# Cellular, Tissue, and Gene Therapies Advisory Committee Meeting

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**FDA Briefing Document**

**Cellular, Tissue, and Gene Therapies**
**Advisory Committee Meeting**

**June 9-10, 2022**

**BLA 125717**
**betibeglogene autotemcel**
**Applicant: bluebird bio, Inc.**

DISCLAIMER STATEMENT

The attached package contains background information prepared by the Food and Drug Administration (FDA) for the members of the advisory committee. The FDA background package often contains assessments and/or conclusions and recommendations written by individual FDA reviewers. Such conclusions and recommendations do not necessarily represent the final position of the individual reviewers, nor do they necessarily represent the final position of the Review Division or Office. We bring the Biologics License Application (BLA) for beti-cel, a first-in-class product, with the Applicant's proposed indication, to this Advisory Committee to gain the Committee's insights and opinions. The background package may not include all issues relevant to the final regulatory recommendation and instead is intended to focus on issues identified by the FDA for discussion by the advisory committee. The FDA will not issue a final determination on the issues at hand until input from the advisory committee process has been considered and all reviews have been finalized. The final determination may be affected by issues not discussed at the advisory committee meeting.

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## ABBREVIATIONS

| | |
|---|---|
| AE | Adverse Event |
| AHSCT | Allogenic hematopoietic stem cell transplantation |
| AML | Acute Myeloid Leukemia |
| ANC | Absolute Neutrophil Count |
| AUC | Area Under the Curve |
| BLA | Biologics License Application |
| BM | bone marrow |
| BMBx | bone marrow biopsy |
| CAR | Chimeric Antigen Receptor |
| CALD | Childhood Cerebral Adrenoleukodystrophy |
| CMC | Chemistry Manufacturing Controls |
| c/dg | copies/diploid genome |
| CTGTAC | Cellular, Tissue, and Gene Therapies Advisory Committee |
| CRF | Case Report Form |
| DAT | Direct Antiglobulin Test |
| DP | Drug Product |
| EMA | European Medicines Agency |
| FDA | Food and Drug Administration |
| FISH | Fluorescence In Situ Hybridization |
| GCP | Good Clinical Practice |
| G-CSF | Granulocyte-colony stimulating factor |
| GT | Gene Therapy |
| GvHD | Graft vs. Host Disease |
| Hb | Hemoglobin |
| HbA | Hemoglobin A (adult hemoglobin) |
| HbA2 | Hemoglobin A2 (minor variant of adult hemoglobin) |
| HbAT87Q | Hemoglobin containing βA-T87Q-globin |
| HBB | β-globin gene |
| HbE | Hemoglobin E |
| HbF | Fetal hemoglobin |
| HSCs | Hematopoietic Stem Cells |
| HSCT | Hematopoietic Stem Cell Transplant |
| HGVS | Human Genome Variation Society |
| HIV | Human immunodeficiency virus |
| HLA | Human leukocyte antigen |
| HRQoL | Health-related quality of life |
| ICF | Informed Consent Form |
| IND | Investigational New Drug Application |
| ISA | Integration site analysis |
| ITT | Intention-to-Treat Population |
| IV | Intravenous |

| | |
|---|---|
| kg | Kilogram |
| LCR | Locus Control Region |
| LTR | Long terminal repeat |
| LVV | Lentiviral vector |
| Mb | Mega-base |
| MDS | Myelodysplastic Syndrome |
| MedDRA | Medical Dictionary for Regulatory Activities |
| mg | milligram |
| MLV | Moloney murine leukemia virus |
| mg | milligram |
| OBE | Office of Biostatistics and Epidemiology |
| ODD | Orphan Drug Designation |
| PB | Peripheral blood |
| PCR | Polymerase Chain Reaction |
| PD | Pharmacodynamics |
| PE | Platelet Engraftment |
| PK | Pharmacokinetics |
| RBC | Red Blood Cell |
| RRE | rev-Response Element |
| SAE | Serious adverse event |
| SCD | Sickle Cell Disease |
| SCID-X1 | X-linked severe combined immunodeficiency |
| SD | Standard Deviation |
| SIN | Self-Inactivating (vector) |
| SNP | Single Nucleotide Polymorphism |
| TDT | Transfusion-dependent (β) thalassemia |
| TEAE | Treatment-Emergent Adverse Event |
| TI | Transfusion independence |
| TIF | Thalassemia International Federation |
| TP | Transplant Population also called Efficacy-Evaluable Population |
| TR | Transfusion reduction |
| TRM | Transplant-related mortality |
| US | United States |
| VAF | Variant Allele Frequency |
| VAS | Visual Analog Score |
| VCN | Vector Copy Number |
| VOC | Vaso Occlusive Crisis |
| VOD | Veno-occlusive liver disease |
| WBC | White blood cell |

# 1 CLINICAL INDICATION

Bluebird bio, Inc., (Applicant) is seeking approval for betibeglogene autotemcel for the treatment of subjects with β-thalassemia who require regular red blood cell (RBC) transfusions.

# 2 EXECUTIVE OVERVIEW

## Topic

This Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC) Meeting is convened to discuss the BLA submitted by the Applicant for betibeglogene autotemcel (beti-cel) for the treatment of subjects with β-thalassemia who require regular RBC transfusions. Beti-cel is a biological product containing genetically modified autologous hematopoietic stem cells (HSCs) transduced with BB305 lentiviral vector (LVV) encoding the βA-T87Q-globin.

## Background

Transfusion dependent β-thalassemia (TDT) is a rare hemoglobinopathy associated with life-long anemia requiring frequent RBC transfusions, complicated by organopathy related to iron overload, reduced quality of life, and shortened survival. Allogenic hematopoietic stem cell transplantation (AHSCT) using HLA matched related donors results in the best outcomes, but few patients have such donors available. Despite supportive care with transfusions, iron chelators and luspatercept, there continues to be a significant unmet need for patients with this disease. Beti-cel is a one-time autologous gene therapy product designed to treat TDT.

## Issues

The primary evidence of effectiveness and safety is generated from two single-arm, open-label, Phase 3 studies (HGB-207 and HGB-212), with supportive safety data from a Phase 1/2 study (HGB 204). Although the studies provide evidence of effectiveness of beti-cel for the treatment of TDT, FDA has concerns regarding the potential risk of insertional oncogenesis, which stem from the following findings in the clinical studies:

- Delayed platelet engraftment and slow platelet count recovery in the context of pathologic bone marrow findings, including one subject with emergent dysplastic megakaryocytes and four subjects with small numbers of potentially emergent ring sideroblasts.
- Insertion Site Analyses (ISA) noting integrations into the vehicle-associated membrane protein-4 (VAMP4) gene noted in >50% of TDT subjects, with two

subjects with insertion sites (IS) into oncogenes including BCR, XPO7, and CBFB at levels that meet the criterion of oligoclonality based on the Applicant's proposed ISA algorithm.
- Delayed platelet engraftment and slow platelet count recovery in the context of pathologic bone marrow findings, including one subject with emergent dysplastic megakaryocytes and four subjects with small numbers of potentially emergent ring sideroblasts.

Although no malignancy or clonal predominance developed in beti-cel recipients, these findings above in the context of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) noted in other bluebird bio lentiviral vector-based products manufactured by the Applicant raise concerns regarding risks of insertional oncogenesis. Treatment with another product manufactured with an identical LVV in a study of sickle cell disease (SCD) has been associated with acute myeloid leukemia (AML) and refractory cytopenias with cytogenetic aberrancy, with one subject with IS into VAMP4. Furthermore, studies evaluating another product manufactured with a related third generation LVV for childhood cerebral adrenoleukodystrophy (CALD) have reported cases of MDS due to insertional oncogenesis. Therefore, the FDA seeks the opinion of the Committee regarding the following issues:

**Topic for Discussion**

- Risk of insertional oncogenesis from LVV integration in beti-cel-treated subjects
- Considerations for screening for germline (hereditary) predisposition for malignancy prior to treatment with beti-cel
- Recommendations on post-treatment monitoring for risk of insertional oncogenesis and risk mitigation

**Discussion Questions**

1. Hematologic malignancies have not been reported among TDT subjects treated with beti-cel. However, considering the similarity of beti-cel LVV with the vector used in SCD and its relatedness to the vector used for CALD, and the diagnosis of hematopoietic malignancies in SCD and CALD patients on these other protocols, does the constellation of delayed platelet reconstitution

with the abnormal marrow morphology findings and insertion site analyses predict future development of hematologic malignancies?

2. Please discuss recommendations for screening patients for potential germline and somatic mutations predisposing to hematologic malignancy prior to administration of beti-cel.

3. Please discuss the adequacy of the proposed postmarketing pharmacovigilance program, including the long-term follow-up study and registry study.

4. Please discuss any additional recommendations for safety monitoring for hematologic malignancies, to include specific testing and frequency of testing, in patients with TDT.

**Voting Question**

1. Do the benefits of betibeglogene autotemcel for the treatment of subjects with β-thalassemia who require regular red blood cell transfusions outweigh the risks, including the potential for insertional oncogenesis?
    a. If you voted "yes," please discuss any recommendations for post-approval risk monitoring and mitigation.
    b. If you voted "no", please discuss what additional information you would consider necessary to support a favorable benefit-risk profile.

# 3 BACKGROUND

## 3.1 Regulatory Background

Table 1 summarizes the main interactions between the FDA and the Applicant.

Table 1. Regulatory Milestones

| Date | Milestones |
|---|---|
| December 18, 2012 | Original Investigational New Drug (IND) application submitted |
| January 31, 2013 | Fast Track Designation granted/ Breakthrough Therapy Designation denied |
| May 15, 2013 | Orphan Drug Designation granted (ODD #13-3905) |
| January 29, 2015 | Breakthrough Therapy Designation granted |
| November 30, 2018 | Rare Pediatric Disease Designation granted (RPD-2018-193) |
| December 18 2019 | Rolling Submission Part 1 (Non-clinical) |
| September 20 2021 | Rolling Submission Part 2 Quality (CMC) and Clinical Modules |
| January 14, 2022 | Major Amendment to BLA |

Abbrev: BLA, Biologics License Application; CMC, Chemistry, Manufacturing, and Control

Betibeglogene autotemcel (Zynteglo) was conditionally approved in Europe in 2019 for patients aged 12 and above with non-β0/β0 TDT.[6] The marketing authorization was withdrawn by the European Commission with effective date of April 15, 2022 following the Applicant's request for withdrawal.

## 3.2 β-thalassemia

Beta (β)-thalassemia is a group of inherited hemoglobinopathies caused by mutations in the β-globin gene on chromosome 11 leading to reduced or absent expression of β-globin in erythropoietic cells. The resulting α to non-α globin chain imbalance in erythrocyte progenitors causes precipitation of unpaired α-globin chains, leading to destruction of erythroid precursors and ineffective erythropoiesis and peripheral hemolysis. Due to resulting impairment of hepcidin regulation, β-thalassemia leads to increased iron absorption and progressive iron overload.

Transfusion dependent β-thalassemia (TDT) is the most severe form, characterized by profound anemia requiring life-long red blood cell (RBC) transfusions. Without RBC transfusions, mortality by age 5 is as high as 80%.

3.3 Treatment

Regular RBC transfusions, typically every 2-5 weeks, remain the mainstay of therapy, but contribute to iron overload and organ damage which manifests as endocrinopathies, cardiomyopathy, and cirrhosis. Chronic anemia can lead to growth retardation, skeletal abnormalities, leg ulcers, and hepatosplenomegaly. RBC transfusions can be associated with transfusion-associated infections, such as hepatitis B and C. Even with standard supportive care with transfusions and iron chelators, patients experience diminished quality of life with only a 55% probability of survival to age 30. Luspatercept, an erythroid maturation agent approved for the treatment of anemia in adults with β -thalassemia, may reduce transfusion needs by up to 33%, but can be associated with thrombosis. Allogeneic hematopoietic stem cell transplantation (AHSCT) is standard therapy for those who have an appropriate donor, and can lead to > 85% disease-free survival in children and about 65% in adults. Unfortunately, fewer than 25% of patients have a human leukocyte antigen (HLA)-matched sibling donor, underscoring the unmet medical need in TDT.

3.4 Product Description

Betibeglogene autotemcel (beti-cel) is comprised of autologous hematopoietic stem cells (HSCs) transduced with BB305 lentiviral vector (LVV) encoding the βA-T87Q-globin, suspended in cryopreservative. Being a β-globin, βA-T87Q-globin is intended to take the place of the deficient binding partner to the unpaired α-globin chain in patients with TDT, thus reconstituting hematopoiesis of stable, functional red blood cells (RBCs) and mitigating the sequelae of β-thalassemia. βA-T87Q globin also strongly inhibits HbS polymerization in SCD, where it can serve as a non-sickling β-globin and therapeutically dilute sickling hemoglobin. Being a distinct β-globin variant, βA-T87Q-globin can serve as a biomarker of treatment response following beti-cel therapy.

3.4.1 Lentiviral vectors for gene therapy

Lentiviral vectors have been used in numerous clinical trials to introduce transgene sequences into HSCs and mature T cells ex vivo [1,2]. Four LVV-based chimeric antigen receptor (CAR) T cell products have received regulatory approval in the US.

3.4.2 BB305 Lentiviral vector

The BB305 LVV genome is comprised of (1) the HIV-1 rev response element (RRE) and central polypurine tract sequences, (2) a genomic copy human bA-globin gene, in reverse orientation, and bearing a mutation in the β -globin coding region, resulting in a T87Q amino acid change [3], (3) the human bA-globin promoter, and (4) a mini-locus control region (LCR) composed of the HS2, HS3, and HS4 DNase I hypersensitive sites to restrict b-globin transgene expression to erythroid cells.

For human clinical trials, safety modifications were made [4,5]. These include a deletion of the viral enhancer and promoter elements in the U3 region of the vector's 3' LTR sequence to generate a SIN vector (Figure 1). The SIN modification reduces the likelihood of propagation of replication competent LVV in target cells [6] and decreases the risk of mobilization of the vector genome upon HIV secondary infection [7]. It also reduces activation of cellular oncogenes by enhancer/promoter activities of integrated LTRs [8].

Figure 1. Diagram of the production plasmid pBB305 LVV

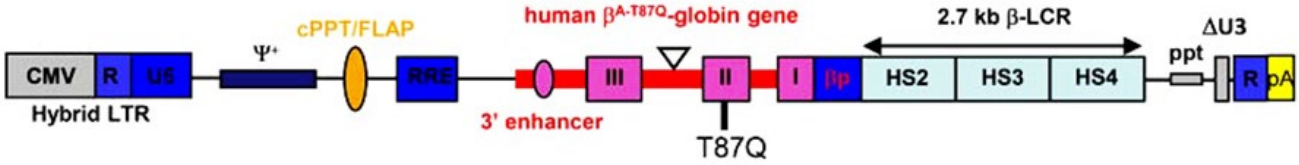



Figure 1. The 3' β-globin enhancer, the 372 base pairs (bp) deletion in intron 2 (triangle), the $\beta^{A\text{-}T87Q}$ mutation (ACA [Thr] to CAG [Gln]) and the DNase I hypersensitive sites (HS) 2, HS3, and HS4 of the human β-globin locus control region (LCR) are indicated. Safety modifications include the 400 bp deletion in the U3 sequence of the 3' LTR. bp: human β-globin promoter; cPPT/flap: central polypurine tract; HIV LTR: human immunodeficiency type-1 virus long-terminal repeat; ppt: polypurine tract; RRE: Rev-responsive element; Ψ+: packaging signal (Modified according to [9]).

3.4.3 betibeglogene autotemcel drug product

Betibeglogene autotemcel (beti-cel) is comprised of autologous HSCs transduced with BB305 lentiviral vector (LVV) encoding the βA-T87Q-globin, suspended in cryopreservative.

3.4.4 Product manufacturing

Beti-cel manufacturing evolved between the version administered in HGB-204 and that used in HGB-207 and HGB-212. Improvements in transduction and cell culture conditions led to increases in the percentage of vector-positive [%LVV+] cells, and integrated copies of the BB305 LVV sequence in beti-cel[17].

3.5 Physiologic Role of $\beta^{A\text{-}T87Q}$-globin

βA-T87Q-globin incorporated within transgenic hemoglobin A (HbA) has a single amino acid substitution of glutamine (Q) for threonine (T) at position 87 (T87Q). The functional transgenic protein binds to endogenous α-globin to form functional Hb, leading to improvement of severe anemia and potentially eliminating the need for chronic transfusions for subjects with β-thalassemia.

3.6 Mechanism of Action of betibeglogene autotemcel

Betibeglogene autotemcel (beti-cel) is a one-time treatment comprised of HSCs that express the βA-T87Q-globin , which has a function similar to that of the native β-globin protein. The functional transgenic protein is intended to bind to endogenous α-globin to form functional Hb, leading to improvement of severe anemia and potentially eliminates the need for chronic transfusions for subjects with β-thalassemia. Expression of βA-T87Q-globin is under the control of the erythroid lineage-specific globin locus control region (LCR), decreasing concerns that inappropriate expression of the introduced βA-T87Q-globin gene in other cell lineages could have adverse consequences. Engraftment of the subject's own HSCs after ex vivo transduction with BB305 LVV is predicted to repair the underlying genetic defect in β-thalassemia.

3.7 Concern of Insertional Oncogenesis

Betibeglogene autotemcel (beti-cel) utilizes a LVV to deliver the gene of interest into hematopoietic stem cells (HSCs). LVVs integrate into the DNA of target cells upon transduction, and thus potentially could affect the expression of nearby genes. After engraftment of transduced HSCs, a progenitor cell derived from transduced HSCs could undergo preferential expansion due to altered expression of nearby genes, resulting in the presence of a predominant clone and subsequent malignancy (insertional oncogenesis). Considering this risk, the development program incorporated measures to assess for clonal expansion in treated subjects using integration site analysis (ISA) of nucleated peripheral blood (PB) cells. No evidence of insertional oncogenesis has been reported in TDT subjects treated with beti-cel; however, cases of myelodysplastic syndrome (MDS), with evidence of pathologic insertions, have been reported in other studies that use the BB305 LVV. Furthermore, cases of AML have occurred in subjects with SCD treated with a

related LVV-based product; however, the relationship between the AML and the LVV has not been established.

Insertional oncogenesis is a major concern when using integrating vectors, including LVVs for permanent cell modification. Recent work has identified four genetic mechanisms resulting in clonal expansion and/or insertional oncogenesis in human clinical trials involving both gamma-retroviral and lentiviral vectors. These include: 1) gene activation by integration of an enhancer sequence present in a vector (enhancer insertion), 2) gene activation by promoter insertion, 3) gene inactivation by insertional disruption, and 4) gene activation by mRNA 3' end substitution. In each example, vector integration in the patient's cells was associated with clonal expansion [10].

The potential for gene activation by integration of an enhancer sequence has been highlighted in infants undergoing gene therapy for X-linked severe combined immunodeficiency (SCID-X1) with gamma-retroviral vectors [11]. A number of SCID-X1 patients developed a T-cell leukemia that was caused by the inserted Moloney murine leukemia virus (MLV) vector switching on an adjacent oncogene [12]. Similar insertional oncogenesis events were observed in patients who were treated for chronic granulomatous disease and Wiskott-Aldrich syndrome [13,14].

LVVs used in clinical trials typically do not carry strong enhancer sequences in their LTRs. However, there are other mechanisms that may impact vector safety [10]. This idea is supported by an observation made in an earlier gene therapy trial for β-thalassemia involving patient CD34+ cells transduced with a LVV bearing a βA-T87Q-globin transgene sequence [9]. Insertion of the LVV within the transcription unit of the proto-oncogene HMGA2 was associated with clonal expansion caused by a cryptic splice site present in LVV's LTR sequence [3]. Although the clonal population of cells detected in the β-thalassemia trial has remained clinically benign [10], it illustrates how LVV insertion can affect adjacent cellular gene expression through non-enhancer-mediated mechanisms.

Clonal dominance caused by LVV insertion into the HMGA2 gene sequence was also observed in a gene therapy trial for older children and young adults with X-linked severe combined immune deficiency involving LVV-transduced CD34+ cells [15]. During a follow-up of vector integration site analysis in blood lineages from patients with SCID-X1, a > 60-fold increase in frequency of forward-orientated integrated LVV sequences within intron 3 of HMGA2 was observed [16]. Some patients demonstrated emergence of dominant HMGA2 clones in progenitor and myeloid lineages, but with no disturbance of hematopoiesis. Molecular analyses revealed a cryptic splice site within the cHS4 insulator sequence present in the LVV's 5' LTR sequence, generating truncated mRNA transcripts for intron 3 HGMA2 inserts. A two base-pair change at the splice site eliminated splicing activity while retaining vector functional capability.

Clonal dominance was also observed in a gene therapy trial for SCD involving autologous transplantation of a subject's hematopoietic stem cells transduced with the BB305 LVV bearing the $\beta^{A\text{-}T87Q}$-globin transgene. Acute myeloid leukemia (AML) developed in a subject approximately 5.5 years after she had received this gene therapy. This is further discussed in section 6.2.4.5 and in Appendix 3.

# 4 CLINICAL DEVELOPMENT

The clinical data to support the safety and efficacy of beti-cel are primarily generated from two Phase 3 studies, Studies HGB-207 and HGB-212. Both studies are open-label, multicenter, single-arm studies that evaluate the safety and efficacy of beti-cel at a target dose of ≥5 x 10^6 CD34+cells/kg. These studies differ chiefly in the studied population genotype. Study HGB-207 enrolled subjects with a non-β0/β0 genotype, whereas Study HGB-212 enrolled subjects with a β0/β0 (including functionally equivalent β0/IVS-I-110 or IVS-I-110/IVS-I-110) genotype.
Supportive safety data from a Phase 1/2 study, HGB-204, were also reviewed. HGB-204 allowed a lower dose of an earlier version of beti-cel manufactured with early version of the manufacturing process (Process 1).

All subjects were required to have detailed retrospective records of transfusion and hemoglobin levels over a period of 2 years before study enrollment to confirm transfusion dependence, which was required for enrollment. Upon completion of the initial studies, subjects were to enroll into the non-interventional long-term, follow-up Study LTF-303, for up to 15 years after beti-cel infusion.

## 4.1 Phase 1/2 Study – Study HGB 204

This was a single-arm, multi-site, single dose (Process 1 beti-cel), Phase 1/2 study of subjects 12 to 35 years of age with β0/β0 and non-β0/β0 genotype transfusion-dependent thalassemia (TDT). Details are in Appendix 1.

## 4.2 Phase 3 Studies HGB-207 and HGB-212

Based on data from Phase 1/2 studies, the manufacturing of beti-cel was modified by improving transduction efficiency[17]. The resulting product used in Phase 3 studies was associated with greater percentages of BB305 LVV vector integration and increased production of the therapeutic protein (hemoglobin containing βA-T87Q-globin). Two nearly identical Phase 3 studies were conducted differing primarily in the thalassemia genotype studied. Study HGB-207 enrolled subjects with a non-β0/β0 genotype at the β-globin gene. Study HGB 212 enrolled subjects with β0/β0 at

the β-globin gene (including functionally equivalent β0/IVS-I-110 or IVS-I-110/IVS-I-110) genotype.

4.2.1 Study Design

HGB-207 and HGB 212 are open-label, single-arm, multicenter studies. The studies are comprised of 4 stages: (1) screening to determine eligibility via detailed history of TDT management including transfusion, hospitalizations, laboratory values and chelation data, (2) apheresis after mobilization with G-CSF and plerixafor to collect HSCs for beti-cel manufacture, (3) myeloablation with busulfan starting at 3.2 mg/kg/day for 4 days with PK analysis to target an AUC of 3800-4500 [μM*min]/day, followed by infusion of beti-cel as a single dose on Day 1, and (4) follow-up of approximately 2 years in the parent study.

4.2.2 Study Objectives

- Primary objective: To evaluate beti-cel efficacy in subjects ≤ 50 years of age with TDT who do not have a β0/β0 genotype at the β-globin gene for study HGB-207; and those with β0/β0 genotype at the β-globin gene for HGB-212
- Secondary objective: To evaluate the safety of treatment with beti-cel in subjects ≤ 50 years of age with TDT without a β0/β0 genotype at the HBB gene (Study HGB-207) and those with β0/β0 genotype at the β-globin gene (HGB-212)

4.2.3 Key Enrollment Criteria

Inclusion Criteria

- Age ≤50 years old
- TDT with a history of ≥ 100 mL/kg/year of pRBCs in the 2 years preceding enrollment (or be managed under standard thalassemia guidelines with ≥ 8 transfusions of pRBCs per year in the 2 years preceding enrollment, subjects ≥ 12 years)

Exclusion Criteria

Genotype Exclusion Criteria

- *HGB-207:* β0 mutation present on both HBB alleles (Functionally severe IVS I-110 (G→A) [Human Genome Variation Society {HGVS} nomenclature: HBB: c.93-21G>A] is considered equivalent to a β0 mutation) is exclusionary
- *HGB-212:* Any mutation other than β0 (e.g., β+, βE, βC) on at least one HBB allele is exclusionary. For the purpose of screening, HBB mutation

IVS-I-110 [HGVS nomenclature: HBB:c.93-21G>A] is considered equivalent to a β0 mutation

Other Exclusion Criteria for the two studies

- Positive test for HIV, hepatitis B or C, and syphilis
- Clinically significant and active bacterial, viral, fungal, or parasitic infection
- WBC count < 3 × 10^9/L, and/or platelet count < 100 × 10^9/L unrelated to hypersplenism
- Uncorrected bleeding disorder
- History of malignancy or myeloproliferative or immunodeficiency disorder
- Immediate family member with Familial Cancer Syndrome; prior HSCT; advanced liver disease; estimated glomerular filtration rate<70ml/min/1.73m2

4.2.4 Treatment Plan

Dose Regimen

Subjects were administered a single infusion of beti-cel manufactured using Process 2 at a target dose of ≥ 5.0 × 10^6 CD34+ cells/kg.

Concomitant Medications Used in the Protocol

Permitted concomitant treatments during conditioning at the investigator's discretion included diuretics, ondansetron and/or metoclopramide (antiemesis), non-phenytoin anticonvulsants for seizure prophylaxis, ursodeoxycholic acid for prevention of hepatic veno-occlusive disease, and anti-infectives for infection prophylaxis or treatment of febrile neutropenia. Iron chelators were to be stopped for 7 days before busulfan to avoid drug-drug interactions; iron chelation resumption was at the investigator's discretion and in accordance with institutional protocols, and with post-transplant chelation guidelines in protocol.

4.2.5 Study Assessments

The key efficacy assessments included:

**Primary Efficacy Endpoint**:

The proportion of subjects meeting the definition of transfusion independence (TI), which was defined as a weighted average Hb $\geqslant$ 9 g/dL without any pRBC transfusions for a continuous period of $\geqslant$ 12 months at any time during the study after drug product infusion.

**Secondary Efficacy Endpoints**

- Characterize subjects who achieve TI by assessing duration of TI, proportion who meet TI definition at Month 24, time from beti-cel infusion to achievement of TI, and weighted average hemoglobin (Hb) during TI
- Characterize transfusion reduction, compare weighted average nadir Hb during 2 years prior to enrollment to that from Month 12 to Month 24, evaluate unsupported Hb levels over time through Month 24
- Characterize iron chelation and therapeutic phlebotomy use along with liver and cardiac iron burden using MRI tools, and quality of life assessment

Safety assessments

- Success and kinetics of HSC engraftment
- Incidence of transplant-related mortality through 100 days and through 365 days after beti-cel infusion
- Overall survival
- Detection of vector-derived RCL in any subject
- Monitoring of laboratory parameters
- Frequency and severity of clinical adverse events (AEs)
- Incidence of acute and/or chronic graft-versus-host disease (GVHD)
- The number of subjects with insertional oncogenesis (myelodysplasia, leukemia, lymphoma, etc.)

4.3 Long-Term Follow Up (LTFU) study, LTF-303

4.3.1 Study Design

This is a non-treatment, multi-center, long-term safety and efficacy follow-up study for subjects with hemoglobinopathies who were treated with beti-cel in the Phase 1/2 and 3 studies. All consenting subjects completing the Applicant's Phase 1/2 and 3 studies were to be enrolled in Study LTF-303 and were followed every 6 months through 5 years after product infusion and annually from Year 5 through Year 15. Safety endpoints were very similar to those of the Phase 3 studies but continued through Year 15 after beti-cel infusion and included: overall survival, beti-cel-related AEs, all SAEs, immune-related AEs, new or worsening hematologic disorders, neurologic disorders, malignancies; incidence of vector-derived RCL, insertional oncogenesis. Other endpoints included: pharmacodynamics (PD) endpoints such as

peripheral blood VCN, βA-T87Q-globin, and Hgb fractions at Year 5, Year 10, and Year 15; and efficacy endpoints as in HGB-207 and HGB-212 but through Year 15.

### 4.4 Registry Study REG-501

#### 4.4.1 Study Design

The Applicant has proposed an observational multicenter, registry in the US to collect longitudinal clinical outcome data in recipients of beti-cel in the postmarketing setting. Objectives are to evaluate safety and effectiveness of beti-cel, thus, the study will monitor for SAEs and adverse events of interest as well as to assess durability of clinical response for 15 years after beti-cel infusion. The key safety outcome measure is to determine the incidence of malignancies after beti-cel infusion.

The clinical management of patients will be at the discretion of the Investigator/HCP; and assessments will be in accordance with clinical and regulatory guidelines applied in the course of current practice. While peripheral blood gene therapy-specific laboratory assessments,including globin analysis (including βA-T87Q-globin), VCN determination, and integration site analysis (ISA), will be requested for all patients enrolled in the registry, no assessments are mandated. Likewise, no study medication is provided and no restrictions on concomitant treatments are imposed in the registry. It is anticipated that approximately 150 patients in the US will be enrolled.

## 5 Efficacy

Due to substantial difference in the potency of the product related to differences in drug product manufacturing in the Phase 1/2 vs. Phase 3 studies, only data from the Phase 3 studies, HGB-207 and HGB-212, were primarily analyzed for efficacy.

### 5.1 Study Population

### 5.2 Efficacy Analysis

Infusion of beti-cel led to detectable LVV vector sequences, measured as vector copy number (VCN) in nucleated peripheral blood (PB) cells of all subjects until the last visit. After a rapid increase in PB VCN levels, subjects reached a plateau. HbAT87Q globin levels increased steadily after beti-cel infusion before reaching a plateau at approximately Month 6. Levels of HbAT87Q globin at Month 6 were similar

in the studies with median (min, max) of 8.7 (1.1, 10.6) g/dL in HGB 207 and 8.9 (0.0,12.0) g/dL in HGB-212; (N = 35). Endogenous Hb levels (e.g., HbA, HbE, HbF, HbA2) tended to stabilize around the time when transgenic HbAT87Q globin levels stabilized.

Determination of efficacy was based on achievement of transfusion independence (TI), which was defined as a weighted average Hb ≥ 9g/dL without any pRBC transfusions for a continuous period of ≥ 12 months at any time during the study after drug product infusion, with no transfusions in the preceding 60 days.

TI was determined in TI-evaluable subjects. The Applicant defined a subject as TI-evaluable if either one of the following was true: 1) the subject achieved TI, 2) completed the Month 24 Visit, or 3) will not reach TI during the study by the following 2 criteria: if the subject received chronic transfusions after 324 days of follow-up or if the subject's Hb level never reached t0 (Hb ≥ 9 g/dL with no transfusions in the preceding 60 days) before 385 days of follow-up.

5.2.1 Primary Efficacy Analysis

Transfusion Independence

The primary efficacy endpoint of TI was determined in TI-evaluable subjects. As the Phase 3 studies are ongoing, not all subjects treated have completed the follow-up period at the time of data lock; therefore, of the efficacy-evaluable analysis set (N=41), 36 (88%) are TI-evaluable. Of the 36 TI-evaluable subjects, 32 (89%) reached TI at any time. TI outcomes are summarized in the table below:

Table 2. Transfusion Independence (Efficacy-Evaluable Analysis Set)

| **Parameter** | **Statistic** | **HGB-207 (N=23)** | **HGB-212 (N=18)** | **Phase 3 Total (N=41)** |
|---|---|---|---|---|
| Overall | | | | |
| TI-evaluable | N | 22 | 14 | 36 |
| Subjects with TI at any time | n (%) 2-sided 95% CI | 20 (91) (71, 99) | 12 (86) (57, 98) | 32 (89) (74, 97) |
| β0/β0 | | | | |
| TI-evaluable Subjects with TI at Any Time | N<br>n (%)<br>2-sided 95% CI | NA<br>NA | 8<br>7 (88)<br>(47, 100) | 8<br>7 (88)<br>(47, 100) |
| Non-β0/β0 | | | | |
| TI-evaluable | N<br>n (%)<br>2-sided 95% CI | 22<br>20 (90)<br>(71, 99) | 6<br>5 (83)<br>(36, 100) | 28<br>25 (89)<br>(72, 98) |

| | | | | |
|---|---|---|---|---|
| Subjects with TI at Any Time | | | | |
| <12 years of Age | | | | |
| TI-evaluable Subjects with TI at Any Time | N<br>n (%)<br>2-sided 95% CI | 7<br>6 (86)<br>(42, 100) | 5<br>4 (80.0)<br>(28, 100) | 12<br>10 (83)<br>(52, 98) |
| ≥12 to<18 years of age | | | | |
| TI-evaluable Subjects with TI at Any Time | N<br>n (%)<br>2-sided 95% CI | 6<br>6 (100)<br>(54, 100) | 4<br>4 (100)<br>(40, 100) | 10<br>10 (100)<br>(69, 100) |
| ≥18 years of age | | | | |
| TI-evaluable Subjects with TI at Any Time | N<br>n (%)<br>2-sided 95% CI | 9<br>8 (89)<br>(52, 100) | 5<br>4 (80)<br>(28, 100) | 14<br>12 (86)<br>(57, 98) |
| <18 years of age | | | | |
| TI-evaluable Subjects with TI at Any Time | N<br>n (%)<br>2-sided 95% CI | 13<br>12 (92)<br>(64, 100) | 9<br>8 (89)<br>(52, 100) | 22<br>20 (91)<br>(71, 99) |
| Male | | | | |
| TI-evaluable Subjects with TI at Any Time | N<br>n (%)<br>2-sided 95% CI | 11<br>11 (100)<br>(72,100.) | 8<br>7 (88)<br>(47, 100) | 19<br>18 (95)<br>(74, 100) |
| Female | | | | |
| TI evaluable Subjects with TI at Any Time | N<br>n (%)<br>2-sided 95% CI | 11<br>9 (82)<br>(48, 98) | 6<br>5 (83)<br>(36, 100) | 17<br>14 (82)<br>(57, 96) |

Source: Reviewer calculations from PDISE ADEF2 dataset. TI outcomes did not differ substantially between subjects under 18 years of age compared with subjects who were ≥ 18 years old, although adolescents ≥12 to < 18-year-old had a higher rate of TI compared with younger and older groups. In the HGB-212 study, β0/β0 subjects had a somewhat higher proportion of reaching TI (87%) than non- β0/β0 (83%) subjects, although the two genotypes achieved approximately the same TI rate in the overall Phase 3 population. Males had a somewhat higher rate of TI than female subjects.

Four (11%) of the 36 TI eligible subjects in the efficacy-evaluable analysis set failed to achieve TI, and 5 additional subjects are not yet TI evaluable. Those TI-evaluable subjects who failed to achieve TI had the lowest PB VCN values of TI-evaluable

subjects and had among the lowest amounts of HbAT87Q globin at Month 6 in their respective studies. These subjects' beti-cel parameters (i.e., DP VCN and %LVV+ cells) were below the median for Phase 3 subjects.

5.2.2 Secondary Efficacy Endpoints

Key secondary endpoints to further characterize the effectiveness and durability of beti-cel included: time to reach TI, weighted average hemoglobin level while in TI, and proportion of subjects meeting definition of transfusion independence over time. These results are summarized in Table 3.

Table 3. Characterization of Transfusion Independence (Subjects achieving TI)

| **Parameter** | **Statistic** | **Total (N=32)** |
|---|---|---|
| Subjects with TI at Month 24[a] | n/N (%) | 27/31 (87) |
| Subjects with TI at Month 36 [a] | n/N (%) | 9/10 (90) |
| Subjects with TI at Month 48 [a] | n/N (%) | 0/1 |
| Subjects with TI Last follow up | n/N (%) | 32/36 (89) |
| Observed duration of TI (months) | Median<br>(range) | 26<br>13, 39 |
| Weighted avg. Hb during TI (g/dL) | Median<br>(range) | 11.5<br>9.3, 13.7 |
| Time from beti-cel infusion to last transfusion prior to TI (months) | Median<br>(range) | 0.84<br>0.0, 2.4 |
| Time from beti-cel infusion to achievement of TI (months) | Median<br>(range) | 16<br>15, 25 |

Source: Reviewer calculations from PDISE ADEF2 dataset. [a]The denominator is based on the number of subjects reaching the noted Month visit.

Transfusion Reduction:

Other secondary endpoints focused on characterization of transfusion reduction (TR). Of subjects with at least 6 months of follow-up, 36/39 (92.3%) achieved ≥ 60% reduction in annualized transfusion volume during the period from 6 months after drug product infusion through last follow-up compared with their annualized baseline pre-treatment transfusion requirements.

There were 9 subjects with at least 6 months of follow-up after beti-cel infusion who either did not achieve TI or were not yet TI-eligible. Four of these did not achieve TI, but had median (min, max) –31.8% (–92, –3.4) decrease in transfusion volume from 6 months after beti-cel infusion to last follow-up. Another 3 subjects with at least six months of follow-up were not yet TI-evaluable but had 100% reduction in transfusion volume compared with baseline.

Unsupported Hemoglobin

Subjects who achieved TI maintained normal, or near-normal Hb levels following successful treatment, and these Hb levels were generally at least as high as the baseline Hb levels previously maintained with ongoing transfusions.

Use of Iron Chelation and Therapeutic Phlebotomy

A key morbidity of TDT is iron overload requiring chronic iron chelation therapy, and all subjects were on chelation at time of screening. Of 32 subjects who achieved TI, 20 (62.5%) were able to stop iron chelation for at least 6 months post beti-cel infusion compared to 1 of 4 (25%) subjects who did not achieve TI. For the 24 subjects who stopped chelation, the median (min, max) time from stopping chelation to last follow-up was 22.7 (6.0, 47.3) months. Eight of the subjects (33%) who stopped chelation underwent phlebotomy, of which one continues with phlebotomy as of last follow up.

Change in Iron Burden in Liver and Heart

Change in iron overload was assessed by evaluating liver iron content (LIC) using magnetic resonance imaging (MRI). A trend suggesting frequent initial worsening of LIC in subjects between beti-cel infusion and the first post-infusion measurement at Month 12, was observed. This was often followed by a return toward baseline levels by Month 24, especially among those who achieved TI. Cardiac iron was assessed using T2 measured with cardiovascular magnetic resonance relaxation parameter R2* (assessed clinically via its reciprocal T2*) measured in the ventricular septum. As in the case of liver iron, cardiac iron burden tended to increase at Month 12 before reversing toward baseline by month 24. Twenty (57%) of the subjects had cardiac iron burden above their baseline levels at last follow-up.

5.3 Efficacy Conclusion

Data contained in the BLA support the effectiveness of beti-cel for treatment of subjects with β-thalassemia who require regular RBC transfusions. Treatment with beti-cel led to TI in 89% of subjects, with a median time from drug product infusion to last pRBC transfusion of 0.8 months and a median (min, max) observed duration of TI of 26 months (13, 39) as of the data cut-off. Fourteen of 15 TI-evaluable subjects (93%) achieved TI in Study HGB-207 Cohort 1 (subjects ≥ 12 years of age), six of seven TI-evaluable subjects (86%) achieved TI in Study HGB-207 Cohort 2 (subjects < 12 years of age), and 12 of 14 TI-evaluable subjects (86%) achieved TI in Study HGB-212, meeting their respective success criteria.

FDA believes that TI represents a clinically meaningful benefit for patients with transfusion-dependent β-thalassemia, and could decrease the risks of iron overload, multi-system organ complications and early mortality. This clinical endpoint is supported by pharmacodyamics data which show rises in βA-T87Q-globin leading to the attainment of target levels of unsupported total Hb, regardless of age or

genotype. Ongoing Phase 3 and long-term follow-up studies suggest durability of beti-cel activity with subjects maintaining their Hb levels to time of last follow-up [median (range) 26 months (13-39)].

# 6 SAFETY

## 6.1 Study Population

The FDA safety analysis includes data from Studies HGB-204, HGB-207, HGB-212, as well as the ongoing long term follow up study, LTF-303. Study subjects were followed for median of 2.5 years with a range of 0.3-7 years. The safety population was comprised of 59 subjects who received beti-cel.

Exposure:
Subjects in the studies generally received target doses of busulfan. Subjects were targeted to receive a beti-cel dose of ≥ 5.0 x 10^6 CD34+ cells/kg in the Phase 3 studies. Subjects in Study HGB-207 received a lower median dose of beti-cel, 8.1 x 10^6 CD34+ cells /kg (range 5.0 to 19.9), compared to Study HGB-212 subjects, who received 10.8 x 10^6 CD34+ cells /kg (range 6, 42). However, vector copy numbers (VCN) per transduced cell (DP VCN/DP% LVV+ cells) were slightly higher in Study HGB-207 subjects (4.4, range of 2.8 to 7.7) compared to Study HGB-212 subjects (3.7, range of 2.6, 7.5). HGB-204 subjects were to receive ≥ 3.0 x 10^6 CD34+ cells/kg and they received median 8.1 × 10^6 CD34+ cells/kg (range 5.2 to 18.1) with median DP VCN of 0.7 (0.3 to 1.5) c/dg).

## 6.2 Adverse Events

Adverse events (AEs), reported between the start of mobilization to before the start of conditioning, reflected toxicities related to pre-requisite mobilization consisting of plerixafor, G-CSF, and line insertion, including transient thrombocytopenia, nausea, vomiting, procedural and musculoskeletal pain, hypocalcemia, and catheter pain.

### 6.2.1 Overall Summary of Treatment-Emergent Adverse Events (TEAEs)

Most AEs were reported after start of conditioning, and included gastrointestinal toxicities such as emesis and mucositis, hepatobiliary (including veno-occlusive disease) and myelosuppression (cytopenias). As such events are expected complications of myeloablative conditioning with busulfan, it is challenging to determine contribution of beti-cel, which was administered 48 hours after completion of conditioning chemotherapy. The observed types and rates of AEs reported during the period between conditioning and before neutrophil engraftment are reasonably expected from busulfan conditioning. While febrile neutropenia was common (54.2%), severe grade infections were not. There were four AEs of sepsis (6.8%),

one of which was grade 3 and one grade 4. The table below summarizes treatment emergent nonlaboratory AEs reported from Day 1 to Month 24.

Table 4. Treatment Emergent Non-laboratory AEs Reported in ≥ 10 % of Subjects, Day 1 to Month 24. N=59 Safety Population

| Preferred Term/ Grouped Term | All Grades (%) | Grades 3 or Higher (%) |
|---|---|---|
| Mucositis* | 55 (93) | 40 (68) |
| Alopecia | 35 (59) | 0 |
| Febrile neutropenia | 32 (54) | 32 (54) |
| Vomiting | 29 (49) | 0 |
| Epistaxis | 26 (44) | 10 (17) |
| Pyrexia | 25 (42) | 6 (10) |
| Abdominal pain* | 22 (37) | 2 (3.4) |
| Musculoskeletal pain* | 22 (37) | 0 |
| Constipation | 18 (31) | 0 |
| Diarrhea | 18 (31) | 1 (1.7) |
| Cough* | 18 (31) | 0 |
| Rash* | 16 (27) | 0 |
| Pigmentation disorder* | 15 (25) | 0 |
| Transfusion reaction | 14 (24) | 1 (1.7) |
| Headache* | 14 (24) | 1 (1.7) |
| Decreased appetite | 13 (22) | 6 (10) |
| Pruritus | 13 (22) | 0 |
| Nausea | 12 (20) | 1 (1.7) |
| Dyspepsia | 10 (17) | 3 (5) |
| Upper respiratory tract infection* | 10 (17) | 0 |
| Fatigue | 9 (15) | 0 |
| Viral infection* | 8 (14) | 2 (3.4) |
| Procedural pain | 8 (14) | 0 |
| Veno-occlusive liver disease | 7 (12) | 5 (9) |
| Insomnia | 7 (12) | 0 |
| Vaginal hemorrhage | 7 (12) | 0 |
| Oropharyngeal pain* | 7 (12) | 0 |
| Nasopharyngitis | 6 (10) | 0 |
| Dyspnea | 6 (10) | 0 |
| Rhinitis | 6 (10) | 0 |

Source: Reviewer calculations ISS ADAE dataset. *FDA defined grouped terms

Most commonly reported laboratory-based AEs were cytopenias and hepatic enzyme abnormalities. The following table summarizes laboratory-based AEs from Day 1 to Month 24.

Table 5. Treatment Emergent Laboratory-based AEs in ≥ 10% Subjects* Day 1 to Month 24. N=59 Safety Population

| **Laboratory-Based Abnormality** | **All grades n (%)** | **Grade 3-4 n (%)** |
|---|---|---|
| Neutrophils decreased | 59 (100) | 59 (100) |
| Platelets decreased | 59 (100) | 59 (100) |
| WBC decreased | 59 (100) | 59 (100) |
| Hgb decreased | 58 (98) | 56 (95) |
| Lymphocytes decreased | 57 (97) | 36 (61) |
| Calcium decreased | 51 (86) | 1 (1.7) |
| ALT increased | 51 (86) | 12 (20) |
| AST increased | 43 (73) | 2 (3.4) |
| Bilirubin increased | 22 (37) | 6 (10) |
| Potassium decreased | 44 (75) | 7 (12) |
| Sodium decreased | 37 (63) | 6 (10) |
| Phosphate decreased* | 35 (61) | 10 (17) |
| ALP increased | 21 (36) | 0 |
| Calcium increased | 14 (24) | 1 (1.7) |
| Potassium increased | 10 (17) | 0 |
| Glucose increased* | 6 (11) | 6 (11) |

Source: Reviewer analysis ISS ADLB dataset. *Due to missing data, the denominator was 57 for Phosphate and 54 for Glucose.

6.2.2 Deaths and Serious Adverse Events (SAEs)

No deaths occurred in any of the studies. Thirty-two (54.2%) of the 59 subjects in the safety population experienced 68 SAEs. Twelve subjects (19.3%) experienced 13 SAEs prior to beti-cel treatment. These events were attributed to either study procedure, apheresis, or other.

Treatment-Emergent SAEs:

Of the 59 subjects who experienced treatment emergent SAEs, 25 subjects (42.4%) experienced 55 SAEs from Day 1 through the last day of follow-up, and are shown in the table below:

Table 6. Treatment Emergent Serious Adverse Events in ≥5%. N=59 Safety Population. Day 1 to Last Follow-up

| **Preferred Term/Group Term** | **n (%) All Grade** | **n (%) ≥ Gr3 SAE** |
|---|---|---|
| Neutropenia | 3 (5) | 3 (5) |
| Thrombocytopenia | 3 (5) | 3 (5) |

| | | |
|---|---|---|
| Pyrexia | 4 (7) | 0 |
| Veno occlusive liver disease | 5 (8) | 5 (8) |
| Thrombosis* | 3 (5) | 2 (3) |

Source: Reviewer calculations from ISS ADAE dataset. * included pulmonary embolism, cardiac thrombus and vein thrombosis.

Most SAEs, such as cytopenias, infections, and veno-occlusive disease are frequently associated with myeloablation with busulfan, and were attributed to study interventions other than beti-cel. Thrombotic events were also attributed by FDA to other factors (indwelling catheter and concomitant medications). However, 2 SAEs: a grade 3 thrombocytopenia from Day 114 to Day 163, as well as 1 SAE of thrombocytopenia leading to epistaxis on Day 69-70 in the context of delayed platelet engraftment (which occurred Day 80), were attributed to beti-cel by the FDA.

6.2.3 AEs of Interest

FDA considered the following AEs of interest: new or worsening hematologic abnormalities, to include cytopenias, delayed platelet engraftment with prolonged thrombocytopenia, bone marrow morphology abnormalities and potential Insertional oncogenesis. These were special focus of our evaluation because of a potential safety signal consisting of prolonged thrombocytopenia and abnormal marrow morphologies among TDT subjects along with hematologic malignancies and insertional oncogenesis in subjects treated with other LVV products for other diseases. The concern that beti-cel may interfere with hematopoietic reconstitution is grounded in the observation that platelet engraftment is delayed relative to what would be expected for autologous HSCT or even allogeneic HSCT, and the failure of platelet counts to return to baseline levels.

*6.2.3.1 Cytopenias:*

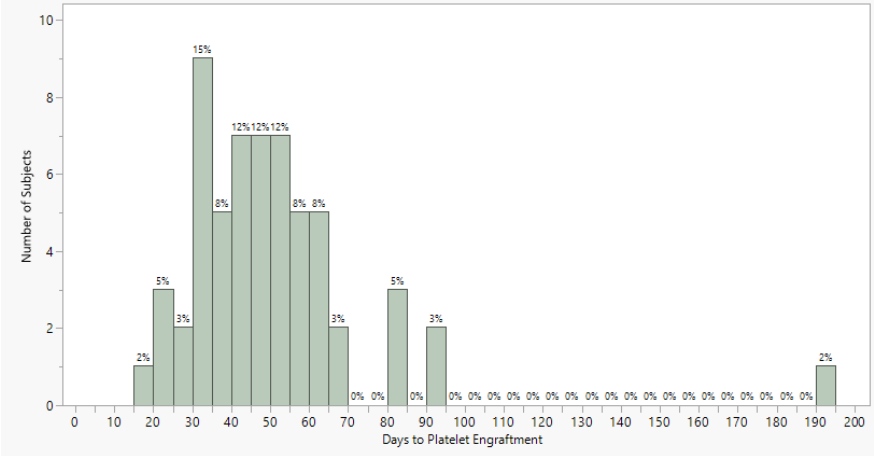
Recipients of myeloablative conditioning chemotherapy are expected to develop severe cytopenias before reconstitution of hematopoiesis after infusion of HSCs. Many dynamics, including the use of growth factors, can affect time to hematopoietic recovery. Prolonged thrombocytopenia was the most notable cytopenia observed, which is further discussed in section 6.2.3.2, below.

Neutrophil engraftment was defined in the protocol as three consecutive absolute neutrophil counts ≥ 0.5 x 10^9/L on 3 different days within 42 days of beti-cel administration. However, in 10 of 59 beti-cel treated subjects (16.9%) the time to neutrophil engraftment (NE) was not possible to determine given that these subjects continued to receive granulocyte-colony stimulating factor (G-CSF) for ≥ 7 days beyond NE. Only 2 subjects remained on continuous G-CSF beyond Day 43, notable, because neutrophil engraftment failure is protocol defined in those cases where it is not reached by Day 43.

*6.2.3.2 Delayed Platelet Engraftment*

Reconstitution of the platelet lineage after beti-cel was notably slower than expected and reported in the literature for patients with TDT undergoing AHSCT[24]. Platelet engraftment (PE) was defined as 3 consecutive platelet values ≥ 20 × $10^9$/L obtained on different days after a post-transplant value of < 20 × $10^9$/L, with no platelet transfusions administered for 7 days immediately preceding and during the evaluation period. The median time to platelet engraftment was 46 days, with range of 19-191, as shown in the figure and table below.

Figure 2. Platelet Engraftment Day for Safety Population N=59




Source: Reviewer Analysis, derived from ADSL from ISS dataset

Table 7. Platelet Engraftment Status (Safety Population)

| **Parameter** | **Statistic** | **TDT (N=59)** |
|---|---|---|
| Subjects with platelet engraftment | n (%) | 59 (100) |
| Day of platelet engraftment | N<br>Median<br>Min, Max | 59<br>49<br>19, 191 |
| ≤Day 30 | n (%) | 7 (12) |
| >Day 30 to ≤Day 60 | n (%) | 40 (68) |
| >Day 60 to ≤Day 90 | n (%) | 9 (15) |
| >Day 90 | n (%) | 3 (5) |

Source: Reviewer Analysis, derived from ISS ADSL dataset

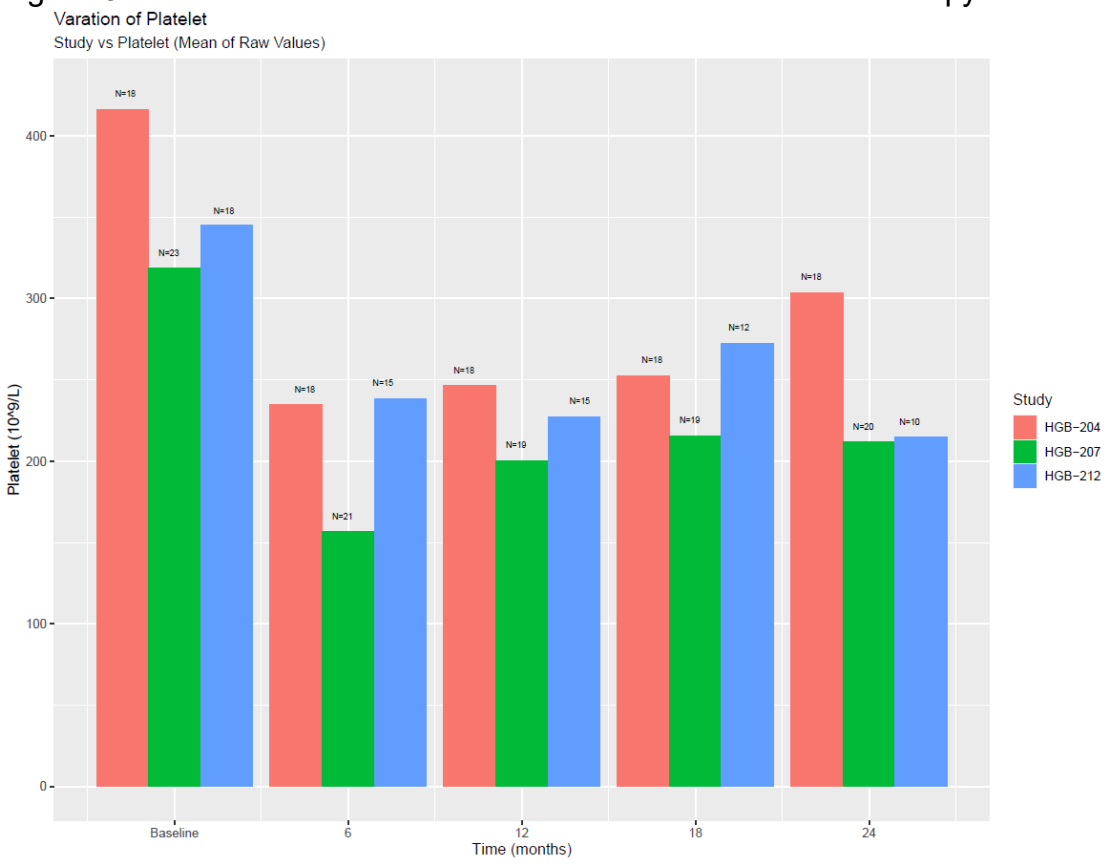
Further recovery of platelets beyond the threshold to reach platelet engraftment was also delayed. Beti-cel treated subjects reached the first of 3 consecutive unsupported platelet counts of ≥ 100 × $10^9$/L at a median (min, max) of Day 86 (24, 891). Most (32/59 [54%]) subjects did not have sustained, unsupported platelet

values ≥ 100 × $10^9$/L as of the data cut. These data demonstrate that prolonged low platelet counts (< 100 × $10^9$/L) are common after beti-cel treatment and may persist past Day 100.

In addition, FDA considered persistent ≥ grade 3 thrombocytopenia lasting substantially beyond that expected following busulfan myeloablation as a hematologic event of interest. Ten of the 59 treated subjects (17%) experienced ≥ grade 3 thrombocytopenia persisting beyond Day 80. Other causes, such as medications and infections were ruled out in these subjects.

Most subjects eventually tended to achieve platelet counts that were normal or mildly thrombocytopenic without associated bleeding events; however, in general subjects did not return to their baseline platelet counts following beti-cel treatment. The figure below illustrates median baseline platelet counts and subsequent mean platelet count trajectory to month 24 in beti-cel recipients. This demonstrates that platelet counts tend not to return to baseline after beti-cel.

Figure 3. Mean Platelet Values at Baseline and Post beti-cel Therapy




Source: Computed by FDA Statistician from ADLB datasets

The mechanism underpinning this post-beti-cel platelet decrement is unknown. However, several reports of bone marrow pathology (e.g., ring sideroblasts, and emergent dysplastic changes in megakaryocytes) in subjects after beti-cel treatment, are of concern, and are discussed in Section 6.2.4.3.

Bleeding Events

In light of delayed platelet engraftment and slow recovery observed after beti-cel therapy with potentially increased risk of hemorrhage, bleeding events in the context of prolonged thrombocytopenia were of special interest. One case of a 5-year-old subject in Study HGB-207 who on Day 69 required hospitalization with pRBC and platelet transfusions for grade 3 epistaxis due to severe thrombocytopenia in the setting of delayed platelet engraftment, was reported. No other significant bleeding events were reported.

*6.2.4.3 Bone Marrow Morphologic Abnormalities*

Dyserythropoiesis, and features related to abnormal iron metabolism in marrow morphology, are known complications of TDT. Consequently, Phase 3 subjects underwent serial (baseline, Month 12 and Month 24) marrow sampling to assess the impact of beti-cel on dyserythropoiesis of thalassemia. Unfortunately, the bone marrow data are limited, as the studies were not designed to evaluate cytopenias, but to evaluate the evolution of dyserythropoiesis changes between baseline and Month 24. Pathologists were not free to order ancillary molecular, cytogenetic or other studies on the marrow samples. Nonetheless, the bone marrow assessments that were conducted did identify several subjects with abnormalities such as ring sideroblasts or dysplastic megakaryocytes.

In Study HGB-207, one subject had emergent abnormal (monolobated) megakaryocytes at Month 12 in concert with borderline thrombocytopenia (158k/mm3 platelets), which were reported as concerning for MDS by an independent pathologist. However, the subject declined subsequent biopsies. The subject's platelet counts slowly increased. Four other post-beti-cel marrow samples contained post beti-cel ring sideroblasts but baseline samples lacked iron stains, making it challenging to attribute the finding to thalassemia or beti-cel treatment. Similarly, in Study HGB-212, although ring sideroblasts and dysmegakaryopoietic changes were observed in a small percentage of subjects, it is challenging to attribute this emergence of ring sideroblasts to beti-cel treatment.

Of note, dysmegakaryopoietic changes were noted in a few subjects following beti-cel administration. The marrow data were limited such that it was not feasible to determine whether some subjects experience emerging or worsening dysplastic features of megakaryocytes. Bone marrow samples were reviewed by outside pathologists who concluded there was no morphologic evidence of developing MDS in the post-beti-cel samples; rather, the abnormalities observed were ascribed to

chronic stress erythropoiesis due to underlying thalassemia. However, FDA cannot conclude if the morphologic changes are a result of chronic stress erythropoiesis. Considering that the great majority of subjects achieved TI with robust erythrocyte production functionally correcting their thalassemia, some decrease in "chronic stress erythropoiesis" would be expected by 24 months post-transplant.

*6.2.4.4 Insertional Oncogenesis*

LVVs integration into target cell genes during transduction is associated with potential risk of disrupting expression of nearby genes, as discussed in section 3.7. Such LVV integrations can lead to oncogenesis after the development of a predominant clone. Beti-cel recipents are monitored regularly for this complication with integration site analysis (ISA) of blood cells, and complete blood counts. The ISA algorithm is described in Appendix 2.

Beti-cel-treated subjects have not experienced malignancies or clonal predominance as of the data cut-off. However, three subjects with TDT meet the oligoclonality definition, based on the proposed ISA algorithm (which is discussed in Appendix 2). Of these, two have demonstrated stable oligoclonality for several years without development of a predominant clone or malignancy, but have concerning cytopenias and IS into proto-oncogenes:

1. One subject continues to have mild thrombocytopenia and has not reached platelet count of ≥ 100 x 10^9/L as of Day 737 after beti-cel infusion. This subject appears to have integration sites that may be within the same clone, including the proto-oncogenes XP07 and CBFB. The most recent IS-specific VCN was 0.0425 c/dg in XPO7 and 0.0461 c/dg in CBFB
2. A second subject remains mildly thrombocytopenic and did not reach platelet count ≥100 x 10^9/L until after Day 501; this subject has integration sites that may be within the same clone including proto-oncogene *BCR*. For *BCR*, most recently measured integration site-specific VCN was 0.1245 c/dg.
3. A third subject had oligoclonality identified recently with a single IS of ≥ 10% relative frequency but achieved unremarkable platelet reconstitution.

A potential relationship between oligoclonal LVV integrations and the delay of platelet reconstitution observed following beti-cel therapy remains unproven but is worrisome given cases of insertional oncogenesis experienced by recipients of other lentiviral vector-based products manufactured by the Applicant with related or identical LVV. LVV integrations into oncogenes are of particular concern, given cases of hematologic malignancy reported in CALD subjects with MECOM oncogene integrations.

LVV Insertions into Other Genes of Potential Concern in TDT subjects

Insertions into *VAMP4* have also been detected in 31 of 55 subjects with TDT (56%), with 59 unique *VAMP4* IS detected in beti-cel treated subjects. The highest

maximum frequency detected was 0.217%. This is of concern because one of the subjects treated with lovo-cel for SCD who developed AML was found to have a predominant clone with an IS in the *VAMP4* gene; however, the role of this integration is not clear; this is further discussed in the next section and in Appendix 3. *VAMP4* integration does not appear to be correlated with delayed platelet reconstitution.

*6.2.4.5 Insertional Oncogenesis Following Other LVV Products*

Lovo-cel in Sickle Cell Disease

Because mutations in the gene coding for the β-globin cause thalassemia and sickle cell disease, introducing a gene that expresses a functional β-globin (such as $\beta^{A\text{-}T87Q}$ globin) may be therapeutic in both diseases. Lovo-cel is a product being developed by the Applicant for treatment of SCD, which is related to beti-cel. Lovo-cel is an autologous CD34+ product that contains HSCs transduced with the BB305 LVV, encoding the βA-T87Q globin protein, which has anti-sickling properties while conserving the function of the wild-type β-globin protein. Lovo-cel and beti-cel share the same BB 305 LVV, and only have modest manufacturing differences. Myeloablative conditioning is also required before lovo-cel infusion. As of the data cut off, 49 subjects have received lovo-cel in clinical studies for SCD.

MDS and AML have been reported after administration of lovo-cel, though a causal role of LVV has not been conclusively demonstrated in the development of these malignancies. Among N=49 subjects with SCD treated with lovo-cel, AML developed in 2 subjects. In one of these cases, analysis of peripheral blood samples revealed that blast cells contained a prominent site for BB305 LVV integration within the VAMP4 gene which encodes vesicle-associated membrane protein 4. Based on available information, FDA cannot conclude that vector insertion into the VAMP4 gene did not contribute to the AML. FDA concerns are further discussed in Appendix 3.

Two additional subjects with SCD were evaluated for suspicion of evolving MDS. One subject with SCD was diagnosed with MDS after marrow biopsy to evaluate severe anemia showed hypoplasia of early myeloid precursors along with trisomy 8 on Fluorescent in situ hybridization (FISH) in 6% of cells. MDS diagnosis was later revised to "transfusion dependent anemia" when no trisomy 8 was seen on repeat marrow biopsy, with improvement in myelopoiesis. Another subject with SCD had bone marrow findings of erythroid dysplasia with 7.7% trisomy 8, "highly suggestive of MDS", and also 6.1% tetrasomy 8; work up is ongoing because of concurrent B12 deficiency.

More detailed description of the two cases of hematologic malignancy and the two cases suspicious for MDS following lovo-cel administration, are described in Appendix 3.

Eli-cel in CALD

Eli-cel is a different LVV-based product intended for the treatment of childhood cerebral adrenoleukodystrophy (CALD), a rare, X-linked neurodegenerative disease caused by mutations in the ATP-binding cassette (ABC), subfamily D, member 1 (ABCD1) gene that encodes the transporter protein adrenoleukodystrophy protein (ALDP). Deficiency of ALDP causes accumulation of very long-chain fatty acids in the central nervous system, Leydig cell of the testes, and the adrenal cortex. Boys with early CALD are treated with allogenic hematopoietic stem cell transplant. An unmet medical need exists for therapeutic options because of insufficient suitable donors. Eli-cel is comprised of autologous CD34+ HSCs transduced with a LVV related to that used in beti-cel, however containing ABCD1 cDNA and a constitutive promoter,.

Of 67 subjects administered eli-cel in clinical studies, 3 have been diagnosed with MDS. Two of these were diagnosed within ≤ 24 months of eli-cel treatment and contain a dominant clone with LVV integration into the MDS1 and EVI1 complex locus, (*MECOM*) oncogene with overexpression of EVI1. The 3rd MDS case appears to be related to integration into another proto-oncogene, *PRDM16*. More detailed description of the cases of MDS and evolving hematologic disorders in four additional subjects where integration sites in proto-oncogenes are increasing in relative frequency following eli-cel administration are described in Appendix 3.

# 7 Benefit-Risk Analysis

## 7.1 Efficacy Summary

Data contained in the BLA support the effectiveness of beti-cel for treatment of subjects with β-thalassemia who require regular RBC transfusions. Treatment with beti-cel led to durable TI in 89% of subjects. FDA believes that TI represents a clinically meaningful benefit for patients with transfusion-dependent β-thalassemia, and could decrease the risks of iron overload, multi-system organ complications and early mortality. This clinical endpoint is supported by pharmacodyamics data which show rises in βA-T87Q-globin leading to the attainment of target levels of unsupported total Hb, regardless of age or genotype.

## 7.2 Safety Summary

No deaths were reported in any of the clinical trials. The treatment regimen comprised of mobilization/ apheresis, conditioning, and infusion of beti-cel and has a safety/tolerability profile dominated by known effects of G-CSF, plerixafor, and busulfan. The majority of AEs were consistent with the known side effects of the

procedures and pharmacotherapy of the prerequisite treatment. Beti-cel was generally well-tolerated with 2 beti-cel-related SAEs of Grade 3 thrombocytopenia, which resolved, and 29 non-serious product-related AEs consisting mostly of infusion-related events on Day 1, and protracted ≥ Gr 3 thrombocytopenias. No subject experienced GVHD, graft rejection, or graft failure.

Events of interest included delayed platelet engraftment with median engraftment at Day 46 (range 19 to 191) and slow/incomplete recovery of platelet counts towards normal. Delayed platelet engraftment is an important identified risk following treatment with beti-cel. Although delayed platelet engraftment has not been associated with an increase in medically significant bleeding, many subjects experience prolonged recovery of platelets, which did not return to baseline. While these findings were not associated with clinical complications, several subjects had findings of emergent bone marrow abnormalities, including ringed sideroblasts and aberrant megakaryocytes. Although no clonal predominance related to vector integration or insertional oncogenesis was reported in studies of TDT, 2 subjects treated for SCD in different trials with a related LVV-based product developed AML, and 2 subjects with SCD had evidence of trisomy 8 at some visits after treatment, one becoming transfusion- dependent. Additionally, 3 subjects treated with a different LVV-based product for CALD developed MDS with integration into a proto-oncogene.

FDA has not drawn definitive conclusions with respect to the role, if any, LVV integrations play in the delayed platelet engraftment experienced by subjects treated with beti-cel; however, the hematologic malignancies observed after treatment with LVV-based products for SCD and CALD increase our concern that delayed platelet engraftment may represent MDS.

Clinical studies of beti-cel treatment demonstrate a clinically meaningful benefit in subjects with TDT, and most subjects achieved transfusion independence across all ages and genotypes. Durability of transfusion independence was demonstrated through approximately 39 months of available follow-up in Phase 3 study subjects. The safety profile is largely consistent with the known effects of G-CSF, plerixafor, and busulfan that comprise the prerequisite mobilization/apheresis conditioning before beti-cel infusion. Among 62 subjects who have commenced mobilization and 59 who have been treated with beti-cel in clinical studies, most reported AEs were consistent with the known side effects of necessary pretreatment procedures and drugs; the AEs were mostly non-serious and resolved. An important risk associated with beti-cel therapy is the delay of platelet engraftment (compared with AHSCT), followed by impaired platelet recovery. While clonal predominance due to LVV integration or LVV-mediated oncogenesis has not been reported in any subject with TDT, MDS and AML have been reported in studies of other LVV-based products in subjects with SCD and CALD.

The benefit-risk profile of beti-cel is not completely characterized for the proposed population due to the pathologic latency of platelet recovery, along with marrow morphology abnormalities in subjects with TDT, and the hematologic malignancies in studies with other LVV-based products.

# 8 REFERENCE LIST

1. Milone, M.C. & O'Doherty, U. Clinical use of lentiviral vectors. *Leukemia* **32**, 1529-1541 (2018).
2. Naldini, L. In vivo gene delivery by lentiviral vectors. *Thromb Haemost* **82**, 552-554 (1999).
3. Cavazzana-Calvo, M.*, et al.* Transfusion independence and HMGA2 activation after gene therapy of human beta-thalassaemia. *Nature* **467**, 318-322 (2010).
4. Negre, O.*, et al.* Gene Therapy of the β-Hemoglobinopathies by Lentiviral Transfer of the β(A(T87Q))-Globin Gene. *Hum Gene Ther* **27**, 148-165 (2016).
5. Magrin, E., Miccio, A. & Cavazzana, M. Lentiviral and genome-editing strategies for the treatment of β-hemoglobinopathies. *Blood* **134**, 1203-1213 (2019).
6. Zufferey, R.*, et al.* Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. *J Virol* **72**, 9873-9880 (1998).
7. Hanawa, H., Persons, D.A. & Nienhuis, A.W. Mobilization and mechanism of transcription of integrated self-inactivating lentiviral vectors. *J Virol* **79**, 8410-8421 (2005).
8. Cesana, D.*, et al.* Uncovering and dissecting the genotoxicity of self-inactivating lentiviral vectors in vivo. *Mol Ther* **22**, 774-785 (2014).
9. Negre, O.*, et al.* Preclinical evaluation of efficacy and safety of an improved lentiviral vector for the treatment of β-thalassemia and sickle cell disease. *Curr Gene Ther* **15**, 64-81 (2015).
10. Bushman, F.D. Retroviral Insertional Mutagenesis in Humans: Evidence for Four Genetic Mechanisms Promoting Expansion of Cell Clones. *Mol Ther* **28**, 352-356 (2020).
11. Hacein-Bey-Abina, S.*, et al.* Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J Clin Invest* **118**, 3132-3142 (2008).
12. Howe, S.J.*, et al.* Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. *J Clin Invest* **118**, 3143-3150 (2008).

13. Stein, S., *et al.* Genomic instability and myelodysplasia with monosomy 7 consequent to EVI1 activation after gene therapy for chronic granulomatous disease. *Nat Med* **16**, 198-204 (2010).
14. Boztug, K., *et al.* Stem-cell gene therapy for the Wiskott-Aldrich syndrome. *N Engl J Med* **363**, 1918-1927 (2010).
15. De Ravin, S.S., *et al.* Lentiviral hematopoietic stem cell gene therapy for X-linked severe combined immunodeficiency. *Sci Transl Med* **8**, 335ra357 (2016).
16. De Ravin, S.S., *et al.* Lentivector cryptic splicing mediates increase in. CD34+ clones expressing truncated HMGA2 in human SCID-X1. *Nature Portfolio* (2022).
17. Locatelli, F., *et al.* Betibeglogene Autotemcel Gene Therapy for Non–β0/β0 Genotype β-Thalassemia. *New England Journal of Medicine* **386**, 415-427 (2021).
18. Ghavamzadeh, A., et al. Comparable Outcomes of Allogeneic Peripheral Blood versus Bone Marrow Hematopoietic Stem Cell Transplantation in Major Thalassemia: Multivariate Long-Term Cohort Analysis, Biology of Blood and Marrow Transplantation **25**, 307-312. (2019)

# 9 Appendices

Appendix 1

Phase 1/2 Study HGB-204

This was a single-arm, multi-site, single dose, Phase 1/2 study of subjects 12 to 35 years of age with β0/β0 and non-β0/β0 genotype transfusion-dependent thalassemia (TDT). Transfusion dependence was defined as having received at least 100 mL/kg/year of pRBCs or ≥8 transfusions of pRBCs per year in each of the 2 years prior to enrollment. The study was comprised of 4 stages: (1) screening to determine eligibility via detailed history of TDT management including transfusion, hospitalizations, laboratory values and chelation data, (2) apheresis after mobilization with granulocyte colony-stimulating factor (G-CSF) and plerixafor to collect HSCs for drug product manufacture, (3) marrow myeloablation with busulfan for 4-5 days, a washout period of ≥ 72h, followed by infusion of beti-cel, and (4) follow-up of approximately 2 years.

Study Objectives

- Evaluate the safety of treatment with beti-cel in subjects with TDT

- Evaluate the efficacy of treatment with beti-cel in subjects with TDT

Key Enrollment Criteria

Inclusion Criteria

1. Age 12-35 years at the time of consent or assent
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. Karnofsky performance status of ≥60; eligible to undergo HSCT
5. Treated and followed for at least the past 2 years in a specialized center that maintained detailed medical records, including transfusion history

Exclusion Criteria

1. Positive for human immunodeficiency virus (HIV), hepatitis B virus (HBV), or hepatitis C virus (HCV)
2. Active bacterial, viral, fungal, or parasitic infection
3. A white blood cell (WBC) count <3 × 10^9/L, and/or platelet count <100 × 109/L
4. Uncorrected bleeding disorder
5. Any prior or current cancer, myeloproliferative or immunodeficiency disorder
6. Prior HSCT
7. Advanced liver, renal, pulmonary, cardiac disease, or severe iron overload

Treatment Plan

Dose Regimen
Beti-cel for each subject was prepared using their autologous cells and each subject received a single intravenous beti-cel dose of ≥3.0 × 106 CD34+ cells/kg on Day 1.

Concomitant Medications
Permitted concomitant treatments during conditioning at the Investigator's discretion included diuretics, ondansetron and/or metoclopramide (antiemesis), anticonvulsants for seizure prophylaxis except for phenytoin, ursodeoxycholic acid for prevention of hepatic veno-occlusive disease, and anti-infectives for infection prophylaxis or treatment of febrile neutropenia. Iron chelators were to be stopped for 7 days before busulfan. Iron chelation resumption was at the investigator's discretion and in accordance with institutional protocols, and with post-transplant chelation guidelines in protocol.

Study Assessments

Primary Efficacy Endpoint:

The proportion of subjects who met the definition of transfusion independence (TI), defined as a weighted average hemoglobin (Hb) ≥ 9 g/dL without pRBC transfusions for a continuous period of ≥ 12 months at any time after beti-cel infusion.

Secondary Endpoints:

- Characterization of TI (duration, time to achievement, time from infusion to last pRBC transfusion, weighted Hb during TI, proportion of subjects with TI at Months 18 and 24)
- Characterization of transfusion reduction (TR; proportion of subjects with defined percentages of TR in transfusion volume compared to the transfusion volume in the 2 years prior to study enrollment; percent reduction in frequency of transfusions; weighted Hb nadir)
- Exploratory endpoints included assessment of iron burden, iron chelation, phlebotomy, hospitalizations, and assessment of Health-Related Quality of Life (HRQoL)

Safety Assessments:

- Success and kinetics of HSC engraftment
- Incidence of transplant-related mortality through 100- and 365-days after beti-cel infusion and overall survival
- Detection of vector-derived replication-competent lentivirus (RCL)
- Integration Site Analysis (ISA) to determine presence of clonal dominance
- Descriptive analysis of adverse events
- Changes in laboratory parameters and frequency and severity of clinical AEs

Appendix 2

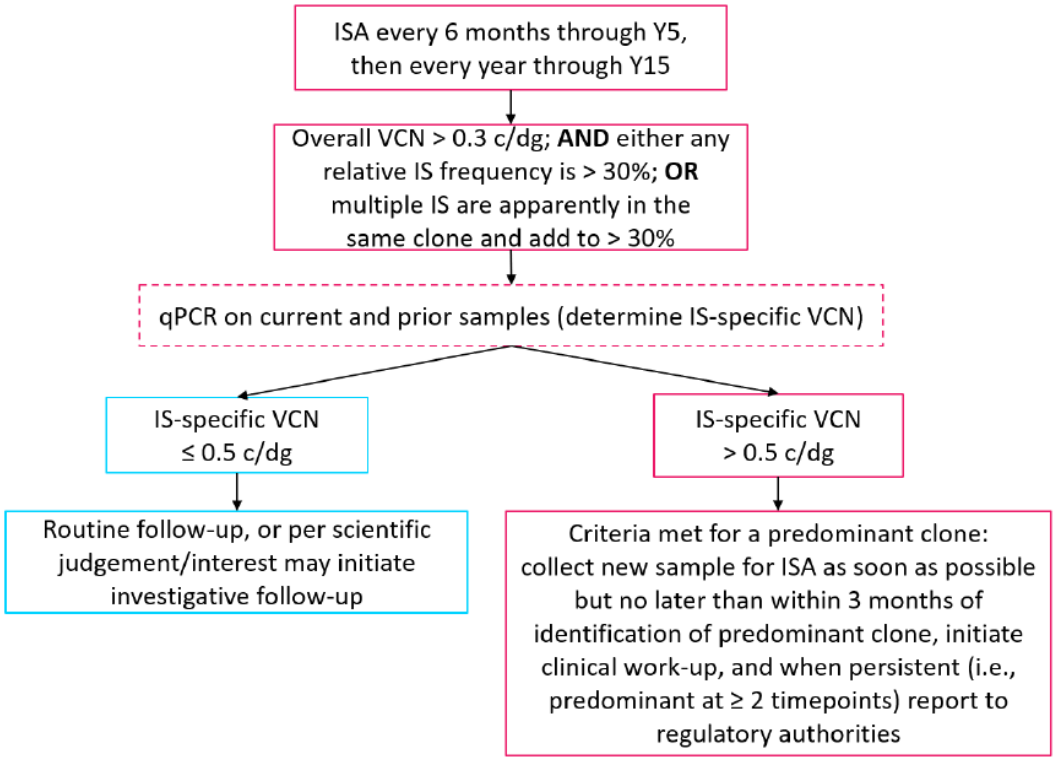
Integration Site Analysis

Lentiviral vectors have the potential to alter the host genome at undesirable locations, thus portending a theoretical risk of malignancy. While insertional oncogenesis has not been observed with beti-cel to date, hematologic malignancy has been reported after treatment with LVV-based products in other diseases.

To evaluate for clonal predominance, beti-cel recipients underwent surveillance with an insertion site analysis (ISA) algorithm. ISA was performed using high-throughput, semi-quantitative methods which identify integration sites (IS) based on vector sequence primers. Identified insertion sites are considered of interest when the overall peripheral blood VCN is > 0.3 c/dg AND either any relative IS frequency is > 30% OR multiple IS are apparently in the same clone and add up to > 30%. Multiple insertion sites apparently in the same clone is defined as more than one relative frequency where values are within 20% of each other (e.g., 5% ± 1%, 10% ± 2%,

15% ± 3%, etc.), as well as any additional cases identified through the Applicant's internal review of ISA reports. When multiple IS appeared in the same clone, a confirmatory bone marrow or peripheral blood colony-forming unit assay was performed. IS of interest would be interrogated, using a quantitative assay (e.g., qPCR) designed to detect the specific IS and determine an IS-specific VCN that will help to estimate clonal contribution. If results of the quantitative, IS-specific follow-up assay reveal an IS-specific VCN > 0.5 c/dg, estimating > 50% clonal contribution, criteria will be met to consider the subject as having a predominant clone. This threshold also applies to individual lineage evaluations (myeloid, lymphoid, etc.) when performed. Clinical work-up would be recommended for a predominant clone, and a repeat sample was to be collected within three months after identification of a predominant clone, with retrospective testing by IS-specific qPCR done on sample(s) previously collected, as available. A predominant clone identified at 2 or more time points is considered persistent. The algorithm is presented in the figure below:

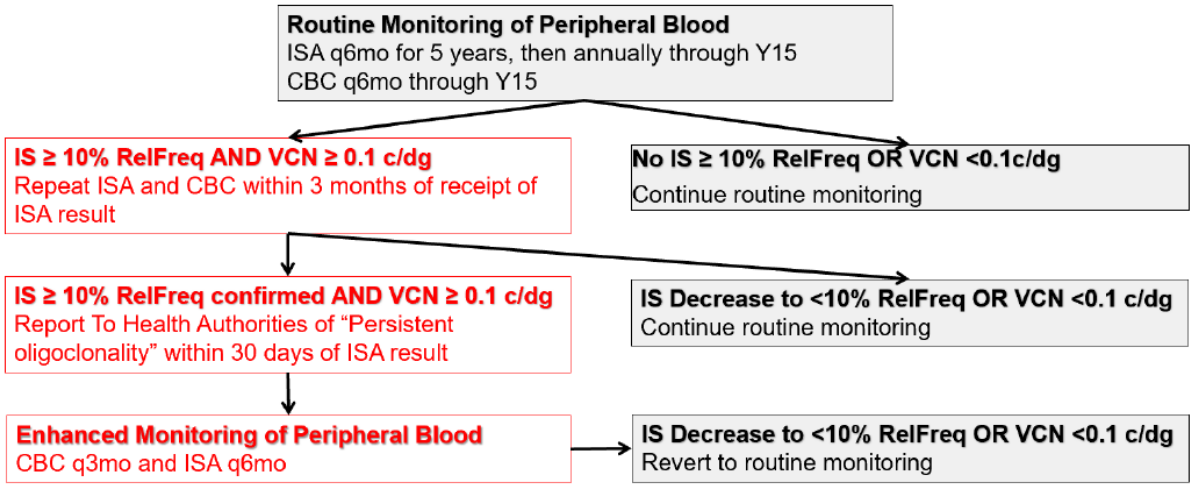
Figure 4. Original ISA Algorithm




Proposed Revision of IS screening strategy

To increase the sensitivity of the IS screening algorithm to detect persistent oligoclonality, a known risk factor for malignancy, the Applicant proposed a new ISA algorithm, lowering the minimum threshold Relative Frequency (RelFreq) to ≥ 10% associated with a vector copy number (VCN) of ≥ 0.1 c/dg and used this as a trigger for increased clinical hematologic monitoring. Persistent oligoclonality (≥ 10% Relative Frequency at two consecutive ISA timepoints) would be reported to Health Authorities. The figure below illustrates the new algorithm whose implementation is currently under discussion.

Figure 5. New ISA Algorithm




Note: If a VCN result is not available for the same visit at which the RelFreq exceeds 10% for the first time, the VCN will be done at the same time that the ISA is repeated. During enhanced monitoring, VCN will be performed on same samples as ISA if not already scheduled as per SOE.

The same ISA algorithm is used across trials for the similar product (lovo-cel) to treat sickle cell disease (SCD), and for a related product (eli-cel) used to treat childhood cerebral adrenoleukodystrophy (CALD).

Appendix 3

Cases of Hematologic Malignancy and Suspected Evolving Malignancy in Clinical Studies of other LVV-Based Products

The following section provides more detailed information on cases of AML and MDS, and po/tentially evolving MDS, which were observed after therapy with lovo-cel for SCD and eli-cel for CALD in the respective development programs. The data were also submitted to this BLA.

Subjects Treated with lovo-cel

SCD Subjects with Hematologic Malignancy

1. Subject 206-A-02: 46-year-old with βS/βS; history of treatment with HU; was treated 2015; engraftment was unremarkable. He had no VOCs after bb1111 but was restarted on HU about a year later. The subject had anemia post-GT requiring transfusions, and EPO was started ~2.5 years after bb1111. Six months later, at 3 yrs. post-GT, pancytopenia was noted, followed by a diagnosis of MDS (RAEB-2). Peripheral blood showed pancytopenia with circulating myeloblasts at 6.7%. BMBx showed 40% cellularity, megakaryocytic dysplasia with small and hypolobated nuclei, and erythroid dysplasia, severe myeloid hypoplasia, and 15% CD34+ myeloblasts and 10% by flow cytometry; flow cytometry shows blasts are dimCD45+, CD34+, CD117+, HLA DR+, CD13+, CD33+. Cytogenetic analysis showed 45,XY,- 7,add(19)(p11)[8]/46,XY[12]. NGS panel testing of BM cells showed RUNX1 p.Asp198Gly at VAF 8.3%, PTPN11 p.Phe71Leu at VAF 5.2%, and KRAS p.Gly12Ala at VAF 2.7% mutations. ISA showed no clonal predominance through Month 36, and that the CD34+ blasts did NOT contain a vector integration. Subject was treated with 5 cycles of hypomethylating agent therapy [2 cycles of 5-Azacitidine and 3 cycles of Decitabine], but he progressed to AML. Despite standard induction chemotherapy with idarubicin days 1-3 and cytarabine days 1-7, he had persistent disease with 15%-19% bone marrow blasts. The subject had re-induction therapy with blasts reduced to 5% prior to AHSCT. Preparative regimen consisted of melphalan, fludarabine, and total body irradiation followed by haploidentical HSCT. Had remission at Day 100: blasts 3%, normal trilineage maturation, no monosomy 7), but relapsed 2 months later. At that point, patient was treated with decitabine and venetoclax for 8 cycles; switched to azacytidine/venetoclax for 3 additional cycles. Following this, was found to have PB blasts and was placed on hospice care, where he died. VCN analysis demonstrated a lack of enrichment of LVV sequences in the blast cell-enriched population, arguing against insertional oncogenesis due to LVV transduction. No VCN was detected in BM cells at the time of relapsed AML post-allogeneic HSCT.

2. Subject 206-A-01: 32-year-old with βS/βS; had prior treatment

with hydroxyurea (HU); received bb1111 dose in 2015 followed by prompt hematopoietic cell engraftment. The subject did not respond to bb1111 and continued to require transfusions and intermittent HU. Blasts were found in blood in December 2020 at 2%; rising to 29% in February 2021. AML was diagnosed at that time, 5.5 years after bb1111. Blasts were CD34+CD33+LVV+; chromosomal microarray analysis showed monosomy 7 in 70% of cells, with partial loss of 11p involving WT1 in 50% of cells. NGS revealed a RUNX1 frameshift mutation p.A149*fs with a VAF of 26% and a PTPN11 missense mutation p.A72V with VAF of 30%. The AML was refractory to induction chemotherapy with idarubicin days 1-3 and cytarabine days 1-7; subject received salvage chemotherapy and haplo-identical transplant, but died of relapsed disease and transplant complications.
ISA at 4.5 years showed an increase in frequency of LVV IS in the VAMP4 (vesicle-associated membrane protein 4) gene, located on chromosome 1. Blasts were positive for a LVV integration in VAMP4. However, VAMP4 expression was not elevated in the blast cell-enriched cell population relative to the blast cell-depleted cell population; Retrospective microarray and myeloid mutation panel testing on PB leukocyte pellets from screening in 2015, and at Months 3, 6, 18 and 24 post-GT, revealed no abnormalities.

Transcriptomic data showed that VAMP4 expression was not elevated in the myeloblast-enriched CD34+ PB sample relative to the myeloblast-depleted CD34- PB sample. Although the Applicant reported that “careful assessment of the genetic region within 10Mb of the VAMP4 locus revealed no remarkable expression level change in the majority of genes”, but bulk RNA- seq was performed without internal control spike-in RNAs which are needed to compare expression levels across samples. Instead, read counts were normalized by sequencing depth, which may not accurately reflect differences in gene expression. Moreover, the gene expression analysis done might be inadequate to assess changes in gene expression for the 16 protein-encoding genes present within 1Mb of VAMP4 and the 127 protein-encoding genes present within 10Mb. The Applicant has stated that the AML is unrelated to the *VAMP4* integration because of the location of the insertion site, the very low transgene expression in blast cells, and no conclusive effect on expression of surrounding genes. However, the

Agency continues to have doubts about these claims, as outlined below:

Potential role of VAMP4 insertion in development of AML of subject 206-A-01

The Applicant supported the opinion that the AML was unrelated to VAMP4 integration with the following:

a) Most analyses reveal that VAMP4 itself has no known role in the development of AML or other cancers [21]
b) Classic driver alterations in AML (RUNX1 and PTPN11) were present
c) Assessment by the Applicant of region within 10 Mb of the VAMP4 locus showed no remarkable expression level change in the majority of genes
d) The IS is unremarkable with respect to cancer associated genes
e) The IS did not disrupt genomic elements
f) Several somatic mutations predisposing to AML were present after diagnosis, consistent with patients with SCD being at increased risk for hematologic malignant conditions after transplantation [22]

FDA believes that there are insufficient data to conclude that LVV integration into VAMP4 gene did not contribute to the AML in this subject. Our concerns with the Applicant's arguments listed above include:

a) Presence of mutations such as RUNX1 and PTPN11 does not preclude a role for VAMP4 IS. One publication suggests VAMP4 binding to CALM, which is involved as a translocation breakpoint in some AMLs[23].
b) The Applicant reported scrutinizing a gene region within 10Mb of the VAMP4 IS and found that most genes did not have expression level change, but the Applicant did not describe the possible impact of the remaining nearby genes that did have remarkable expression level change; furthermore, the methodology used for bulk RNA-seq did not use internal spike-in controls, which are necessary for internal quantification of gene expression levels.

c) The IS may be of significance with respect to cancer-associated genes, based on publication mentioned above[23].
d) FDA identified a number of genes near VAMP4, including 33 genes within 1Mb (16 of which are protein-encoding); 256 genes (127 protein-encoding) within 10Mb. Among these, at least one IS was present in 69% and 83% within 1Mb and 10Mb, respectively, in protein encoding genes. The Applicant did not substantiate the claim that no disruption of genomic elements occurred due to the VAMP4 IS.
e) Certain variants >20Mb away from the *VAMP4* integration site in chr1q have not been accounted for by the Applicant, including:
    - A 2.33Mb deletion and replacement by 10nt at position 147,036,771
    - A 1.01 KB deletion at position 231,212,613
    - Four copy number alterations:
        - o Copy-neutral loss of heterozygosity at loc 143208875, 64313 nt
        - o Loss at loc 221,570,410, 10,502 nt
        - o Gain at 248,404,967, 122,696 nt
        - o Loss at 248,575,123, 71,733 nt

Subjects Sickle Cell Disease with Suspected Evolving MDS

1. Subject 206-C-27: 20-year-old with βS/βS and deletion of two α-globin genes; history of treatment with HU for 2 years. She was treated in 2020. The subject had persistent, severe anemia requiring transfusions post-GT, and was DAT positive for IgG. About 6.5 months remained anemic, and a scheduled bone marrow biopsy revealed hypoplasia of early myeloid precursors with progressive maturation, reduced cellularity with relative erythroid hypoplasia, megakaryocytes without dysplasia; M:E ratio of 1.1:1, and no abnormal blast population. Karyotype was normal, but FISH showed trisomy 8 in 6% (12/200) cells; 4.5-9.5% of cells showed signals consistent with tetraploidy. The subject was given a tentative diagnosis of MDS. However, a repeat bone marrow biopsy performed one month later did not show trisomy 8 by FISH; karyotype and SNP microarray were both normal. NGS panel showed no mutations.

Diagnosis was changed to "transfusion dependent anemia". Subject has episodic exacerbations of chronic pain and persistent anemia for which she is transfused. FISH, SNP microarray, and molecular NGS panel testing were performed on the retained bb1111 pre-conditioning; all were reported to be normal. VCN of 4.6 c/dg has remained stable in PB since Months 3-6, with increasing levels of HbAT87Q (~3.8g/dL at Month 6) detected. ISA at 6 and 12-months did not reveal any IS > 0.15% relative frequency. Repeat BM in July 2021 showed improvement in myelopoiesis, but qualitative changes in erythroid series persist. Karyotype and FISH studies were normal; no trisomy 8 was seen. NGS (UCSF500 Gene Panel) showed no somatic abnormalities although a heterozygous *ATM* mutation was found in bone marrow and buccal swab. Her diagnosis was changed to "transfusion dependent anemia" and the subject has episodic exacerbations of chronic pain and persistent anemia for which she is transfused. Cytogenetics at Year 1 (karyotype, FISH and SNP microarray) were normal. At Year 1.5, the following abnormalities were identified on NGS (Rapid Heme Panel): ATM (VAF 26.8%), TERT (VAF 58.8%), IKZF1 (VAF 37.8%), and TET2 (VAF 40.2%). Cytogenetics were otherwise normal.
It appears the ATM, TERT, IKZF1, and TET2 variants were present prior to DP administration, having been retrospectively found with NGS for a specimen from November 2019. In addition, testing on the retained DP and on CD34- cells from bone marrow remaining after CD34+ enrichment, although initially reported to be normal, identified variants in TET2 and IKZF1.

2. Subject 206-C-32: 14-year-old with βS/βS and deletion of two α-globin genes; was treated in 2020. Did not have a baseline marrow biopsy, and engraftment data are not available. PB at screening and Month 9 were reported as normal by SNP microarray. About one year later in July 2021, the subject was noted to have mild pancytopenia and vitamin B12 deficiency: 130 pg/mL; PB showed a WBC 4.05 K/uL, hemoglobin 9.2 g/dL; MCV 78; plts 130 K/uL. Occasional sickle cells were seen in the PB. Patient was placed on vitamin B12 supplementation, and several weeks later, the B12 level was 262 pg/mL. Within two months, levels declined again to 154 pg/mL; B12 supplements continued. BM aspirate was collected per protocol. Many tri-and bi-nucleate erythroid progenitors were

noted, and dysplasia was described as present in ~15%-20% of erythroid cells; blasts < 5%, and ring sideroblasts < 5%. Karyotype was normal, but a FISH panel had 7.7% trisomy 8 (10/130 nuclei scored) and 6.1% tetrasomy 8 (8/130). Marrow sample was interpreted as "erythroid dysplasia with trisomy 8, highly suggestive of MDS". A definitive diagnosis of MDS was not given. Follow-up studies were performed two months later in September 2021: PB showed WBC 4.78 K/uL, hemoglobin 9.2 g/dL, MCV 75, plt count 127 K/uL, ANC 1710; BMBx (this time including core) showed a normocellular marrow with trilineage hematopoiesis, erythroid hyperplasia and 10-20% of erythroid cells showed dysplasia, with nuclear budding, binucleation, and irregular nuclear contours. Blasts of 1%. The morphologic dysplasia in the erythroid lineage, especially the observed binucleation, was concerning for an evolving MDS . No dysplasia was seen in the megakaryocytic or granulocytic lineages. A normal karyotype was seen, but FISH studies showed persistence of trisomy and tetrasomy for chromosome 8: 5% trisomy (10/200 nuclei scored), and 4% tetrasomy (8/200 nuclei scored), which are essentially stable.

Molecular NGS performed later on all samples showed no variants consistent with a myeloid malignancy. A TET2 c.4946A>G variant of unknown significance at VAF 47.5% was reported; a retrospective NGS panel performed on PB samples at screening and Month 9 post-GT revealed the same TET2 variant at VAF 50%, suggesting its germline nature. PB ISA at 6 and 12 months was polyclonal, with no clone >1%.

MDS and hematologic abnormalities related to eli-cel treatment for CALD

Subjects with MDS

Eli-cel (elivaldogene autotemcel) is intended for the treatment of CALD. Eli-cel shares the same vector backbone as beti-cel and contains a copy of the ATP-binding cassette, sub-family D, member 1 (ABCD1) cDNA, driven by an internal enhancer/promoter derived from the unique 3' (U3) region of murine myeloproliferative sarcoma virus with a negative control region deletion (MNDU3).

Three subjects who received eli-cel have developed MDS, two of whom had integrations into *MECOM* and additional genes that met criteria for clonal predominance before MDS was diagnosed.

1. Subject 104-18: 11-year-old treated with eli-cel in May 2020, who developed MDS with dysmegakaryopoiesis 14 months post-GT. He had a clone with integration into *SLC6A16* and *MECOM*, accompanied by increased *EVI1* expression, with a clonal contribution of approximately 43% in $CD15^+$ cells at Month 12. His bone marrow biopsy revealed 10-20% cellularity with dysmegakaryopoiesis, meeting criteria for MDS.
2. Subject 104-08: 13-year-old treated with eli-cel in July 2019, who developed MDS with dysmegakaryopoiesis 22 months post-GT. He had a clone with integration into *MECOM* (accompanied by increased *EVI1* expression), *ACTR*, *RAP2C*, and *STGAL6* with a mean clonal contribution of 86% in $CD15^+$ cells at one-year post-GT.
3. Subject 102-03: 5-year-old treated with eli-cel in March 2014, who developed MDS with excess blasts 7.5 years after treatment. Blasts were positive for the LVV. Based on S-EPTS/LM-PCR, it appears the clone has integrations into *PRDM16*. His bone marrow biopsy demonstrated 60-70% cellularity with 15% myeloid blasts, and $CD34^+$ cells making up 20-30% of cells in some discrete foci on immunohistochemistry. Flow cytometry demonstrated 15% blasts. FISH and karyotype were normal. A rapid heme panel showed *KRAS* and *NRAS* mutations at 14% and 3% VAF.

Other eli-cel Recipients with Findings Concerning for Evolving Insertional Oncogenesis

1. Subject 102-31: 4-year-old treated with eli-cel in April 2018. ISA shows LVV integrations into *MECOM* and *EVI5* that has risen in relative frequency at but the integration-site-specific VCN is below the Applicant's current threshold for clonal predominance. His bone marrow biopsy demonstrated no evidence of malignancy on flow cytometry or rapid heme panel.
2. Subject 102-11: 7-year-old treated with eli-cel in February 2015. ISA shows LVV integrations in *MECOM* with *EVI* overexpression; *ACER3*, and *RFX3* with an average clonal contribution of 83% in $CD15^+$ cells. However, he has no clinical signs of malignancy with a moderately hypocellular bone marrow. Flow cytometry, FISH, and molecular NGS panels do not suggest any malignancy.

3. Subject 104-09: 9-year-old treated with eli-cel in August 2019. He has had prolonged cytopenias since GT. He required platelet and PRBC transfusions, until Day 69; and received filgrastim and eltrombopag support until Day 111

and Day 310, respectively. A bone marrow biopsy and aspirate at 2 months post-GT demonstrated 5% cellularity with normal erythroid and granulocytic maturation; parvovirus was detected by PCR and has remained positive in the bone marrow at all time points to date despite ten months of treatment with IVIG. At one year, ANC was 1400, Hb normal and platelet count was 108K/uL. At 23 months post-GT, the bone marrow cellularity had increased to 60-70%, with trilineage hematopoietic maturation, and atypical megakaryocytes but no increase in blasts. There was no evidence of malignancy on flow cytometry, and karyotype was normal. An MDS-focused NGS panel revealed one likely pathogenic loss of function heterozygous variant detected in the *MPL* gene (p.R102P) and an alteration of uncertain significance in *CALR* (D165G). Twenty-six months post-GT, the bone marrow cellularity is patchy and variable, but estimated overall to be 30%, with trilineage hematopoiesis, atypical megakaryopoiesis, and 2% blasts. Flow cytometry showed no increase in myeloid blasts or any abnormal myeloid blast population. At month 26, the ANC is 1600, Hb is normal and platelet count is 100K/uL. ISA demonstrated integration into *MECOM,* although the relative frequency has declined from 7.1% at Month 12 to 4.3% at Month 26, which was the most recent assessment. ISA shows the clones with the highest relative frequency and a slight upward trend at 26 months are *LINC00982* and *SMG6*, at 10.4% and 8.6%, respectively. The subject also has integrations into *MPL* that are at a comparatively low but increasing in frequency.

4. Subject 104-22: 13-year-old with concerning integration site pattern because of a rising relative frequency of integration into the proto-oncogenes *MECOM* and *MPL*. He has mildly low platelet counts but blood counts are otherwise normal.