI patients

Non-β^0/β^0 patients from Studies HGB-204 and HGB-205 who have achieved TI at any time reported median (min, max) total Hb levels in the absence of transfusions of 10.70 (8.6, 13.4) g/dL at Month 6 (N = 9), of 10.70 (9.1, 13.7) g/dL at Month 24 (N = 11), 11.40 (9.1, 13.5) g/dL at Month 36 (N = 10), and 11.10 (10.4, 13.7) g/dL at Month 48 (N = 5).

For the 4 non-β^0/β^0 patients from Study HGB-207 who have achieved TI (which included a patient homozygous for the severe β^+ variant IVS-I-5), median (min, max) total Hb in the absence of transfusions at Month 6 was 12.00 (11.5, 13.3) g/dL. Patients from Study HGB-207 do not yet have Hb data for Month 24 or beyond, but available data at Month 12 for 8 patients from Study HGB-207 (TI and non-TI) reported median (min, max) total Hb values in the absence of transfusions of 12.40 (8.4, 13.1) g/dL. Median total haemoglobin over time for non-β^0/β^0 patients who have achieved TI is presented in Figure 13.

Figure 13: Median total haemoglobin over time in non-β^0/β^0 patients with TDT treated with Zynteglo who have achieved transfusion independence (Studies HGB-204, HGB-205, HGB-207, LTF-303)



Bars represent interquartile ranges
Data as of 13 December 2018

Transfusion reduction in non-TI patients

As of 13 December 2018, 5 out of 8 patients (including 2 out of 3 non-β^0/β^0 patients) in Phase 1/2 studies who did not achieve TI attained a >60% reduction in pRBC transfusion volumes. Median (min, max) change in pRBC transfusion volume for the 8 non-TI patients was −66.55% (−100.0%, −10.3%) from 6 months post-drug product infusion through last follow-up as compared to their baseline pretreatment transfusion requirements. In the 3 Phase 1/2 non-β^0/β^0 patients who did not achieve TI, reductions of 100%, 86.9% and 26.8% in transfusion volume requirements and of 100%, 85.3% and 20.7% in transfusion frequency were observed between Month 6 through Month 24 visit when compared to their pre-study levels of RBC transfusions. For these patients, the >60% reduction in transfusion requirements were associated with weighted average nadir Hb similar to pre-treatment weighted average nadir Hb. Thus, while the transduction efficiency in Phase 1/2 studies was not sufficient to provide adequate expression of HbA^{T87Q} to support TI in these patients, they still received clinical benefit with transfusion reduction.

For the 4 patients in Study HGB-207 who have completed at least their Month 12 Visit but have not achieved TI, 3 patients demonstrated 100% reduction in annualized transfusion volume, while the remaining patient had a 75.8% reduction.

Most non-β^0/β^0 patients required pRBC transfusions for less than 6 months after drug product infusion in Phase 1/2 studies HGB-204 and HGB-205 and less than 3 months after drug product infusion in Phase 3 study HGB-207. Median (min, max) time from drug product infusion to last pRBC transfusions for non-β^0/β^0 patients treated in Studies HGB-204 and HGB-205 was 1.95 (0.2, 31.6) months (N = 14) and for non-β^0/β^0 patients in Study HGB-207 was 0.95 (0.5, 19.7) months (N = 15).

Use of Iron Removal Therapy

After Zynteglo infusion, patient iron levels were managed at physician discretion.

All patients in HGB-204 restarted iron chelation and continue to use iron chelators. One patient in HGB-205 restarted iron chelation and continues to use iron chelators. Three patients in HGB-205 started phlebotomy.

For HGB-207, of the 11 patients followed for at least 6 months after Zynteglo infusion, 6 patients did not restart iron chelation or receive phlebotomy, 3 patients restarted iron chelation, and 2 patients received phlebotomy to reduce iron levels. Of the 9 patients who have not received phlebotomy, 8 of these patients have maintained a weighted average nadir Hb of >11 g/dL from hospital discharge post-drug product infusion through last follow-up, which would be sufficient to stop chelation therapy and support phlebotomy.

Change in Iron Burden

The majority of non-β^0/β^0 patients who have achieved TI in the Phase 1/2 studies are beginning to demonstrate iron burden levels that are lower than their pre-treatment baseline values at last follow-up.

At 48 months after infusion of Zynteglo for patients who achieved TI, the median reduction (min, max) in serum ferritin levels from baseline was 75.02% (39.2, 84.8) (N=3, HGB-204; N=2, HGB-205). The median reduction in LIC from baseline was 67.14%, ranging from an 83.3% reduction to a 269.2% increase (N=3, HGB-204; N=2, HGB-205). Six of the 11 non-β^0/β^0 patients from Studies HGB-204 and HGB-205 who have achieved TI (with range of follow-up of 29.3 to 58.6 months post-drug product infusion) had an LIC value at last follow-up that was lower than their pre-treatment baseline value.

All 11 non-β^0/β^0 patients from Studies HGB-204 and HGB-205 who have achieved TI continue to have normal cardiac T2* values (i.e., >20 ms) from baseline through last follow-up, with 6 patients demonstrating a cardiac T2* value at last follow-up that was higher than their pre-treatment baseline.

Additionally, all 11 non-β^0/β^0 patients from Studies HGB-204 and HGB-205 who have achieved TI had a serum ferritin value at last follow-up that was lower than their pre-treatment baseline value (median [min, max] serum ferritin at Baseline was 3885 (1643, 8629) pmol/L.

There is not yet sufficient follow-up in Phase 3 studies to make a determination on changes in iron burden.

Dyserythropoiesis

Exploratory analyses were performed to confirm resolution of dyserythropoiesis, the fundamental physiologic characteristic of TDT, in the bone marrow. Bone marrow biopsies taken before treatments were consistent with a diagnosis of TDT, including a low myeloid/erythroid ratio (N=15, HGB-207), reflective of erythroid hyperplasia. For 8 patients who had sufficient on-study follow-up to obtain a 12-month follow-up bone marrow assessment, myeloid/erythroid ratios for 7 patients increased from a range of 0.1 to 0.5 at baseline to a range of 0.6 to 1.9 approximately 12 months after Zynteglo infusion, suggesting that Zynteglo improves erythropoiesis in patients with TDT.

Additionally, of the 11 non-β^0/β^0 patients who have achieved TI in Phase 1/2 studies. 4 patients had normal percent reticulocytes, 4 patients had no nucleated RBCs, and 5 patients had serum transferrin receptor levels within normal range at last follow-up, indicating normalization of dyserythropoiesis for these patients.

In summary, key pharmacodynamic and efficacy outcomes for non-β^0/β^0 TDT patients treated with Zynteglo in Studies HGB-204, HGB-205, HGB-207, and LTF-303 are included in Table 35.

Table 35: Efficacy outcomes for non-β^0/β^0 TDT patients treated with Zynteglo (Studies HGB-204, HGB-205, HGB-207, LTF-303)

HbA^{T87Q} (g/dL) at 6 months n median (min, max)	**HbA^{T87Q} (g/dL) at 24 months** n median (min, max)	**Hb (g/dL) at 6 months*** n median (min, max)	**Hb (g/dL) at 24 months*** n median (min, max)	**TI**** n/N^ (%) [95% CI]	**WA Hb during TI (g/dL)** n median (min, max)	**Duration of TI (months)** N median (min, max)
Study HGB-205						
4 7.543 (4.94, 9.59)	4 8.147 (6.72, 10.13)	4 10.73 (7.6, 13.4)	4 10.91 (8.8, 13.6)	3/4 (75.0%) [19.4, 99.4]	3 11.30 (10.5, 13.0)	3 NR (34.9+, 56.3+)
Study HGB-204						
10 4.153 (1.03, 8.52)	10 5.418 (1.10, 9.60)	7 9.20 (7.7, 13.3)	8 10.35 (9.1, 13.7)	8/10 (80.0%) [44.4, 97.5]	8 10.27 (9.3, 13.2)	8 NR (21.2+, 45.3+)
Study HGB-207						
11 9.494 (3.35, 10.60)	NA***	11 11.90 (8.4, 13.3)	NA***	4/5 (80.0%) [28.4, 99.5]	4 12.42 (11.5, 12.6)	4 NR (12.0+, 18.2+)

*Patients who have not received transfusions in the prior 60 days.
**Transfusion independence (TI): a weighted average Hb ≥9 g/dL without any RBC transfusions for a continuous period of ≥12 months at any time during the study after medicinal product infusion.
***No patients are currently evaluable for these endpoints.
^N represents the total number of patients evaluable for TI, defined as patients who have completed their parent study (i.e., 24 months of follow-up), or achieved TI, or won't achieve TI in their parent study.
NR= Not reached. Hb=Total Hb. WA Hb = Weighted average Hb
Data as of 13 December 2018

2.5.4. Discussion on clinical efficacy

Design and conduct of clinical studies

Efficacy results have been presented for HGB-204, 205 and 207 presenting data from 32 patients of the non-β^0/β^0 genotype and 9 β^0/β^0 genotype patients 12 years and older. The pivotal phase 3 study for patients with a non-β^0/β^0 genotype is the ongoing study HGB-207 conducted in Europe, US and Thailand. During the clinical studies the process development has been optimised to generate maximum HbA^{T87Q} expression, this resulted in variations in the drug product between the studies.

All three efficacy studies were single arm studies. The natural course of β-thalassaemia is well known with a clinically stable presentation over time, and patients do not spontaneously generate clinically meaningful levels of HbA or become transfusion independent. This notion together with the within-patient comparison where two years of retrospective haematological data was collected from medical records for each patient to be enrolled in the study, and each patient will serve as his/her own control for evaluation of transfusion requirements, reduces the need for a concurrent internal control to isolate the treatment effect. This approach was discussed under the PRIME scheme. Allogeneic HSCT would not be an appropriate control given that there are different conditioning regimens required; therefore allogeneic HSCT is associated with a different benefit/risk as compared to autologous transplantation due to the regimens' different safety profile. The currently applied-for target population for Zynteglo are patients for whom a HLA-matched related donor is not available. Standard supportive care with transfusion and chelation would also not be an appropriate comparator for the primary and key secondary study endpoints of transfusion independence and transfusion reduction because patients with TDT receiving supportive care do not spontaneously achieve transfusion independence or have significant reductions

in their transfusion requirements. Altogether, the single arm study design was agreed in a scientific advice (EMEA/H/SAH/038/1/2015/PA/SME/ADT/II).

This Marketing Authorisation Application (MAA) applies for the treatment of patients with TDT who have a non-β^0/β^0 genotype. All patients were transfusion-dependent in accordance with study entrance criteria for Studies HGB-204, HGB-205, HGB-207, and HGB-212, which included a requirement for patients to have a history of pRBC transfusions of at least 100 mL/kg/year in the 2 years preceding enrolment, or to be managed under standard thalassaemia guidelines with ≥8 pRBC transfusions per year in the 2 years preceding enrolment.

The primary endpoint, transfusion independence (TI) requires 12 months without any pRBC transfusion while maintaining a weighted average Hb of ≥9 g/dL after a preceding 60 days without any pRBC transfusion. The definition was similar across all clinical studies. For comparison, the known rate of achieving TI in patients with β-thalassaemia who have not received an allogeneic HSCT is zero, as TDT patients do not spontaneously become transfusion independent. Therefore, achieving and maintaining TI is attributable to the therapeutic effect of Zynteglo which is considered clinically relevant. The proposed secondary endpoints are considered adequate to provide additional support. To reduce bias in the open-label design the post-treatment transfusion guidelines were pre-specified in the protocol. As patient serves as its own control and for reliability of clinical endpoints, it was of utmost importance to have a well-documented (and standardised) information on pre-baseline transfusion requirements. Thus, centres were carefully selected in clinical trials. Only patients treated and followed for at least the past 2 years in a specialized centre that maintained detailed medical records on RBC transfusions, in-patient hospitalisation and iron chelation history could be included in clinical studies.

Treatment was first offered to adults, then expanded to adolescents, and then children, in a staggered fashion, depending upon safety results in each age group. Age limits varied per study, e.g. study HGB-205 included only patients with age between 16-19 years and study HGB-204 included patients 12-35 years of age. Patients homozygous or heterozygous for the β^+ allele IVS I-110 (HGVS nomenclature: *HBB*:c.93-21G>A) were excluded from study HGB-207. Similar to β^0 alleles, the IVS-I-110 mutation is widely recognized as producing little to no β-globin (Borgna-Pignatti and Galanello, 2009), thus patients with β^0/IVS-I-110 or IVS-I-110/IVS-I-110 genotypes were grouped with the β^0/β^0 patients in Study HGB-212.

Efficacy analysis is based on 32 adult and adolescent patients with TDT and a non-β^0/β^0 genotype treated with Zynteglo (N=10, HGB-204; N=4, HGB-205; N=15, HGB-207; N=3, HGB-212). The studied patient number is relatively small given the substantial geographic, ethnic and genetic variation encompassed by β-thalassaemia globally. It is noted that the patients in the study were of multiple races and nationalities, and had multiple different genotypes with varying levels of endogenous β-globin production, and that the included patients do seem to reflect global diversity of TDT. Further, while it is not possible to include all hundreds of mutations at the *HBB* locus which have been associated with the phenotype of β-thalassaemia in a clinical trial, it is noted that patients with wide range of endogenous β-globin production (from 0.4 to 5.1 g/dL; still meeting criteria for transfusion dependence) were treated.

These endogenous β-globin production levels could be seen as representative for the range expected in real world TDT patients.

Current treatment experience is limited to patients aged 35 years or younger. The comparison of the <18 years vs ≥18 years of age subpopulations do not suggest a change in efficacy or safety by age (see also discussion on clinical safety).

Efficacy data and additional analyses

The majority of patients with TDT who have a non-β^0/β^0 genotype treated with Zynteglo in study HGB-204 and HGB-205 have achieved TI (11 out of 14; 78.6%). The achievement of TI is clinically meaningful eliminating the need for regular blood transfusions and associated comorbidities due to iron overload. Out of the 3 non-β^0/β^0 patients from Studies HGB-204 and HGB-205 who had not achieved TI, transfusion requirements were reduced by more than 60% in annualised transfusion volume and annualised transfusion frequency in 2 patients. The percent change in annualized transfusion volume from the period from 6 months post-drug product infusion through Month 24 as compared to annualised baseline pre-treatment transfusion volume was 26.8%, 86.9%, or 100%. These patients reported similar reductions in annualized transfusion frequency for the period from 6 months post-drug product infusion through Month 24 as compared to baseline, with 3, 11.9, and 13 fewer transfusions per year (i.e., reductions of 20.7%, 85.3%, and 100.0%).

Support of efficacy of Zynteglo is derived from the β^0/β^0 patients. Even though only a minority of β^0/β^0 patients achieved TI, the 6 β^0/β^0 patients in Study HGB-204 who have not achieved TI did demonstrate a median transfusion volume reduction of -63.30% (-76.3%, -8.3%).

The primary endpoint of the phase 3 study HGB-207 has not been reached due to limited follow-up for patients in this ongoing study. From all included patients (N=15), TI was evaluable for 5 patients of whom 4 (80%) achieved TI. For the 6 non-β^0/β^0 patients ≥12 years of age (in Study HGB-207) with Month 6 HbA^{T87Q} data, the logistic regression model was applied to predict the probability of achieving TI by Month 24 and at any time. Results predict that they have an approximately 98% or higher probability of achieving TI, including 1 patient with an IVS-I-5/IVS-I-5 genotype and 1 patient with an IVS-I-5/β^0 genotype who produce approximately 0.3 g/dL or less of endogenous HbA in the absence of pRBC transfusions (1 patient who has achieved TI and the other predicted to achieve TI). Therefore, the results appear to be in line with those of studies HGB-204 and HGB-205. Even so, it is important to keep in mind that the prediction is derived from a limited number of patients and hence accompanied with uncertainty.

For the 4 patients in Study HGB-207 who have completed at least their Month 12 Visit but have not achieved TI, 3 patients demonstrated 100% reduction in annualized transfusion volume, while the remaining patient had a 71.4% reduction.

Due to the limited follow-up time, the majority of secondary endpoints could not be analysed in HGB-207 as these compared data from the 2 years prior to enrolment with data from 12 months after drug product infusion to Month 24. Moreover, it is expected that secondary endpoint analyses will be further substantiated through the long-term follow-up study LTF-303 (specific obligation) and registry (Annex II condition). Data with respect to a decrease in iron overload is of interest. It is acknowledged that reduction of the iron burden is a continuing process which takes time and thus requires long-term follow up. Further, data from patients treated in Study HGB-204 showed that LIC transiently increases after transplantation, with elevated levels observed at Month 12 before declining towards baseline at Month 24. Updated data indicate lower LIC and higher cardiac T2* values when compared to baseline levels in 6 of 11 patients treated in Studies HGB-204 and HGB-205 who have achieved TI, and lower serum ferritin

levels in all 11 of these patients at last follow-up. These data suggest a trend towards decreasing iron levels.

Of note, patients with severe iron overload for whom iron accumulation is hard to reverse were excluded from the studies, e.g. patients with a cardiac T2* <10 ms by MRI (severe cardiac iron storage) because of safety considerations.

One patient in study HGB 207, showed a 90%-lower PB VCN than DP VCN. Findings from additional analyses were consistent with the hypothesis that there may be an uneven distribution of LVV transduction between short-lived and long-lived CD34+ cells during manufacturing of the drug product. Even though this effect seems to be an outlier, the negative clinical implication of this sharp decrease is worth keeping PB VCN under close scrutiny in the ongoing clinical trials.

Efficacy results across studies have been durable within the duration of follow-up. Once TI is achieved, TI status was durable for non-β^0/β^0 patients with a range for the duration of TI to date (up to 60 months follow up). No non-β^0/β^0 patient across all studies who achieved TI has since required a transfusion, so TI status was maintained. Non-β^0/β^0 patients who have completed at least their Month 30 Visit (N = 7) all had their last pRBC transfusion in the parent study and remained transfusion-free throughout Study LTF-303. The fact that stem cells are transduced and the consideration that immunisation against the transgene is unlikely, provide some level of confidence in the sustainability of the correction of the β thalassaemia. This is further reinforced by the 10 years follow-up of a patient with β-thalassaemia being treated with a similar approach (LG001 HPV569, Cavazzana-Calvo 2010) and preliminary evidence of long-term haemoglobin stability over time in two patients from study HGB-205 with approximately 4-5 years of follow up. Overall, the follow-up time for a substantial number of patients is limited.

During the clinical studies, the drug product manufacturing process was optimised for transgene expression. This change in manufacturing process in studies HGB-205, HGB-204, and HGB-207 had an influence in several PD parameters. In line with the PD section of this report, the manufacturing processes used to make DP impacted the DP VCN, PB VCN and HbA^{T87Q} expression and will impact efficacy parameters to improve efficacy outcomes. Only limited data for the commercial manufacturing process are available. Additional data obtained with the commercial manufacturing process will be provided and is a specific obligation of the marketing authorisation.

In addition, in order to further confirm the appropriateness of the acceptance criteria, the Marketing Authorisation Holder (MAH) should re-evaluate the acceptance criteria for attributes related to potency tests using batch release data and clinical results after 6 months follow-up of 20 patients treated with commercial batches.

Assessment of paediatric data on clinical efficacy

This application is for patients 12 years and older and in total 11 adolescents have been treated as of December 2018. Overall, the population consisted of young patients with age range 12-35 years. Currently, there is no reason to assume differences in efficacy of Zynteglo based on age.

Additional efficacy data needed in the context of a conditional MA

Comprehensive data will be provided by proposing the following specific obligations: the HGB-207 study with two-year follow-up on adults and adolescents (N=15) enrolled in the study, the HGB-212 with additional data in patients with the non-β^0/β^0 genotype and the LTF-303 interim study report that will include data on patients treated with Zynteglo with at least 5 years of follow-up.

2.5.5. Conclusions on the clinical efficacy

The number of patients is low with limited follow-up time for patients in Phase 3 studies, but the overall presented data show clinically relevant and meaningful results by achieving TI in 11 out of 14 patients in studies HGB-204 and HGB-205 and 4 of 5 evaluable patients in study HGB-207. The preliminary data from study HGB-207 are consistent with early results and demonstrate improvement in total Hb levels to be achieved.

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 Zynteglo in patients 12 years and older with TDT who do not have a β^0/β^0 genotype, the MAH should submit interim and final data on Study HGB-207.
- In order to confirm the efficacy and safety of Zynteglo in patients 12 years and older with TDT who do not have a β^0/β^0 genotype, the MAH should submit interim and final data from patients with a severe non-β^0/β^0 genotype such as IVS-I-110/IVS-I-110 and IVS-I-110/β^0 from Study HGB-212.
- In order to confirm the efficacy and safety of Zynteglo in patients 12 years and older with TDT who do not have a β^0/β^0 genotype, the MAH should submit interim data and the 5 years follow-up results of study LTF-303.

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

- Non-interventional post-authorisation safety and efficacy study: In order to further characterise and contextualise the long-term safety and efficacy of Zynteglo in patients 12 years and older with TDT who do not have a β^0/β^0 genotype, the MAH should conduct and submit the results of a study based on data from a product registry (REG-501) and use data on patients treated with transfusions and/or HLA-matched allogenic HSCT treated patients from an established European registry as a comparator group.

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

2.6. Clinical safety

The information presented in Section 2.6 includes safety data from multiple data cuts as noted throughout. Safety analyses focused on patients with TDT (across all genotypes) and included patients with SCD treated with LentiGlobin for SCD (drug product manufactured with the same BB305 LVV as Zynteglo) in the assessment of gene therapy-related risks.

Treatment paradigm

Treatment with Zynteglo is preceded by procedural and medical interventions, namely haematopoietic stem cell collection (mobilisation with G-CSF and plerixafor followed by apheresis), and myeloablative conditioning using busulfan, that carry their own risks. Thus, describing the overall extent of the safety profile of Zynteglo in patients with TDT also includes exposure to mobilisation and conditioning agents.

Patient exposure

The primary safety analysis population is based on one completed Phase 1/2 clinical study (HGB-204) in adult and adolescent patients (≥12 years of age) with TDT and five ongoing studies: HGB-205, HGB-207, HGB-212, HGB-206, and the long-term follow up study LTF-303 (see Table 36). LTF-303 captures

data for all Zynteglo-treated patients from the Month 24 visit in the parent study through 15 years post drug product infusion.

Data in patients with sickle cell disease (SCD) treated with LentiGlobin for SCD were submitted as supportive data.

Table 36: Overall Extent of Exposure to Zynteglo or LentiGlobin for SCD

Study Identifier (Status)	Study Design	Study Population	Primary Objective(s)	Number of Patients[1]	Data Cut Dates	Test Product(s); Dosage Regimen; Manufacturing Process for Zynteglo
HGB-204 (completed)	Open-label, uncontrolled	Patients with TDT	safety and efficacy	18 TDT treated (all ≥12 years of age)	CSR Data Lock: 07 Mar 2018 Module 2.7.4 Data Cut: 07 Mar 2018 Additional Data Cut: 13 Dec 2018	Zynteglo; ≥3.0×10^6 CD34+ cells/kg (apheresis); Base manufacturing process
HGB-205 (ongoing)	Open-label, uncontrolled	Patients with SCD or TDT	safety, tolerability, and success of engraftment	4 TDT and 3 SCD treated (all ≥12 years of age)	Interim CSR Data Cut: 11 Oct 2017 Module 2.7.4 Data Cut: 07 Mar 2018 Additional Data Cut: 13 Dec 2018	Zynteglo; ≥3.0×10^6 CD34+ cells/kg (apheresis; TDT) ≥2.0×10^6 CD34+ cells/kg (BM harvest; SCD); Original manufacturing process
HGB-207 (ongoing)	Open-label, uncontrolled	Patients with TDT with non-β^0/β^0 genotypes	efficacy and safety	20 TDT treated (15 ≥12 years of age and 5 <12 years of age)	Interim CSR Data Cut: 22 Feb 2018 Module 2.7.4 Data Cut: 22 Feb 2018 Module 2.7.4 Late-Breaking Data Cut: 15 May 2018 Additional Data Cut: 13 Dec 2018	Zynteglo; ≥5.0×10^6 CD34+ cells/kg (apheresis); Refined or commercial manufacturing process
HGB-212 (ongoing)	Open-label, uncontrolled	Patients with TDT with β^0/β^0, IVS-I-110/ β^0, and IVS-I-110/IVS-I-110 genotypes	efficacy and safety	6 TDT treated (2 β^0/β^0 ≥12 years of age; 3 non-β^0/β^0 ≥12 years of age; 1 non-β^0/β^0 <12 years of age)	CSR Data Cut: Not applicable Module 2.7.4 Data Cut: 07 Mar 2018 Additional Data Cut: 13 Dec 2018	Zynteglo; ≥5.0×10^6 CD34+ cells/kg (apheresis); Refined or commercial manufacturing process
LTF-303 (ongoing)	Long-term follow-up; safety and efficacy	Patients with haemoglobinopathies who have been treated with the drug product in the parent studies	monitor for the long-term safety and efficacy of the drug product	22 TDT and 8 SCD enrolled	Interim CSR Data Cut: 21 Nov 2017 Module 2.7.4 Data Cut: 07 Mar 2018 Additional Data Cut: 13 Dec 2018	Not applicable

Study Identifier (Status)	Study Design	Study Population	Primary Objective(s)	Number of Patients[1]	Data Cut Dates	Test Product(s); Dosage Regimen; Manufacturing Process for Zynteglo
HGB-206 (ongoing)	Open-label, uncontrolled	Adult patients with SCD	safety	19 SCD treated	CSR Data Cut: Not applicable Module 2.7.4 Data Cut: 07 Mar 2018 Additional Data Cut: 13 Dec 2018	LentiGlobin for SCD; ≥2.0×10^6 CD34+ cells/kg (BM harvest) ≥3.0×10^6 CD34+ cells/kg (apheresis); Not applicable

[1] Number of patients treated as of 13 December 2018

Subgroups for safety analyses included the NB0 pool (all patients with TDT with a non-β^0/β^0 genotype) and the TDT pool (all patients with TDT).

As of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies, the main safety analysis of the ITT population consists of 39 patients with TDT who initiated mobilisation, 33 (85%) of whom had received Zynteglo (i.e., transplant population, TP). Of the 6 patients in the TDT pool who were not treated with drug product, 2 patients discontinued after 1 cycle of mobilization and apheresis (due to inadequate stem cell mobilisation and pregnancy) and 4 were pending treatment. Several other subgroups were defined in order to evaluate variations in safety and tolerability in major categories of genotype and drug manufacturing processing.

Patients were followed for a median (min, max) of 24.15 (0.8, 47.7) months after drug product infusion in the TDT pool, and 23.7 (0.8, 47.7) months after drug product infusion in the NB0 pool (see Table 37).

Table 37: Length of Follow-Up Post-Drug Product Infusion (TP)

Parameter	Statistic	*NB0* (N=24)	*TDT* (N=33)
Last Follow-up (Months post drug product infusion) [1]	Mean (SD)	21.86 (15.247)	23.75 (14.563)
	Median	23.7	24.15
	Min, Max	0.8, 47.7	0.8, 47.7
Follow-up Data Available (Months post drug product infusion)[1]			
3 Months	n (%)	21 (87.5)	30 (90.9)
6 Months	n (%)	18 (75.0)	26 (78.8)
9 Months	n (%)	17 (70.8)	25 (75.8)
12 Months	n (%)	15 (62.5)	23 (69.7)
15 Months	n (%)	14 (58.3)	22 (66.7)
18 Months	n (%)	14 (58.3)	22 (66.7)
24 Months	n (%)	11 (45.8)	17 (51.5)
30 Months	n (%)	7 (29.2)	11 (33.3)
36 Months	n (%)	4 (16.7)	7 (21.2)
42 Months	n (%)	2 (8.3)	2 (6.1)
48 Months	n (%)	0	0

Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies

Patient demographics were reflective of that for patients with TDT in Europe and globally. Certain patients were excluded from clinical studies for safety reasons, i.e. patients with hypersensitivity to the active substance or excipients, current or previous history of leukaemia or lymphoma, positive test for human immunodeficiency virus (HIV)-1, HIV-2, HTLV-I, and HTLV-2, pregnancy or breastfeeding, or unable to undergo HSCT safely, as determined by a healthcare professional.

Adverse events

For all studies, AEs were coded using Medical Dictionary for Regulatory Activities (MedDRA) (Version 19.0) and collected according to each protocol. For data analysis of AEs, the studies have been divided into periods corresponding to study milestones and by duration of time post-Zynteglo infusion.

The following study periods have been defined:

- ICF to <MB: Date of informed consent until either date of initiation of mobilization (TDT patients or some SCD patients, as applicable) or bone marrow harvest (some SCD patients, as applicable)*
- MB to <C: date of initiation of mobilization/bone marrow harvest until date of initiation of conditioning
- C to <NE: Date of initiation of conditioning until the date of neutrophil engraftment (NE)
- NE to <M24: Date of NE through Month 24
- Day 1 (date of drug product infusion) through Month 24; annual periods thereafter
- D1 to last visit: Day 1 through Last Visit+
- Total: Date of informed consent through Last Visit*+

* Excluded from laboratory summaries.
+ Last visit defined to include all safety data, even if it is after a scheduled visit; could occur either in the parent study or in the LTF-303 study.

AEs are summarised by category for the TDT pool and the NB0 pool (see Table 38). TEAE was defined as those events that occur during or after the drug product infusion, i.e. Day 1. This is at least 7 days after start of conditioning with busulfan (4-5 days conditioning, at least 72 hours washout).

All but 1 patient in the TDT pool had experienced at least 1 AE, with 12.8% experiencing at least 1 AE related to drug product; all of these latter events were Grade 1. Nineteen patients (48.7%) experienced at least 1 SAE; no drug product-related SAEs were reported. No deaths were reported and no patients discontinued from their respective studies due to an AE.

Table 38: Overall Summary of Adverse Events, NB0 and TDT Pools (ITT)

Parameter	Statistic	*NB0* (N=30)	*TDT* (N=39)
Number of Subjects with ≥1 Adverse Event (AE)	n (%)	29 (96.7)	38 (97.4)
Number of Subjects with ≥1 Treatment-Emergent (TE) AE	n (%)	24 (80.0)	33 (84.6)
Number of Subjects with ≥1 AE related to Drug Product	n (%)	4 (13.3)	5 (12.8)
Number of Subjects with ≥1 Serious Adverse Event (SAE)	n (%)	15 (50.0)	19 (48.7)
Number of Subjects with ≥1 TESAE	n (%)	10 (33.3)	13 (33.3)
Number of Subjects with ≥1 TESAE Related to Drug Product	n (%)	0	0
Number of Subjects with ≥1 ≥Grade 3 AE	n (%)	25 (83.3)	34 (87.2)
Number of Subjects with ≥1 ≥Grade 3 TEAE	n (%)	24 (80.0)	33 (84.6)
Number of Subjects with ≥1 ≥Grade 3 AE Related to Drug Product	n (%)	0	0
Number of Subjects with ≥1 AE Resulting in Death	n (%)	0	0

TE, treatment-emergent. Study Pools: NB0 = non-β^0/β^0 genotype; TDT = All TDT.
Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies

Table 39: Incidence of All AEs Occurring in ≥3 Patients: TDT Pool (ITT) by system organ class and preferred term

System Organ Class Preferred Term	ICF to <MB, n (%)	MB to <C, n (%)	C to <NE, n (%)	NE to M24, n (%)	D1 to Last Visit, n (%)	Total (ICF to Last Visit), n (%)
Number of Subjects at Risk	39	39	33	32	33	39
Number of Subjects with at Least 1 AE	21 (53.8)	37 (94.9)	33 (100.0)	31 (96.9)	33 (100.0)	38 (97.4)
Blood and lymphatic system disorders	0	15 (38.5)	33 (100.0)	15 (46.9)	32 (97.0)	35 (89.7)
Thrombocytopenia	0	10 (25.6)	31 (93.9)	3 (9.4)	31 (93.9)	33 (84.6)
Anaemia	0	3 (7.7)	29 (87.9)	10 (31.3)	26 (78.8)	30 (76.9)
Neutropenia	0	1 (2.6)	22 (66.7)	3 (9.4)	22 (66.7)	22 (56.4)
Febrile neutropenia	0	0	15 (45.5)	0	15 (45.5)	15 (38.5)
Leukopenia	0	0	13 (39.4)	3 (9.4)	13 (39.4)	13 (33.3)
Gastrointestinal disorders	5 (12.8)	14 (35.9)	31 (93.9)	16 (50.0)	33 (100.0)	37 (94.9)
Stomatitis	0	0	26 (78.8)	0	25 (75.8)	26 (66.7)
Nausea	1 (2.6)	9 (23.1)	20 (60.6)	2 (6.3)	5 (15.2)	24 (61.5)
Vomiting	1 (2.6)	7 (17.9)	17 (51.5)	4 (12.5)	13 (39.4)	23 (59.0)
Abdominal pain	2 (5.1)	2 (5.1)	12 (36.4)	4 (12.5)	14 (42.4)	17 (43.6)
Constipation	0	0	13 (39.4)	2 (6.3)	12 (36.4)	15 (38.5)
Diarrhoea	0	0	10 (30.3)	3 (9.4)	12 (36.4)	12 (30.8)
Dyspepsia	0	2 (5.1)	5 (15.2)	2 (6.3)	7 (21.2)	8 (20.5)
Abdominal pain upper	0	1 (2.6)	3 (9.1)	0	2 (6.1)	4 (10.3)
Anal inflammation	0	0	2 (6.1)	2 (6.3)	4 (12.1)	4 (10.3)
Gingival bleeding	0	1 (2.6)	0	3 (9.4)	3 (9.1)	4 (10.3)
Toothache	0	1 (2.6)	1 (3.0)	1 (3.1)	2 (6.1)	3 (7.7)
General disorders and administration site conditions	1 (2.6)	22 (56.4)	20 (60.6)	13 (40.6)	22 (66.7)	32 (82.1)
Pyrexia	1 (2.6)	1 (2.6)	12 (36.4)	6 (18.8)	15 (45.5)	15 (38.5)
Catheter site pain	0	10 (25.6)	4 (12.1)	0	2 (6.1)	12 (30.8)
Fatigue	0	3 (7.7)	2 (6.1)	4 (12.5)	5 (15.2)	8 (20.5)
Pain	0	3 (7.7)	2 (6.1)	1 (3.1)	1 (3.0)	6 (15.4)
Non-cardiac chest pain	0	1 (2.6)	2 (6.1)	2 (6.3)	3 (9.1)	4 (10.3)
Catheter site haemorrhage	0	3 (7.7)	0	0	0	3 (7.7)
Chest discomfort	0	1 (2.6)	2 (6.1)	0	1 (3.0)	3 (7.7)
Hepatobiliary disorders	1 (2.6)	1 (2.6)	3 (9.1)	5 (15.6)	7 (21.2)	9 (23.1)
Veno-occlusive liver disease	0	0	1 (3.0)	3 (9.4)	4 (12.1)	4 (10.3)
Hepatomegaly	0	0	2 (6.1)	1 (3.1)	3 (9.1)	3 (7.7)
Infections and infestations	9 (23.1)	7 (17.9)	9 (27.3)	23 (71.9)	24 (72.7)	30 (76.9)
Upper respiratory tract infection	1 (2.6)	2 (5.1)	0	6 (18.8)	6 (18.2)	8 (20.5)
Folliculitis	0	1 (2.6)	2 (6.1)	2 (6.3)	4 (12.1)	5 (12.8)
Cellulitis	0	0	1 (3.0)	2 (6.3)	3 (9.1)	3 (7.7)
Injury, poisoning and procedural complications	11 (28.2)	13 (33.3)	12 (36.4)	10 (31.3)	16 (48.5)	24 (61.5)
Procedural pain	9 (23.1)	10 (25.6)	2 (6.1)	0	2 (6.1)	16 (41.0)
Transfusion reaction	0	1 (2.6)	9 (27.3)	1 (3.1)	10 (30.3)	11 (28.2)
Skin abrasion	0	0	1 (3.0)	3 (9.4)	4 (12.1)	4 (10.3)
Contusion	0	1 (2.6)	0	2 (6.3)	2 (6.1)	3 (7.7)
Investigations	1 (2.6)	3 (7.7)	11 (33.3)	16 (50.0)	16 (48.5)	18 (46.2)
Alanine aminotransferase increased	0	0	3 (9.1)	9 (28.1)	10 (30.3)	10 (25.6)
Aspartate aminotransferase increased	0	0	3 (9.1)	7 (21.9)	9 (27.3)	9 (23.1)
Gamma-glutamyltransferase increased	0	0	3 (9.1)	4 (12.5)	7 (21.2)	7 (17.9)
Blood bilirubin increased	0	0	5 (15.2)	2 (6.3)	5 (15.2)	6 (15.4)
Metabolism and nutrition disorders	2 (5.1)	7 (17.9)	13 (39.4)	4 (12.5)	12 (36.4)	20 (51.3)
Hypokalaemia	1 (2.6)	2 (5.1)	5 (15.2)	2 (6.3)	5 (15.2)	8 (20.5)
Decreased appetite	0	0	6 (18.2)	1 (3.1)	6 (18.2)	7 (17.9)
Hypocalcaemia	0	5 (12.8)	1 (3.0)	0	1 (3.0)	6 (15.4)
Hypomagnesaemia	0	2 (5.1)	1 (3.0)	0	1 (3.0)	3 (7.7)
Hyponatraemia	0	0	3 (9.1)	1 (3.1)	2 (6.1)	3 (7.7)
Musculoskeletal and connective tissue disorders	0	10 (25.6)	9 (27.3)	7 (21.9)	10 (30.3)	19 (48.7)
Pain in extremity	0	1 (2.6)	3 (9.1)	3 (9.4)	4 (12.1)	6 (15.4)
Back pain	0	3 (7.7)	2 (6.1)	1 (3.1)	1 (3.0)	5 (12.8)
Bone pain	0	3 (7.7)	2 (6.1)	0	0	5 (12.8)
Myalgia	0	1 (2.6)	3 (9.1)	1 (3.1)	2 (6.1)	5 (12.8)
Musculoskeletal discomfort	0	1 (2.6)	2 (6.1)	0	2 (6.1)	3 (7.7)
Nervous system disorders	2 (5.1)	11 (28.2)	11 (33.3)	0	6 (18.2)	17 (43.6)
Headache	1 (2.6)	5 (12.8)	7 (21.2)	0	2 (6.1)	12 (30.8)
Peripheral sensory neuropathy	0	6 (15.4)	0	0	0	6 (15.4)
Dizziness	0	4 (10.3)	2 (6.1)	0	2 (6.1)	4 (10.3)

System Organ Class Preferred Term	ICF to <MB, n (%)	MB to <C, n (%)	C to <NE, n (%)	NE to M24, n (%)	D1 to Last Visit, n (%)	Total (ICF to Last Visit), n (%)
Psychiatric disorders	0	3 (7.7)	12 (36.4)	4 (12.5)	11 (33.3)	15 (38.5)
Insomnia	0	1 (2.6)	9 (27.3)	3 (9.4)	8 (24.2)	11 (28.2)
Anxiety	0	0	5 (15.2)	0	3 (9.1)	5 (12.8)
Agitation	0	2 (5.1)	1 (3.0)	0	0	3 (7.7)
Renal and urinary disorders	0	0	5 (15.2)	1 (3.1)	5 (15.2)	6 (15.4)
Haematuria	0	0	3 (9.1)	0	2 (6.1)	3 (7.7)
Reproductive system and breast disorders	1 (2.6)	1 (2.6)	8 (24.2)	8 (25.0)	11 (33.3)	11 (28.2)
Vaginal haemorrhage	0	0	5 (15.2)	2 (6.3)	6 (18.2)	6 (15.4)
Menstruation irregular	0	0	1 (3.0)	3 (9.4)	4 (12.1)	4 (10.3)
Respiratory, thoracic and mediastinal disorders	1 (2.6)	6 (15.4)	20 (60.6)	11 (34.4)	24 (72.7)	27 (69.2)
Epistaxis	0	1 (2.6)	11 (33.3)	5 (15.6)	14 (42.4)	15 (38.5)
Pharyngeal inflammation	0	0	9 (27.3)	0	9 (27.3)	9 (23.1)
Cough	0	0	2 (6.1)	4 (12.5)	5 (15.2)	5 (12.8)
Hypoxia	0	1 (2.6)	1 (3.0)	1 (3.1)	2 (6.1)	3 (7.7)
Skin and subcutaneous tissue disorders	2 (5.1)	4 (10.3)	28 (84.8)	11 (34.4)	27 (81.8)	29 (74.4)
Alopecia	0	0	23 (69.7)	2 (6.3)	24 (72.7)	25 (64.1)
Pruritus	0	0	9 (27.3)	0	5 (15.2)	9 (23.1)
Skin hyperpigmentation	0	0	4 (12.1)	1 (3.1)	5 (15.2)	5 (12.8)
Petechiae	0	0	4 (12.1)	0	4 (12.1)	4 (10.3)
Urticaria	0	0	2 (6.1)	2 (6.3)	3 (9.1)	4 (10.3)
Dry skin	0	0	3 (9.1)	0	2 (6.1)	3 (7.7)
Rash	0	2 (5.1)	0	1 (3.1)	1 (3.0)	3 (7.7)
Skin hypopigmentation	0	0	0	3 (9.4)	3 (9.1)	3 (7.7)

Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies

Abbrev: C, Initiation of conditioning; ICF, informed consent form (date of signature); MB, initiation of mobilization; NE, neutrophil engraftment; TDT, transfusion-dependent thalassaemia.
Study Pool: TDT = All TDT.
Note: Study periods are mutually exclusive, with the exception of Day 1 to Last Visit and ICF to Last Visit study periods. Last visit defined to include all relevant safety data, even if it is after a scheduled visit; could occur either in the parent study or in the LTF-303 study;
Note: Hematologic abnormalities reported as AEs that were coded to PTs in the Investigations SOC (e.g., Platelet count decreased) have been pooled with appropriate terms in the Blood and Lymphatic System SOC (e.g., Thrombocytopenia) for tabulation;
Note: If event started in the reporting period and continues into the next reporting period, it will be counted only in the first period. If event starts and stops in the reporting period and then recurs in the next reporting period, it will be counted in both periods;
Note: Number of patients at risk for each period includes patients who enter the period;
Note: C to <NE includes AEs occurring during D1 to <NE.
No adverse events have been reported during the >M24 to M36 (N=17) and >M36 to M48 (N = 7) or >M48 to M60 (N = 0) periods so these columns are not displayed in the table.

From ICF to M24, the highest percentage of patients with AEs was during the C to <NE period, and the lowest during the ICF to <MB period. No events were reported between M24 and M60.

For the ICF to Last Visit study period, most patients experienced events of thrombocytopenia (84.6%), anaemia (76.9%), stomatitis (66.7%), alopecia (64.1%), nausea (61.5%), vomiting (59.0%), and neutropenia (56.4%). The events of thrombocytopenia, anaemia, stomatitis, and neutropenia were primarily reported at a severity grade of ≥Grade 3, whereas the events of alopecia, nausea, and vomiting were primarily reported at a maximum of Grade 1 or Grade 2.

All patients (33/33, 100%) in the TDT pool had an AE ≥Grade 3 in severity in the 'Blood and lymphatic system disorders' SOC during the C to <NE study periods. AEs reported as ≥Grade 3 that occurred in at least 20 patients (50%) were thrombocytopenia, anaemia, neutropenia, and stomatitis.

Infections:

Eleven (28.2%) patients in the TDT pool reported a total of 19 ≥Grade 3 and/or serious infections, of which 7 were non-serious and 12 were serious. Three serious infections occurred prior to Zynteglo finished product infusion. Of the remaining 9 serious infections, 1 occurred prior to NE (Appendicitis) and 8 occurred after NE (Cat scratch disease, Gastroenteritis, Diarrhoea infectious, Asymptomatic HIV infection, Salmonella sepsis, Cellulitis, Tooth infection, and Pneumonia). Non-serious ≥Grade 3 infections included cystitis, herpes simplex, influenza, lung infection, oral herpes, pseudomonal bacteraemia, and Staphylococcal infection. All but the events of asymptomatic HIV infection (wildtype etiology confirmed by western blot) and Salmonella sepsis (both in the same patient) resolved as of 07 March 2018.

Bleeding-related events:

All patients in the TDT pool experienced significant thrombocytopenia and 22 (56.4%) patients in the ITT population experienced a bleeding event, with most events occurring following busulfan treatment. Almost all bleeding events were non-serious and did not require blood transfusion. One SAE of Hypotension (secondary to recurrent epistaxis) occurred 11 days after drug product infusion in a patient whose platelet level was <30,000. This patient required multiple blood and platelet transfusions and local intranasal therapy.

The non-serious bleeding events occurring after infusion of Zynteglo finished product included: Epistaxis (14 patients), Vaginal haemorrhage (6 patients), Gingival bleeding (3 patients), Haematuria (2 patients), and 1 patient each with Conjunctival haemorrhage, Rectal haemorrhage, Metrorrhagia, Anal haemorrhage, and Menorrhagia. These events occurred within 60 days of treatment with Zynteglo, except for the event of Rectal haemorrhage which occurred on Day 718.

All events resolved with either standard treatment or were self-limited.

Thrombotic events:

Five thrombotic events were experienced by 3 patients, all occurring after treatment with Zynteglo. Two events were serious and 3 were non-serious and included: Intracardiac thrombus (splenectomised;

Grade 3 SAE on Day 357); 2 events of Vena cava thrombosis related to central venous catheter placement (Grade 2 AE on Day 51 which became an SAE on Day 58 in the same patient) and 2 events of Deep vein thrombosis in the same patient (both related to oral contraceptive use, splenectomised; Grade 2 AEs on Days 91 and 661).

Hepatic events

In the TDT pool, 9 patients (23.1%) reported 15 hepatic events. There were no cases reported as Drug-induced liver injury or cases that met Hy's Law criteria. Four (4) patients (10.3%) experienced a serious hepatic event (veno-occlusive disease; VOD).

Reproductive and fertility events

A review of the 'Reproductive system and breast disorders' SOC in the TDT pool resulted in 3 reproductive and fertility events in the ITT population: 1 case of Premature menopause and 2 cases of Ovarian failure. All 3 were non-serious, attributed to busulfan by the Investigator, and were ongoing as of 07 March 2018.

Drug product-related adverse events

Five patients in the TDT pool reported 8 events (all nonserious and Grade 1) assessed by the Investigator as related or possibly related to Zynteglo (3 events of Abdominal pain, 2 events of Hot flush, and 1 event each of Dyspnoea, Non-cardiac chest pain, and Dysplasia). All events, except for dysplasia, occurred on Day 1 in 4 patients and resolved on the same day without treatment.

The event of dysplasia was reported at the Month 24 Visit (Day 723) and remained ongoing as of 07 March 2018. The Month 24 bone marrow biopsy revealed mild dysplastic changes in the erythroid series which were more prominent than in the previous bone marrow aspirate at Month 12. The pathology report stated that no significant neoplastic blast population was identified; normal cytology was observed for lymphocytes, monocytes, and neutrophils; WBC and platelet counts were normal. Integration Site Analysis (ISA) from peripheral blood did not indicate emergence of a dominant clone derived from LVV-transduced cells.

Procedure-related adverse events

The treatment with Zynteglo is accompanied by procedures involved in preparation for Zynteglo administration, such as mobilisation with G-CSF and/or plerixafor, apheresis and conditioning with busulfan. The Investigators had the option of attributing an AE to a study procedure, specifically 'Mobilisation/Apheresis', or to the drugs used for mobilisation or conditioning.

Events attributed by the Investigator to mobilisation and apheresis were mostly non-serious and typical of the intervention and treatment. Specifically, very common adverse reactions attributed to mobilisation/apheresis included thrombocytopenia, peripheral sensory neuropathy, headache, nausea, and hypocalcaemia. Two SAEs were attributed to mobilisation/apheresis: one event each of Thrombocytopenia and Hypokalaemia, which occurred in the same patient. All AEs occurred in the period between mobilization and conditioning and resolved prior to conditioning.

The majority of events attributed by the Investigator to conditioning with busulfan were non-serious. Specifically, very common adverse reactions attributed to busulfan included thrombocytopenia, anaemia, neutropenia, febrile neutropenia, leukopenia, decreased appetite, headache, insomnia, epistaxis, pharyngeal inflammation, stomatitis, nausea, vomiting, diarrhoea, abdominal pain, constipation, anal inflammation, dyspepsia, veno-occlusive liver disease (VOD), alopecia, pruritus, skin hyperpigmentation, petechiae, vaginal haemorrhage, pyrexia, ALT increased, AST increased, blood bilirubin increased, and gamma-glutamyltransferase (GGT) increased. Six events in 5 patients were reported as SAEs: 4 events

of veno-occlusive liver disease, 1 event of hypotension (secondary to epistaxis), and 1 event of hypoxia. All events resolved.

Adverse Events of Special Interest

The EOI pre-specified in the safety SAP were:

- Neutrophil engraftment failure
- HIV infection (defined using a custom MedDRA query: HLT='Acquired immunodeficiency syndromes' or 'Retroviral infections')
- Autoimmune disorders (defined using a custom MedDRA query: HLGT='Autoimmune disorders' or HLT= 'Autoimmunity analyses)
- Lack of efficacy (defined using the Lack of efficacy/effect SMQ)
- Malignancies (defined using the following SMQs: Malignant tumors, Malignant lymphomas, Myelodysplastic syndromes [MDS], and Blood premalignant disorders)

No cases of neutrophil engraftment failure, autoimmune disorders, or lack of efficacy were noted in the TP population (n=33). No events or laboratory values indicative of graft-vs-host disease (GVHD) or other autoimmune diseases were noted.

Platelet Engraftment (PE): As of 07 March 2018, the 33 patients who achieved PE had a median (min, max) Day of PE of 41.0 (19, 191). This time to platelet engraftment is longer than previously reported for TDT in the allogenic transplant setting where a median time to PE was reported from published literature to be within 30 days of transplant. Delayed time to PE did not appear to be associated with an increased risk of bleeding.

HIV infection: One patient (3%) in Study HGB-204 was found to have asymptomatic HIV infection at the Month 24 study visit. The Investigator assessed that the asymptomatic HIV infection was not related to drug product or procedures and was attributed to conventional routes of transmission. The event was ongoing at the time of the report. The patient remained in the study.

Malignancy: No malignancies were noted in the TP population (n=33). One patient treated with drug product manufactured with the same BB305 LVV as Zynteglo experienced an SAE of myelodysplasia. A male patient with SCD participating in Study LTF-303 presented with cytopenia and peripheral blast cells approximately 3 years after busulfan conditioning. A bone marrow aspirate suggested MDS. The patient's cytogenetic analysis revealed a chromosomal abnormality which has been associated with secondary leukaemia. An assessment of a tumour cell-enriched cell population for LVV insertion was negative.

Serious adverse event/deaths/other significant events

No deaths have been reported in the Zynteglo program to date, with 100% overall survival and no transplant related mortality including in 23 TDT patients with at least 365 days of follow-up.

Nineteen (48.7%) of TDT patients (ITT) reported a total of 31 SAEs, none of which were assessed as drug-product related. Of the 31 SAEs, 10 events occurred in 8 patients prior to drug product infusion and were attributed to study procedures, mobilization, apheresis, or other unknown reasons.

The incidence of all SAEs in patients in the TDT pool is presented in Table 40 by study period, with SOCs listed alphabetically and PTs within each SOC listed in descending order of occurrence in the Total (ICF to Last Visit) column.

Table 40: Incidence of All Serious Adverse Events: TDT Pool (ITT)

System Organ Class Preferred Term	ICF to <MB n (%)	MB to <C n (%)	C to <NE n (%)	NE to M24 n (%)	D1 to Last Visit n (%)	ICF to Last Visit n (%)
Number of Subjects at Risk	39	39	33	32	33	39
Number of Subjects with at Least 1 Serious AE	5 (12.8)	3 (7.7)	3 (9.1)	11 (34.4)	13 (39.4)	19 (48.7)
Blood and lymphatic system disorders	0	1 (2.6)	0	1 (3.1)	1 (3.0)	2 (5.1)
Anaemia	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
Thrombocytopenia	0	1 (2.6)	0	0	0	1 (2.6)
Cardiac disorders	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
Intracardiac thrombus	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
General disorders and administration site conditions	0	1 (2.6)	0	0	0	1 (2.6)
Catheter site haemorrhage	0	1 (2.6)	0	0	0	1 (2.6)
Hepatobiliary disorders	0	0	1 (3.0)	2 (6.3)	3 (9.1)	3 (7.7)
Veno-occlusive liver disease	0	0	0	2 (6.3)	2 (6.1)	2 (5.1)
Hepatomegaly	0	0	1 (3.0)	0	1 (3.0)	1 (2.6)
Infections and infestations	2 (5.1)	1 (2.6)	1 (3.0)	6 (18.8)	6 (18.2)	9 (23.1)
Appendicitis	0	0	1 (3.0)	0	1 (3.0)	1 (2.6)
Asymptomatic HIV infection	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
Cat scratch disease	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
Catheter site infection	1 (2.6)	0	0	0	0	1 (2.6)
Cellulitis	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
Diarrhoea infectious	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
Gastroenteritis	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
Gastroenteritis viral	1 (2.6)	0	0	0	0	1 (2.6)
Pneumonia	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
Salmonella sepsis	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
Tooth infection	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
Viral infection	0	1 (2.6)	0	0	0	1 (2.6)
Injury, poisoning and procedural complications	2 (5.1)	0	0	1 (3.1)	1 (3.0)	3 (7.7)
Accidental overdose	1 (2.6)	0	0	0	0	1 (2.6)
Post procedural haemorrhage	1 (2.6)	0	0	0	0	1 (2.6)
Transfusion reaction	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
Metabolism and nutrition disorders	1 (2.6)	1 (2.6)	0	1 (3.1)	1 (3.0)	3 (7.7)
Hyperglycaemia	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
Hypokalaemia	0	1 (2.6)	0	0	0	1 (2.6)
Hypophosphataemia	1 (2.6)	0	0	0	0	1 (2.6)
Nervous system disorders	1 (2.6)	0	0	0	0	1 (2.6)
Seizure	1 (2.6)	0	0	0	0	1 (2.6)
Psychiatric disorders	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
Major depression	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
Respiratory, thoracic and mediastinal disorders	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
Hypoxia	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
Vascular disorders	0	0	1 (3.0)	2 (6.3)	3 (9.1)	3 (7.7)
Hypotension	0	0	1 (3.0)	0	1 (3.0)	1 (2.6)
Vena cava thrombosis	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)
Venoocclusive disease	0	0	0	1 (3.1)	1 (3.0)	1 (2.6)

Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies
Abbrev.: C; Initiation of conditioning; ICF, informed consent form (date of signature); MB, initiation of mobilization or bone marrow harvest; NE, date of neutrophil engraftment; TDT, transfusion-dependent thalassaemia.
Study Pool: TDT = All TDT.
Note: Study periods are mutually exclusive, with the exception of Day 1 to Last Visit and ICF to Last Visit study periods. Last visit defined to include all relevant safety data, even if it is after a scheduled visit; could occur either in the parent study or in the LTF-303 study.
Note: Hematologic abnormalities reported as AEs that were coded to PTs in the Investigations SOC (e.g., platelet count decreased) have been pooled with appropriate terms in the Blood and Lymphatic System SOC (e.g., thrombocytopenia) for tabulation.
Note: If event started in the reporting period and continues into the next reporting period, it will be counted only in the first period. If event starts and stops in the reporting period and then recurs in the next reporting period, it will be counted in both periods.

Note: Number of patients at risk for each period includes patients who enter the period.
Note: C to <NE includes AEs occurring during D1 to <NE.
No adverse events have been reported during the >M24 to M36 (N=17) and >M36 to M48 (N = 7) or >M48 to M60 (N = 0) periods so these columns are not displayed in the table.

Of the 31 SAEs, the following 21 SAEs were treatment-emergent and occurred in 13 patients:

Infections (9): Of the 9 treatment-emergent serious infections, 1 occurred prior to NE (Appendicitis) and 8 occurred after NE (cat scratch disease, gastroenteritis, diarrhoea infectious, asymptomatic HIV infection, salmonella sepsis, cellulitis, tooth infection, and pneumonia). All but the events of HIV infection and salmonella sepsis (both in the same patient) were resolved as of 07 March 2018.

Bleeding Events (1): One event of hypotension (secondary to epistaxis) occurred 11 days after drug product infusion in a patient whose platelet level was <30,000. This patient required multiple blood and platelet transfusions and local intranasal therapy. The event of epistaxis was attributed to conditioning and resolved.

Thrombotic Events (2): One event each of intracardiac thrombus and Vena cava thrombosis, both of which resolved. In addition to the risk factor of having TDT, these patients had risk factors of asplenia and an in-dwelling catheter, respectively.

Hepatic Events (4): All were veno-occlusive liver disease (VOD) and assessed by the Investigator as related to busulfan conditioning; all resolved after treatment with defibrotide. VOD is a reported, severe AE associated with the use of busulfan.

Lab Findings (1): One event of Hyperglycaemia which resolved.

Other (4): One event each of Transfusion reaction (platelets), Anaemia, Major depression, and Hypoxia, all of which resolved.

Laboratory findings

Haematology parameters

Potentially clinically significant (PCS) haematology values are summarized by parameter for the TDT pool in Table 41. All patients had low PCS values for leukocytes ($<3 \times 10^9$/L), erythrocytes ($\leq 3.5 \times 10^{12}$/L), and platelets ($\leq 75 \times 10^9$/L) in the C to NE period. This significant pancytopenia occurred following busulfan conditioning and improved with subsequent bone marrow reconstitution and engraftment.

Following NE and subsequent PE, most PCS abnormalities were in the erythrocyte line. Following PE, all patients maintained platelet levels of $>20 \times 10^9$/L in the absence of platelet transfusions.

Table 41: Summary of Potentially Clinically Significant Haematology Laboratory Abnormalities: TDT Patients (ITT)

Laboratory test	PCS Threshold	Statistic	Time Period: MB to <C	Time Period: C to <NE	Time Period: NE to M24	Time Period: Day 1 to Last Visit
Leukocytes	<3.0 x 10^9/L	n/N (%)	0/39	33/33 (100.0)	30/32 (93.8)	33/33 (100.0)
	>=16 x 10^9/L	n/N (%)	39/39 (100.0)	5/33 (15.2)	3/32 (9.4)	5/33 (15.2)
Lymphocytes	<0.8 x 10^9/L	n/N (%)	6/39 (15.4)	26/33 (78.8)	21/32 (65.6)	30/33 (90.9)
	>12 x 10^9/L	n/N (%)	17/39 (43.6)	0/33	0/32	0/33
Neutrophils	<1.5 x 10^9/L	n/N (%)	2/39 (5.1)	33/33 (100.0)	32/32 (100.0)	33/33 (100.0)
	>13.5 x 10^9/L	n/N (%)	38/39 (97.4)	4/33 (12.1)	2/32 (6.3)	4/33 (12.1)
Monocytes	>2.5 x 10^9/L	n/N (%)	33/39 (84.6)	2/33 (6.1)	4/32 (12.5)	4/33 (12.1)
Eosinophils	>1.6 x 10^9/L	n/N (%)	19/39 (48.7)	0/33	4/32 (12.5)	4/33 (12.1)
Basophils	>1.6 x 10^9/L	n/N (%)	4/39 (10.3)	0/33	0/32	0/33
Erythrocytes	<=3.5 x 10^12/L	n/N (%)	21/39 (53.8)	33/33 (100.0)	32/32 (100.0)	33/33 (100.0)
	>=6.4 x 10^12/L	n/N (%)	0/39	0/33	1/32 (3.1)	1/33 (3.0)
Platelets	<=75 x 10^9/L	n/N (%)	12/39 (30.8)	33/33 (100.0)	30/32 (93.8)	33/33 (100.0)
	>=700 x 10^9/L	n/N (%)	2/39 (5.1)	0/33	1/32 (3.1)	1/33 (3.0)

Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies
PCS = Potentially Clinically Significant
Note: n/N is the number of patients with a potentially clinically significant laboratory abnormality divided by the number of patients that enter the period with at least one post-baseline value in the period.
Note: The laboratory value has to represent a worsening from baseline in order to qualify as potentially clinically significant in this analysis. Baseline is defined as value closest but prior to date of mobilization.

Clinical chemistry

Clinical chemistry values are summarized by parameter for the TDT pool in Table 42. Changes to clinical chemistry values were observed frequently during the NE to M24 period, including elevated phosphate (17/32, 53.1%) and elevated glucose (6/32, 18.8%) levels. Transient elevations of ALT (13/32, 40.6%) and bilirubin (10/32, 31.3%) were also observed, often attributed by the Investigator to concurrent treatment with busulfan, azole antifungals, and sulfa antibiotics.

Table 42: Summary of Potentially Clinically Significant Chemistry Values: TDT Pool (ITT)

Laboratory Category	Laboratory Test	PCS Threshold	Statistic	Time Period: MB to < C	Time Period: C to <NE	Time Period: NE to M24	Time Period: >M24 to M36	Time Period: D1 to Last Visit
Liver	Alanine Aminotransferase	≥3× ULN	n/N (%)	0/36	1/33 (3.0)	13/32 (40.6)	0/11	13/33 (39.4)
	Aspartate Aminotransferase	≥3× ULN	n/N (%)	0/35	1/33 (3.0)	8/32 (25.0)	0/11	8/33 (24.2)
	Alkaline Phosphatase	≥3× ULN	n/N (%)	0/36	1/33 (3.0)	1/32 (3.1)	0/11	1/33 (3.0)
	Bilirubin	≥34.2 umol/L	n/N (%)	10/36 (27.8)	12/33 (36.4)	10/32 (31.3)	0/10	12/33 (36.4)
Renal	Urea Nitrogen	≥10.7 mmol/L	n/N (%)	0/34	0/33	1/32 (3.1)	0/11	1/33 (3.0)
	Creatinine	≥176.8 umol/L	n/N (%)	0/36	0/33	0/32	0/11	0/33
Electrolytes	Sodium	≤126 mmol/L	n/N (%)	0/36	0/33	1/32 (3.1)	0/11	1/33 (3.0)
		≥156 mmol/L	n/N (%)	0/36	0/33	0/32	0/11	0/33
	Potassium	≤3 mmol/L	n/N (%)	0/36	5/33 (15.2)	4/32 (12.5)	0/11	8/33 (24.2)
		≥6 mmol/L	n/N (%)	0/36	0/33	0/32	0/11	0/33
	Chloride	≤90 mmol/L	n/N (%)	0/35	0/33	0/32	0/11	0/33
		≥118 mmol/L	n/N (%)	0/35	0/33	0/32	0/11	0/33
Other	Phosphate	≤0.55 mmol/L	n/N (%)	0/36	2/33 (6.1)	1/32 (3.1)	0/9	3/33 (9.1)
		≥1.71 mmol/L	n/N (%)	7/36 (19.4)	17/33 (51.5)	17/32 (53.1)	0/9	23/33 (69.7)
	Glucose	≤2.22 mmol/L	n/N (%)	0/34	0/32	0/32	0/11	0/33
		≥9.71 mmol/L	n/N (%)	0/34	15/32 (46.9)	6/32 (18.8)	1/11 (9.1)	13/33 (39.4)
	Protein	≤45 g/L	n/N (%)	0/35	2/33 (6.1)	1/32 (3.1)	0/11	3/33 (9.1)
		≥100 g/L	n/N (%)	0/35	0/33	0/32	0/11	0/33

Data as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies
Abbrev.: ULN, upper limit of normal.
Study Pool: TDT = All TDT.
Note: n/N is the number of patients with a potentially clinically significant laboratory abnormality divided by the number of patients that enter the period with at least one post-baseline value in the period.
Note: The laboratory value has to represent a worsening from baseline in order to qualify as potentially clinically significant in this analysis. Baseline is defined as value closest but prior to date of mobilization.

Replication Competent Lentivirus

Two methods were used for screening blood samples from patients receiving Zynteglo for the detection of replication-competent Lentivirus (RCL). Initially an enzyme-linked immunosorbent assay (ELISA) for HIV-1 p24 capsid protein was used. However, as there were some caveats with the ELISA assay, a qPCR for detection of VSV-G DNA was adopted as an alternative method for detection of RCL.

Blood was tested for LVV-derived RCL at protocol-defined visits up to month 24. No LVV-derived RCL was detected in any patient treated with Zynteglo (RCL testing results available for 46 patients).

Integration Site Analysis

Integration site analysis (ISA) was based on published methods (Schmidt et al. 2007), performed as a qualified method at Genewerk (Heidelberg, Germany) according to Testing Facility SOPs. ISA was performed using replicate linear amplification-mediated polymerase chain reaction (LAM-PCR) and the non-restrictive (nr) LAM-PCR variant, followed by subsequent deep sequencing. The method was not validated nor GLP compliant, however, according to the Applicant the methods were "fit-for-purpose" and conducted with a focus on high quality and reliability.

BB305 LVV integration site (IS) monitoring was performed in 40 patients (TDT and SCD) who were enrolled in the open-label Phase 1/2 clinical Studies HGB-204 (n=18), HGB-205 (n=7), and HGB-206 (n=9), and in Phase 3 Study HGB-207 (n=6), from 3 to 6 months after drug product infusion up to the most recent time point (with 2 patients in Study HGB-205 being followed for approximately 48 months after drug product infusion as of 07 March 2018). Highly polyclonal repopulation was observed in all patients assayed for ISA as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies (N=37), detected as the presence of multiple unique integration sites in genomic DNA isolated from peripheral blood cells (minimum and maximum of 207 and 21,149 unique sites). In total, >550,000 unique IS were identified. Results showed that all patients were repopulated with a highly polyclonal population of HSCs. None of these IS showed an individual contribution of >30% of all integration sites at any specific time point. Clonal selection or outgrowth was not observed.

Safety in special populations

Intrinsic and extrinsic factors

There is no experience in patients older than 35 years of age.

The Applicant has analysed the potential impact of age (adult, n=23 vs adolescent, n=9; ≥18 years vs ≥12 and < 18 years of age), race (white, n=23 vs Asian, n=10), gender and genotype (non-β^0/β^0, n=21 vs β^0/β^0, n=9) on the safety profile (neutrophil and platelet engraftment, AEs, and SAEs) of Zynteglo.

Platelet engraftment (PE) differed between genotypes (median day of PE on Day 44.0 (19, 191; N=21) vs Day 36.0 (28, 55; N=9) for non-β^0/β^0 patients and β^0/β^0 patients respectively), age (Day 37.0 (19, 191, N=23) versus 53.0 (20, 65, N=9) for adults vs adolescents and race (Day 41.0 [20,191, n=23] versus Day 36.0 [19,51, n=10] for Asian and White patients).

Further, 3 of the 4 cases of VOD occurred in patients < 18 years of age.

The Applicant also analysed the potential impact of the manufacture process (extrinsic factor) on the

safety profile (neutrophil engraftment (NE) and platelet engraftment (PE), AEs, and SAEs) of Zynteglo.

The day of NE was fastest (median [min, max; N]) with the original manufacturing process (Day 16.5 [14, 29; N=4]), followed by the base manufacturing process (Day 18.5 [14, 30; N=18]) and the refined or commercial manufacturing processes (Day 21.5 [14, 28;N=11]). The day of PE occurred earliest with the original manufacturing process (Day 23.0 [20,26]) followed by the base manufacturing process (Day 39.5 [19,191]) and the refined or commercial manufacturing processes (Day 44.0 [28,51]).

No notable differences in the incidence or severity of AEs were observed between groups that differed by the manufacturing drug process used. However, there were differences in numbers of patients and varying lengths of follow-up by group (up to 4 years for patients with the original manufacturing process, 2 years for patients with the base manufacturing process, and 12 months for patients with the refined manufacturing process as of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies).

Immunological events

The formation of antibodies against transgenic $\beta^{A\text{-}T87Q}$-globin have not been studied in Studies HGB-204, HGB-205, HGB-207, HGB-212 and LTF-303.

No immunogenicity-related events (e.g., accelerated clearance or destruction of erythrocytes containing $\beta^{A\text{-}T87Q}$-globin that would result in decreases in $\beta^{A\text{-}T87Q}$-globin concentrations over time, and/or hypersensitivity reactions) were noted in any of the clinical trials conducted with Zynteglo.

Safety related to drug-drug interactions and other interactions

No formal drug-drug interaction studies were submitted. There were no AEs observed in patients suggestive of any potential drug-drug interaction with Zynteglo.

Discontinuation due to adverse events

There were no AEs leading to discontinuation or withdrawal from study.

Post marketing experience

Zynteglo is not approved for any indication in any region. Therefore, no post-marketing data are available.

Supportive safety data from patients with sickle cell disease (SCD)

As of 07 March 2018, 24 patients with SCD underwent mobilization or bone marrow harvest; 2 patients discontinued prior to drug product infusion (reasons: withdrawal by patient and physician decision); 6 patients had not yet received the drug product infusion; 16 patients were infused with drug product manufactured with the same BB305 LVV as Zynteglo (TP), 7 patients completed their parent study and have enrolled in long-term follow-up Study LTF-303.

All patients in the TP of the SCD pool (16/16, 100%) achieved NE on median (min, max) Day 22.5 (17, 38). Fourteen of 16 treated patients (87.5%) had platelet engraftment (defined as an unsupported platelet count of $\geq 50 \times 10^9$/L in SCD) on median (min, max) Day 46.5 (12, 92). The 2 patients who had not had PE had been followed for 28 and 92 days as of07 March 2018.

Of the 16 SCD patients infused with drug product manufactured with the same BB305 LVV as Zynteglo, 1 (6.3%) patient experienced an AE related to drug product (hot flush), and 14 (87.5%) patients experienced an SAE, 11 (68.8%) patients experienced TESAEs.

The majority of TESAEs (n=7) in SCD patients were pain crises which led to hospitalization, an event specific to the SCD population. Excluding TESAEs considered by the Investigator to be related to sickle cell disease, 9/16 (56.3%) patients experienced 20 TESAEs. Pyrexia, Bacteraemia, and Hepatic enzyme increased were the only PTs unrelated to SCD reported as TESAEs by more than 1 patient. No drug product-related SAEs were reported in SCD patients. All SAEs resolved (one with sequalae: a pre-treatment event of Device related thrombosis), except for an event of Patellofemoral pain syndrome and of Device related infection, which were not resolved as of 07 March 2018.

An SAE of myelodysplasia was reported. A patient with SCD participating in Study LTF-303 presented with cytopenia and peripheral blast cells approximately 3 years after busulfan conditioning. A bone marrow aspirate suggested MDS. The patient's cytogenetic analysis reveals a chromosomal abnormality which has been associated with secondary leukaemia. An assessment of tumour cell-enriched cell populations for LVV insertion was negative, providing convincing evidence against insertional oncogenesis. The event is attributed by the Investigator and the Sponsor to busulfan conditioning.

2.6.1. Updated data on safety: 13 December 2018 (Studies HGB-205, HGB-206, HGB-207, HGB-212, and LTF-303)

As of 13 December 2018, the Intent-to-Treat (ITT) population included 57 patients in the TDT pool who initiated mobilisation. All 57 ITT patients in the TDT pool underwent mobilization with G-CSF and plerixafor. Forty-eight of these patients (84.2%) underwent myeloablative conditioning with busulfan as a single agent and received Zynteglo. Patients were followed for a median (min, max) of 18.71 (0.5, 58.6) months after drug product infusion in the TDT pool, and 15.16 (0.5, 58.6) months after drug product infusion in the NB0 pool.

All patients who were followed to at least Day 43 post drug product infusion had neutrophil engraftment (NE). For patients 12 years and older (N=42), neutrophil engraftment occurred on median (min, max) Day 19.5 (13, 38) after medicinal product infusion. Platelet engraftment was recorded for 42/48 (87.5%) patients in the TDT pool. Patients with TDT 12 years and older treated with Zynteglo who achieved platelet engraftment had a median (min, max) platelet engraftment on Day 41.0 (19, 191) (N=39).

The AEs and clinical laboratory findings remain consistent with the known effects of mobilization/apheresis, conditioning, and HSCT. All patients remain alive at last follow-up, with a median (min, max) follow-up duration of 18.71 (0.5, 58.6) months for patients with TDT treated with Zynteglo. The safety profile was in line with what was reported at the initial submission.

The following additional product-related AEs were reported:

- One serious event of Thrombocytopenia assessed as possibly related to Zynteglo was reported in a patient in Study HGB-207. The patient achieved PE on Day 53, but platelets remained persistently below 35,000/µL and an SAE of Thrombocytopenia was reported on Day 114. The event was considered medically significant but was not life-threatening and did not require hospitalization. The Investigator considered the SAE of Thrombocytopenia resolved when the patient's platelet count was >50,000/uL on Day 162.- One additional patient in the TDT pool reported an event of Grade 2 Abdominal pain on Day 1 assessed by the Investigator as related or possibly related to drug product. The event resolved without treatment.
- One patient experienced an event of Grade 1 Pain in extremity on Day 14, a case of leg pain assessed by the Investigator as related to neutrophil engraftment; the event resolved on Day 39 without treatment.

The following additional SAEs were reported:

- One new event each of Bacillus bacteraemia, Neutropenic sepsis, Sepsis, and Lower respiratory tract infection. These events resolved.
- One new event of VOD which resolved after treatment with defibrotide.
- New events of Pyrexia (3), Neutropenia (2), Thrombocytopenia (2) one of which one was assessed as possibly related to drug product, as discussed above, and 1 event each of Febrile neutropenia, Major depression, and Stomatitis. All of these events have resolved except for 1 event each of Thrombocytopenia, Neutropenia, and Pyrexia, which were all reported in the same patient and attributed to the conditioning agent.

All patients remain alive at last follow-up, with a median (min, max) follow-up duration of 18.71 (0.5, 58.6) months as of 13 December 2018 (N = 48). There were no cases of graft-versus-host disease (GVHD). There were no cases of transplant-related mortality including in 29 patients with at least 1 year of follow-up.

No cases of insertional mutagenesis leading to clonal dominance, leukaemia or lymphoma, or LVV-derived RCL have been reported (including approximately 20 patients with at least 2 years of follow-up).

2.6.2. Discussion on clinical safety

Safety database

The analysis of the safety profile of Zynteglo as of 13 December 2018 is limited to 48/57 TDT patients that have been treated. Support is provided by the experience gained in 16 patients with SCD treated with LentiGlobin for SCD manufactured with the same BB305 LVV as Zynteglo.

Treatment with Zynteglo is preceded by haematopoietic stem cell collection (mobilisation with G-CSF and plerixafor followed by apheresis), and myeloablative conditioning using busulfan. Due to the limited sample size, the single-arm design of the studies and concomitant treatment, it is difficult to establish whether exposure to Zynteglo may have contributed to the occurrence of AEs and thus to the overall safety profile of the whole treatment procedure. Notably, as Zynteglo treatment is inherently linked to a HSCT procedure also the well-known risks associated with this procedure should be taken into account when determining the benefit-risk of Zynteglo treatment. Adverse reactions have been described specifically for the mobilisation/apheresis, myeloablative conditioning and product related in the SmPC.

As patient numbers are limited, safety data are pooled across all studies, TDT genotypes, cell doses and drug product manufacturing processes. The refinements made to the drug product manufacturing process aimed to increase transgene expression. No substantial difference in drug product composition is noted between the overall TDT population and the non-β^0/β^0 population patients when treated with batches made from several improved manufacturing processes.

Patients were followed for a median (min, max) of 18.71 (0.5, 58.6) months after drug product infusion in the TDT pool, and 15.16 (0.5, 58.6) months after drug product infusion in the NB0 pool. The duration of follow-up for patients treated with the refined or commericial manufacturing process is more limited than the follow-up for patients treated with the original or base manufacturing processes.

Safety profile

As of 13 December 2018, all but 1 patient in the TDT pool had experienced at least 1 AE, with 14.6% experiencing at least 1 AE related to drug product. Twenty-nine patients with TDT in the ITT population (50.9%) reported a total of 51 SAEs, one of which was assessed as possibly related to drug product. Of

the 51 SAEs, 15 events occurred in 13 patients prior to drug product infusion and were attributed to study procedures, mobilisation, apheresis, or other unknown reasons. No deaths were reported and no patients discontinued from their respective studies due to an AE. The only case of drug product-related SAE concerned one event of thrombocytopenia. The event was considered medically significant but was not life-threatening and did not require hospitalisation.

As of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies, most patients experienced events of thrombocytopenia (84.6%), anaemia (76.9%), stomatitis (66.7%), alopecia (64.1%), nausea (61.5%), vomiting (59.0%), and neutropenia (56.4%, with 33% febrile neutropenia). The events of thrombocytopenia, anaemia, stomatitis, and neutropenia were primarily reported at a severity grade of ≥Grade 3, whereas the events of alopecia, nausea, and vomiting were primarily reported at a maximum of Grade 1 or Grade 2.

As of 22 February 2018 for Study HGB-207 and 07 March 2018 for all other studies, 30 patients in the TDT pool experienced at least one event in the SOC infection or infestation, of which 11 (28.2%) patients reported a total of 19 ≥Grade 3 and/or serious infections, with 7 non-serious and 12 serious infections. Of the 9 serious infections occurring after Zynteglo treatment, 1 occurred prior to neutrophil engraftment (NE) (appendicitis) and 8 occurred after NE (cat scratch disease, gastroenteritis, diarrhoea infectious, asymptomatic HIV infection, salmonella sepsis, cellulitis, tooth infection, and pneumonia). All but the event of asymptomatic HIV infection (wildtype aetiology confirmed by western blot) and salmonella sepsis (both in the same patient) resolved as of 07 March 2018.

As of 22nd February 2018 for Study HGB-207 and 07 March 2018 for all other studies, thrombocytopenia was accompanied by a bleeding event in 22 (56.4%) patients in the ITT population. Most events occurred following busulfan treatment, were considered non-serious and did not require blood transfusion.

As of 22nd February 2018 for Study HGB-207 and 07 March 2018 for all other studies, five thrombotic events were experienced by 3 patients; 2 events were serious (Intracardiac thrombus, Vena cava thrombosis). In the TDT pool 9 patients (23.1%) reported 15 hepatic events, 4 patients (10.3%) experienced a serious hepatic event (VOD). It is noted that 3/4 serious VOD observed occurred in paediatric population (3 adolescents with VOD in 9 adolescents treated), all related to busulfan that is known to induce such events.

As of 22nd February 2018 for Study HGB-207 and 07 March 2018 for all other studies, most of the AEs were attributed to the HSCT procedure and not the Zynteglo product. Only 8 events in 5 patients in the TDT pool were assessed by the Investigator as related or possibly related to Zynteglo. Of these, 7 events occurred on Day 1 in 4 patients (3 events of abdominal pain, 2 events of hot flush, and 1 event each of dyspnoea and non-cardiac chest pain). These events were Grade 1 and all resolved on the same day without treatment and were attributed by the Applicant to the presence of the dimethyl sulfoxide (DMSO) cryoprotectant. This is agreed, such infusion-related reactions have often been attributed to DMSO.

One case of dysplasia was also attributed to Zynteglo treatment by the Investigator. This patient had an AE of Grade 1 dysplasia at the Month 24 Visit. This event was more likely a consequence of longstanding dyserythropoiesis considering that no dysplasia was noted at Month 30.

Many of the AEs were attributed to the HSCT procedure and confirm the toxicity profile associated with HSCT.

As of 13 December 2018, the median time (min, max) to neutrophil engraftment (NE) of Day 20.5 (13, 38)(N=46) is in line with what is usually seen in TDT patients receiving allogeneic HSCT, but the time to platelet engraftment (PE) seems substantially prolonged in the studied population with a median (min, max) of Day 41.5 (19, 191), while usually PE is recorded within 20-30 days in TDT patients receiving allogeneic HSCT, per published literature. The cause of this apparent delayed engraftment could not be identified. As prolonged time to PE carries the risk for bleeding, it is reassuring that no excess of bleeding

events has occurred in the patients with prolonged PE time. Delayed platelet engraftment is listed as identified risk in the Risk Management Plan (RMP) and neutrophil engraftment failure as a potential risk.

Substantial changes in the haematology and clinical chemistry parameters were frequently observed, but these can be expected in patients undergoing HSCT. No unexpected effects were noted.

No cases of malignancies were noted in the TDT population, however one case of myelodysplasia was reported in a patient with SCD participating in Study LTF-303. As no LVV insertion was found in tumour cell-enriched cell population and the cytogenetic analysis revealed a chromosomal abnormality which has been associated with secondary leukaemia, it is considered likely that this event is caused by exposure to busulfan.

The potential for generation of replication competent virus and insertional mutagenesis are concerns associated with retroviral gene therapies (i.e. therapies using an integrating LVV), and thus also with Zynteglo. It is noted that these risks have already been mitigated by the choice for and design of the LVV (i.e. replication-defective, SIN, lentiviral vector lacking enhancer/promoter long terminal repeat sequences), the manufacture process (screening each LVV batch for potential RCL, extensive post-transduction washing), and patient selection (exclusion of patients with pre-treatment evidence of HIV-1 or HIV-2 infection). No RCL was detected in blood samples up to 24 months after Zynteglo administration. As the risk for the occurrence of recombination in patients infected with HIV is hypothetical no contraindication for HIV and HTLV were considered necessary. Still seropositive patients will currently not be treated as seropositive samples are not accepted for manufacturing. The integration site analyses did not reveal any data suggesting that oncogenesis occurred due to integration site mutagenesis. Section 4.4 of the SmPC provides recommendations to monitor annually for leukaemia or lymphoma (including with a complete blood count) for 15 years post treatment with Zynteglo and that patients who have received Zynteglo should not be screened for HIV infection using a polymerase chain reaction (PCR)-based assay.

The Applicant performed an analysis of the impact of some intrinsic (age, race, gender, genotype) and extrinsic factor (manufacturing process) on the safety profile. However, population size is too small and there are too many variables to allow any meaningful comparisons. The most remarkable difference might be the fact that most cases of VOD occurred in the adolescent population. However, considering the adult population is still rather young (no patients >35 years were enrolled and median age of overall TDT population is 20 years), it may be questioned whether age is such a discriminating factor and this could just as well be a chance finding.

At the time of MAA review, the safety and efficacy of plerixafor in children less than 18 years was not yet established according to the SmPC of Mozobil. Based on the review of publicly available data and preliminary data in the < 18 years of age patients mobilised with plerixafor submitted by the applicant, plerixafor appears to add minimal safety risks in children beyond the risks associated with the use of single agent G-CSF and does seem to decrease the rate of failed mobilisations. One exception may be a concern regarding the spleen as splenomegaly is a common feature in TDT patients and mobilisation with plerixafor has been implicated in splenic rupture. It is reassuring that apart from a Grade 1 non-SAE of splenomegaly no AEs related to the spleen have been observed, yet it is noted patient numbers are limited to 34 non-splenectomised patients (as of 13 December 2018). Therefore, the proposal to monitor for AEs of splenic rupture is endorsed. Splenic rupture has been added as potential risk in the safety specifications of the RMP.

Further with regards to age, it is noted that current treatment experience is limited to patients aged 35 years or younger. The observed benefit-risk in the studied population can be extrapolated to the older population (i.e. >35) as long as transplant eligibility criteria for patients with TDT are met. It is agreed that the decision to start treatment should be based on specific co-morbid factors known to impact the safety of treatment including myeloablative conditioning, rather than a strict upper age limit in the

indication. Therefore, section 4.4 of the SmPC includes specific criteria to help health care providers identify patients with TDT for whom HSC transplantation is appropriate. Moreover, patients older than 35 has been included as "missing information" in the safety specifications of the RMP.

The following restrictions on concomitant medications are recommended in the SmPC:

- Iron chelation should be stopped at least 7 days prior to myeloablative conditioning. Prophylaxis for hepatic veno-occlusive disease (VOD) is recommended. Depending on the myeloablative conditioning agent administered, prophylaxis for seizures should be considered.
- Patients should not take anti-retroviral medications or hydroxyurea from at least one month prior to mobilisation until at least 7 days after Zynteglo infusion.

As of 07 March 2018, a separate high-level analysis of the safety profile in patients with SCD treated with drug product manufactured with the same BB305 LVV as Zynteglo (n=16) was provided. Apart from the events of hospitalisation due to pain crises (attributable to the underlying disease) only numerical differences in AE frequencies were noted. However, no conclusions can be drawn on the differences considered the small populations. Overall the available safety data in the SCD population are generally supportive of that seen in the TDT population.

No studies aiming to investigate the immunogenicity of Zynteglo have been performed that is justified as risk assessment indicated a lack of immunogenic potential of the gene product. This claim is supported by the fact that no immunogenicity-related events were noted in the studies. The lack of immunogenic potential can be explained by the fact that $\beta^{A\text{-}T87Q}$-globin is highly similar to naturally occurring proteins: the modification of the single amino acid (T87Q) was chosen because of its presence of the Q in the γ- and δ-globin chains.

Pregnancy and breast-feeding as well as previous treatment with HSC gene therapy have been added as a contraindication (please see section 4.3 of the SmPC.

In addition, contraindications to the mobilisation agents and the myeloablative conditioning agents must be considered (see section 4.3 of the SmPC).

From the safety database all the adverse reactions reported in clinical trials have been included in the SmPC.

Additional safety data needed in the context of a conditional MA

There are still some uncertainties on the safety profile of Zynteglo due to the limited data on long-term safety of Zynteglo, the limited data in patients treated with the drug product manufactured with the commercial manufacturing process, and the limitations of single-arm trial design, in particular considering that treatment is irreversible and with an intended life-long effect. The current analyses provide an idea of the safety profile associated with Zynteglo, in part due to the already well-known safety profile of the mobilisation/apheresis regimen and of the conditioning regimen with busulfan.

Additional data will be obtained with the commercial drug product manufacturing process in study HGB-207 and additional safety data will also be made available with studies HGB-212 and LTF-303 which are specific obligations of the marketing authorisation.

2.6.3. Conclusions on the clinical safety

Overall, due to the limitations in the safety database it is not possible to fully discriminate between effects caused by Zynteglo and those by the concomitant treatment/HSCT procedure. Nevertheless, adverse reactions have been described specifically for the mobilisation/apheresis, myeloablative conditioning and product related in the SmPC. Common AEs that may be caused by Zynteglo may also

be missed due to the limited number of patients included in the safety database. Furthermore, the safety data were pooled across studies, TDT genotypes, cell doses and manufacturing processes. No apparent differences in the safety profile have been observed based on these factors, although patient numbers are low. Similarly, a conclusion on the effect of intrinsic factors (age, race, gender, genotype) on the safety profile of Zynteglo is complicated by low patient numbers.

Overall, the safety profile seems to be in line with that what is known for HSCT. As treatment with Zynteglo encompasses an HSCT procedure, the risks associated with mobilisation and conditioning are also part of the benefit:risk of Zynteglo. This includes the risk for secondary malignancies, as is illustrated by the event of myelodysplasia in an SCD patient, VOD (5 serious events) and impairment of fertility.

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

- In order to confirm the efficacy and safety of Zynteglo in patients 12 years and older with TDT who do not have a β^0/β^0 genotype, the MAH should submit interim and final data on Study HGB-207.
- In order to confirm the efficacy and safety of Zynteglo in patients 12 years and older with TDT who do not have a β^0/β^0 genotype, the MAH should submit interim and final data from patients with a severe non-β^0/β^0 genotype such as IVS-I-110/IVS-I-110 and IVS-I-110/β^0 from Study HGB-212.
- In order to confirm the efficacy and safety of Zynteglo in patients 12 years and older with TDT who do not have a β^0/β^0 genotype, the MAH should submit interim data and the 5-year follow-up results of study LTF-303.

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

- In addition, as a condition to the MA, a non-interventional post-authorisation safety and efficacy study should be conducted in order to further characterise and contextualise the long-term safety and efficacy of Zynteglo in patients 12 years and older with transfusion-dependent β thalassaemia (TDT) who do not have a β^0/β^0 genotype. The MAH should conduct and submit the results of a study based on data from a product registry (REG-501) and use data on patients treated with transfusions and/or HLA-matched allogenic HSCT treated patients from an established European registry as a comparator group.

The registry will specifically address the risk of delayed platelet engraftment, insertional oncogenesis, loss of response to gene therapy and neutrophil engraftment failure. Interim data on the registry should also be submitted at each annual renewal of the conditional marketing authorisation.

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

2.7. Risk Management Plan

Safety concerns

Summary of safety concerns	
Important identified risks	Delayed platelet engraftment
Important potential risks	Insertional oncogenesis Loss of response to gene therapy Neutrophil engraftment failure Splenic rupture
Missing information	Long-term safety and efficacy Use in patients over 35 years of age

Pharmacovigilance plan

Study / Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Category 1 - Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
Long-term observational registry study (including product registry REG-501) Planned	To evaluate long-term safety and effectiveness of treatment with Zynteglo finished product in patients 12 years and older with transfusion-dependent β thalassaemia (TDT) who do not have a β^0/β^0 genotype.	Delayed platelet engraftment Insertional oncogenesis Loss of response to gene therapy Neutrophil engraftment failure Splenic rupture Long-term safety and efficacy	Protocol submission Interim data analysis Final study report submission	February 2020 At each annual renewal - December 2024 - December 2034 4Q 2039
Category 2 – Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
Study HGB-207 Ongoing	To evaluate the efficacy of treatment with Zynteglo finished product in patients ≥50 years of age with transfusion-dependent β thalassaemia (TDT) who do not have a β^0/β^0 genotype at the β globin (*HBB*) gene To evaluate the safety of treatment with Zynteglo finished product in patients ≤50 years of age with TDT who do not have a β^0/β^0 genotype at the *HBB* gene	Delayed platelet engraftment Insertional oncogenesis Loss of response to gene therapy Neutrophil engraftment failure Splenic rupture Long-term safety and efficacy	Interim results Interim study report when all included adolescents and adults have completed the study	At each annual renewal of the conditional marketing authorisation December 2021

Study / Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Study HGB-212 Ongoing	To evaluate the efficacy of treatment with Zynteglo finished product in patients ≤50 years of age with transfusion-dependent β-thalassaemia (TDT) who have a β^0/β^0 genotype at the HBB gene To evaluate the safety of treatment with Zynteglo finished product in patients ≤50 years of age with TDT who have a β^0/β^0 genotype at the *HBB* gene	Delayed platelet engraftment Insertional oncogenesis Loss of response to gene therapy Neutrophil engraftment failure Splenic rupture Long-term safety and efficacy	Annual data update Interim study report when all included adolescents and adults with an IVS-I-110/IVS-I-110 or IVS-I-110/β^0 genotype have completed the study	At each annual renewal of the conditional marketing authorisation December 2021
Study LTF-303 Ongoing	Monitor for long-term safety of the gene therapy drug product (i.e., the "drug product") used in bluebird bio-sponsored clinical studies (i.e., the "parent studies") in treated patients with haemoglobinopathies. Monitor for long-term efficacy of the drug product	Insertional oncogenesis Loss of response to gene therapy Long-term safety and efficacy	Annual data update Interim Study Report	At each annual renewal of the conditional marketing authorisation December 2024

Risk minimisation measures

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Delayed platelet engraftment	Routine risk minimisation measures - SmPC sections 4.4, and 4.8 - PL section 2 Sign and symptoms of bleeding and recommendations if present included in PL section 2. Restricted prescription medicine Additional risk minimisation measures - Educational materials for healthcare professionals - Educational materials for patients - Patient alert card	Routine pharmacovigilance beyond adverse reactions reporting and signal detection: - Quarterly review of aggregate safety data Additional pharmacovigilance - Study REG-501 - Study HGB-207 - Study HGB-212
Insertional oncogenesis	Routine risk minimisation measures - SmPC sections 4.3, 4.4, and 5.3 - PL section 2 Recommendations for annual monitoring for leukaemia/lymphoma given in SmPC section 4.4 Collection of blood samples for testing if leukaemia/lymphoma is diagnosed given in SmPC section 4.4 Restricted prescription medicine Additional risk minimisation measures - Educational materials for healthcare professionals - Educational materials for patients	Routine pharmacovigilance beyond adverse reactions reporting and signal detection: - Quarterly review of aggregate safety data Additional pharmacovigilance - Study REG-501 - Study HGB-207 - Study HGB-212 - Study LTF-303

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Loss of response to gene therapy	Routine risk minimisation measures – SmPC section 4.4 Restricted prescription medicine Additional risk minimisation measures – Educational materials for healthcare professionals – Educational materials for patients – Patient alert cards	Routine pharmacovigilance beyond adverse reactions reporting and signal detection: - none Additional pharmacovigilance - Study REG-501 - Study HGB-207 - Study-HGB-212 - Study LTF-303
Neutrophil engraftment failure	Routine risk minimisation measures – SmPC section 4.4 – PL sections 2 and 3 Restricted prescription medicine Additional risk minimisation measures – Educational materials for healthcare professionals	Routine pharmacovigilance beyond adverse reactions reporting and signal detection: - Quarterly review of aggregate safety data Additional pharmacovigilance - Study REG-501 - Study HGB-207 - Study HGB-212
Splenic rupture	Routine risk minimisation measures: - SmPC section 4.4 Restricted prescription medicine Additional risk minimisation measures - Educational materials for healthcare professionals	Routine pharmacovigilance beyond adverse reactions reporting and signal detection: - Quarterly review of aggregate safety data Additional pharmacovigilance - Study REG-501 - Study HGB-207 - Study HGB-212

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Long-term safety and efficacy	Routine risk minimisation measures - SmPC section 4.4 Restricted prescription medicine Additional risk minimisation measures - Patients alert cards - Controlled distribution	Routine pharmacovigilance beyond adverse reactions reporting and signal detection: - Quarterly review of aggregate safety data Additional pharmacovigilance - Study REG-501 - Study HGB-207 - Study HGB-212 - Study LTF-303
Use in patients over 35 years of age	Routine risk minimisation measures - SmPC section 5.1 Restricted prescription medicine Additional risk minimisation measures - none	Routine pharmacovigilance beyond adverse reactions reporting and signal detection: - Subpopulation analysis of safety in patients over the age of 35 years will be included with each PSUR. Additional pharmacovigilance - none

The CHMP, CAT and PRAC considered that the risk management plan version 1.0 is acceptable.

2.8. Pharmacovigilance

Pharmacovigilance system

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

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 new EURD list entry will therefore use the EBD to determine the forthcoming Data Lock Points.

2.9. New Active Substance

Autologous CD34+ cell-enriched population that contains hematopoietic stem cells transduced with lentiviral vector encoding the $\beta^{A\text{-}T87Q}$-globin gene has not been previously authorised in a medicinal product in the European Union (EU).

2.10. Product information

2.10.1. User consultation

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

2.10.2. Labelling exemptions

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

The QRD agreed to use the particulars for small immediate packaging units for the infusion bags. Whilst this labelling text is only intended for small containers of 10 mL or less and the Zynteglo infusion bag has a volume of 20 mL, a large amount of information needs to be included on the primary packaging label for this autologous ATMP. The limitations of the available space for the label of the infusion bag only allow for the inclusion of the labelling text for small immediate containers. The QRD agreed that a Lot Information Sheet containing the strength (cell concentration) of each lot, the dose, the number of lots, the total number of infusion bags for the individual patient, would supplement the minimum particulars to appear on the infusion bag label.

2.10.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Zynteglo (autologous CD34+ cell-enriched population that contains hematopoietic stem cells (HSCs) transduced with lentiviral vector (LVV) encoding the $\beta^{A\text{-}T87Q}$-globin gene) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU and it is approved under a conditional marketing authorisation.

Therefore, the SmPC 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

3.1. Therapeutic Context

3.1.1. Disease or condition

The claimed indication is the treatment of patients 12 years and older with transfusion-dependent β-thalassaemia (TDT) who do not have a β^0/β^0 genotype, for whom haematopoietic stem cell (HSC) transplantation is appropriate but a human leukocyte antigen (HLA)-matched related HSC donor is not available.

3.1.2. Available therapies and unmet medical need

TDT is most frequently managed with supportive care that includes lifelong regular RBC transfusions, given every 2 to 5 weeks, aimed at maintaining Hb levels ≥ 9 g/dL (Cappellini *et al.* 2014) to mitigate symptoms of anaemia and suppress ineffective erythropoiesis. Due to excess iron introduced through chronic transfusions, patients with TDT also require an intensive regimen of iron chelation using oral and/or injectable chelating agents to minimize iron overload in organs, particularly in the liver, heart,

and endocrine system.

No medicinal product has been approved to date to address the underlying cause of TDT. At the time of MAA review, allogeneic HSCT was the only potential cure for TDT where cure is defined as a treatment that may achieve near normal or normal levels of total haemoglobin and eliminate the need for transfusions. Allogeneic HSCT addresses the underlying genetic cause of TDT and can restore production of functional RBCs. If allogeneic HSCT is performed early enough, it is also possible to prevent iron overload and its associated complications. Allogeneic HSCT is associated with risks for serious complications, such as graft-rejection, graft-versus-host disease (GVHD), and infection. Due to well-documented safety concerns, allogeneic HSCT is offered primarily to the minority of children and adolescents who have an available human leukocyte antigen (HLA)-matched sibling donor [<25% of cases (Angelucci *et al.* 2014)]. However, in practice, a far smaller proportion of eligible patients actually undergo allogeneic HSCT due in part to persistent concerns over procedure-related toxicity.

3.1.3. Main clinical studies

The clinical development program of Zynteglo for the treatment of patients with TDT includes 5 single arm clinical studies: 2 Phase 1/2 studies (Studies HGB-205 and HGB-204), 2 Phase 3 studies (Studies HGB-207 and HGB-212), and 1 long-term follow-up study (Study LTF-303).

The manufacturing process was refined to ultimately achieve higher levels of HbA^{T87Q} values in patients following the clinical finding from study HGB 204 that the base manufacturing process would not be sufficient in patients with no endogenous HbA, i.e. patients with β^0/β^0 genotype. The refined and commercial manufacturing process have only been used in the pivotal studies HGB-207 and HGB-212 in patients with TDT.

As of 13 December 2018, a total of 32 non-β^0/β^0 patients have been treated, of which 11 were adolescents and 21 were adults. The age range was 12 to 35 years, the youngest patient (age 12 years) was enrolled in study HGB-207.

The ongoing long-term follow-up Study LTF-303 includes patients treated with Zynteglo who have completed any of the above parent studies for an additional 13 years of follow-up, for a total of 15 years of follow-up post-drug product infusion. As of 13 December 2018, the follow-up time has been up to approximately 60 months post-drug product infusion.

3.2. Favourable effects

The natural history of β-thalassaemia is well known and patients do not spontaneously produce higher levels of HbA or become transfusion independent. The mechanism of action of Zynteglo addresses the underlying genetic cause of the disease by the administration of HSCs containing functional copies of the $\beta^{A\text{-}T87Q}$-globin gene through an autologous HSCT procedure.

All non-β^0/β^0 patients who received Zynteglo produced Hb containing $\beta^{A\text{-}T87Q}$-globin (HbA^{T87Q}). Median HbA^{T87Q} levels increase from month 1 through approximately month 6 to month 9 post-HSCT, after which stabilisation is observed. For patients with the non-β^0/β^0 genotype, HbA^{T87Q} expression level at any time point was variable between patients, but kinetics were similar between patients.

A correlation between PB VCN and transgene expression (HbA^{T87Q}) and efficacy outcomes (total Hb, transfusion independence and reduction) has been shown. Although limited clinical data are available for study HGB-207 performed with drug product produced from the refined and commercial manufacturing processes, the preliminary results showed an improved efficacy with a similar safety.

The majority of patients with TDT who do not have a β^0/β^0 genotype treated with Zynteglo in study HGB-204 and HGB-205 have achieved TI (11 out of 14; 78.6%). For study HGB-207, 4 out of 5 TI-evaluable

patients achieved TI (data cut: 13 December 2018), and based on a developed prediction model (generated from Studies HGB-204, HGB-205 and the 5 TI-evaluable patients in Study HGB-207), an additional 6 patients from Study HGB-207 produced close to 8 g/dL or more HbA^{T87Q} at Month 6 and are predicted to achieve TI with ≥98% probability. Therefore the results appear to be in line with the results of studies HGB-204 and HGB-205. Achievement of TI is clinically meaningful, eliminating the need for regular blood transfusions and associated comorbidities due to iron overload.

In patients with TDT who do not have a β^0/β^0 genotype who have not achieved TI (n=4), reductions in transfusion volume requirements and/or transfusion frequency were observed, ranging from 26.8% to 100% reduction in transfusion volume (compared to baseline pre-treatment requirements). While the transduction efficiency was not sufficient to provide adequate expression of HbA^{T87Q} to support TI in these patients, they still received clinical benefit by meaningful reduction in transfusions.

Support of efficacy of Zynteglo is partially derived from the β^0/β^0 patients. Although only a minority of β^0/β^0 patients achieved TI, the 6 out of 8 β^0/β^0 patients in Study HGB-204 who have not achieved TI achieved a median transfusion volume reduction of -63.3% (-76.3%, -8.3%).

Clinical responses across studies have been durable. Once TI was achieved, TI status was durable for non-β^0/β^0 patients with the minimum duration of TI to date ranging from 12.0+ to 56.3+ months (depending on the time of follow-up). No non-β^0/β^0 patient who achieved TI has since required a transfusion and TI status was maintained. Non-β^0/β^0 patients who achieved TI and who have completed at least their Month 30 Visit (N = 7) all had their last pRBC transfusion in the parent study and remained transfusion-free throughout the follow-up Study LTF-303, demonstrating stable integration of LVV provirus into long-term progenitor HSCs and stable expression of the transgene in erythroid cells.

PD parameters and efficacy results showed consistency across studies (HGB-204 and HGB-205) irrespective of the drug process change. HGB-207 is performed with the refined and commercial drug manufacturing processes, which are expected to increase achievement of TI in even a larger portion of treated patients, due to increased DP VCNs. For a number of patients, a higher expression of HbA^{T87Q} was observed in study HGB-207. As of December 2018, all patients who were predicted to achieve TI based on pharmacodynamic modelling and who have become evaluable for TI have achieved TI.

Across all clinical studies to date, no significant or clinically relevant differences in effect of treatment were observed across gender, age, or race, although the sample size is small.

A main long-term benefit of TI and the normalisation of Hb levels is expected to be a limitation or reduction in organ damage from iron overload. The follow-up is too short to accurately determine whether a decrease in iron burden in the liver and heart are obtained in all patients who achieved TI status but the preliminary data do seem to indicate a reduction in serum iron burden-related parameters, as indicated by improvements in ferritin (7/8 patients with Baseline values), serum transferrin (7/9 patients), serum iron (7/9 patients), and transferrin saturation (5/7 patients with Baseline and M24 values).

3.3. Uncertainties and limitations about favourable effects

Endogenous Hb fractions contribute to total Hb in patients with TDT with non-β^0/β^0 genotype and may complement HbA^{T87Q} production and thus help to achieve TI. Two non-β^0/β^0 patients in Study HGB-207 with the severe non-β^0 mutation IVS-I-5 (1 patient with IVS-I-5/IVS-I-5 and 1 patient with IVS-I-5/β^0) have <1 g/dL of endogenous HbA. At the opposite end of the spectrum, a patient with a *HBB*:c.92+1G>T β^0 mutation compound heterozygous with an unknown second *HBB* mutation is an outlier with high endogenous β-globin production of 5.1 g/dL of endogenous HbA. In spite of these substantial differences

in endogenous β-globin production, these 3 patients met the criteria for TI, thus the level of endogenous Hb influences only to a small extent the total Hb level post-HSCT with Zynteglo transduced cells.

Products manufactured using the base manufacturing process cannot be considered fully comparable with those manufactured with the refined or commercial manufacturing processes, and thus data obtained with material from the original or base manufacturing processes can only be considered as supportive for a full MAA. Ongoing Phase 3 Studies (HGB-207 and HGB-212), which used the refined and commercial manufacturing processes, have treated 3 patients of the β^0/β^0 genotype (in Study HGB-212). To guarantee that no patient is treated with a sub-optimal batch, tight control of the drug product potency attributes was implemented to ensure that the benefit of treatment with the commercial process is clinically in line with that of Phase 3 studies, which is of particular importance for patients with very low endogenous Hb production. Furthermore, it is proposed to re-evaluate the acceptance criteria for attributes related to drug product potency tests using batch release data and clinical results after 6 months follow-up of 20 patients treated with commercial batches.

Overall, the number of patients is limited and patients were selected using an extensive list of inclusion and exclusion criteria. Even so, the efficacy is consistent over the clinical studies and has shown to be durable, the follow-up time for a substantial number of patients in Phase 3 studies is limited. As this application concerns a conditional approval, specific obligations have been proposed in order to obtain more efficacy and safety results from studies HGB-207, HGB-212 and LTF-303.

3.4. Unfavourable effects

48 patients with TDT have received Zynteglo. The overall safety profile seems to be in line with HSCT procedures. The most common AEs were thrombocytopenia (84.6%), anaemia (76.9%), stomatitis (66.7%), alopecia (64.1%), nausea (61.5%), vomiting (59.0%), and neutropenia (56.4%). The AEs related to Zynteglo are thrombocytopenia, abdominal pain, non-cardiac chest pain, pain in the extremities, dyspnoea and hot flush.

SAEs included infections, bleeding events, thrombotic events, hepatic events (including VOD), hyperglycaemia and others (transfusion reaction, anaemia, depression and hypoxia). One SAE (thrombocytopenia) was considered due to drug product (Zynteglo) infusion.

The safety profile of Zynteglo also takes into consideration the AEs of the mobilisation/apheresis and especially conditioning regimen, carrying a particular risk of veno-occlusive disease and infertility. It is noted that pyrexia was only reported in patients <18 years (n=3, 16%) and that VOD occurred relatively more frequent in < 18 years old patients (16% vs 7%). Please refer to section 4.8 of the SmPC for the list of AEs related to the mobilisation, apheresis and myeloablative conditioning. Contraindications to the mobilisation agents and the myeloablative conditioning agent must be considered.

Zynteglo is contraindicated in pregnant and breast-feeding women (see section 4.3 and 4.6 of the SmPC). In addition, Zynteglo is contraindicated in previous treatment with HSC gene therapy.

When compared to allogeneic HSCT a delay in platelet engraftment was noted. This has been reflected in section 4.4 of the SmPC.

The parameters indicative of iron load in patients in the study are rather variable. The values are within the range reported in literature. No analyses on potential relationship between the other parameters for iron overload (T2* and ferritin) was provided. The risk of iron load has been adequately described section 4.4 in the SmPC and there is currently no indication for a relation between baseline iron load and clinical outcome.

3.5. Uncertainties and limitations about unfavourable effects

Due to the limitations in the safety database (such as the limited number of patients, single arm trial design) it is difficult to fully discriminate between adverse effects caused by Zynteglo and those by the concomitant treatment/HSCT procedure, or determine whether Zynteglo exposure may have contributed to the occurrence of AEs. Nevertheless, adverse reactions have been described specifically for the mobilisation/apheresis, myeloablative conditioning and product in the SmPC. Also common AEs that may be caused by Zynteglo treatment may be missed. As patient numbers are limited, safety data were pooled across studies, TDT genotypes, cell doses and manufacturing processes. No apparent differences in the safety profile have been observed based on these factors, although patient numbers are low. Similarly, a conclusion on the effect of intrinsic factors (age, race, gender, genotype) on the safety profile of Zynteglo is complicated by low patient numbers. Long-term follow up is limited, in particular considering the expected life-long activity of the product. This is especially the case for patients treated with the commercial manufacturing process. Study HGB-207 was performed with product made using the refined or commercial manufacturing processes; the preliminary results show an improved efficacy with a similar safety. Additional safety data are expected with studies HGB-207, HGB-212 and LTF-303 as well as with the registry (REG-501).

With respect to the treatment protocol, the mobilisation was conducted with plerixafor and G-CSF. Plerixafor was not authorised in the EU for use in paediatric patients at the time of MAA review and did not have an indication for use in non-malignant diseases (Mozobil SmPC). The applicant has committed to continue to monitor the safety of using G-CSF and plerixafor for mobilisation, through the Registry REG-501 to collect all serious adverse reactions related to mobilisation agents, including plerixafor.

A later platelet engraftment in the younger age group (median day of engraftment 51 days in younger vs 36 days in adult patients) has been shown, as well as neutrophil engraftment which seems to be somewhat later in the younger age group (median 26 d vs 19 d <18y vs ≥18y). As there are confounding factors and population is limited, it cannot be established whether indeed age was a contributing factor for this apparent delayed engraftment. The SmPC provides recommendations to monitor patients for thrombocytopenia and bleeding according to standard guidelines. Blood cell count determination and other appropriate testing should be promptly considered whenever clinical symptoms suggestive of bleeding arise.

Section 4.4 of the SmPC recommends that patients should receive rescue treatment with the back-up cell collection when experiencing neutrophil engraftment failure (see also section 4.2 of the SmPC). In clinical trials, no patients failed to engraft bone marrow, as measured by neutrophil engraftment (N=42).

Zynteglo is a gene therapy product using an LVV to genetically modify HSCs. Theoretical safety concerns mainly consist of insertional oncogenesis and replication competent retrovirus, these have been minimised by the design of the lentiviral construct and through the manufacturing process. Integration site analyses (ISA) have been performed through the non-clinical investigations and repeatedly analysed using linear amplification mediated PCR (LAM-PCR) in patients included in clinical studies. Of the patients with available ISA data (N=59), no evidence of clonal dominance has been reported. Potential effects due to transgene overexpression, loss of efficacy and the possibility for the formation of replication competent lentiviral cannot be fully excluded. No clinical symptoms of myeloproliferation have been seen in patients treated with Zynteglo. The SmPC recommends that patients should be monitored annually for leukaemia or lymphoma (including with a complete blood count) for 15 years post treatment with Zynteglo. If leukaemia or lymphoma is detected in any patient who received Zynteglo, blood samples should be collected for ISA. Additional long-term safety data will be provided by a 15-year long-term follow-up observational registry for patients treated with Zynteglo which will monitor the identified and potential risks in an increased number of patients and according to the safety specifications of the RMP.

3.6. Effects Table

Table 43: Effects Table for Zynteglo for Patients with TDT (data as of 13 December 2018)

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
Transfusion Independence (TI) for evaluable non-β^0/β^0 patients	Proportion of patients with a weighted average haemoglobin (Hb) ⩾9 g/dL without any pRBC transfusions for a continuous period of ⩾ 12 months at any time during the study after Zynteglo infusion	n/N (%)	15/19 (79%; 95% CI: 54% to 94%)	NA	The single patient in Study HGB-207 who has not achieved TI exhibited a substantial decline from DP VCN to PB VCN, resulting in low HbA^{T87Q} production Study HGB-205 was performed with the base manufacturing process, HGB-204 with the original manufacturing process, HGB-207 with the refined or commercial manufacturing process.	Studies HGB-204, HGB-205, HGB-207, LTF-303.
Observed duration of TI for non-β^0/β^0 patients		min, max	12.0+ to 56.3+ months. * median duration of TI not reached		All non-β^0/β^0 patients who have achieved TI at any time have maintained their TI status through all Hb assessments. True duration of TI is unknown as follow up time is limited.	Studies HGB-204, HGB-205, HGB-207, LTF-303.
Transfusion Reduction (TR) in non-β^0/β^0 patients that did not achieve TI at any time (n=4)	% change in annualized transfusion volume from the period from 6 months post-DPI through Month 24 as compared to annualized baseline pretreatment transfusion requirements	%	-26.8%, -71.4% -86.9%, -100%	NA	Small number of patients	Studies HGB-204, HGB-205, HGB-207.
Unfavourable Effects						
AE for all patients with TDT (ITT)	Patients with at Least 1 AE	% (n)	98.2% (n=56)	NA	Limited safety database, contribution of Zynteglo to the overall safety profile cannot be distinguished from that of concomitant treatment/HSCT procedure	

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
SAE for all patients with TDT (ITT)	Patients with at Least 1 SAE	% (n)	50.9% (n=29)	NA	Limited safety database, one AE was assessed as possibly related to drug product	
Common AE for all patients with TDT (ITT)	AEs occurring in at least 50% of patients	%	Thrombocytopenia (82.5%), Anaemia (71.9%), Stomatitis (64.9%), Vomiting (50.9.%), Neutropenia (57.9%), Alopecia (52.6%)	NA	Limited safety database, contribution of Zynteglo to the overall safety profile cannot be distinguished from that of concomitant treatment/HSCT procedure	
Delayed platelet engraftment for all patients with TDT	Time to platelet values $\geqslant 20 \times 10^9$/L on 3 consecutive days	Median, days	41.5 (min 19, max 191).	< 30 days	contribution of Zynteglo to the delayed platelet engraftment remains unexplained	

Abbreviations: DPI = drug product infusion, ITT = Intent-to-Treat, TI= transfusion independence

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

TDT is a severe condition in which patients require regular pRBC transfusions for survival, resulting in unavoidable iron overload which in turn, in the absence of appropriate and maintained iron chelation, can cause serious cardiac, liver, and endocrine comorbidities, and shortened lifespan compared to the general population. At the time of MAA review, the only potential curative treatment option was allogeneic HSCT. Despite the fact that allogeneic HSCT is associated with significant risks, this has been the preferred treatment option for TDT patients who have an HLA-matched sibling stem cell donor, indicating the benefit obtained by the transplantation process, i.e. becoming transfusion free and avoiding the risk of iron overload and the need for iron chelation therapy.

15/19 (79%) patients with TDT, who do not have a β^0/β^0 genotype and who were eligible for HSCT but had no matched sibling donor, were treated with Zynteglo in study HGB-204, HGB-205, and HGB-207, and achieved TI. This can be considered as a major clinical benefit of Zynteglo treatment. This clinically relevant effect is supported by pharmacodynamic parameters.

While the benefit of treatment in the studied population is evident, uncertainties regarding this benefit remain. The safety profile also includes the AEs related to mobilisation, apheresis and myeloablative conditioning. There is a theoretical risk associated with genetically modified cells involving an integrative LVV (and notably the risk of insertional oncogenesis). The limited patient numbers and long-term safety data complicate the evaluation of the contribution of Zynteglo to the safety profile of HSCT.

Additional efficacy and safety data will be further substantiated by the ongoing clinical development (study HGB-207 and study HGB-212) and the long-term follow-up monitoring of patients (LTF-303 and registry REG-501). Finally, further support for the assumption that the commercial manufacturing process is clinically in line with the refined manufacturing process batches is expected to be obtained by

a re-evaluation of the acceptance criteria for attributes related to potency tests using batch release data and clinical results after 6 months follow-up of patients treated with commercial batches.

3.7.2. Balance of benefits and risks

The benefit-risk balance of Zynteglo for the indication: "*Zynteglo is indicated for the treatment of patients 12 years and older with transfusion-dependent β-thalassaemia (TDT) who do not have a β^0/β^0 genotype, for whom haematopoietic stem cell (HSC) transplantation is appropriate but a human leukocyte antigen (HLA)-matched related HSC donor is not available"* is positive.

3.7.3. Additional considerations on the benefit-risk balance

Conditional marketing authorisation

The product falls within the scope of Regulation (EC) No 507/2006 concerning conditional marketing authorisations, as it aims at the treatment of a seriously debilitating disease and is duly recognised by the EU as an orphan medicinal product. Zynteglo has been accepted as PRIME, acknowledging that it addresses an unmet medical need and that its early availability would be considered beneficial.

The development has been the patient of several protocol assistance advices to determine the most appropriate time and endpoint for the submission of the marketing authorisation and to support the design of the post-authorisation studies. These included two parallel advices with Health Technology assessment bodies.

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/CHMP considers that the product fulfils the requirements for a conditional marketing authorisation:

- The benefit-risk balance is positive.
- It is likely that the applicant will be able to provide comprehensive data. The applicant is likely to be able to provide comprehensive data relevant to the initial indication including long-term safety and efficacy data for adult and adolescent patients with TDT who have a non-β^0/β^0 genotype, post initial approval from the following sources: Phase 3 Study HGB-207, Phase 3 study HGB-212 and Long-term follow-up Study LTF-303.
- The unmet medical need has been addressed. The therapeutic armamentarium for the treatment of TDT can be considered insufficient. The symptomatic approach of transfusions combined with iron chelation therapy may result in a certain improvement of life expectancy. The challenges with regard to side effects, insufficiency of a non-curative approach as well as non-adherence as a result of the burden of treatment remain. The only curative treatment option on the other hand, allogeneic HSCT, is associated with severe side-effects and reactions. The treatment of TDT, therefore, represents an unmet medical need.
- The benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required. The majority of patients with TDT who have a non-β^0/β^0 genotype treated with Zynteglo in study HGB-204 and HGB-205 have achieved TI (11 out of 14; 78.6%). For study HGB-207, 4 out of 5 TI-evaluable patients achieved TI (data cut: 13 December 2018), and based on a developed prediction model (generated from Studies HGB-204, HGB-205 and the 5 TI-evaluable patients in Study HGB-207), an additional 6 patients from Study HGB-207 produced close to 8 g/dL or more HbA^{T87Q} at Month 6 and are predicted to achieve TI with ≥98% probability. Achievement of TI is clinically meaningful, eliminating the need for regular blood

transfusions and associated comorbidities due to iron overload. These effects are expected to be life-long, following successful engraftment in the patient.

As comprehensive data on the product are not available, a conditional marketing authorisation was requested by the applicant in the initial submission and is agreed by the CAT and CHMP.

Because of the limited number of patients in the clinical studies; comprehensive data is required to be provided by the applicant as laid down in the following specific obligations:

- In order to confirm the efficacy and safety of Zynteglo in patients 12 years and older with TDT who do not have a β^0/β^0 genotype, the MAH should submit interim and final data on Study HGB-207.
- In order to confirm the efficacy and safety of Zynteglo in patients 12 years and older with TDT who do not have a β^0/β^0 genotype, the MAH should submit interim and final data from patients with a severe non β^0/β^0 genotype such as IVS I-110/IVS-I-110 and IVS I-110/β^0 from Study HGB-212.
- In order to confirm the efficacy and safety of Zynteglo in patients 12 years and older with TDT who do not have a β^0/β^0 genotype, the MAH should submit interim data and the 5 years follow-up results of study LTF-303.

In addition, the following two Annex II conditions are requested:

- In order to further confirm the appropriateness of the acceptance criteria, the MAH should re-evaluate the acceptance criteria for attributes related to potency tests using batch release data and clinical results after 6 months follow-up of 20 patients treated with commercial batches.
- Non interventional post-authorisation safety and efficacy study: In order to further characterise and contextualise the long-term safety and efficacy of Zynteglo in patients 12 years and older with TDT who do not have a β^0/β^0 genotype, the MAH should conduct and submit the results of a study based on data from a product registry (REG-501) and use data on patients treated with transfusions and/or HLA-matched allogenic HSCT treated patients from an established European registry as a comparator group.

Accelerated assessment

This application has been assessed on an accelerated timetable.

3.8. Conclusions

The overall benefit-risk profile of Zynteglo is positive.

The CHMP endorse the CAT conclusion on benefit-risk balance as described above.

4. Recommendations

Outcome

Based on the CAT review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Zynteglo is favourable in the following indication:

"Zynteglo is indicated for the treatment of patients 12 years and older with transfusion-dependent β-thalassaemia (TDT) who do not have a β^0/β^0 genotype, for whom haematopoietic stem cell (HSC) transplantation is appropriate but a human leukocyte antigen (HLA)-matched related HSC donor is not available."

The CHMP therefore recommends the granting of the conditional marketing authorisation patient to the following conditions:

Conditions or restrictions regarding supply and use

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

Additional risk minimisation measures

Prior to launch of Zynteglo in each Member State the Marketing Authorisation Holder (MAH) must agree about the content and format of the educational and controlled distribution programme, including communication media, distribution modalities, and any other aspects of the programme, with the National Competent Authority.

The educational and controlled distribution programme is aimed at providing information on the safe use of Zynteglo.

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

- Physician educational material
- Patient information pack

The physician educational material should contain:

- The Summary of Product Characteristics
- Guide for healthcare professionals
- Guide for handling and method of administration

The Guide for healthcare professionals shall contain the following key elements:

- Warnings and precautions of the mobilisation agents and the myeloablative conditioning agent must be considered.
- Treatment with Zynteglo in the clinical trials was associated with delayed platelet engraftment. No correlation was observed between incidence of bleeding AE and time to platelet engraftment. Precautions regarding bleeding consequences of thrombocytopenia need to be taken. Patients should be made aware of the risk of bleeding events that are not easily identifiable, such as internal bleeding.
- Treatment with Zynteglo is in theory associated with the risk of insertional mutagenesis, potentially leading to development of malignancy. All patients must be advised on signs of leukaemia and to seek immediate medical attention if these signs are present.
- A negative serology test for HIV is necessary to ensure acceptance of apheresis material for Zynteglo manufacturing.
- The potential risk of loss of response to gene therapy may lead to loss of transfusion independence or increase transfusion needs for patients who did not reach transfusion-independence.
- All patients should receive annual monitoring of complete blood counts and total haemoglobin levels to monitor for leukaemia/lymphoma and maintenance of efficacy, respectively.
- The short-term potential risk of treatment with Zynteglo represents failure of engraftment, which shall be managed by administration of rescue cells.
- The need to explain and to ensure that patients understand:
  - potential risks of treatment with Zynteglo
  - signs of leukaemia/lymphoma and what action to take
  - content of patient's guide
  - the need to carry the patient alert card and show it to every healthcare professional
  - enrolment in the drug product Registry
- Scope of the Registry and how to enrol patients

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

- Instructions on receiving and storing of Zynteglo and how to check Zynteglo prior to administration
- Instructions about the thawing of Zynteglo
- Instructions on protective equipment and treatment of spills.

The patient information pack should contain:

- Package leaflet
- A patient/carer guide
- A patient alert card

The patient/carer guide shall contain the following key messages:

- Treatment with Zynteglo is in theory associated with the risk of development of malignancy. Signs of leukaemia and the need to obtain urgent medical care if these signs are present.
- Patient alert card and the need to carry it on their person and tell any treating healthcare professional that they were treated with Zynteglo.
- The potential risk of loss of response to gene therapy may lead to loss of transfusion independence or increase transfusion needs for patients who did not reach transfusion-independence.
- The importance of annual check-ups.
- Treatment with Zynteglo is associated with the risk of delayed platelet engraftment that could lead to an increased tendency for bleeding.
- Signs and symptoms of bleeding and the need to contact the physician if any signs of unusual or prolonged bleeding or any other relevant signs are present.
- Enrolment in the drug product Registry.

The patient alert card shall contain the following key messages:

- Information of risk of delayed platelet engraftment, potentially leading to bleeds, and theoretical risks.
- Statement that the patient was treated with gene therapy and should not donate blood, organs, tissues, or cells.
- Statement that the patient was treated with Zynteglo, including LOT number and treatment date(s).
- Details on reporting of adverse effects.
- Information on the possibility of false positivity of certain commercial HIV tests because of Zynteglo.
- Contact details where a health care professional can receive further information.

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

- Zynteglo will only be available through bluebird bio qualified treatment centres to ensure traceability of the patient's cells and manufactured drug product between the treating hospital and manufacturing site. The selection of the treatment centres will be conducted in collaboration with national health authorities as appropriate.

Obligation to conduct post-authorisation measures

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

Description	Due date
In order to further confirm the appropriateness of the acceptance criteria, the MAH should re-evaluate the acceptance criteria for attributes related to potency tests using batch release data and clinical results after 6 months follow-up of 20 patients treated with commercial batches.	Interim report: at each annual renewal When 20 patients have been treated with 6 months follow-up
Non interventional post-authorisation safety and efficacy study: In order to further characterise and contextualise the long-term safety and efficacy of Zynteglo in patients 12 years and older with transfusion-dependent β-thalassaemia (TDT) who do not have a β^0/β^0 genotype, the MAH should conduct and submit the results of a study based on data from a product registry (REG-501) and use data on patients treated with transfusions and/or HLA-matched allogenic HSCT treated patients from an established European registry as a comparator group.	Protocol submission: February 2020 Interim results: - at each annual renewal - Dec. 2024 - Dec. 2034 Final results: Q4 2039

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 Zynteglo in patients 12 years and older with transfusion-dependent β-thalassaemia (TDT) who do not have a β^0/β^0 genotype, the MAH should submit interim and final data on Study HGB-207	Interim results: at each annual renewal Final results: December 2021
In order to confirm the efficacy and safety of Zynteglo in patients 12 years and older with transfusion-dependent β-thalassaemia (TDT) who do not have a	Interim results: at each annual

Description	Due date
β^0/β^0 genotype, the MAH should submit interim and final data from patients with a severe non-β^0/β^0 genotype such as IVS-I-110/IVS-I-110 and IVS-I-110/β^0 from Study HGB-212.	renewal Final results: December 2021
In order to confirm the efficacy and safety of Zynteglo in patients 12 years and older with transfusion-dependent β-thalassaemia (TDT) who do not have a β^0/β^0 genotype, the MAH should submit interim data and the 5 years follow-up results of Study LTF-303.	Interim results: at each annual renewal Final results: December 2024

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 CHMP review of the available data, the CHMP considers that autologous CD34+ cell-enriched population that contains hematopoietic stem cells transduced with lentiviral vector encoding the $\beta^{A\text{-}T87Q}$-globin gene is a new active substance as it is not a constituent of a medicinal product previously authorised within the European Union.

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/0257/2015 and the results of these studies are reflected in the SmPC and, as appropriate, the Package Leaflet.