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<b>Division / Office</b>	DCEPT/OTAT
<b>Committee Chair</b>	Jakob Reiser, PhD
<b>Clinical Reviewer(s)</b>	Karl Kasamon, MD
<b>Project Manager</b>	Mona Badawy & Cara Pardon
<b>Priority Review</b>	Yes
<b>Reviewer Name(s)</b>	Jiang Hu, Ph.D.
<b>Review Completion Date / Stamped Date</b>	
<b>Supervisory Concurrence</b>	Lin Huo, Ph.D., Acting Team Lead, TEB2/DB/OBPV
	Lihan Yan, Ph.D., Acting Branch Chief, TEB2/DB/OBPV
	Shiowjen Lee, Ph.D., Deputy Director, DB/OBPV
<b>Applicant</b>	Bluebird bio, Inc.
<b>Established Name</b>	Betibeglogene autotemcel
<b>(Proposed) Trade Name</b>	ZYNTEGLO
<b>Pharmacologic Class</b>	$\beta^{A-T87Q}$ -globin gene addition therapy
<b>Formulation(s), including Adjuvants, etc</b>	Autologous CD34+ Hematopoietic Stem Cells Transduced with Lentiviral Vector, BB305 LVV, Encoding the Human $\beta^{A-T87Q}$ -Globin Gene.
<b>Dosage Form(s) and Route(s) of Administration</b>	Cell suspension for intravenous infusion
<b>Dosing Regimen</b>	A single dose of ZYNTEGLO contains a minimum of $5.0 \times 10^6$ CD34+ cells/kg
<b>Indication(s) and Intended Population(s)</b>	Treatment of $\beta$ -Thalassemia for subjects who require regular red blood cell transfusions

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## GLOSSARY

AE	adverse event
allo-HSCT	allogeneic hematopoietic stem cell transplantation
AUC	area under the curve
BCG	Bacillus-Calmette Guérin
BLA	Biologics License Application
DMC	Data Monitoring Committee
FDA	Food and Drug Administration
GVHD	graft-versus-host disease
Hb	hemoglobin
HbA	hemoglobin A (i.e., adult hemoglobin)
HBB	$\beta$ -globin gene
HLA	Human Leukocyte Antigen
HSC	Hematopoietic stem cells
ICH	International Conference on Harmonization
IND	investigational new drug
INN	International non-proprietary name
ITT	intent-to-treat
IV	intravenous
LVV	lentiviral vector (LVV)
NE	neutrophil engraftment
PD	pharmacodynamics
PRBC	packed red blood cell(s)
RBC	red blood cell
SAE	serious adverse event
SAP	statistical analysis plan
SCD	sickle cell disease
SEP	Successful Engraftment Population
TDT	transfusion-dependent $\beta$ -thalassemia
TEAE	treatment-emergent adverse event
TI	transfusion independence
TP	Transplant Population

## 1. Executive Summary

This original Biologics License Application (BLA) is submitted for Zynteglo (betibeglogene autotemcel) for the treatment of subjects with  $\beta$ -thalassemia who require regular red blood cell (RBC) transfusions.

This BLA is supported by two on-going pivotal studies HGB-207 and HGB-212, two completed supportive phase 1/2 studies HGB-204 and HGB-205, as well as one long-term follow-up study LTF-303. The safety and efficacy of Zynteglo are being evaluated in subjects with transfusion-dependent  $\beta$ -thalassemia (TDT).

The primary efficacy endpoint in the pivotal studies was the proportion of subjects who achieve transfusion independence (TI) (a weighted average Hb  $\geq$  9 g/dL without any pRBC transfusions for a continuous period of  $\geq$ 12 months) at any time after the product infusion. In study HGB-207, of the 22 subjects who were evaluable for TI, 20 subjects (90.9%; 2-sided 95% confidence interval [CI] of 70.8% to 98.9%) achieved TI at any time post the product infusion. In Cohort 1 (subjects  $\geq$ 12 years old) of study HGB-207, 14 out of 15 TI-evaluable subjects (93.3%, 2-sided 95% CI of 68.1% to 99.8%) achieved TI at any time post infusion. In Cohort 2 (subjects  $<$ 12 years old), 6 out of 7 TI-evaluable subjects (85.7%, 2-sided 95% CI of 42.1% to 99.6%) achieved TI at any time post infusion. In Study HGB-212, of the 14 subjects who were evaluable for TI, 12 subjects (85.7%; 2-sided 95% CI of 57.2% to 98.2%) achieved TI at any time post infusion. Results of Cohort 1 in study HGB-207 and study HGB-212 met the pre-specified success criterion of exceeding 30% TI rate. Although the success criterion for Cohort 2 in study HGB-207 was not pre-specified, the lower bound of 2-sided 95% CI of 42.1% is deemed acceptable.

The safety was evaluated through subjects from all five studies. No death occurred in all studies.

Overall, there were no statistical issues identified during the review of this BLA. The efficacy analyses of studies HGB-207 and HGB-212 at the time of data cutoff met the pre-specified success criteria. I defer to the clinical reviewer on the acceptance of the safety profile based on the expected nature of the disease and the adverse events observed in these studies.

## 2. Clinical and Regulatory Background

Zynteglo is a gene therapy product developed for the treatment of  $\beta$ -thalassemia. The active substance consists of an autologous CD34+ enriched cell population that contains hematopoietic stem cells (HSC) transduced with lentiviral vector (LVV) encoding human  $\beta^A$ -T87Q-globin gene.

### 2.1 Disease or Health-Related Condition(s) Studied

$\beta$ -thalassemia is a rare, genetic blood disease caused by mutations in the  $\beta$ -globin gene (HBB), resulting in reduced or absent production of functional adult

hemoglobin (HbA) that normally accounts for > 95% of the total hemoglobin (Hb) in the blood of adults. Patients lack sufficient RBCs and Hb to effectively transport oxygen throughout the body, resulting in severe anemia, and have ineffective erythropoiesis which can lead to morbidities via splenomegaly, marrow expansion, concomitant bone deformities, and iron overload.

TDT is defined as a requirement for either  $\geq 100$  mL/kg/year of packed red blood cells (pRBCs) in the 2 years, or being managed under standard thalassemia guidelines, with  $\geq 8$  transfusions of pRBCs per year in the 2 years. The standard of care for adult patients with TDT is lifelong treatment with frequent blood transfusions and iron chelation.

## 2.2 Currently Available, Pharmacologically Unrelated Treatment(s) / Intervention(s) for the Proposed Indication(s)

Treatment options currently available in the US for patients with  $\beta$ -thalassemia include regular RBC transfusions with iron chelation, REBLOZYL (luspatercept-aamt), and allogeneic hematopoietic stem cell transplantation (allo-HSCT).

According to current guidelines, the standard medical care for patients with  $\beta$ -thalassemia who require regular transfusions consists of transfusions typically every 2 to 5 weeks. Transfusions are time-consuming and lifelong, and they do not restore normal erythropoiesis.

REBLOZYL (luspatercept-aamt) was approved by the Food and Drug Administration (FDA) in November 2019 for the treatment of anemia in adult patients with  $\beta$ -thalassemia who require regular RBC transfusions and is an additional chronic treatment option for patients with TDT in the US.

Allo-HSCT is a potentially curative therapy for patients with  $\beta$ -thalassemia and it may enable thalassemia-free survival in the absence of chronic RBC transfusions. However, allo-HSCT carries significant risks of graft rejection, graft-versus-host disease (GVHD), severe infections, and mortality, and most subjects are not considered optimal candidates for allo-HSCT due to lack of donor match, disease complications, and/or age.

Given the limitations of available treatment options for subjects with  $\beta$ -thalassemia who require regular RBC transfusions, there is a significant unmet medical need for a curative treatment.

## 2.4 Previous Human Experience with the Product (Including Foreign Experience)

The clinical development program for Zynteglo includes two completed phase 1/2 studies (HGB-204 and HGB-205), and two ongoing phase 3 studies (HGB-207 and HGB-212). A total of 66 TDT subjects initiated any study procedures and 63 TDT subjects received Zynteglo. The target indication of Zynteglo in this BLA is for subjects with TDT.

Zynteglo was granted conditional approval in the E.U. and United Kingdom. Zynteglo has been indicated in Europe for patients 12 years and older with non-

$\beta 0/\beta 0$ -genotype TDT, for whom HSCT is appropriate but a Human Leukocyte Antigen (HLA)-matched related donor is unavailable.

## 2.5 Summary of Pre- and Post-submission Regulatory Activity Related to the Submission

Two type B pre-investigational new drug (pre-IND) meetings were held in 2002 and 2012. The applicant discussed CMC, preclinical and clinical issues in the pre-IND meetings (CRMTS 8350, PS001613). The applicant submitted the IND 15324/0 on 12/19/2012.

A list of all meetings between the applicant and FDA during the development of Zynteglo under IND 15324 are provided in Table 1. Meeting minutes from these interactions are included in this BLA submission.

Table 1: List of Meetings with FDA for Zynteglo

<i>Meeting Number</i>	<i>Meeting Date</i>	<i>Meeting Type</i>
CRMTS 9702	3/25/2015	Type B Multidisciplinary Meeting
CRMTS 9823	7/09/2015	Type B CMC Meeting
CRMTS 10156	5/31/2016	Type B Multidisciplinary Meeting
CRMTS 10484	12/08/2016	Type C Meeting with Lonza Biologics
CRMTS 11253	7/11/2018	Type B Multidisciplinary Meeting
CRMTS 11650	3/5/2019	Type B Written Response
CRMTS 11979	9/19/2019	Type B CMC Meeting
CRMTS 12028	11/7/2019	Type B Pre-BLA Meeting

Source: Adapted from BLA 125717/0/0001 Module 1.6.3: Meeting-correspondence.pdf, page 1.

During the pre-BLA meeting, FDA agreed with the applicant to submit the BLA with interim results from HGB-207 and HGB-212 if study subjects had a reasonable follow-up time.

This product was granted Fast Track designation in 2013 and Breakthrough Therapy designation in 2015.

## 3. SUBMISSION QUALITY AND GOOD CLINICAL PRACTICES

### 3.1 Submission Quality and Completeness

This submission was adequately organized for conducting a complete statistical review without unreasonable difficulty.

## 5. SOURCES OF CLINICAL DATA AND OTHER INFORMATION CONSIDERED IN THE REVIEW

### 5.1 Review Strategy

The Zynteglo clinical development program for the treatment of patients with TDT includes five clinical studies: two completed Phase 1/2 studies (Studies HGB-204 and HGB-205), two on-going Phase 3 studies (Studies HGB-207 and HGB-212), and one long-term follow-up study (Study LTF-303).

The primary efficacy analysis for this BLA focuses on the subjects enrolled in Phase 3 Studies HGB-207 and HGB-212 and results are presented in Section 7. Demographics and baseline information for the completed Phase 1/2 studies HGB-204 and HGB-205 are also included in Section 7. The safety analysis of this BLA includes all 5 clinical studies in Section 8.

## 5.2 BLA/IND Documents That Serve as the Basis for the Statistical Review

The following documents in BLA submission 125717 were reviewed and served as the basis for this statistical memo:

- 125717/0001
  - Module 1
- 125717/0002
  - Module 2.2: Introduction
  - Module 2.5: Clinical overview
  - Module 2.7: Clinical summary
  - Module 5.3.5.2: Clinical study reports, protocols, and SAPs for Study HGB-204, HGB-205, HGB-207, HGB-212, and LTF-303
  - Module 5.3.5.3: Pooled analyses of efficacy and safety of all 5 studies
  - Datasets of single studies and pooled datasets
- 125717/0011
  - Module 1: Response to IR 05 (dated 12/03/21)
- 125717/0013
  - Module 5: 90-day safety update report

## 5.3 Table of Studies/Clinical Trials

Table 2 summarizes five clinical studies in the clinical development program of Zynteglo reviewed in this memo.



Table 2: Overview of Clinical Studies Evaluating Zynteglo in subjects with TDT

<i>Type of study</i>	<i>Study Identifier</i>	<i>Objective of the study</i>	<i>Study Design and Type of Control</i>	<i>Test Product(s); Dosage Regimen; Route of Administration</i>	<i>Number of TDT Subjects enrolled</i>	<i>Diagnosis of Subjects</i>	<i>Duration Of Treatment</i>	<i>Study Status; Type of Report</i>
Phase 1/2	HGB-204	Safety and efficacy	Non-randomized, open label, multi-site, single dose and uncontrolled	$\geq 3.0 \times 10^6$ autologous transduced CD34+ hematopoietic stem cells/kg; Intravenous infusion	19	$\beta$ -thalassemia major	Single dose	Completed; Clinical Study Report
Phase 1/2	HGB-205*	Safety and efficacy	Non-randomized, open label, multi-site, single dose and uncontrolled	$\geq 3.0 \times 10^6$ autologous transduced CD34+ hematopoietic stem cells/kg; $\geq 2.0 \times 10^6$ autologous transduced CD34+ hematopoietic stem cells/kg (bone marrow harvest); Intravenous infusion	4 $\beta$ -thalassemia major	$\beta$ -thalassemia major or severe SCD	Single dose	Completed; Clinical Study Report
Phase 3	HGB-207	Efficacy and safety	Non-randomized, open label, multi-site, single dose and uncontrolled	$\geq 5.0 \times 10^6$ autologous transduced CD34+ hematopoietic stem cells/kg; Intravenous infusion	24	TDT	Single dose	Ongoing; Interim Report
Phase 3	HGB-212	Efficacy and safety	Non-randomized, open label,	$\geq 5.0 \times 10^6$ autologous transduced CD34+	19	TDT, non- $\beta^0/\beta^0$ genotypes	Single dose	Ongoing; Interim Report

			multi-site, single dose and uncontrolled	hematopoietic stem cells/kg; Intravenous infusion				
Long-term follow-up	LTF-303	Long-term follow-up from parent studies; safety and efficacy	Multi-site, long-term follow-up	N/A: Subjects dosed in parent studies	51	TDT	N/A	Ongoing; Interim Report

Source: adapted from 125717/0002/m2/27-clin-sum, synopses-indiv-studies.pdf, page 1-2

\*: Additional three sickle cell disease subjects were also enrolled in Study HGB-205.

## 6. DISCUSSION OF INDIVIDUAL STUDIES/CLINICAL TRIALS

Because of many common features in study procedures and design between studies, efficacy review for the pivotal studies is presented together in Section 7 and safety review for all studies in Section 8.

## 7. INTEGRATED OVERVIEW OF EFFICACY

The review of efficacy in this section is intended to discuss efficacy results from the two pivotal studies (HGB-207 and HGB-212). Background information and baseline summaries also include the two supportive phase 1/2 studies (HGB-204 and HGB-205) prior to the safety overview in Section 8.

### 7.1 Major Inclusion Criteria

Subjects must meet all the following criteria to be considered eligible for enrollment in each study:

1. Subjects  $\leq 50$  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). Pediatric subjects ( $< 12$  years of age) may only be enrolled at a given site if approved by the relevant regulatory authority. Provided that the Data Monitoring Committee (DMC) has approved enrolling subjects younger than 5 years of age, subjects younger than 5 years of age may be enrolled at sites with regulatory approval for the specified age range 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.
2. Diagnosis of TDT with a history of at least 100 mL/kg/year of pRBCs in the 2 years preceding enrollment (all subjects), or be managed under standard thalassemia guidelines (e.g., with  $\geq 8$  transfusions of pRBCs per year in the 2 years preceding enrollment (subjects  $\geq 12$  years).
3. Clinically stable, had a Karnofsky performance status of  $\geq 80$  for adults ( $\geq 16$  years of age) or a Lansky performance status of  $\geq 80$  for adolescents or children ( $< 16$  years of age), and eligible to undergo HSCT.
4. Treated and followed for at least the past 2 years in a specialized center 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.

### 7.2 Design Overview

All studies were single-arm, open label, and single-dose studies.

HGB-204 and HGB-205 were phase 1/2 studies to evaluate the safety and efficacy of Zynteglo for subjects with TDT. HGB-207 and HGB-212 were single-arm, multi-site, single-dose phase 3 studies to evaluate efficacy and safety of Zynteglo in subjects with TDT.

All 4 studies have four distinct stages:

1. Screening and eligibility assessment
2. Autologous CD34+ cell collection, drug product manufacture and disposition

Subjects were to undergo HSC mobilization with granulocyte colony-stimulating factor and plerixafor. Peripheral blood mononuclear cells were collected by apheresis (up to 2 mobilization cycles separated by at least 2 weeks) for both drug product manufacture and to provide back-up cells for rescue (e.g., in the event of an engraftment failure).

3. Myeloablative conditioning and drug product infusion (Day 1)

After the drug product was dispositioned for clinical use, and the subject's eligibility was re-confirmed, the subject was to undergo myeloablative conditioning with intravenous (IV) busulfan for 4 consecutive days followed by at least 48 hours of washout. On Day 1, thawed Zynteglo was administered via IV infusion.

4. Follow-up through engraftment and until approximately 24 months after drug product infusion

Subjects were to be followed until approximately 24 months after Zynteglo infusion (Month 24 Visit). After completion of the Month 24 Visit, and after provision of written informed consent (and assent if applicable), subjects were to be enrolled in long-term follow-up Study LTF-303, to be followed for up to an additional 13 years, for a total of 15 years after drug product infusion.

#### Study Treatments or Agents Mandated by the Protocol

The investigational product is Zynteglo, which is an autologous CD34+ cell-enriched population that contains HSCs transduced with BB305 lentiviral vector (LVV) encoding the  $\beta^{A-T87Q}$ -globin gene.

Zynteglo was administered via a single IV infusion at a dose of  $\geq 3.0 \times 10^6$  CD34+ cells/kg in studies HGB-204 and HGB-205, and a dose of  $\geq 5.0 \times 10^6$  CD34+ cells/kg in studies HGB-207 and HGB-212.

#### Sites and Centers

There were six sites for HGB-204, one site for HGB-205, nine sites for HGB-207, and eight sites for HGB-212. Overall, there were 14 sites from 8 countries (Australia, Germany, France, UK, Greece, Italy, Thailand, and US) involved for all 4 clinical trials.

#### Surveillance/Monitoring

In each study, there was an independent DMC that comprised of members with appropriate scientific and medical expertise to monitor the safety of the study.

#### Endpoints

The primary efficacy endpoint was the proportion of subjects who meet the definition of TI (a weighted average Hb  $\geq 9$  g/dL without any pRBC transfusions for a continuous period of  $\geq 12$  months) at any time after drug product infusion. The determination of TI is as follows:

- Calculation of time period of TI will start when the subject achieves a Hb  $\geq 9$  g/dL with no transfusions in the preceding 60 days.
- To meet the initial TI criteria, the weighted Hb must be  $\geq 9$  g/dL at the end of the 12-month period.
- To remain in the TI state beyond the 12-month period, the treated subject needs to maintain a weighted Hb of  $\geq 9$  g/dL from that point forward, without receiving a pRBC transfusion.
- A transfusion of pRBCs for a single acute event (e.g., surgery, trauma, parvovirus infection, or sepsis) will not be counted towards the definition of TI. For the calculation of the weighted Hb when an allowed transfusion has occurred, the Hb that triggered the acute pRBC transfusion would be carried forward for 60 days after the acute pRBC transfusion, or until next acute pRBC transfusion, and Hb values during those 60 days would be imputed by the carried-forward value. Post 60 days, the actual Hb drawn would again be used in the calculation of TI. When determining TI among subjects with acute pRBC transfusions, a subject can still achieve TI within the 60 days of Hb imputation. In the case there were more than one acute transfusion, the Hb that triggered the acute transfusion will be carried forward for 60 days or until next acute transfusion within 60 days. Then the Hb that triggered the next acute transfusion will be carried forward for another 60 days.

The weighted average Hb for determining TI was defined as follows. Let  $t_0, t_1, t_2, \dots$  represent the consecutive time points for assessment of Hb, where  $t_0$  denotes the time when Hb is first  $\geq 9$  g/dL with no transfusions in the preceding 60 days, and the  $t_i$  are continuing as long as no transfusions are given. Further, let  $h_0, h_1, h_2, \dots$  represent the Hb level at each of these time points. Then the weighted average Hb is calculated as:

$$[(t_1 - t_0) \times ((h_0 + h_1)/2) + (t_2 - t_1) \times ((h_1 + h_2)/2) + \dots + (t_k - t_{k-1}) \times ((h_{k-1} + h_k)/2)] / (t_k - t_0)$$

where  $t_k$  represents the time point such that  $(t_k - t_0)$  represents at least 12 consecutive months. This calculation is invariant to the metric used for the time points, e.g., calendar dates or days from drug product infusion, since the consecutive differences in times would always be measured as a number of days. Note that the weighted average may be considered as an average area under the curve (AUC) calculation for Hb. To determine if a subject remains TI beyond 12 months, the calculation of weighted average Hb will always start at  $t_0$ . If a subject loses TI status, defined as starting pRBC transfusions again or the weighted Hb falls below 9 g/dL, a new  $t_0$  will be identified to determine future TI status.

The primary efficacy point was analyzed as a point estimate with the associated 2-sided 95% CI calculated using the Clopper-Pearson exact binomial method.

Secondary endpoints that characterize TI include:

- Proportion of subjects who met the definition of TI at Months 24, 36, 48, 60, Year 6, Year 7, and the last follow-up.

- Duration of TI (TI subjects only).
- Time from drug product infusion to achievement of TI (TI subjects only).
- Weighted average Hb during TI (TI subjects only).

There were no statistical inferences planned for the secondary endpoints in the statistical analysis plan (SAP).

#### Success criteria

In Study HGB-207, the success criterion for the primary efficacy endpoint was the lower bound of the 2-sided 95% exact CI  $\geq 30\%$  for Cohort 1 (subjects  $\geq 12$  years old). No success criterion was pre-specified for Cohort 2.

In Study HGB-212, the success criterion was the lower bound of the 2-sided 95% exact CI  $\geq 30\%$ .

#### Sample size considerations

In HGB-207, a study size of 15 in Cohort 1 with a point estimate of 60% (9 out of 15 subjects) would yield a lower 1-sided 97.5% exact confidence bound of 32.3%, exceeding the 30% minimal success criterion. And a point estimate of 62.5% (5 out of 8 subjects) in Cohort 2 would yield a lower 1-sided 97.5% exact confidence bound of 24.5%.

In HGB-212, a study size of 18 with a point-estimate of 55.6% (10 out of 18 subjects) would yield a lower 1-sided 97.5% exact confidence bound of 30.8%, exceeding the 30% minimal success criterion.

#### Study Populations

Intent-to-Treat Population (ITT): All subjects who initiated any study procedures, beginning with mobilization. The ITT would be used to summarize subject disposition and safety.

Transplant Population (TP): All subjects who underwent Zynteglo infusion. Subject disposition, demographics, efficacy analysis and pharmacodynamics (PD) analysis were planned to be performed based on the TP.

TI-evaluable Population: Subjects who have completed their parent study (i.e., 24 months of follow-up), or achieved TI, or wouldn't achieve TI in their parent study. A subject was deemed 'will not reach TI in the parent study' if he/she was receiving chronic transfusions after 324 days (750 days –  $14 \times 30.4375$  days) of follow-up (less than 14 months of follow-up in parent study), or if his/her Hb level never reached  $t_0$  (Hb  $\geq 9$  g/dL with no transfusions in the preceding 60 days) by 385 days (750 days – 365.25 days) of follow-up. The primary efficacy analysis in this submission was performed with the TI-evaluable population.

*Remarks: Due to studies HGB-207 and HGB-212 are still on-going at the data cutoff of the submission, five subjects (see remarks under Table 3) were not included in the TI-evaluable population. These subjects had not achieved TI but were not classified as non-TI subjects either. The applicant performed primary efficacy analysis based on the TI-evaluable population rather than the pre-*

*specified TP, which after discussion with clinical review team was deemed acceptable. In this review, additional analyses based on TP were included along with results using the TI-evaluable population.*

All subjects in Studies HGB-204 and HGB-205 have completed their last study visit in the parent studies; Studies HGB-207 and HGB-212 are ongoing. A subset of subjects from the four studies followed for longer than 24 months post drug product infusion forms the on-going Study LTF-303. All observed data at the time of analysis were utilized to conduct the analyses pre-specified in the SAP.

### 7.3 Demographics and Baseline Characteristics

The TP analysis set includes 63 subjects with TDT treated across 4 studies: 18 in Study HGB-204, 4 in Study HGB-205, 23 in Study HGB-207, 18 in Study HGB-212. Table 3 summarizes the number of subjects in various analysis populations for the four studies.

Table 3: Analysis Populations

<i>Study</i>	<i>Screened</i>	<i>ITT</i>	<i>TP</i>	<i>TI-evaluable</i>
HGB-204	23	19	18	18
HGB-205	4	4	4	4
HGB-207	32	24	23	22
HGB-212	19	19	18	14
Total	78	66	63	58

Source: FDA statistical reviewer's analysis

*Remark: In studies HGB-207 and HGB-212, by the data cut-off date of the BLA submission, five subjects (HGB-207-(b) (6), HGB-212-(b) (6), HGB-212-(b) (6), HGB-212-(b) (6), HGB-212-(b) (6)) had not been followed for 24 month and had not achieved TI either. Therefore, these five subjects were included in the TP population but not in the TI-evaluable population.*

Table 4 shows the pooled demographics for TDT subjects based on the ITT and TP population respectively.

Table 4. Pooled demographics based on ITT and TP (Phase 1/2 and 3 studies)

	ITT N=66	TP N=63
Sex (%)		
Female	37 (56.06%)	35 (55.56%)
Male	29 (43.94%)	28 (44.44%)
Races (%)		
Asian	38 (57.58%)	36 (57.14%)
White	24 (36.56%)	24 (38.10%)
Others	3 (4.55%)	2 (3.17%)
Not provided	1 (1.52%)	1 (1.59%)
Age at Informed Consent or Assent		
Mean (SD)	18.09 (8.22)	18.09 (8.22)
Median (min, max)	18 (4, 36)	18 (4, 36)
Age group (%)		
< 12 years	16 (24.24%)	16 (25.40%)
≥ 12 to < 18 years	17 (25.76%)	16 (25.40%)
≥ 18 years	33 (50.00%)	31 (49.21%)
Ethnicity (%)		
Hispanic or Latino	2 (3.03%)	2 (3.17%)
Not Hispanic or Latino	60 (90.91%)	57 (90.48%)
Not Provided	4 (6.06%)	4 (6.35%)
Hb Genotype (%)		
$\beta^0/\beta^0$	21 (31.8%)	20 (31.7%)
Non- $\beta^0/\beta^0$	45 (68.2%)	43 (68.3%)

Source: FDA statistical reviewer's analysis

As the primary efficacy analysis based on the TP population was also presented in this review memo for phase 3 studies HGB-207 and HGB-212, Table 5 summarizes the demographics and medical history for TP population for each of the phase 3 studies.



Table 5: Demographics and Characteristics for Phase 3 studies (TP)

	HGB-207 (N=23)	HGB-212 (N=18)	Overall (N=41)
Sex (%)			
Female	12 (52.17%)	8 (44.44%)	20 (48.78%)
Male	11 (47.83%)	10 (55.56%)	21 (51.22%)
Races (%)			
Asian	13 (56.52%)	7 (38.89%)	20 (48.78%)
White	8 (8.70%)	10 (55.56%)	18 (43.90%)
Others	2 (34.78%)	0	2 (4.88%)
Not provided	0	1 (5.56%)	1 (2.44%)
Age at Informed Consent or Assent			
Mean (SD)	15.61 (7.41)	15.78 (8.95)	15.68 (8.01)
Median (min, max)	15 (4, 34)	12.5 (4, 33)	13 (4, 34)
Age group (%)			
< 12 years	8 (34.78%)	8 (44.44%)	16 (39.02%)
≥ 12 to < 18 years	6 (26.09%)	5 (27.78%)	11 (26.83%)
≥ 18 years	9 (39.13%)	5 (27.78%)	14 (34.15%)
Ethnicity (%)			
Hispanic or Latino	1 (4.35%)	0	1 (2.44%)
Not Hispanic or Latino	21 (91.30%)	17 (94.44%)	38 (92.68%)
Not Provided	1 (4.35%)	1 (5.56%)	2 (4.88%)
Baseline pRBC transfusion volume median (mix, max)	207.88 (142.1, 274.4)	194.21 (74.6, 289.0)	190.37 (74.6, 289.0)
Baseline pRBC transfusion frequency median (mix, max)	16.00 (11.5, 37.0)	17.25 (11.0, 39.5)	17.00 (11.0, 39.5)
Baseline Liver iron content (mg/g) median (mix, max)	5.30 (1.00, 41.00)	3.55 (1.20, 13.20)	4.90 (1.00, 41.00)
Baseline Cardiac T2* (msec) median (mix, max)	36.7 (21, 57)	37.0 (15, 75)	36.7 (15, 75)
Baseline Serum Ferritin (pmol/L) median (mix, max)	4438.2 (784, 22517)	3275.0 (1279, 8874)	3671.9 (784, 22517)

Source: FDA statistical reviewer's analysis, 125717/0002/m2/27-clin-sum, summary-clin-efficacy-beta-thalassemia.pdf, Section 3, Table 7, page 67-68.

#### 7.4 Analysis of Primary Endpoint

In Study HGB-207, 23 subjects were treated with Zynteglo, with all 23 had completed the Month 12 Visit, and 20 had completed Month 24 Visit. All treated subjects had successful neutrophil and platelet engraftment. Of the 22 subjects who were evaluable for TI, 20 subjects (90.9%; 2-sided 95% CI of 70.8% to 98.9%) achieved TI at any time post infusion. In Cohort 1 of the study, 14 out of 15 TI-evaluable subjects (93.3%, 2-sided 95% CI of 68.1% to 99.8%) achieved TI at any time post infusion and met the pre-specified success criterion. In Cohort 2, 6 out of 7 TI-evaluable subjects (85.7%, 2-sided 95% CI of 42.1% to

99.6%) achieved TI at any time post infusion. Table 6 summarizes the efficacy analysis for study HGB-207 based on the TI-evaluable population and TP.

Table 6: Proportion of Subjects Who Have Achieved TI at any time post infusion in Study HGB-207 (TI-evaluable population and TP)

Group	Statistic	Cohort 1 (N=15)	Cohort 2 (N=8)	Overall (N=23)
TI-evaluable	N	15	7	22
Subjects with TI at any time among TI-evaluable subjects	n (%)	14 93.3	6 85.7	20 90.9
	2-sided 95% CI (%)	68.1, 99.8	42.1, 99.6	70.8, 98.9
Subjects with TI at any time among TP	%	93.3	75.0	87.0
	2-sided 95% CI (%)	68.1, 99.8	34.9, 96.8	66.4, 97.2

Source: FDA statistical reviewer's analysis, BLA 125717/0 Module 5: hgb-207-study-report-body.pdf, Table 19, page 131.

In Study HGB-212, 18 subjects were treated with Zynteglo with 12  $\beta^0/\beta^0$  subjects and 6 non- $\beta^0/\beta^0$  subjects. Of the 14 subjects who were evaluable for TI, 12 subjects (85.7%; 2-sided 95% CI of 57.2% to 98.2%) achieved TI at any time post infusion. Therefore, the success criterion for HGB-212 has been met. Table 7 summarizes the efficacy analysis for study HGB-212 based on the TI-evaluable population and TP.

Table 7: Proportion of Subjects Who Have Achieved TI at any time post infusion in Study HGB-212 (TI-evaluable population and TP)

Group	Statistic	$\beta^0/\beta^0$ (N = 12)	Non- $\beta^0/\beta^0$ (N = 6)	Overall (N=18)
TI-evaluable	N	8	6	14
Subjects with TI at any time among TI-evaluable	n (%)	7 87.5	5 83.3	12 85.7
	2-sided 95% CI (%)	47.3, 99.7	35.9, 99.6	57.2, 98.2
Subjects with TI at any time among TP	%	58.3	83.3	66.7
	2-sided 95% CI (%)	27.7, 84.8	35.9, 99.6	41.0, 86.7

Source: FDA statistical reviewer's analysis, BLA 125717/0 Module 5: hgb-212-study-report-body.pdf, Table 20, page 132.

## 7.5 Analysis of Secondary Endpoint(s)

Table 8-9 summarize results of the secondary efficacy endpoints for study HGB-207 and study HGB-212, respectively. Results are descriptive in nature as there were no statistical inferences pre-specified in the SAP.

Table 8: Characterization of Transfusion Independence (TI Subjects only)  
HGB-207

<i>Group</i>	<i>Statistic</i>	<i>Cohort 1 (N=14)</i>	<i>Cohort 2 (N=6)</i>	<i>Overall (N=20)</i>
Duration of TI (months)	Median	20.53	19.89	20.37
	Min, Max	19.2, 21.6	15.7, 20.8	15.7, 21.6
Weighted average Hb during TI (g/dL)	Median	12.146	9.989	11.659
	Min, Max	11.26, 12.83	9.54, 11.46	9.54, 12.83
Time from drug product infusion to last pRBC (months)	Median	0.94	0.80	0.87
	Min, Max	0.5, 2.2	0.5, 2.4	0.5, 2.4
Time from drug product infusion to initial achievement of TI (months)	Median	15.39	16.05	15.39
	Min, Max	15.0, 17.9	14.8, 19.4	14.8, 19.4

Source: Adapted from BLA 125717/0 Module 5: hgb-207-study-report-body.pdf, Table 20, page 134.

Table 9: Characterization of Transfusion Independence (TI Subjects only)  
HGB-212

<i>Parameter</i>	<i>Statistic</i>	<i><math>\beta^0/\beta^0</math> (N=7)</i>	<i><i>Non-<math>\beta^0/\beta^0</math></i> (N=5)</i>	<i>Overall (N=12)</i>
Subjects with TI at Month 24	N (%)	6 (85.7)	3 (60.0)	9 (75.0)
	2-sided 95% CI	42.1, 99.6	19.4, 99.4	48.2, 97.7
Duration TI (months)	Median	20.80	18.83	19.45
	Min, Max	13.1, 21.7	12.5, 20.5	12.5, 21.7
Weighted average Hb during TI (g/dL)	Median	10.021	10.323	10.179
	Min, Max	9.35, 13.45	9.95, 12.96	9.35, 13.45
Time from drug product infusion to last pRBC (months)	Median	0.79	1.28	0.82
	Min, Max	0.0, 1.5	0.5, 1.9	0.0, 1.9
Time from drug product infusion to initial achievement of TI (months)	Median	15.67	15.67	15.67
	Min, Max	14.8, 24.5	15.3, 16.1	14.8, 24.5

Source: Adapted from BLA 125717/0 Module 5: hgb-223-study-report-body.pdf, Table 21, page 135.

## 7.6 Subpopulations

Subpopulation analyses by age, sex, race, and genotype were performed on the pooled TI-evaluable population and TP for the two phase 3 studies in Table 10

Table 11, respectively. Numerically different proportions of subjects with TI at any time post infusion were observed across age, sex, race, or genotype subgroups. However, because of the small sample sizes in the subgroups, the interpretation of the observed differences may be limited. I defer to the clinical reviewer on determining the clinical meaningfulness of the observed differences.

Table 10: TI at Any Time post infusion - subgroup analysis (phase 3 studies, pooled TI-evaluable population)

Group	N	n	%	2-sided 95% CI (%)
Age group				
< 12	12	10	83.3	51.6, 97.9
≥ 12 and < 18	10	10	100.0	69.2, 100.0,
≥ 18	14	12	85.7	57.2, 98.2
Sex				
Female	17	14	82.4	56.6, 96.2
Male	19	18	94.7	74.0, 99.9
Race				
Asian	15	14	93.3	68.1, 99.8
White	18	16	88.9	65.3, 98.6
Other	2	1	50.0	1.3, 98.7
Not provided	1	1	100.0	2.5, 100.0
Genotype				
non-β <sup>0</sup> /β <sup>0</sup>	28	25	89.3	71.8, 97.7
β <sup>0</sup> /β <sup>0</sup>	8	7	87.5	47.3, 99.7

Source: Adapted from BLA 125717/0002 Module 2: summary-clin-efficacy-beta-thalassemia.pdf, Section 3.3.

Table 11: TI at Any Time post infusion - subgroup analysis (phase 3 studies, pooled TP)

Group	N	n	%	2-sided 95% CI (%)
Age group				
< 12	16	10	62.5	35.4, 84.8
≥ 12 and < 18	11	10	90.9	58.7, 99.8
≥ 18	14	12	85.7	57.2, 98.2
Sex				
Female	20	14	70.0	45.7, 88.1
Male	21	18	85.7	63.7, 97.0
Race				
Asian	20	14	70.0	45.7, 88.1
White	18	16	88.9	65.3, 98.6
Other	2	1	50.0	1.3, 98.7
Not provided	1	1	100.0	2.5, 100.0
Genotype				
non-β <sup>0</sup> /β <sup>0</sup>	29	25	86.2	68.3, 96.1
β <sup>0</sup> /β <sup>0</sup>	12	7	58.3	27.7, 84.8

Source: FDA statistical reviewer's analysis

## 7.7 Efficacy Conclusions

In Study HGB-207, 20 of 22 TI-evaluable subjects achieved TI at any time post infusion. Fourteen out of 15 TI-evaluable subjects (93.3%, 2-sided 95% CI of 68.1% to 99.8%) achieved TI at any time post infusion in Cohort 1 and 6 out of 7 TI-evaluable subjects (85.7%, 2-sided 95% CI of 42.1% to 99.6%) achieved TI at any time post infusion in Cohort 2. The success criterion for Cohort 1 has been met.

In Study HGB-212, 12 out of 14 TI-evaluable subjects (85.7%; 2-sided 95% CI of 57.2% to 98.2%) achieved TI at any time post infusion. The success criterion of HGB-212 has been met.

## 8. INTEGRATED OVERVIEW OF SAFETY

The review of safety in this section is intended to discuss integrated safety results combining all five studies: completed Phase 1/2 studies HGB-204 and HGB 205, ongoing Phase 3 studies HGB-207 and HGB-212, as well as the long-term follow-up Study LTF-303.

Descriptive statistics were used to summarize safety data for a total of 66 subjects in all five studies.

### 8.1 Deaths

No death was reported in the completed studies HGB-204 and HGB-205. As of the last follow-up, there have been no deaths in the ongoing studies HGB-207, HGB-212, and LTF-303.

### 8.2 Nonfatal Serious Adverse Events (SAEs)

In HGB-204, a total of 15 SAEs were observed during the study, experienced by 10/19 (52.6%) subjects. None of the SAEs were assessed by the Investigator to be possibly related or related to Zytiglo.

In HGB-205, three subjects with TDT experienced a total of 6 SAEs. No drug related SAEs.

In HGB-207, 14 subjects experienced 30 serious adverse events (SAEs). Three subjects experienced SAEs of Veno-occlusive liver disease that were attributed to busulfan conditioning and resolved. One subject experienced a drug product-related SAE of Thrombocytopenia, which was resolved without sequelae.

In HGB-212, 5 subjects experienced 12 SAEs, none of which were considered to be related to the drug product.

Eight subjects experienced 12 SAEs during Study LTF-303, none of which were related to drug product.

Safety results for all studies are summarized in Table 12.

Table 12: SAEs in HGB-204, HGB-205, HGB-208, HGB-212

	<i>Number of subjects with SAEs (n%)</i>	<i>Number of SAEs</i>
HGB-204 (N=19)	10 (52.6)	15
HGB-205 (N=4)	3 (75.0)	6
HGB-207 (N=24)	14 (58.3)	30
HGB-212 (N=19)	5 (26.3)	12
LTF-303 (N=51)	8 (15.7)	12

Source: FDA statistical reviewer's analysis

### 8.3 Adverse Events of Special Interest (AESI)

No events of transplant-related mortality or graft-versus-host disease (GVHD) were reported in any subjects treated with Zynteglo. No engraftment failure or graft rejection was observed in any subject treated with Zynteglo.

## 10. CONCLUSIONS

### 10.1 Statistical Issues and Collective Evidence

Zynteglo is a gene therapy product developed for the treatment of TDT, a rare, genetic blood disease resulting in reduced or absent production of functional HbA that normally accounts for > 95% of the total Hb in the blood of adults. This BLA submission consists of data from two ongoing pivotal studies HGB-207 and HGB-212, completed phase 1/2 studies HGB-204 and HGB-205, as well as ongoing long-term follow-up study LTF-30.

For efficacy assessment, the primary endpoint was the proportion of subjects who meet the definition of TI (a weighted average Hb  $\geq$  9 g/dL without any pRBC transfusions for a continuous period of  $\geq$ 12 months) at any time after drug product infusion in both HGB-207 and HGB-212. The endpoint was analyzed as a point-estimate with a 2-sided 95% CI calculated using the Clopper Pearson exact binomial method.

In Study HGB-207, the success criterion for Cohort 1 was prespecified as the lower 1-sided 97.5% exact confidence bound exceeding 30%. Among the 22 subjects who were evaluable for TI, 20 subjects (90.9%; 2-sided 95% CI of 70.8% to 98.9%) achieved TI at any time post infusion which included 14 out of 15 TI-evaluable subjects (93.3%, 2-sided 95% CI of 68.1% to 99.8%) in Cohort 1, and 6 out of 7 TI-evaluable subjects (85.7%, 2-sided 95% CI of 42.1% to 99.6%) in Cohort 2. The pre-specified success criterion for Cohort 1 has been met. Although the success criterion for Cohort 2 was not pre-specified, the lower bound of 2-sided 95% CI of 42.1% is deemed acceptable.

In Study HGB-212, the success criterion was the lower 2-sided 95% exact confidence bound exceeding 30%. Of the 14 subjects who were evaluable for TI, 12 subjects (85.7%; 2-sided 95% CI of 57.2% to 98.2%) achieved TI at any time post infusion, so the pre-specified success criterion has been met.

The safety evaluation included safety data from all five studies. There was no death observed in the studies. Since all studies were single-arm studies, it's difficult to conduct comparative analysis to detect safety issues. I defer to the clinical reviewer on the acceptance of the safety profile based on the expected nature of the disease and the adverse events observed in these studies.

## 10.2 Conclusions and Recommendations

The efficacy analyses of study HGB-207 and HGB-212 at the time of data cutoff show that the pre-specified success criteria had been met. I defer to the clinical reviewer on the acceptance of the safety profile based on the expected nature of the disease and the adverse events observed in these studies.