Cellular, Tissue, and Gene Therapies Advisory Committee Meeting

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ADVISORY COMMITTEE

BETIBEGLOGENE AUTOTEMCEL BRIEFING DOCUMENT

BLA 125717

CELLULAR, TISSUE, AND GENE THERAPIES ADVISORY COMMITTEE

Meeting Dates: 09 June and 10 June 2022

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AE	adverse event
AIDS	acquired immunodeficiency syndrome
ALDP	adrenoleukodystrophy protein
allo-HSCT	allogeneic hematopoietic stem cell transplantation
AML	acute myeloid leukemia
ANC	absolute neutrophil count
AUC	area under the curve
beti-cel	betibeglogene autotemcel
BL	baseline
BLA	Biologics License Application
BMC	bone marrow cells
C	initiation of conditioning
CAF	Cooley's Anemia Foundation
CALD	cerebral adrenoleukodystrophy
CBC	complete blood count
c/dg	copies per diploid genome
CI	confidence interval
CHF	congestive heart failure
COI	chain of identity
CSR	clinical study report
DLco	diffusion capacity of carbon monoxide
DNA	deoxyribonucleic acid
DP	drug product
DPI	drug product infusion
DP VCN	drug product vector copy number
E	event
EBMT	European Society for Blood and Marrow Transplantation
eli-cel	elivaldogene autotemcel
EPO	erythropoietin
EQ-5D	EuroQol- 5 Dimension
FACT	Foundation for the Accreditation of Cellular Therapy
FACT-BMT	Functional Assessment of Cancer Therapy-Bone Marrow Transplantation
FDA	Food and Drug Administration
FEV1	forced expiratory volume in 1 second
FVC	forced vital capacity
G-CSF	granulocyte colony-stimulating factor
gDNA	genomic DNA

GRV	gamma retroviral vectors
GI	gastrointestinal
GVHD	graft-versus-host disease
Hb	hemoglobin
HbA	hemoglobin A (i.e., adult hemoglobin)
HbA2	hemoglobin A2 (i.e., minor variant of adult hemoglobin)
HbA^{T87Q}	hemoglobin containing $\beta^{A\text{-}T87Q}$-globin
HBB	β-globin gene
HbE	hemoglobin containing β^{E}-globin
HbF	hemoglobin containing g-globin (i.e., fetal hemoglobin)
hCG	human chorionic gonadotropin
HCP	health care provider
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HPC-A	hematopoietic progenitor cells obtained by apheresis
HPLC	high performance liquid chromatography
HRQoL	health-related quality of life
HSC	hematopoietic stem cell (in this briefing document, the CD34+ cell-enriched population which contains hematopoietic stem/progenitor cells, are referred to as HSCs)
HSCT	hematopoietic stem cell transplantation
ICF	informed consent form
IND	Investigational New Drug (application)
IS	insertion site
ISA	integration site analysis/analyses
ITT	Intent-to-Treat
IV	intravenous
LDL-R	low density lipoprotein receptor
LIC	liver iron concentration
lovo-cel	lovotibeglogene autotemcel
LTFU	long-term follow-up
LVEF	left ventricular ejection fraction
LVV	lentiviral vector
M	initiation of mobilization
MDS	myelodysplastic syndrome
MedDRA	Medical dictionary for Regulatory Activities
MRI	magnetic resonance imaging
MSD	matched sibling donor

NA	not applicable
NCT	National Clinical Trial (number)
NE	neutrophil engraftment
NGS	next generation sequencing
PB	peripheral blood
PB VCN	peripheral blood vector copy number
PCR	polymerase chain reaction
PD	pharmacodynamic(s)
PE	platelet engraftment
PI	package insert
PK	pharmacokinetic(s)
qPCR	quantitative polymerase chain reaction
RBC	red blood cell
RCL	replication-competent lentivirus
RelFreq	relative frequency
RNA	ribonucleic acid
RP-HPLC	high performance liquid chromatography
RV	residual volume
SAE	serious adverse event
SCD	sickle cell disease
SD	standard deviation
S-EPTS/LM-PCR	extension primer tag selection ligation-mediated PCR
SIN	self-inactivating
SOS	sinusoidal obstruction syndrome
TDT	transfusion-dependent β-thalassemia
TEAE	treatment-emergent adverse event
TESAE	treatment-emergent serious adverse event
TI	transfusion independence
TIF	Thalassemia International Federation
TP	Transplant Population
TR	transfusion reduction
TRM	transplant-related mortality
TSS	transcriptional start site
UIS	unique mappable integration site
US	United States
VAF	variant allele frequency
VCN	vector copy number
VOD	veno-occlusive liver disease
VSV	vesicular stomatitis virus

VSV-G	vesicular stomatitis virus-G protein
VUS	variant of unknown significance
WBC	white blood cells

1. EXECUTIVE OVERVIEW

1.1. Introduction

β-thalassemia is a rare genetic blood disease caused by mutations in the β-globin *(HBB)* gene and is characterized by reduced or absent production of the functional β-globin protein necessary to form adult hemoglobin (HbA), the primary form of hemoglobin (Hb) in human red blood cells (RBCs) after approximately 6 months of age. Insufficient β-globin production results in an imbalance of α-globin to β-globin and accumulation of α-globin that precipitates in maturing erythroid cells, leading to premature cell death (apoptosis), ineffective erythropoiesis, and hemolysis, which cause the anemia characteristic of this disease (Galanello and Origa 2010).

β-thalassemia patients with severe anemia require lifelong, regular RBC transfusions every 2 – 5 weeks for survival (Cappellini et al. 2021). Without regular transfusions, these individuals typically experience significant morbidity and die in early childhood. In addition to regular transfusions, iron chelation therapy is critical to mitigate the toxic effects of iron overload related to regular RBC transfusions (Taher et al. 2021; Cappellini et al. 2021). Optimized transfusions and more effective chelation have resulted in improved survival. However, patients continue to be affected by co-morbidities due to iron overload. Furthermore, complications related to these therapies have resulted in a significant disease management burden (Borgna-Pignatti et al. 2004; Tubman et al. 2015). Median age of death among known reported deaths in patients with transfusion-dependent β-thalassemia (TDT) was 37 years of age in the US population from 2011 to 2021 (Chieco and Butler 2022).

While allogeneic hematopoietic stem cell transplant (allo-HSCT) can be considered a curative therapy for some patients with TDT, its successful outcome is effectively limited to children with a related human leukocyte antigen (HLA)-matched sibling donor (MSD). As such, allo-HSCT is a valid option for only 25 – 30% of patients who may have a MSD (Angelucci et al. 2014) and 40-50% of patients who may have a matched donor (Cappellini et al. 2021). In addition, serious risks such as graft versus host disease (GVHD), graft failure and transplant-related mortality occur frequently (Galambrun et al. 2013; Angelucci et al. 2014; Baronciani et al. 2016; Chaudhury et al. 2017; Caocci et al. 2017).

betibeglogene autotemcel (beti-cel) is an innovative one-time, autologous gene addition therapy that can potentially cure patients with β-thalassemia who require regular RBC transfusions: beti-cel can increase the amount of total Hb to normal or near-normal levels to eliminate the need for chronic RBC transfusions and iron management therapies. The clinical benefits of this one-time beti-cel treatment are expected to last for the patient's lifetime.

beti-cel consists of a CD34+ cell enriched population that contains hematopoietic stem cells (HSCs) transduced with a self-inactivating (SIN), replication-incompetent lentiviral vector (LVV) encoding the $\beta^{A\text{-}T87Q}$-globin gene. In this briefing document, the CD34+ cell-enriched population which contains hematopoietic stem and progenitor cells, are referred to as HSCs. The expressed $\beta^{A\text{-}T87Q}$-globin protein is structurally identical to endogenous adult β-globin with the exception of a glutamine substitution for threonine at position 87 (T87Q), which enables tracking via reverse phase high performance liquid chromatography (RP-HPLC) of the β-globin produced by the transgene in patients' peripheral blood (Negre et al. 2016). The adult hemoglobin containing $\beta^{A\text{-}T87Q}$-globin has comparable oxygen kinetics to adult hemoglobin containing wild-type β-globin (Pawliuk et al. 2001; Negre et al. 2016). As $\beta^{A\text{-}T87Q}$-globin gene expression is

regulated by the β-globin locus control region and erythroid-specific promoter, this enables its expression only in erythroid cells and not in other hematopoietic cell types. The $\beta^{A\text{-}T87Q}$-globin protein combines with α-globin to correct the α/β-globin imbalance in erythroid cells, producing functional gene therapy-derived HbA^{T87Q}.

The patient's HSCs are first mobilized into the bloodstream for collection by apheresis and then enriched for CD34+ cells, which are then transduced with the BB305 LVV to create the beti-cel drug product (DP). Following successful DP manufacture, the patient undergoes full myeloablation to eliminate existing bone marrow HSCs containing the underlying β-globin mutation and to permit engraftment of the transduced cells. beti-cel is then administered to patients via a single intravenous infusion, where the transduced CD34+ HSCs engraft into the bone marrow and reconstitute the hematopoietic system with the production of functional HbA^{T87Q}.

The minimum recommended dose of beti-cel is 5.0×10^6 CD34+ cells/kg patient weight, with no maximum dose.

As an autologous product, beti-cel does not require a donor, is not associated with the risk of graft rejection or GVHD and does not require lymphodepletion during myeloablative conditioning or immunosuppression post-transplant as needed for allo-HSCT.

The Investigational New Drug (IND) application for beti-cel was submitted to the FDA in December 2012 and cleared on 17 January 2013. beti-cel was granted Fast Track, Breakthrough Therapy, and Rare Pediatric Disease designations. Orphan Drug Designation was granted for the treatment of β-thalassemia major and intermedia, which covers the intended indication for beti-cel. On December 2019, the rolling Biologics License Application (BLA) was initiated with the submission of the nonclinical module. The complete BLA was submitted to FDA on September 2021, and beti-cel was granted Priority Review on November 2021.

The proposed beti-cel indication is for the treatment of patients with β-thalassemia who require regular red blood cell (RBC) transfusions.

1.2. Disease Overview

HbA, the primary form of hemoglobin in adults, is a hetero-tetramer of 2 α- and 2 β-globins, each globin having a heme molecule capable of binding one oxygen molecule. β-thalassemia is caused by mutations in the β-globin gene, which can be classified as either β^0, where there is no expression and consequently no β-globin production, β^+, where there is some but variably reduced production of β-globin, or β^E, where there is reduced β-globin production and production of a variant Hb (HbE). Without adequate β-globin, there is a reduction or absence of HbA production.

Patients with β-thalassemia are described as having a β^0/β^0 genotype or non-β^0/β^0 genotype. Patients with a β^0/β^0 genotype have β^0 mutations in both alleles and no functional β-globin is produced. Patients with non-β^0/β^0 genotypes have at least 1 copy of a β^+ or β^E mutation (primarily found in patients of Southeast Asian origin), which is associated with some degree of functional β-globin production. In all, > 350 mutations in the β-globin gene have been associated with β-thalassemia (Taher et al. 2021), and the type of underlying β-globin mutation is one of the determinants of the severity of disease.

In β-thalassemia, insufficient β-globin production results in an accumulation of excess uncomplexed α-globins that precipitate in maturing erythroid cells, leading to premature cell death, ineffective erythropoiesis, and hemolysis (Galanello and Origa 2010). The marrow is not able to maintain Hb production leading to anemia of variable severity based on the type of mutation in the β-globin gene. As a result, oxygen delivery to tissues is compromised leading to organ dysfunction and clinical manifestation of symptoms.

Anemia can range from mild to severe, with severe forms requiring the initiation of regular RBC transfusions at an early age. In clinical practice, patients with β-thalassemia are managed and classified by their transfusion needs and not by their genotypes. Patients with TDT (β-thalassemia major or severe HbE-β–thalassemia) require lifelong, regular transfusions for survival, whereas patients with non-TDT (β-thalassemia intermedia or mild-to-moderate HbE-β-thalassemia) require no transfusions or occasional transfusions because of specific circumstances (Taher et al. 2021). Individuals with non-TDT may progress over time to becoming TDT patients (Cappellini et al. 2021).

Patients with the most severe form of the disease almost always exhibit symptoms between the ages of 6 months and 2 years. They experience symptoms of severe anemia and ineffective erythropoiesis that can cause marrow expansion with bone deformities that distort facial features, splenomegaly, and iron overload culminating in organ damage (Cappellini et al. 2021). Although regular RBC transfusions correct severe anemia and prolong survival, Cooley's Anemia Foundation (CAF) reported that the median age of death among known reported deaths of 50 patients with TDT was 37 years in the US from 2011 – 2021 (Chieco and Butler 2022). The CAF database includes 792 patients with TDT as of 2021.

TDT is associated with many disease and treatment-related complications. Complications from iron overload predominate in TDT patients, primarily affecting the heart, liver, and endocrine organs. Consequently, hypopituitarism, hypothyroidism, hypoparathyroidism, heart failure and arrhythmias, hepatic failure, development of carcinoma, diabetes, hypogonadism, and osteoporosis are all comorbidities which can develop secondary to iron overload. Cardiovascular complications resulting from iron overload remain the leading cause of death, although the rate has declined over time with improved disease management (Ladis et al. 2011; Sayani and Kwiatkowski 2015; Betts et al. 2020; Cappellini et al. 2021; Vitrano et al. 2021). Other causes of death include hepatic failure and liver disease, infections, and vascular events; among cancers, hepatocellular carcinoma had the highest mortality in these patients (Ladis et al. 2011; Betts et al. 2020; Vitrano et al. 2021).

The epidemiology of β-thalassemia has changed dramatically over the years. The thalassemia belt extends from the Mediterranean and Northern Africa through the Middle East and Asia to the Western Pacific. It is estimated that about 1.5 percent of the world's population carries a single β-thalassemia mutation, and that accounts for about 80 – 90 million individuals. Approximately 40,000 infants are born each year with both β-globin genes mutated (Taher et al. 2021). Latest estimates in the literature, which partially captures the total population, refer to a prevalence of 2600 patients with α-thalassemia and β-thalassemia in the US (Lal et al. 2021). Assumptions of the percent of thalassemia patients who are transfusion dependent are cited at 35% (Lal et al. 2018) and of the percent of β-thalassemia patients globally who are transfusion dependent (TDT) at 50% (Taher et al. 2021). Based on these estimates, the number of patients in

the US with TDT is approximately 1000 – 1300. This prevalence range is further supported by estimates from CAF (Chieco and Butler 2022).

1.3. Current Treatment Options and Unmet Medical Need

The current standard of care for patients with TDT in the US is regular RBC transfusions with iron chelation therapy. Patients ≥ 18 years of age receiving transfusions and chelation may also receive luspatercept-aamt which is a chronic option to treat anemia in those requiring regular transfusions. Allo-HSCT is a curative option mainly available to a small subset of patients, predominantly children with a MSD. Each of these therapies are associated with risks for serious comorbidities (Cappellini et al. 2021).

Transfusion and Iron Chelation

Regular RBC transfusions with iron chelation are the standard-of-care for patients with TDT, typically consisting of transfusions every 2 – 5 weeks to mitigate symptoms of anemia and suppress ineffective erythropoiesis. Although regular transfusions are life-saving treatment for patients with TDT, they impose significant burdens and risks to patients. Globally, risks associated with regular RBC transfusions include alloimmunization (5 – 10%), reactions to mismatched blood components, adverse transfusion reactions (1/100 – 1/50,000), and infections transmitted through the blood product. Most significantly, transfusions lead to iron overload and toxicity, which must be managed with iron chelation therapy to prevent early death from cardiac, liver, or endocrine failure. However, iron chelation treatment is associated with side effects and requires continuous monitoring, adding to the significant treatment burden (Cappellini et al. 2021).

The requirement of lifelong regular RBC transfusions places a demanding burden on the everyday lives of patients and caregivers. Even with regular RBC transfusions, patients may experience pain and fatigue at Hb nadirs (Paramore et al. 2021; Cappellini et al. 2021). Results of an international study of adults with TDT and parents of adolescents with TDT found that the mean daily time spent on disease management and coordination was 592 min (SD 349 min) or 9.8 hours on actual transfusion days. On non-transfusion days, the mean daily time spent on TDT management was 91 min (SD 221 min) or approximately 11 hours each week without a transfusion to manage the various practical aspects of TDT treatment, such as filling out insurance forms, blood typing, and arranging for childcare and transportation around medical visits (Paramore et al. 2021). Depending on the type of chelation therapy required, these ongoing time and resource commitments could be even greater for many patients and families.

Rigorous continuous monitoring of the iron burden and ongoing adherence to iron chelation treatment are critical for patients with TDT (Cappellini et al. 2021). An iron chelation regimen is burdensome to patients and caregivers, may be associated with adverse reactions, and is not tolerated by all patients, resulting in suboptimal compliance with rates of reported nonadherence ranging from 3 – 30% (Goulas et al. 2012; Kwiatkowski et al. 2012; Trachtenberg et al. 2012, 2014; Casale et al. 2014). Chelation therapy itself requires careful clinical and regular investigational monitoring for side effects, including ophthalmologic and audiologic toxicities, in addition to the annual iron overload assessments via cardiac and liver magnetic resonance imaging (MRI) (Tubman et al. 2015).

Despite chelation therapy, some patients with TDT remain significantly iron overloaded (Dessi et al. 2015; Aydinok et al. 2015; Vitrano et al. 2016; de Montalembert et al. 2017; Shah et al. 2022). Patients who have been inadequately transfused and/or chelated may develop serious cardiac, hepatic, endocrine, and bone complications (Angelucci et al. 2002; Rund and Rachmilewitz 2005; Tubman et al. 2015; Farmakis et al. 2020; Cappellini et al. 2021).

The advent of oral chelators has improved the standard-of-care, resulting in increased survival duration in recent decades (Taher et al. 2021). Despite these and other treatment improvements such as pathogen-free blood transfusions, noninvasive iron monitoring techniques, reduced incidence of alloimmunization, improved treatment of hepatitis, and improved outcomes for allo-HSCT, patients with TDT remain at increased risk of early death and live a life with chronic reliance upon the healthcare system (Taher et al. 2021; Cappellini et al. 2021; Chapin et al. 2022).

Luspatercept-aamt

Luspatercept-aamt (REBLOZYL®) was approved by the US Food and Drug Administration (FDA) in November 2019 for the treatment of anemia in adult patients with β-thalassemia who require regular RBC transfusions. It is an erythroid maturation agent that treats anemia in those requiring regular transfusions. However, it is another chronic therapy (administered once every 3 weeks by a healthcare professional) and does not address the underlying genetic cause of disease. In the primary endpoint analysis of a Phase 3 study, 21.4% (vs. 4.5% in placebo group) patients who received luspatercept-aamt achieved at least 33% reduction in transfusion burden from baseline during weeks 13 through 24 (Cappellini et al. 2020; Reblozyl 2021). The ongoing burden of β-thalassemia management with luspatercept-aamt is high, as hospital administration of the product is required, and transfusions and iron chelation are still required for most patients (Cappellini et al. 2020; Reblozyl 2021). Warnings and precautions for use of luspatercept-aamt include thrombosis/thromboembolism, hypertension, and embryo-fetal toxicity. Data on the long-term outcomes of chronic luspatercept-aamt therapy are limited.

Allogeneic-Hematopoietic Stem Cell Transplantation

For a limited proportion of patients with TDT, allo-HSCT can be considered as a potentially curative treatment option, enabling thalassemia-free survival after engraftment of donor HSCs (Caocci et al. 2017). However, treatment with allo-HSCT is usually limited to children with a matched donor, typically a sibling, due to suboptimal clinical outcomes for adolescents and adults, including patients without a related HLA-matched donor (Baronciani et al. 2016; Li et al. 2019). On average, only 25 – 30% of TDT patients have a MSD (Angelucci et al. 2014)). When successful, allo-HSCT can establish long-term normal hematopoiesis and alleviate the need for transfusions and chelation, by replacing the patient's HSCs that carry the β-thalassemia genetic mutation with those from a healthy donor.

Allo-HSCT carries known and serious risks, including morbidity/mortality due to GVHD or graft failure (Galambrun et al. 2013; Angelucci et al. 2014; Baronciani et al. 2016; Chaudhury et al. 2017; Caocci et al. 2017; Rattananon et al. 2021). With allo-HSCT, GVHD has been a common cause of graft failure, with rates of acute GVHD ranging from 12 – 35% and chronic GVHD ranging from 8 – 24% (Li et al. 2019). The risk of GVHD can be reduced in patients who receive a matched donor transplant compared with an alternative donor transplant (Baronciani et al.

2016; Li et al. 2019). Yet even with optimal HLA-matching, graft failure may occur in a reported 5 – 9% of allo-HSCT patients followed for up to 5 years after treatment (Li et al. 2019). In a recent study assessing 1,110 TDT patients who received allo-HSCT, the 5-year probabilities of overall survival in patients receiving matched donor was in the range of 89-87% and 73-83% in those receiving unmatched donor (Li et al. 2019).

Allo-HSCT procedures, including those with matched donors, still typically require lymphocyte-depleting chemotherapy and immunosuppressive agents to limit immune reactions between the donor and host cells, and can be associated with early and late complications, including graft failure, gastrointestinal (GI) complications, alopecia, acute GVHD, early infections, chronic GVHD, late infections, relapse of the disease, infertility, endocrine complications, neoplasms, and other secondary complications (Carreras et al. 2019). Haploidentical HSCT from HLA-disparate relatives is considered as an experimental treatment (Cappellini et al. 2021). Despite the serious risks associated with allo-HSCT, patients and family still consider allo-HSCT as a treatment option due to the life limiting nature of TDT.

Unmet Medical Need

TDT is a severe and life-limiting disease. Given the limitations of current therapeutic options for TDT patients, there is a significant unmet need for a curative treatment that has a favorable benefit/risk profile and is not constrained by patient age, genotype, or donor availability. Such treatment should address the underlying genetic cause of β-thalassemia, with the goals of eliminating transfusion dependency and associated complications such as iron overload, restoring normal or near-normal Hb levels, and improving not just the survival but the quality-of-life of TDT patients.

1.4. Clinical Development Program

The beti-cel clinical development program consists of 2 completed Phase 1/2 studies (HGB-205 and HGB-204), 2 ongoing Phase 3 studies (HGB-207 and HGB-212) at the time of the data-cut, and 1 ongoing long-term follow-up study (LTF-303) for patients who completed Phase 1/2 and Phase 3 studies.

All Phase 1/2 and Phase 3 studies are open-label, single-arm studies. Two years' pre-enrollment retrospective RBC transfusion and total Hb data were collected for each patient in the studies so that each patient could serve as their own control. Patients with TDT do not spontaneously become transfusion independent without intervention, and it would be unethical to expose a patient to myeloablative conditioning if they were going to receive a placebo comparator and not beti-cel; therefore, comparing parameters before and after treatment is the most appropriate study design in this population.

Consistent with Thalassemia International Federation (TIF) Guidelines (Cappellini et al. 2014), all patients with TDT in the beti-cel clinical studies had a requirement for ≥ 100 mL/kg/year of RBCs in the 2 years preceding enrollment; and/or were managed under standard thalassemia guidelines, with ≥ 8 transfusions of RBCs per year in the 2 years preceding enrollment for patients ≥ 12 years of age. Patients < 12 years of age needed to have a history of transfusion of at least 100 mL/kg/year of RBCs in the 2 years preceding enrollment.

Patients in clinical studies using beti-cel underwent HSC mobilization using granulocyte-colony stimulating factor (G-CSF) and plerixafor, followed by apheresis to obtain a CD34+ cell-enriched population that contains HSCs for transduction. Mobilization using G-CSF and plerixafor is well established in the scientific literature (Yannaki et al. 2012, 2013; Karponi et al. 2015; Maschan et al. 2015; Teusink et al. 2016). Myeloablation with busulfan was conducted before DP infusion to deplete endogenous HSCs, enabling therapeutic repopulation of the patient's bone marrow with HSCs containing the transgene. Myeloablation using alkylating chemotherapeutic agents like busulfan is used in allo-HSCT and is an acceptable therapeutic option for patients with TDT undergoing allo-HSCT (Parkman et al. 1978; Kapoor et al. 1981; Hobbs et al. 1986).

The completed Phase 1/2 studies are:

1. **HGB-205** (N = 4 patients with TDT treated with beti-cel; non-β^0/β^0 genotypes; ages ≥ 5 to ≤ 35 years), which followed each patient for 2 years. This study also included 3 patients with SCD
2. **HGB-204** (N =18 patients treated with beti-cel; all genotypes; ages ≥ 12 to ≤ 35 years), which followed each patient for 2 years

The ongoing Phase 3 studies are:

3. **HGB-207** (N = 23 patients treated with beti-cel; non-β^0/β^0 genotypes; Cohort 1: N = 15, ages ≥ 12 to ≤ 50 years; Cohort 2: N = 8, ages < 12 years), which follows each patient for 2 years and was ongoing at the time of the datacut.
4. **HGB-212** (N = 18 patients treated with beti-cel; β^0/β^0, β^0/IVS-I-110, or IVS-I-110/IVS-I-110 genotypes; ages ≤ 50 years, including 13 patients < 18 years and 12 patients with a β^0/β^0 genotype), which follows each patient for 2 years and is ongoing.

Patients treated with beti-cel who have completed or withdrawn from any of the above parent studies are asked to enroll in ongoing long-term follow-up Study **LTF-303** for a total of 15 years of follow-up post-DP infusion.

The Phase 1/2 Study HGB-204 was analyzed by genotype, and better efficacy outcomes were observed in the non-β^0/β^0 patients. The Phase 3 Studies HGB-207 (non-β^0/β^0) and HGB-212 (β^0/β^0, β^0/IVS-I-110, or IVS-I-110/IVS-I-110) were therefore designed to evaluate beti-cel by genotype. β^0/IVS-I-110 and IVS-I-110/IVS-I-110 genotypes were included in Study HGB-212 because they are considered to be a more severe genotype, with little to no endogenous HbA production. Sequentially, the Phase 3 Study HGB-207 in non-β^0/β^0 patients initiated first and was designed with 2 age-based cohorts in order to evaluate older patients before proceeding to younger patients. Phase 3 Study HGB-212 was initiated in a staged manner in patients of all ages approximately 1 year after Study HGB-207.

The DP manufacturing process was optimized between the Phase 1/2 and Phase 3 studies, which increased the number of transduced HSCs for infusion in the Phase 3 studies, resulting in improved and consistent efficacy across genotypes in the Phase 3 studies.

1.5. Efficacy

Across all studies, 66 patients have initiated a study procedure, beginning with mobilization [intent-to-treat (ITT) population]. There have been 3 discontinuations, all before myeloablative conditioning, due to investigator decision secondary to inadequate mobilization, pregnancy, or patient withdrawal. A total of 63 patients have been treated with beti-cel (transplant population); 58 were evaluable for Transfusion Independence (TI-evaluable - see Section 5.3.1 for more details) at the time of the BLA submission, of whom 36 were TI-evaluable from the Phase 3 studies. All study patients had a significant burden of disease, as indicated by a median baseline transfusion volume of 190 mL/kg/year and a median transfusion frequency of 15 transfusions per year. Overall:

- 32 of 36 patients (88.9%) who were evaluable for TI (definition in Section 5.3.1) achieved TI in the Phase 3 studies. The median (min - max) weighted average total Hb during TI for Phase 3 patients who achieved TI was 11.52 (9.3 – 13.7) g/dL. Patients who achieved TI all maintained TI through the most recent follow-up, including up to 4 years duration of follow-up for the Phase 3 patients, and 7 years for the Phase 1/2 patients (Figure 5, Figure 7).
- Consistent and direct relationships between DP characteristics (vector copy number [VCN] and % transduced cells), pharmacodynamic (PD) parameters (peripheral blood [PB] VCN and HbA^{T87Q}), and clinical outcomes (total Hb and TI) were observed.
- The best association with optimal clinical outcomes was the percentage of transduced cells in the DP. In a post-hoc analysis, all Phase 3 patients treated with beti-cel who met the proposed commercial threshold for % transduced cells achieved TI (N = 26).
- Ongoing production of HbA^{T87Q} demonstrates the stable integration of the $\beta^{A\text{-}T87Q}$-globin transgene into the patient's long-lived HSCs, enabling transgene expression in their erythroid progeny.
- Many patients who achieved TI following treatment with beti-cel treatment had decreased iron burden over time and were eventually able to stop using iron chelation therapy. Following DP infusion and a protocol-defined delay period, use of iron removal therapy was at the investigator's discretion.
- The percentage of TI patients who had liver iron concentration (LIC) below 7 mg/g increased over time.
- Results of exploratory analyses suggest that achieving TI after treatment with beti-cel leads to improvements in erythropoiesis.

For a more detailed discussion of efficacy results, please see Section 5.

1.6. Safety

Of the 66 patients in the ITT population, the safety cohort includes 63 patients who received DP (Transplant Population) in the clinical development program. The median follow-up is approximately 3 years, and at present, survival remains 100%. Overall:

- The peri-transplant adverse events were generally dominated by myeloablation with busulfan, including hepatic veno-occlusive disease (sinusoidal obstructive syndrome) and severe cytopenias. These events will require careful patient monitoring. The deliberate roll out of beti-cel to Centers of Excellence (the Qualified Treatment Centers) will help mitigate these risks as the prescribers will be well versed in identifying and managing these risks and reporting adverse events.
- Delay in platelet engraftment, as compared to standard engraftment times for allo-HSCT, was observed. It is unclear if this is specific to beti-cel or consistent with what would be expected for autologous transplantation in β-thalassemia in general. bluebird bio proposes communicating this risk to the prescribers and facilitating appropriate education.
- Drug product related reactions were mostly nonserious and included cytopenias, and infusion related reactions that were mild and transient.
- To date no cases of insertional oncogenesis or other hematological malignancies have been reported following treatment with beti-cel.

Insertional oncogenesis has long been recognized as a potential safety concern for ex vivo gene addition-modified HSC products using retroviral vectors, including LVV-transduced HSCs. Accordingly, patients in the beti-cel development program have been routinely monitored with hematologic assessments and measures of clonal dynamics. Integration site analysis (ISA) generally showed robust polyclonal reconstitution of the hematopoietic cell system.

bluebird bio is committed to robust long-term follow-up of patients in Study LTF-303 and the proposed post-marketing registry (REG-501) to capture potential cases of malignancy during long-term follow-up. The registry is designed to satisfy recommendations in the FDA Guidance for Industry, "Long Term Follow-Up After Administration of Human Gene Therapy Products" (FDA 2020), regarding capture of key safety events, including malignancy, during long term follow-up of gene therapy patients.

For a more detailed discussion of safety results, please see Section 6.

1.7. Benefit-Risk Assessment

With a total of 221 patient-years of follow-up and up to 7 years of follow-up for individual patients, beti-cel treatment has demonstrated efficacy for children, adolescents, and adults with all genotypes of TDT, and an acceptable and manageable safety profile. The benefit:risk assessment is positive for the proposed indication.

TDT is a severe and burdensome disease. Despite major advances, with the current standard of care, consisting of regular pRBC transfusion and iron chelation therapy, most adult patients are still expected to have a shortened life expectancy (Chieco and Butler 2022). Allo-HSCT from a matched related donor is generally not recommended for patients > 15 years (Baronciani et al. 2016); therefore, most adolescents and adults with TDT have limited curative options. In these patients, while the current standard of care is designed to maintain hemoglobin levels adequate for a reasonable level of activity and quality of life, the regular transfusions exacerbate iron overload with attendant long-term complications, including end organ damage mainly in the

heart and endocrine organs. beti-cel offers a curative treatment that is highly effective with approximately 90% of patients achieving transfusion independence, regardless of age or genotype. The near complete correction of ineffective erythropoiesis ameliorates the anemia and reduces excessive iron absorption resulting in cessation of transfusions and normalization of iron levels over time.

The safety profile of the beti-cel regimen largely reflects the known effects of autologous HSCT using the mobilization agents (G-CSF and plerixafor), and myeloablative conditioning (busulfan) necessary for the transplant. Overall, autologous HSCT has fewer and less severe toxicities than allogeneic HSCT, most notably GVHD. Veno-occlusive disease (VOD) of the liver (sinusoidal obstructive syndrome) occurred in limited numbers of patients undergoing treatment with beti-cel and responded to treatment without sequelae. Some beti-cel treated patients experienced delayed platelet engraftment with limited bleeding complications. The long-term risk of insertional oncogenesis following treatment with beti-cel is unknown and will be monitored over time, but to date there have been no cases of insertional oncogenesis nor any other hematologic malignancy in 63 treated patients across studies in the beti-cel clinical development program, with a total of 221 patient-years of follow-up and up to 7 years of follow-up for individual patients. Given the increased risks associated with allo-HSCT in adolescents and adults with TDT, there is an unmet need for safe and effective curative therapies. The positive benefit:risk profile for adolescents and adults with TDT using the autologous approach of beti-cel is demonstrated by high rates of transfusion independence and acceptable rates of adverse events in adults and adolescents with TDT.

The benefit-risk profile of beti-cel treatment for children with TDT allows for expansion of curative options beyond HLA-matched sibling donor (MSD) allo-HSCT, which has a long and positive track record. However, a majority of children with TDT will lack a suitably matched related donor and outcomes using alternate donor stem cell sources have been inferior, with higher rates of graft failure, graft versus host disease, graft rejection, and transplant related mortality; therefore these transplants are not routinely performed. These children without a MSD would be good candidates for beti-cel therapy, which has comparable efficacy to allo-HSCT from a MSD, but without the risks of GVHD or transplant-related mortality (TRM). Earlier intervention in children prior to excess transfusion-related iron deposition and organ dysfunction may be an additional benefit and results in the Phase 3 beti-cel studies have demonstrated high rates of transfusion independence in children similar to adults. As with adults and adolescents, the beti-cel safety profile is acceptable and manageable in children, and patients who become transfusion independent will stop loading iron as well.

Many families with younger children with TDT might prefer to wait for a transplant-based therapy for a variety of reasons, an important one being the option for fertility preservation once the child has achieved puberty. With the regular use of oral chelation, most young children with TDT who are compliant with chelation, currently do not have significant iron overload, and waiting to proceed with a transplant based curative treatment should not increase the risk for adverse events. For those without a matched-sibling donor, the long-term risks of delaying curative therapy must be balanced with the potential long-term risks of beti-cel therapy. All of these factors must be weighed in considering the optimal course of treatment for individual children with TDT.

In conclusion, given the high burden of disease and complications in TDT, and the limited availability of curative options, bluebird bio believes that the efficacy and safety demonstrated to date support the use of beti-cel as a curative treatment option for adults and adolescents, and for children. Treatment decisions should be made on an individual basis. The positive observed treatment benefits of beti-cel are considered to outweigh any identified and potential risks for patients with β-thalassemia who require regular RBC transfusions.

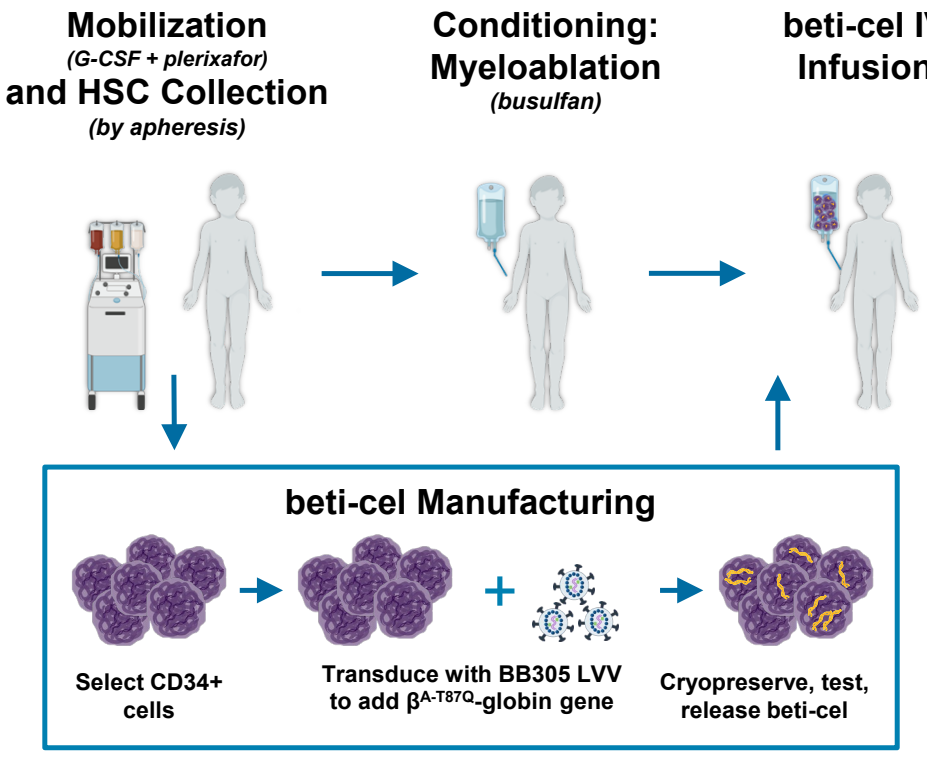
2. DRUG PRODUCT

The drug product, beti-cel, consists of an autologous CD34+ cell-enriched population that contains the patient's own HSCs transduced ex-vivo with BB305 LVV encoding the $\beta^{A\text{-}T87Q}$-globin gene. The beti-cel drug product is supplied frozen as a suspension in cryopreservation solution for intravenous infusion in 20 mL fluoro-ethylene-propylene bags. Each bag contains 1.2 – 20 × 10^6 CD34+ cells/mL, frozen in approximately 20 mL of solution. The minimum dose is 5.0 × 10^6 CD34+ cells/kg patient weight.

Each patient undergoes HSC mobilization with G-CSF and plerixafor in combination, followed by apheresis to harvest the cells. The collected cells are shipped to the manufacturing site where CD34+ cells are selected and then transduced with BB305 LVV to manufacture beti-cel. After myeloablative conditioning and beti-cel infusion, transduced HSCs engraft in the bone marrow and differentiate to reconstitute the hematopoietic system, including RBCs that contain HbA^{T87Q} to treat the patient's β-thalassemia (Figure 1).

More than one drug product lot may be required to achieve the minimum beti-cel dose. As such, a patient may undergo additional mobilization cycles to provide hematopoietic progenitor cells obtained by apheresis (HPC-A) for use in the manufacture of an additional drug product lot to provide a total CD34+ cell count that meets or exceeds the target dose. Shipment of drug product lot(s) to the Qualified Treatment Center for infusion occurs once the dose requirement has been met and all product lot(s) have been released.

Figure 1. Overview of beti-cel Treatment



G-CSF, granulocyte-colony stimulating factor; HSC, hematopoietic stem cell; IV, intravenous; LVV, lentiviral vector

2.1. BB305 Lentiviral Vector

BB305 LVV is a self-inactivating, third-generation, replication incompetent, human immunodeficiency virus (HIV) type 1-based LVV. To efficiently transduce patient mobilized CD34+ cells, the HIV envelope protein is replaced by the vesicular stomatitis virus glycoprotein G (VSV-G) envelope.

The structure of an LVV particle comprises an external lipid envelope and an internal protein core that includes 2 copies of the viral RNA genome complexed with nucleocapsid proteins and three viral enzymes: reverse transcriptase, integrase, and protease. All viral genes are absent from the BB305 LVV genome, rendering the LVV incapable of replication. The BB305 LVV genome consists of a positive-strand RNA that carries key viral elements necessary for LVV function, as well as sequences encoding the $\beta^{A\text{-}T87Q}$-globin gene. The only protein-coding element in the vector genome is the $\beta^{A\text{-}T87Q}$-globin transgene.

The expression of the transgene is controlled by the erythroid specific human β-globin promoter.

The function of BB305 LVV is to integrate the therapeutic $\beta^{A\text{-}T87Q}$-globin transgene into the genome of the patient's own HSCs. Harvested CD34+ cells are transduced ex vivo with BB305 LVV, and during the transduction process, viral enzymes present in the LVV core reverse-transcribe the vector RNA into double-stranded DNA and facilitate the integration of the proviral DNA into the CD34+ cell genome. This integration step is critical because it allows the therapeutic transgene to be inherited by all daughter cells that derive from the transduced HSCs. The LVV itself is not directly administered to patients.

2.2. Quality and Control of Drug Product

Manufacture and release testing of beti-cel drug product is well-controlled and validated. Throughout beti-cel development, FDA advice has been sought and followed to establish appropriate analytical methods for measuring, monitoring, and characterizing product quality.

A multivariate analysis of associations between drug product attributes and clinical parameters was performed to determine which of the former are predictive of clinical efficacy, based on relationships between the clinical PD parameter, PB VCN, circulating HbA^{T87Q} levels, and the potential achievement of transfusion independence, that are discussed in Section 5.5. The drug product attribute most predictive of PB VCN levels linked with positive clinical outcomes was the percentage of cells containing integrated LVV sequences (% transduced cells).

A second multivariate analysis was performed to further understand which variables were predictive of meeting % transduced cells attribute targets for drug product that were chosen based on the first multivariate analysis. Patient medical history data at the time of beti-cel infusion and manufacturing parameters were included as input features. The second multivariate analysis showed that combinations of transduction process parameters exercised during manufacturing are predictive of achieving the targeted % transduced cells. Results from the multivariate analyses combined with knowledge gained in Phase 3 clinical manufacturing were used to set the normal operating and proven acceptable ranges for commercial manufacturing transduction step critical process parameters. Additionally, proposed commercial product specification acceptance criteria for the percentage of transduced cells consider clinical patient

experience and are reflective of the level in drug product above which positive clinical outcomes (i.e., transfusion independence) were consistently observed.

For a more detailed description of product characteristics and manufacturing processes and control, please see Section 9, Appendix A.

3. NONCLINICAL FINDINGS

The pharmacology, toxicology, and genotoxicity of the BB305 LVV used for manufacturing beti-cel were evaluated in vitro and in vivo. In the in vitro studies, BB305 LVV transduction of mobilized peripheral blood CD34+ HSCs resulted in expression of high levels of $\beta^{A\text{-}T87Q}$-globin in erythroid cells.

An in vitro immortalization assay demonstrated that the BB305 LVV exhibited a strongly reduced risk of immortalization of murine bone marrow cells (BMCs), as compared with a positive control gammaretroviral vector and a positive control LVV containing a strong viral promoter. Thus, the BB305 LVV was determined to have minimal genotoxic potential.

The most relevant nonclinical study conducted in support of beti-cel development utilized a specific animal model of β-thalassemia in a pivotal, combined therapeutic proof-of-concept, pharmacology, biodistribution, single-dose toxicity, and genotoxicity study. The β-thalassemic (C57BL/6 $Hbb^{th1/th1}$) mice used in this study have a homozygous deletion of the mouse β-major-globin gene (*Hbb1*), the mouse equivalent to the human adult β-globin gene, and manifest clinical and biological features like those observed in humans with TDT (Skow et al. 1983; Rouyer-Fessard et al. 1990).

In this study, β-thalassemic mice received primary transplantation of β-thalassemic mouse BMCs that were either mock-transduced (no LVV) or transduced with HPV569 LVV (another β-globin transgene containing vector) or BB305 LVV. In this mouse model of homozygous β-thalassemia (similar to β^0/β^0 patients), transplantation of LVV-transduced β-thalassemic mouse BMCs resulted in durable bone marrow engraftment, production of $\beta^{A\text{-}T87Q}$-globin in red blood cells and correction of the disease phenotype.

Additional pharmacology, biodistribution and general safety studies with BB305 LVV-transduced human CD34+ HSCs administered to myeloablated, immunodeficient mice demonstrated long-term engraftment of transduced cells and durable production of $\beta^{A\text{-}T87Q}$-globin with no toxicity or tumorigenicity associated with BB305 LVV.

Collectively, there was no evidence of toxicity, genotoxicity, or oncogenesis associated with BB305 LVV integration and no toxicity related to production of $\beta^{A\text{-}T87Q}$-globin transgenic protein. ISA of post-transplantation BMCs demonstrated no preferred integration in the proximity of or within genes associated clinically (for gammaretroviral vectors) with either clonal expansion or leukemia. No evidence of clonal expansion was observed, integrations were present across the genome, and there was no enrichment for integrations near cancer-related genes.

All other in vitro and in vivo nonclinical studies conducted to support beti-cel corroborated these pivotal findings. These findings support the hypothesized mechanism of action by which transplantation of beti-cel to patients with TDT may be an effective gene therapy.

4. CLINICAL DEVELOPMENT PROGRAM

In the beti-cel clinical development program, consideration was given to the rarity of TDT, the burden and complications associated with standard of care (transfusions and chelation), availability of a curative treatment option (i.e., allo-HSCT) for patients, and the risks associated with current treatment options.

Formal comparisons to allo-HSCT were not conducted because patients in the beti-cel clinical studies serve as their own control through comparison of parameters before treatment versus after treatment and allo-HSCT is not standard of care for all TDT patients (see also Section 4.2).

4.1. Clinical Studies

The clinical development program for beti-cel includes 2 Phase 1/2 studies, HGB-205 and HGB-204, and 2 Phase 3 studies HGB-207, and HGB-212 (Table 1). HGB-205 was initiated before HGB-204 for operational reasons. The duration of each study was 2 years and patients who completed these studies were asked to enroll in a long-term follow-up study, LTF-303.

Study HGB-204 was analyzed by genotype and better efficacy outcomes were observed in patients with non-β^0/β^0 genotypes versus β^0/β^0 genotypes. Thus, DP manufacturing was optimized, and 2 Phase 3 studies were initiated to re-evaluate outcomes by genotype.

Study HGB-212 schedule of events and endpoints are included in Section 10, Appendix B. Details on Study HGB-212 are representative of the other Phase 3 study, HGB-207.

Table 1. Clinical Studies Evaluating beti-cel in Patients With TDT

Study Identifier (Status) NCT #	Study Title	Number of Patients With TDT[a] and Genotype	Recommended Cell Dose	Recommended Busulfan Average Daily AUC	Primary Efficacy Endpoint(s) from Study Protocol	Data Cut-Off Date for Ongoing Studies
HGB-205 (completed 26 Feb 2019) NCT02151526	A Phase 1/2 Open Label Study Evaluating the Safety and Efficacy of Gene Therapy of the β-Hemoglobinopathies (Sickle Cell Anemia and β-Thalassemia Major) by Transplantation of Autologous $CD34^+$ Stem Cells Transduced Ex Vivo with a Lentiviral $\beta^{A\text{-}T87Q}$-globin Globin Vector (LentiGlobin BB305 Drug Product)	7 planned; aged ≥ 5 to ≤ 35 years (TDT or SCD)[b] 4 TDT treated; 4 TDT completed (all non-β^0/β^0; aged 16 to 19 years)	$\geq 3.0 \times 10^6$ CD34+ cells/kg	4000 to 5200 μM*min	RBC transfusion requirements (measured in milliliters [mL] per kilogram [kg]) per month and per year post-transplant. Number of total in-patient hospitalization days (post-transplant discharge) at 6, 12, and 24 months. Note: CSR included the same TI endpoint as for HGB-207	NA; study complete
HGB-204 (completed 21 Feb 2018) NCT01745120	A Phase 1/2 Open Label Study Evaluating the Safety and Efficacy of Gene Therapy in Subjects with β-thalassemia Major by Transplantation of Autologous $CD34^+$ Stem Cells Transduced Ex Vivo with a Lentiviral $\beta^{A\text{-}T87Q}$-globin Vector (LentiGlobin BB305 Drug Product)	18 planned; TDT of all genotypes; aged ≥ 12 to ≤ 35 years 18 treated; 18 completed (10 non-β^0/β^0; 8 β^0/β^0; (3 aged ≥ 12 to < 18 years, 15 aged ≥ 18 to ≤ 35 years) 1 discontinued before conditioning	$\geq 3.0 \times 10^6$ CD34+ cells/kg	3600 to 5000 μM*min	The sustained production of ≥ 2.0 g/dL of hemoglobin A (HbA) containing $\beta^{A\text{-}T87Q}$-globin for the 6 months between month 18 and month 24 post-transplant. Note: CSR included the same TI endpoint as for HGB-207	NA; study complete

Study Identifier (Status) NCT #	Study Title	Number of Patients With TDT[a] and Genotype	Recommended Cell Dose	Recommended Busulfan Average Daily AUC	Primary Efficacy Endpoint(s) from Study Protocol	Data Cut-Off Date for Ongoing Studies
HGB-207[c] (ongoing) NCT02906202	A Phase 3, Single Arm Study Evaluating the Efficacy and Safety of Gene Therapy in Subjects with Transfusion-dependent β-Thalassemia, who do not have the β^0/β^0 Genotype, by Transplantation of Autologous $CD34^+$ Stem Cells Transduced Ex Vivo with a Lentiviral $\beta^{A\text{-}T87Q}$-Globin Vector in Subjects ≤ 50 Years of Age	23 planned; non-β^0/β^0 genotype; 15 aged ≥ 12 and ≤ 50 years (Cohort 1); 8 aged < 12 years (Cohort 2) 23 treated (all non-β^0/β^0); 15 Cohort 1, 8 Cohort 2 20 completed; 3 ongoing 1 discontinued before conditioning due to pregnancy	≥ 5.0×10^6 CD34+ cells/kg	3800 to 4500 μM*min[d]	Proportion of patients who achieve TI, defined as a weighted average Hb ≥ 9 g/dL without any RBC transfusions for a continuous period of ≥ 12 months at any time during the study after DP infusion.	09 March 2021
HGB-212 (ongoing) NCT03207009	A Phase 3 Single Arm Study Evaluating the Efficacy and Safety of Gene Therapy in Subjects with Transfusion-dependent β-Thalassemia by Transplantation of Autologous $CD34^+$ Stem Cells Transduced Ex Vivo with a Lentiviral $\beta^{A\text{-}T87Q}$-Globin Vector in Subjects ≤ 50 Years of Age	18 planned; β^0/β^0, β^0/IVS-I-110, or IVS-I-110/IVS-I-110 genotype; at least 10 < 18 years of age; 12 without an IVS-I-110 mutation 18 treated (5 aged ≥ 18 years, 5 aged ≥ 12 and < 18 years, and 8 aged < 12 years; 12 β^0/β^0, 6 non-β^0/β^0) 9 completed, 9 ongoing 1 withdrew consent before conditioning	≥ 5.0×10^6 CD34+ cells/kg	3800 to 4500 μM*min[d]	Proportion of patients who achieve TI, defined as a weighted average Hb ≥ 9 g/dL without any RBC transfusions for a continuous period of ≥ 12 months at any time during the study after DP infusion.	09 March 2021

Study Identifier (Status) NCT #	Study Title	Number of Patients With TDT[a] and Genotype	Recommended Cell Dose	Recommended Busulfan Average Daily AUC	Primary Efficacy Endpoint(s) from Study Protocol	Data Cut-Off Date for Ongoing Studies
LTF-303 (ongoing) NCT02633943	A Long-term Follow-up of Subjects with Hemoglobinopathies Treated with Ex Vivo Gene Therapy Using Autologous Hematopoietic Stem Cells Transduced with Lentiviral Vector	Planned to include all consenting/assenting patients who were treated with DP for therapy of a hemoglobinopathy in a bluebird bio-sponsored clinical study[e] 51 patients with TDT treated with beti-cel (18 from HGB-204, 4 from HGB-205, 19 from HGB-207, and 10 from HGB-212) enrolled in LTF-303, and all continue to participate as of the data cut-off.	Not applicable	Not applicable	No primary efficacy endpoint; for patients with TDT, assessments include interval transfusions required (RBC mL/kg), hemoglobin levels, therapeutic phlebotomies, iron chelator use, and measures of iron overload. Note: CSR included the same TI endpoint as for HGB-207	09 March 2021

NCT, National Clinical Trial (number); AUC, area under the curve; DP, drug product; CSR, clinical study report; RBC, red blood cell; SCD, sickle cell disease; TDT, transfusion-dependent β-thalassemia; TI, transfusion independence.

a. As of the data cut-off date: 09 March 2021.

b. Three patients with SCD were treated with lovotibeglogene autotemcel (lovo-cel) in Study HGB-205, however these patients were not included in any efficacy analyses for beti-cel. These patients were included in some safety analyses for beti-cel to support evaluation of the overall safety profile of the BB305 LVV.

c. Study HGB-207 completed on 31 March 2022, after the data cut-off date.

d. Changed from 4000 to 5000 µM*min in Protocol HGB-207 Version 3.0 (19 June 2018) and in Protocol HGB-212 Version 2.0 (19 June 2018).

e. Data from patients with SCD are included in some beti-cel safety analyses to support evaluation of the safety profile of the BB305 LVV. Patients with SCD are not included in any beti-cel efficacy analysis.

4.2. Rationale for Single-Arm Study Design

All beti-cel clinical studies are open-label, single-arm studies with the patients serving as their own control through comparison of parameters before treatment versus after treatment. An inactive (placebo) control cannot be used because conditioning with busulfan is fatal without hematopoietic reconstitution and it would not be appropriate to put patients through mobilization and conditioning only to return back their own non-transduced cells. Allo-HSCT is also not an appropriate control for several reasons, including, variability in the conditioning regimens and the need for lymphodepletion during allo-HSCT, patient ineligibility for allo-HSCT due to age and/or availability of an HLA-matched donor, and the association of allo-HSCT with significant morbidity and mortality related to GVHD and other complications.

In summary, for most patients with TDT, with the potential exception of children with an MSD, allo-HSCT risks are too severe to provide a benefit compared to the standard of care which consists of transfusions and chelation. Standard supportive care with transfusion/chelation would also not be an appropriate control, because patients with TDT receiving supportive care do not spontaneously achieve TI (primary endpoint) or have significant reductions in their transfusion and chelation requirements.

4.3. Patients Enrolled in the Clinical Studies

Consistent with TIF guidelines at the time (Cappellini et al. 2014), patients ≥ 12 years of age enrolled in the beti-cel clinical program were transfusion-dependent as defined by either ≥ 100 mL/kg/year of RBCs in the 2 years preceding enrollment, or were managed under standard thalassemia guidelines, with ≥ 8 transfusions of RBCs per year in the 2 years preceding enrollment. Patients < 12 years of age had a transfusion history of at least 100 mL/kg/year of RBCs in the 2 years preceding enrollment.

All patients met study criteria and were clinically stable and eligible to undergo HSCT, but without a known and available HLA-matched family donor, or a matched unrelated donor if required by regional regulatory authorities.

Patients up to 50 years of age were eligible for inclusion in HGB-207 and HGB-212. Treatment with beti-cel was first investigated in adults, then expanded in a staggered manner to include patients 12 to < 18 years of age, and then patients < 12 years of age, depending upon safety results in each age category. Efficacy and safety in patients < 12 years of age have been evaluated in ongoing Phase 3 studies HGB-207 and HGB-212 and the LTF-303 study.

In HGB-204, 10 patients had non-β^0/β^0 genotypes and 8 patients had a β^0/β^0 genotype. HGB-207 enrolled only patients with non-β^0/β^0 genotypes and HGB-212 enrolled patients with β^0/β^0 or the severe β^0/IVS-I-110 or IVS-I-110/IVS-I-110 genotypes (at least 12 patients were without an IVS-I-110 mutation).

The distribution of transfusion requirements, ages, and genotypes of patients treated with beti-cel across the clinical program reflects the global population of patients with transfusion-dependent β-thalassemia.

4.4. Common Methodologies and Procedures Across Studies

4.4.1. Mobilization and Apheresis Procedure

Patients in the beti-cel clinical studies underwent HSC mobilization through G-CSF (e.g., filgrastim, lenograstim) and plerixafor, followed by apheresis to obtain a CD34+ cell population containing HSCs for transduction. The combination of G-CSF and plerixafor has been studied in adults with β-thalassemia and has been shown to be safe and highly effective at mobilizing HSCs, with more robust and replicable mobilization compared with either single agent, without additional toxicity (Yannaki et al. 2012, 2013). Published data indicate that plerixafor is also safe and effective for the mobilization of HSCs for autologous transplantation in pediatric patients (Karponi et al. 2015; Teusink et al. 2016) and has also resulted in successful mobilization of patients who fail to mobilize with single agent G-CSF, as well as produced larger yields of CD34+ cells (Maschan et al. 2015).

The patient's recommended Hb level during mobilization and apheresis was ≥ 11 g/dL (≥ 10 g/dL in HGB-204). The target minimum number of CD34+ cells to be collected for manufacturing DP in the Phase 3 studies was 12×10^6 CD34+ cells/kg. Back-up cells were collected at the time of apheresis to be used for rescue in case of engraftment failure or failure to infuse DP for any reason after myeloablation.

Up to 2 mobilization cycles were to be performed per patient to obtain sufficient cells for DP manufacture and back-up, however if the manufactured DP failed to meet specifications for any reason, a patient could undergo repeat mobilization and apheresis to manufacture new DP. Additional mobilization cycles were only to be performed if the initial mobilization cycle was tolerated, and in the opinion of the study physician, would not jeopardize the patient's medical condition.

Potential risks related to G-CSF, plerixafor, and apheresis were included in the informed consent forms (ICFs).

4.4.1.1. Mobilization Regimen

G-CSF dosing recommendations were adjusted based on splenectomy status. Lower doses of G-CSF were recommended for splenectomized patients because different responses to G-CSF have been described in splenectomized and non-splenectomized patients (Yannaki et al. 2012). In particular, splenectomized patients with TDT could theoretically be at increased risk for thromboembolic events during G-CSF mobilization. In the Phase 3 studies and in HGB-204, a G-CSF dose of 10 µg/kg/day was recommended for non-splenectomized patients. In HGB-205 and for splenectomized patients in HGB-204 and the Phase 3 studies, a G-CSF dose of 5 µg/kg/day was recommended.

For each cycle of mobilization and apheresis, it was recommended that patients receive G-CSF for 5 consecutive days with a 6th day if needed and then plerixafor (at 0.24 mg/kg) on the 4th and 5th days of G-CSF dosing. Recommendations were for complete blood counts to be performed on each day of G-CSF dosing and peripheral CD34+ cell counts to be performed daily starting on the 4th day of G-CSF dosing until the cycle was completed. Each mobilization cycle could include up to 3 apheresis procedure days, which were recommended to start on the 5th day of G-CSF dosing, and the mobilization regimen after 2 days of apheresis was to be discussed with

the medical monitor. If additional mobilization cycles were needed, either for DP manufacture or for back-up cells, the mobilization cycles were to be separated by at least 2 weeks.

4.4.2. Myeloablation/Conditioning

Myeloablation of patients with busulfan was conducted before DP infusion with a 48-hour washout to deplete endogenous HSCs and allow repopulation of the patient with HSCs containing the transgene. As myeloablation therapy is required prior to transplant, oocyte banking and sperm preservation were offered to all patients.

Although busulfan is usually administered with lymphocyte-depleting agents (e.g., (Busulfex 2020)), general guidelines in the busulfan prescribing information (PI) for dosing regimen and monitoring of busulfan AUC were followed when busulfan was used as a single agent in clinical studies with beti-cel. A dose of 3.2 mg/kg daily, or 0.8 mg/kg every 6 hours, for 4 consecutive days was recommended for ongoing Phase 3 studies, with dose adjustment as needed based on pharmacokinetics monitoring to achieve a target daily busulfan AUC of 4200 (range 3800 to 4500) uM*min. Per protocol, it was recommended that busulfan be administered at 0.8 mg/kg every 6 hours in children and adolescents with a target AUC of 1050 (range 950 to 1125) uM*min for every 6-hour dosing to avoid higher peak concentrations, while still providing equivalent daily exposure.

4.4.3. Drug Product Analysis

The presence of vector sequences in the genomic DNA (gDNA) of cells was detected using quantitative polymerase chain reaction (qPCR) and results were expressed as vector copy number (VCN; with units of vector copies per diploid genome [c/dg]). VCN in drug product was referred to as DP VCN and was measured in gDNA from pooled hematopoietic colonies after in vitro culture. Note that PB VCN, a pharmacodynamic parameter and not a DP attribute, was measured using a similar process except that the gDNA was extracted from peripheral blood cells. The percentage of transduced cells in the DP was measured by qPCR on single sorted cells after in vitro culture. DP VCN testing was performed on every DP lot. Testing for DP % transduced cells was performed on DP lots for HGB-204, HGB-207, and HGB-212, but was not performed for HGB-205.

4.4.4. Transfusion Independence

The proportion of patients meeting the definition of TI was the primary endpoint in the Phase 3 studies and was also evaluated in the Phase 1/2 studies, thus evaluation of efficacy was consistent across the clinical program. TI was defined as a weighted average Hb $\geq$ 9 g/dL without any RBC transfusions for a continuous period of $\geq$ 12 months at any time after DP infusion, starting 60 days after the last RBC transfusion. This starting point for TI was chosen to avoid confounding of the Hb measurement from recent transfusions.

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

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

where t_k represents the time point such that (t_k-t_0) represents at least 12 consecutive months. To determine if a patient remained TI beyond 12 months, the weighted average Hb needed to be maintained at ≥ 9 g/dL without any RBC transfusions.

A weighted average Hb was utilized given Hb levels were collected at varying sample time intervals across follow-up.

TI was analyzed as a point-estimate of the proportion of patients achieving TI at any time during the study, with a 2-sided 95% CI calculated using the Clopper-Pearson exact binomial method. The duration of TI was analyzed using Kaplan-Meier methods.

Exact logistic regression models built on data from patients across the clinical program who are TI-evaluable were used to predict achievement of TI by Month 24 for Phase 3 patients not yet TI-evaluable using Month 6 HbA^{T87Q}. A Month 6 unsupported total Hb of ≥ 9 g/dL was also used to predict achievement of TI for these patients.

4.4.5. Hemoglobin Parameters

The relative levels of α-globin and of the β-like-globins (including $\beta^{A\text{-}T87Q}$-globin) in peripheral blood hemolysates were measured by RP-HPLC using standards containing $\beta^{A\text{-}T87Q}$-globin and other globins with known retention times. The relative levels, along with total Hb (g/dL), were used to calculate the amount of each Hb fraction (g/dL) in the peripheral blood. Specifically, the value for total Hb was multiplied by the fraction of each β-like-globin (including β^{A}-, $\beta^{A\text{-}T87Q}$-, β^{E}-, δ-, γ^{A}-, and γ^{G}-globins) relative to all the others to calculate their contribution. Unsupported total Hb was defined as the total Hb measurement level without any acute or chronic RBC transfusions within 60 days prior to the measurement date.

4.5. Sample Size and Success Criteria Justification

TDT is a rare and orphan disease in the US, with approximately 1000 – 1300 patients in total.

The FDA provides guidance for sponsors who are developing gene therapy products for rare and serious or life-threatening diseases such as β-thalassemia with significant unmet need (FDA 2020a). The design and sample size of the beti-cel clinical program was in accordance with this FDA guidance and was discussed with the Agency over the years; general agreement was reached on the design of each study.

HGB-207 Cohort 1 consisted of patients with non-β^0/β^0 genotypes and age ≥ 12 years. A sample size of 15 patients was generally agreed upon with an objective to claim superiority over a 30% null hypothesis for the primary endpoint, the proportion of patients achieving TI. The justification to select the 30% threshold was 2-fold:

- TI is unlikely to occur spontaneously; therefore, 30% is a valuable threshold for testing the proportion of TI responders. A success criterion of 60% (9 out of 15 patients) gives a lower 1-sided 97.5% exact confidence bound of 32.3%, clearing the 30% threshold. Key opinion leaders with expertise in the treatment of β-thalassemia also supported a minimal 60% success rate for the proportion of patients who become TI as being clinically meaningful

- The upper exact confidence bound for no events in a hypothetical untreated control of 15 patients is 22%. The 30% threshold exceeds this upper bound

HGB-207 Cohort 2 consisted of 8 patients with non-β^0/β^0 genotypes aged < 12 years. Its success criterion is similar to the point estimate for Cohort 1 (see above): 62.5% (5 out of 8 patients).

The TI success criterion for HGB-212 in 18 TDT patients (N = 12 β^0/β^0 genotype; N = 6 β^0/IVS-1-110 or IVS-1-110/IVS-1-110 genotypes) utilizes the same 30% null hypothesis. The success criterion is 55.6% (10 out of 18 patients) with TI, giving a lower 1-sided 97.5% exact confidence bound of 30.8%, which clears the 30% null hypothesis. Although HGB-212 was not designed with separate age cohorts, there are 8 patients in HGB-212 who are < 12 years, giving a total sample size of 16 TDT patients who are < 12 years in the Phase 3 program.

In addition to the 41 patients treated in Phase 3 studies, 22 patients in Phase 1/2 Studies HGB-205 and HGB-204, all ≥ 12 years of age, have also been treated with DP and have provided supportive efficacy and safety data, for a total of 63 beti-cel-treated patients.

In keeping with FDA guidance recommending long-term follow-up study goals and duration (FDA 2020b), patients who complete the beti-cel clinical trials are enrolled in study LTF-303 and will be followed for up to 15 years after beti-cel infusion. Currently 51/63 (81.0%) have enrolled in LTF-303. No patient has declined participation in LTF-303.

5. EFFICACY

The efficacy data from the clinical program demonstrate that in the great majority of patients with β-thalassemia who require regular RBC transfusions, one-time treatment with beti-cel leads to lasting TI and durable normal or near-normal total Hb levels, and can lead to improvements in disease parameters, reductions in iron concentration, reduced use of iron chelation therapy, and potential improvements in erythropoiesis and quality of life.

As described in Table 1, Section 4.1, the Phase 1/2 Studies HGB-204 and HGB-205 are completed, and Phase 3 Studies HGB-207 and HGB-212 are ongoing. Long-term follow-up Study LTF-303 is also ongoing. Data from completed studies are presented as of their respective database lock dates. Data from ongoing studies are presented as of the BLA data cut-off date of 09 March 2021.

5.1. Disposition, Demographic, and Baseline Characteristics

Sixty-six patients have initiated a study procedure, beginning with mobilization, and comprise the ITT Population. Three patients discontinued from the clinical studies prior to conditioning: 1 patient each due to investigator decision secondary to inadequate mobilization, pregnancy, and patient withdrawal. In total, 63 patients were treated with beti-cel and comprise the Transplant Population (TP).

For the 63 treated patients, median (min, max) follow-up time was 35.48 (4.1, 86.5) months post-DP infusion. Thirty-two patients have enrolled and been treated in clinical study sites located in the US, 2 in Australia, 26 in Europe, and 3 in Thailand.

Median (min, max) pre-enrollment baseline transfusion requirements in the 63 treated patients were 190.37 (74.6, 289.0) mL/kg/year for transfusion volume and 15.00 (10.0, 39.5) transfusions per year for transfusion frequency, indicating that all study patients have a significant burden of disease within the spectrum of TDT.

In the Phase 3 Studies HGB-207 and HGB-212, 41 patients were treated with beti-cel, and these 41 patients have a median (min, max) duration of follow-up of 27.24 (4.1, 48.2) months. Most patients treated in Phase 3 studies (29/41; 70.7%) have completed their parent study, including 20/23 (87.0%) patients in study HGB-207 and 9/18 (50.0%) patients in HGB-212. The 23 patients treated in HGB-207 had median (min, max) follow-up of 29.54 (13.0, 48.2) months and the 18 patients treated in HGB-212 had median (min, max) follow-up of 24.62 (4.1, 35.5).

All 22 patients treated with beti-cel in the Phase 1/2 studies have completed these studies and enrolled in the long-term follow-up study LTF-303. These 22 patients have a median (min, max) follow-up of 67.56 (59.2, 86.5) months.

Demographically, the 63 patients (Table 2) who have been treated with beti-cel are similar to the global population of patients with β-thalassemia. β-thalassemia is found most frequently in persons of Mediterranean, Middle Eastern, Indian, and Southeast Asian descent (Colah et al. 2010). Therefore, the predominance of Asian and White patients is expected in these studies (57.1% identified as Asian, and 38.1% identified as White). 55.6% of patients were female. The median (min, max) age across the studies was 17.00 (4.0, 35.0) years and as such the median age of the patients is older than that which is usually recommended for allo-HSCT.

In the Phase 1/2 studies, more adults than pediatric patients were treated, as safety was first determined in adults before treating pediatric patients; in the Phase 3 studies, the relative numbers of patients are more balanced between the age groups.

The beti-cel clinical studies enrolled a representative sample of common *HBB* variants found in North America and Europe. The β^E-globin mutation is common in patients of Southeast Asian descent, and thus the presence of the β^0/β^E genotype is expected in these studies. The global expanse of the clinical development program for beti-cel resulted in the treatment of patients with different *HBB* mutations and a wide range of endogenous β-globin expression. Despite the differences in endogenous β-globin production, all patients enrolled in the clinical studies met the criteria for transfusion dependence.

Demographic parameters for patients treated in all of the beti-cel clinical studies are summarized in Table 2.

Table 2. Demographic Parameters by Study Phase, Parent Study, and Overall (TP)

Parameter		Statistic	Phase 1/2 Studies HGB-204	Phase 1/2 Studies HGB-205	Phase 1/2 Studies Pooled	Phase 3 Studies HGB-207	Phase 3 Studies HGB-212	Phase 3 Studies Pooled	All Studies
Patients treated with beti-cel (TP)		N	18	4	22	23	18	41	63
Age[a], y		N	18	4	22	23	18	41	63
		Median	20.00	17.50	20.00	15.00	12.50	13.00	17.00
		Min, Max	12.0, 35.0	16.0, 19.0	12.0, 35.0	4.0, 34.0	4.0, 33.0	4.0, 34.0	4.0, 35.0
Age category	≥ 18 y	n (%)	15 (83.3)	2 (50.0)	17 (77.3)	9 (39.1)	5 (27.8)	14 (34.1)	31 (49.2)
	< 18 y	n (%)	3 (16.7)	2 (50.0)	5 (22.7)	14 (60.9)	13 (72.2)	27 (65.9)	32 (50.8)
	≥ 12 – < 18 y	n (%)	3 (16.7)	2 (50.0)	5 (22.7)	6 (26.1)	5 (27.8)	11 (26.8)	16 (25.4)
	< 12 y	n (%)	0	0	0	8 (34.8)	8 (44.4)	16 (39.0)	16 (25.4)
Sex	Male	n (%)	5 (27.8)	2 (50.0)	7 (31.8)	11 (47.8)	10 (55.6)	21 (51.2)	28 (44.4)
	Female	n (%)	13 (72.2)	2 (50.0)	15 (68.2)	12 (52.2)	8 (44.4)	20 (48.8)	35 (55.6)
Race	Asian	n (%)	14 (77.8)	2 (50.0)	16 (72.7)	13 (56.5)	7 (38.9)	20 (48.8)	36 (57.1)
	White	n (%)	4 (22.2)	2 (50.0)	6 (27.3)	8 (34.8)	10 (55.6)	18 (43.9)	24 (38.1)
	Other[b]	n (%)	0	0	0	2 (8.7)	0	2 (4.9)	2 (3.2)
	Not reported	n (%)	0	0	0	0	1 (5.6)	1 (2.4)	1 (1.6)
HBB genotype	β^0/β^0	n (%)	8 (44.4)	0	8 (36.4)	0	12 (66.7)	12 (29.3)	20 (31.7)
	β^E/β^0	n (%)	6 (33.3)	3 (75.0)	9 (40.9)	6 (26.1)	0	6 (14.6)	15 (23.8)
	β^0/β^+	n (%)	1 (5.6)	0	1 (4.5)	12 (52.2)	3 (16.7)	15 (36.6)	16 (25.4)
	β^+/β^+	n (%)	2 (11.1)	1 (25.0)	3 (13.6)	5 (21.7)	3 (16.7)	8 (19.5)	11 (17.5)

Other[c]	n (%)	1 (5.6)	0	1 (4.5)	0	0	0	1 (1.6)

BL, baseline; Hb, hemoglobin; *HBB*, β-globin gene; TP, Transplant Population.
a. Age at consent or date of assent applicable for patients < 18 years old.
b. Other race reported as Asian Pakistani and Caucasian/Thai.
c. Other genotype reported as: *HBB*:c.92+1G>T and Unknown. The unknown allele is an unidentified β^+ mutation because the patient is able to produce some endogenous HbA.

Select baseline parameters for patients treated in the beti-cel clinical studies are summarized in Table 3. There was a wide range in iron burden at baseline amongst the patients, spanning across the normal range to iron overload. The patients treated in the beti-cel clinical studies had baseline characteristics that were representative of patients with β-thalassemia who are eligible to undergo HSCT.

Table 3. Select Baseline Parameters by Study Phase, Parent Study, and Overall (TP)

		Phase 1/2 Studies			Phase 3 Studies			
Baseline Parameter	**Statistic**	**HGB-204**	**HGB-205**	**Pooled**	**HGB-207**	**HGB-212**	**Pooled**	**All Studies**
Patients treated with beti-cel	N	18	4	22	23	18	41	63
LIC, mg/g	Median	5.7	11.2	7.1	5.3	3.6	4.9	5.3
	Min, Max	0.4, 26.4	3.9, 14.0	0.4, 26.4	1.0, 41.0	1.2 13.2	1.0, 41.0	0.4, 41.0
Cardiac T2*, msec	Median	35.0	33.0	34.0	36.7	37.0	36.7	36.1
	Min, Max	10.0, 54.0	29.0, 46.0	10.0, 54.0	21.0, 57.0	15.0, 75.0	15.0, 75.0	10.0, 75.0
Serum ferratin, pmol/L	Median	3146.8[a]	4185.4	3146.8[a]	4438.2	3275.0	3671.9	3519.1[a]
	Min, Max	748, 8629	2139, 7097	748, 8629	784, 22517	1279, 8874	784, 22517	748, 22517
Splenectomy	n (%)	6 (33.3)	3 (75.0)	9 (40.9)	4 (17.4)	3 (16.7)	7 (17.1)	16 (25.4)
No splenectomy	n (%)	12 (66.7)	1 (25.0)	13 (59.1)	19 (82.6)	15 (83.3)	34 (82.9)	47 (74.6)
Baseline performance score[b]	Median	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	Min, Max	90, 100	100, 100	90, 100	80, 100	90, 100	80, 100	80, 100

LIC, liver iron content; TP, Transplant Population.
[a] One patient in Study HGB-204 did not have a baseline serum ferritin value available.
[b] The baseline performance score is the Karnofsky performance score or Lansky performance score as applicable based on age.

5.2. Dosing Characteristics in the Clinical Studies

Comparison of observed values across the 4 clinical studies (Table 4) show similar median cell doses, with an overall median (min, max) total cell dose of beti-cel administered to patients of 9.00 (5.00, 42.10) × 10^6 CD34+ cells/kg. Due to manufacturing improvements (see Section 9, Appendix A), the median DP VCN and DP % transduced cells were higher in the Phase 3 studies than in the Phase 1/2 studies. All treated patients successfully engrafted with beti-cel (see information on neutrophil and platelet engraftment in Section 6.3).

Given that beti-cel is administered on a weight-based cell dose, it is likely that this approach normalizes exposure across the age range, supporting use in patients of a younger age. No consistent differences were observed between patients with non-β^0/β^0 genotypes and those with a β^0/β^0 genotype for DP VCN and DP % transduced cells (Table 7). Results within and across studies suggest that age, sex, race, and genotype did not impact pharmacodynamic parameters; therefore, dosing recommendations are the same for all subpopulations of patients evaluated, including pediatric populations aged < 18 years.

Table 4. Dosing Characteristics in the Clinical Studies of beti-cel (TP)

Parameter	HGB-204 N = 18	HGB-205 N = 4	HGB-207 N = 23	HGB-212 N = 18	All Phase 3 N = 41
Total DP Dose, × 10^6 CD34+ cells/kg					
N	18	4	23	18	41
Median	8.10	10.46	8.10	10.75	9.40
Min, Max	5.20, 18.10	8.79, 13.60	5.00, 19.90	5.90, 42.10	5.00, 42.10
DP VCN[a], c/dg					
N	18	4	23	18	41
Median	0.70	1.30	3.26	3.00	3.00
Min, Max	0.30, 1.50	0.80, 2.10	1.90, 5.60	1.20, 7.00	1.20, 7.00
DP % transduced cells[a],%					
N	18	0[b]	23	18	41
Median	31.5	-	79.3	78.0	78.0
Min, Max	17, 58	-	34, 90	34, 94	34, 94
DP VCN/DP % transduced cells[a], vector copies/transduced cell					
N	18	0[b]	23	18	41
Median	2.35	-	4.44	3.71	4.00
Min, Max	1.52, 3.75	-	2.84, 7.65	2.59, 7.45	2.59, 7.65

c/dg, copies per diploid genome; DP, drug product; TP, Transplant Population; VCN, vector copy number.
a. When more than a single lot of drug product was administered, a weighted average was calculated by considering the relative contribution of each lot to the final dose.
b. % transduced cells values were not evaluated for drug product used for HGB-205.

5.3. Clinical Pharmacology

5.3.1. Background

beti-cel is a gene therapy product and conventional pharmacokinetic (PK) methods cannot be used to monitor the presence of the DP; therefore, no dedicated PK/PD studies have been conducted.

The measurement of PD parameters that detect the presence of integrated vector sequences and the expression of the transgene in differentiated cells can be used to determine successful delivery and persistence of the transduced HSCs. The progeny of successfully engrafted transduced HSCs will also contain the transgene, as measured by PB VCN.

For beti-cel, the primary PD effect after engraftment and differentiation of the HSCs is production of $\beta^{A\text{-}T87Q}$-globin. The T87Q amino acid substitution enables measurement of the transgene production ($\beta^{A\text{-}T87Q}$-globin) in peripheral blood, which allows direct demonstration of the mechanism of action.

All HSCs transduced with BB305 LVV will have 1 or more copies of the transgene within their gDNA, as will all nucleated daughter cells derived from HSCs that engraft as bone marrow cells. Nucleated cells derived from LVV-transduced HSCs will contain gDNA and transgenes. However, only nucleated immature erythroid cells will produce $\beta^{A\text{-}T87Q}$-globin protein. Similar to RBCs from individuals who do not have thalassemia, mature RBCs derived from LVV-transduced HSCs will lack nuclei and will not contain gDNA or transgenes, although they will contain $\beta^{A\text{-}T87Q}$-globin protein. Thus, integrated transgenes and $\beta^{A\text{-}T87Q}$-globin production only exist concurrently in nucleated immature erythroid cells, and not in mature white blood cells or RBCs.

The DP contains a heterogeneous population of CD34+ cells including both short-term progenitor cells and true long-term repopulating stem cells. In these DP lots, there are variable proportions of early (more stem-like) progenitors, which are less easily transduced but give rise to long-term engraftment, and late (less stem-like) progenitors, which are more easily transduced but give rise to short-term engraftment (de la Brunet Grange et al. 2013). Thus, the DP VCN may not precisely reflect the VCN in the sub-population that is responsible for true long-term repopulation. However, both DP VCN and DP % transduced cells were positively correlated with HbA^{T87Q} levels in patients after treatment with beti-cel; a multivariate analysis indicated that the best correlation between PB VCN levels in beti-cel–treated patients was with DP % transduced cells.

5.3.2. Pharmacodynamic Findings

PB VCN

Median PB VCN in patients with TDT was higher in the Phase 3 Studies HGB-207 and HGB-212 than in Phase 1/2 studies, consistent with higher drug product VCNs and higher DP % transduced cells.

Median PB VCNs are stable by Month 6 and remain stable through last follow-up (Table 5). PB VCN levels were durable; the patient with the longest follow-up had stable values through approximately Year 7 after DP infusion. These results demonstrate long-term persistence of transduced repopulating HSCs.

Table 5. PB VCN Over Time (TP)

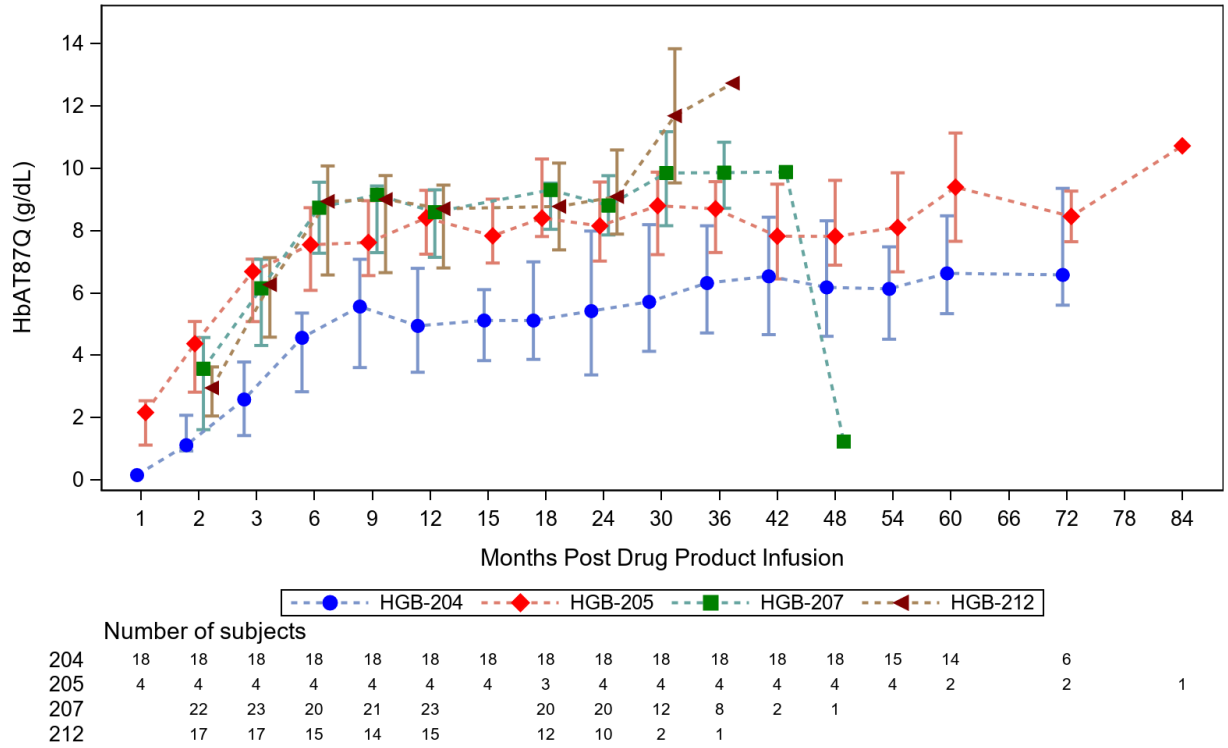
	Phase 1/2 Studies			Phase 3 Studies			All Studies
Parameter Visit	**HGB-204 N = 18**	**HGB-205 N = 4**	**Phase 1/2 N = 22**	**HGB-207 N = 23**	**HGB-212 N = 18**	**All Phase 3 N = 41**	**All TDT N = 63**
DP VCN, c/dg[a]							
N	18	4	22	23	18	41	63
Median	0.70	1.30	0.80	3.26	3.00	3.00	2.25
Min, max	0.30, 1.50	0.80, 2.10	0.30, 2.10	1.90, 5.60	1.20, 7.00	1.20, 7.00	0.30, 7.00
PB VCN, c/dg							
Month 6							
N	18	4	22	22	15	37	59
Median	0.36	1.77	0.39	1.40	0.88	1.29	0.85
Min, max	0.08, 0.95	0.29, 4.69	0.08, 4.69	0.18, 4.52	0.16, 3.33	0.16, 4.52	0.08, 4.69
Month 24							
N	18	4	22	20	10	30	52
Median	0.31	1.74	0.41	1.62	0.90	1.43	0.84
Min, max	0.06, 1.04	0.38, 3.31	0.06, 3.31	0.20, 3.00	0.14, 3.49	0.14, 3.49	0.06, 3.49
Month 36							
N	18	4	22	8	1	9	31
Median	0.34	1.77	0.39	1.93	2.73	2.19	0.75
Min, max	0.07, 1.82	0.30, 3.48	0.07, 3.48	0.13, 3.74	2.73, 2.73	0.13, 3.74	0.07, 3.74
Month 48							
N	18	4	22	1	0	1	23
Median	0.33	2.01	0.38	0.11	–	0.11	0.35
Min, max	0.07, 2.45	0.28, 4.06	0.07, 4.06	0.11, 0.11	–	0.11, 0.11	0.07, 4.06
Month 60							
N	14	4	18	0	0	0	18
Median	0.51	2.37	0.58	–	–	–	0.58
Min, max	0.14, 1.80	0.36, 3.98	0.14, 3.98	–	–	–	0.14, 3.98
Year 6							
N	6	2	8	0	0	0	8
Median	0.43	2.68	0.70	–	–	–	0.70
Min, max	0.21, 0.89	0.95, 4.41	0.21, 4.41	–	–	–	0.21, 4.41
Year 7							
N	0	1	1	0	0	0	1
Median	–	3.86	3.86	–	–	–	3.86
Min, max	–	3.86, 3.86	3.86, 3.86	–	–	–	3.86, 3.86

c/dg, copies per diploid genome; DP, drug product; NA, not applicable; PB, peripheral blood; TDT, transfusion-dependent thalassemia; TP, Transplant Population; VCN, vector copy number.
a. When more than a single lot of drug product was administered, a weighted average was calculated by considering the relative contribution of each lot to the final dose.

HbA^{T87Q}

HbA^{T87Q} levels increased steadily after DP infusion, before reaching a plateau by approximately Month 6 after DP infusion. Thereafter, HbA^{T87Q} levels in peripheral blood were stable and durable for the patients who stopped receiving RBC transfusions (Figure 2, Table 6). Although median HbA^{T87Q} levels remained stable for patients who stopped receiving RBC transfusions, HbA^{T87Q} levels fluctuated for patients who received RBC transfusions, an expected result due to suppression of erythropoiesis by transfused Hb. No evidence of decline in HbA^{T87Q} once TI is achieved was observed out to last follow up (Phase 1/2: up to 7 years; Phase 3: up to 4 years).

Figure 2. Median HbA^{T87Q} Over Time, by Study (TP)



PB VCN, peripheral blood vector copy number; RBC, red blood cell; TP, transplant population.
The markers represent the medians. The bars represent the interquartile ranges.
Only 1 patient in HGB-207 completed the Month 48 Visit. This patient had relatively low PB VCN and HbA^{T87Q} values and restarted regular RBC transfusions approximately 14 months after DP infusion, which accounts for the low HbA^{T87Q} for HGB-207 at Month 48.

Table 6. HbA^{T87Q} Over Time (TP)

	Phase 1/2 Studies			Phase 3 Studies			All Studies
HbA^{T87Q}, g/dL Visit	**HGB-204 N = 18**	**HGB-205 N = 4**	**All Phase 1/2 N = 22**	**HGB-207 N = 23**	**HGB-212 N = 18**	**All Phase 3 N = 41**	**All TDT N = 63**
Month 6							
N	18	4	22	20	15	35	57
Median	4.56	7.54	4.78	8.73	8.93	8.74	7.77
Min, max	0.43, 8.89	4.94, 9.59	0.43, 9.59	1.12, 10.60	0.00, 12.01	0.00, 12.01	0.00, 12.01
Month 24							
N	18	4	22	20	10	30	52
Median	5.42	8.15	6.44	8.75	9.09	8.75	8.15
Min, max	0.47, 9.60	6.72, 10.13	0.47, 10.13	0.34, 11.40	0.71, 12.43	0.34, 12.43	0.34, 12.43
Month 36							
N	18	4	22	8	1	9	31
Median	6.32	8.70	6.71	9.86	12.72	10.62	8.17
Min, max	0.38, 10.10	6.37, 9.96	0.38, 10.10	2.23, 11.79	12.72, 12.72	2.23, 12.72	0.38, 12.72
Month 48							
N	18	4	22	1[a]	0	1	23
Median	6.18	7.81	6.60	1.23	–	1.23	6.48
Min, max	0.45, 9.43	6.72, 10.65	0.45, 10.65	1.23, 1.23	–	1.23, 1.23	0.45, 10.65
Month 60							
N	14	2	16	0	0	0	16
Median	6.63	9.39	6.79	–	–	–	6.79
Min, max	2.84, 9.97	7.63, 11.15	2.84, 11.15	–	–	–	2.84, 11.15
Year 6							
N	6	2	8	0	0	0	8
Median	6.58	8.45	7.42	–	–	–	7.42
Min, max	3.10, 9.49	7.62, 9.29	3.10, 9.49	–	–	–	3.10, 9.49
Year 7							
N	0	1	1	0	0	0	1
Median	–	10.71	10.71	–	–	–	10.71
Min, max	–	10.71, 10.71	10.71, 10.71	–	–	–	10.71, 10.71

TP, Transplant Population.

a. Only 1 patient in HGB-207 completed the Month 48 Visit as of the data cut-off. This patient had relatively low PB VCN and HbA^{T87Q} values and restarted regular RBC transfusions approximately 14 months after DP infusion, which accounts for the low HbA^{T87Q} for HGB-207 at Month 48

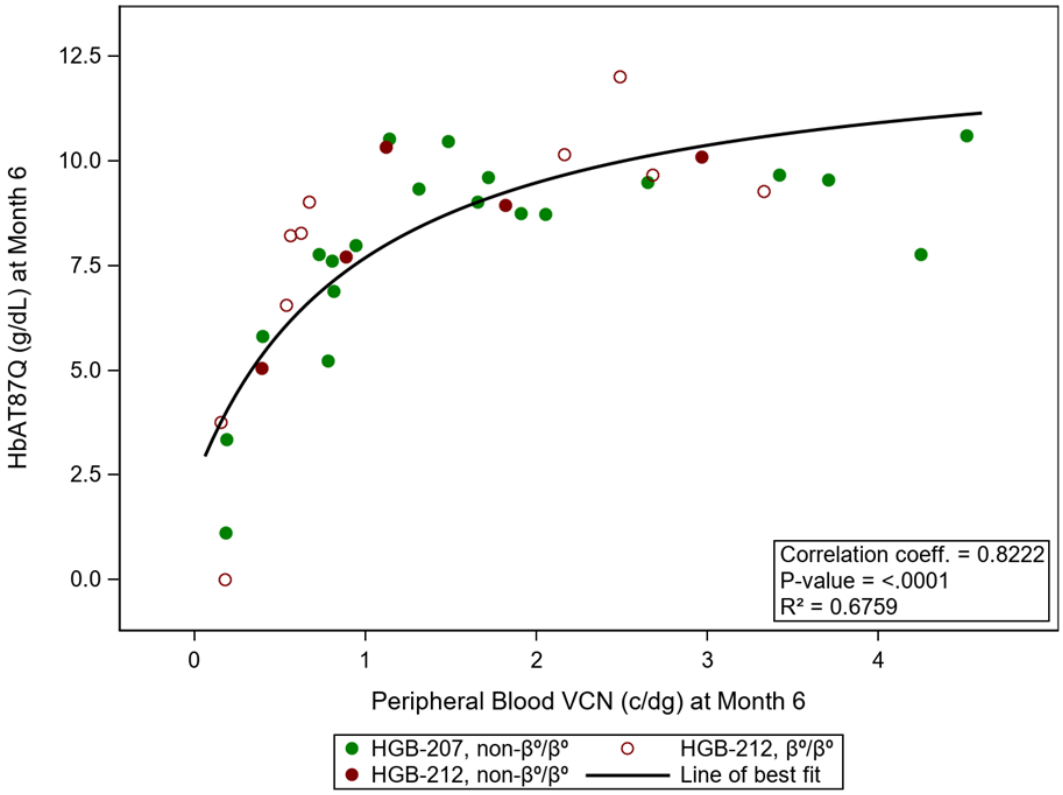
A repeated measures mixed model analysis demonstrated that the HbA^{T87Q}/unsupported total Hb at Month 6 is significantly associated with HbA^{T87Q}/unsupported total Hb levels at later visits over time with a coefficient close to 1. The Fisher's exact test and Kappa score also show that unsupported total Hb ≥ 9 g/dL at Month 6 corresponded to achieving TI at later time points in all cases. These results demonstrate HbA^{T87Q} production is durable, which leads to durable unsupported total Hb and durable TI. Month 6 HbA^{T87Q} and unsupported total Hb are therefore robust predictors for TI.

Exact logistic regression models from TI-evaluable patients in the pooled Phase 3 studies evaluated the relationship between the probability of achieving TI at any time (N = 33) and HbA^{T87Q} at Month 6. The fitted model using this data found a significant association between the probability of achieving TI at any time and the HbA^{T87Q} at Month 6. The exact odds ratio at Month 6 increased 3.3 times (95% confidence interval [CI]: 1.46 to 29.13; $p < 0.0001$) when

HbA^{T87Q} levels increased by 1 g/dL. Patients in the pooled Phase 3 studies who achieved TI usually had higher Month 6 HbA^{T87Q} than those who did not achieve TI, which also demonstrates the predictive power of HbA^{T87Q} at Month 6 for achieving TI at any time. Thus, by Month 6, it is generally apparent whether a patient will achieve TI based on HbA^{T87Q} levels.

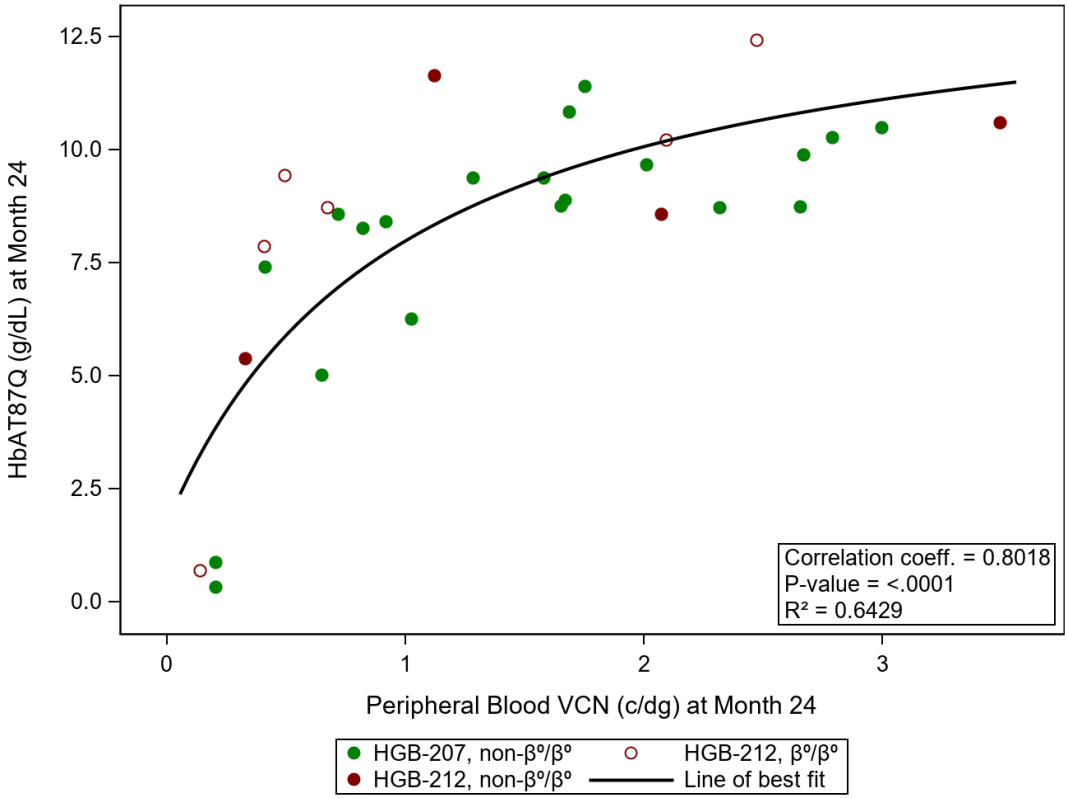
In general, patients with higher PB VCN values produced more HbA^{T87Q} at a given time point. The positive correlation between HbA^{T87Q} expression and PB VCN in the Phase 3 studies is shown at Month 6 (Figure 3) and Month 24 (Figure 4). HbA^{T87Q} is observed to increase quickly with PB VCN at lower PB VCN levels, then plateau at higher PB VCN levels, represented by a transformation of VCN/(1+VCN). In the plateau phase, further increases in PB VCN do not increase HbA^{T87Q} expression. This most likely reflects both the selection of erythroid cells producing $\beta^{A\text{-}T87Q}$-globin with more balanced α-globin/β-globin ratios, as well as the underlying biology of regulation of the β-globin levels within erythroid cells, where there may be an upper level of β-globin or total Hb within a cell beyond which excess β-globin is either metabolized or further production is down-regulated (Khandros and Weiss 2010). Patients with HbA^{T87Q} levels on the plateau generally are producing ≥ 9 g/dL of HbA^{T87Q}, a sufficient level of HbA^{T87Q} alone to achieve TI, regardless of any potentially supplemental endogenous Hb.

Figure 3. Correlation Between HbA^{T87Q} Expression and Peripheral Blood VCN in Phase 3 Patients at Month 6



VCN, vector copy number.
The fitted curve is based on linear regression between HbA^{T87Q} and transformed peripheral blood VCN, VCN/(1+VCN).

Figure 4. Correlation Between HbA^{T87Q} Expression and Peripheral Blood VCN in Phase 3



VCN, vector copy number.
The fitted curve is based on linear regression between HbA^{T87Q} and transformed peripheral blood VCN, VCN/(1+VCN).

Drug Product and Pharmacodynamic Parameters by *HBB* Genotype

No consistent differences were observed between patients with non-β^0/β^0 genotypes and those with a β^0/β^0 genotype for DP VCN, DP % transduced cells, PB VCN, and HbA^{T87Q} values in the Phase 3 studies (Table 7).

Table 7. Comparison of Drug Product Characteristics and Pharmacodynamic Parameters Across Genotypes in the Phase 3 Studies

Parameter	Genotype	
	Non-β^0/β^0	β^0/β^0
DP VCN, c/dg[a]		
N	29	12
Mean (SD)	3.34 (1.05)	3.15 (1.72)
Median (min, max)	3.263 (1.90, 5.60)	2.750 (1.20, 7.00)
DP % transduced cells, %[a]		
N	29	12
Mean (SD)	73.4 (13.43)	74.4 (17.45)
Median (min, max)	78.0 (34, 90)	78.0 (34, 94)
PB VCN at Month 6, c/dg		
N	27	10
Mean (SD)	1.7 (1.2)	1.3 (1.2)
Median (min, max)	1.31 (0.18, 4.52)	0.646 (0.16, 3.33)
HbA^{T87Q} at Month 6, g/dL		
N	13	10
Mean (SD)	7.2 (2.7)	7.7 (3.5)
Median (min, max)	7.77(1.12, 9.66)	8.66 (0[b], 12.01)

c/dg, copies per diploid genome; DP VCN, drug product vector copy number; PB VCN; peripheral blood vector copy number; SD, standard deviation.
a. When more than a single lot of DP was administered, a weighted average was calculated by considering the relative contribution of each lot to the final dose.
b. Note that 1 patient had a stable low PB VCN (e.g., PB VCN of 0.179 c/dg at Month 6) and received chronic RBC transfusions approximately monthly after treatment. Although this patient produced some HbA^{T87Q} after treatment (i.e., highest HbA^{T87Q} g/dL of 2.03 at Month 2), there was no measurable HbA^{T87Q} at Month 6, and only low levels thereafter (e.g., 0.35 g/dL at Month 12 and 0.71 g/dL at Month 24, last visit to date), likely due to the chronic transfusions and resulting suppression of their endogenous hematopoiesis.

5.4. Primary Efficacy Endpoint of Transfusion Independence

The primary endpoint for both HGB-207 and HGB-212 Phase 3 studies is the proportion of patients who meet the definition of TI (defined in Section 4.4.4). As TI does not spontaneously occur in untreated patients, any demonstration of TI is evidence of meaningful therapeutic effect in patients with β-thalassemia who require regular RBC transfusions. The primary efficacy analysis is based on treated Phase 3 patients who are evaluable for TI as of the data cut-off, defined as those who have achieved TI, completed their parent study (Month 24 Visit), or will not achieve TI during their parent study (for example the patient is receiving chronic transfusions 1 year after treatment).

The number and percentage of patients treated in the Phase 3 studies who met the definition of TI at any time with the corresponding 2-sided 95% CIs, are presented in Table 8, with summary statistics presented overall and separated by study, *HBB* genotype, age, sex, and race (Table 9). Both Phase 3 studies have prespecified success criteria: 60% (9 out of 15 patients) for HGB-207 Cohort 1, 62.5% (5 out of 8 patients) for HGB-207 Cohort 2, and 55.6% (10 out of 18 patients) for HGB-212 (Section 4.5). Phase 3 and data from all beti-cel studies were additionally pooled to provide sensitivity for the efficacy analyses.

The prespecified success criteria have all been met (Table 8). In HGB 207 Cohort 1, 14/15 TI-evaluable patients (93.3%; 2-sided 95% CI of 68.1% to 99.8%) achieved TI, and in Cohort 2, 6/7 TI-evaluable patients (85.7%; 2-sided 95% CI of 42.1% to 99.6%) achieved TI. In Study HGB-212, 12/14 TI-evaluable patients (85.7%; 2-sided 95% CI of 57.2% to 98.2%) achieved TI.

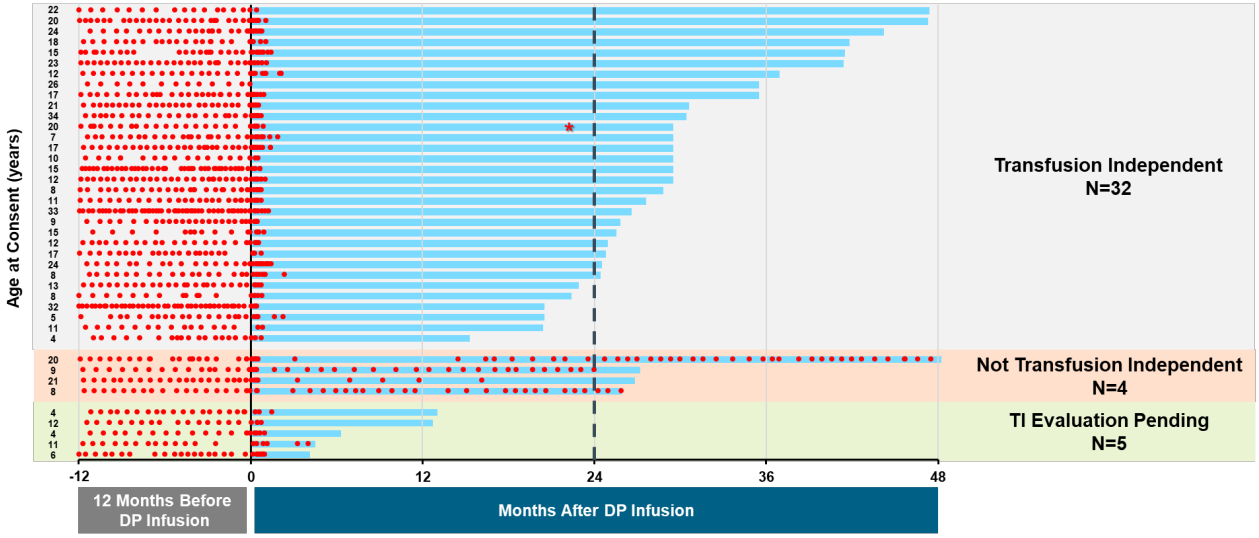
Table 8. Proportion of Patients Who Have Achieved Transfusion Independence at Any Time in the Phase 3 Studies (TP)

Parameter	Statistic	HGB-207 Cohort 1 N = 15	HGB-207 Cohort 2 N = 8	HGB-212 N = 18	Pooled N = 41
TI-evaluable[a]	N	15	7	14	36
Patients with TI at any time	n (%)[b]	14 (93.3)	6 (85.7)	12 (85.7)	32 (88.9)
	2-sided 95% CI	68.1, 99.8	42.1, 99.6	57.2, 98.2	73.9, 96.9

CI, confidence interval; TI, transfusion independence; TP, Transplant Population
a. Patients evaluable for TI are defined as those who have achieved TI, completed their parent study (Month 24 Visit), or will not achieve TI in their parent study.
b. Denominator based on the number of patients evaluable for TI.

Figure 5 demonstrates patient transfusion needs before and after treatment with beti-cel in the Phase 3 studies, with duration of follow-up presented with a blue bar and RBC transfusions presented with red dots.

Figure 5. Transfusion Status in Phase 3 Patients



RBC, red blood cell; TI, transfusion independence.
*Patient received acute transfusion for serious blood loss due to orthopedic surgery.
Red dots demonstrate RBC transfusions, blue bar demonstrate duration of follow-up.
Vertical dotted line at 24 months represents end of HGB-207 and HGB-212 and rollover into LTF-303.

The majority of evaluable patients (88.9%) in the Phase 3 studies achieved TI, regardless of age, sex, race, and *HBB* genotype (Table 9).

Table 9. Proportion of Patients Who Have Achieved Transfusion Independence at Any Time in Phase 3 Subgroups (TP)

Subgroup		Parameter	Statistic	HGB-207 N = 23	HGB-212 N = 18	Pooled N = 41
Overall		TI-evaluable[a] Patients with TI at any time	N n (%)[b] 2-sided 95% CI	22 20 (90.9) 70.8, 98.9	14 12 (85.7) 57.2, 98.2	36 32 (88.9) 73.9, 96.9
HBB genotype	β^0/β^0	TI-evaluable[a] Patients with TI at any time	N n (%)[b] 2-sided 95% CI	0 0 NA	8 7 (87.5) 47.3, 99.7	8 7 (87.5) 47.3, 99.7
	Non-β^0/β^0	TI-evaluable[a] Patients with TI at any time	N n (%)[b] 2-sided 95% CI	22 20 (90.9) 70.8, 98.9	6 5 (83.3) 35.9, 99.6	28 25 (89.3) 71.8, 97.7

Subgroup		Parameter	Statistic	HGB-207 N = 23	HGB-212 N = 18	Pooled N = 41
Age (years)[c]	≥ 18	TI-evaluable[a] Patients with TI at any time	N n (%)[b] 2-sided 95% CI	9 8 (88.9) 51.8, 99.7	5 4 (80.0) 28.4, 99.5	14 12 (85.7) 57.2, 98.2
	< 18	TI-evaluable[a] Patients with TI at any time	N n (%)[b] 2-sided 95% CI	13 12 (92.3) 64.0, 99.8	9 8 (88.9) 51.8, 99.7	22 20 (90.9) 70.8, 98.9
	≥ 12 to < 18	TI-evaluable[a] Patients with TI at any time	N n (%)[b] 2-sided 95% CI	6 6 (100.0) 54.1, 100.0	4 4 (100.0) 39.8, 100.0	10 10 (100.0) 69.2, 100.0
	≥ 12	TI-evaluable[a] Patients with TI at any time	N n (%)[b] 2-sided 95% CI	15[d] 14 (93.3) 68.1, 99.8	9 8 (88.9) 51.8, 99.7	24 22 (91.7) 73.0, 99.0
	< 12	TI-evaluable[a] Patients with TI at any time	N n (%)[b] 2-sided 95% CI	7[e] 6 (85.7) 42.1, 99.6	5 4 (80.0) 28.4, 99.5	12 10 (83.3) 51.6, 97.9
Sex	Male	TI-evaluable[a] Patients with TI at any time	N n (%)[b] 2-sided 95% CI	11 11 (100.0) 71.5, 100.0	8 7 (87.5) 47.3, 99.7	19 18 (94.7) 74.0, 99.9
	Female	TI-evaluable[a] Patients with TI at any time	N n (%)[b] 2-sided 95% CI	11 9 (81.8) 48.2, 97.7	6 5 (83.3) 35.9, 99.6	17 14 (82.4) 56.6, 96.2
Race[d]	Asian	TI-evaluable[a] Patients with TI at any time	N n (%)[b] 2-sided 95% CI	12 11 (91.7) 61.5, 99.8	3 3 (100.0) 29.2, 100.0	15 14 (93.3) 68.1, 99.8
	White	TI-evaluable[a] Patients with TI at any time	N n (%)[b] 2-sided 95% CI	8 8 (100.0) 63.1, 100.0	10 8 (80.0) 44.4, 97.5	18 16 (88.9) 65.3, 98.6

CI, confidence interval; *HBB*, β-globin gene; NA, not applicable; TI, transfusion independence; TP, Transplant Population

a. Patients evaluable for TI are defined as those who have achieved TI, completed their parent study (Month 24 Visit), or will not achieve TI in their parent study.

b. Denominator based on the number of patients evaluable for TI.

c. Age at consent or date of assent applicable for patients < 18 years old.

d. The Other and Not reported race subgroups are excluded from this table to protect patient privacy and because there are too few patients in those groups to support meaningful summary analyses.

The patients who achieved TI in the Phase 3 studies were producing between 3.75 to 12.01 g/dL of HbA^{T87Q} at Month 6 (N=29), with the remainder of their total Hb made up of other endogenous Hb fractions depending on their genotype, primarily HbF (0.18 to 5.95 g/dL, N = 29), HbA_2 (0.24 to 0.78 g/dL, N = 29), HbE (2.21 to 2.73 g/dL, N = 4) in patients with one

or more β^E allele, and HbA (0.06 to 2.90 g/dL, N = 18) in patients with 1 or more β^+ allele other than β^E. These data demonstrate that even patients producing very low levels of endogenous Hb can achieve TI regardless of genotype.

Consistent and direct relationships were observed between DP characteristics (DP VCN and DP % transduced cells; Table 7, Section 5.3.2), PD parameters (PB VCN and HbA^{T87Q}; Section 5.3.2), and clinical outcomes (total Hb and TI). In a post-hoc analysis, all patients who were evaluable for TI after treatment with beti-cel in the Phase 3 studies and received DP with a % transduced cells value that met the proposed commercial specification, achieved TI (N = 26). Of the 10 patients who were evaluable for TI and who received DP with a % transduced cells value below the proposed commercial specification, 6 patients achieved TI. The 4 patients who did not achieve TI are discussed further below.

Across the Phase 3 studies, 4 of the 36 TI-evaluable patients did not achieve TI (2 patients in HGB-207 and 2 patients in HGB-212; see orange shaded area of Figure 5). These patients had the lowest PB VCN values of all TI-evaluable patients and were producing the lowest amounts of HbA^{T87Q} at Month 6 in their respective studies. Although they were not all the lowest in the Phase 3 studies, the DP VCNs (1.90, 2.10, 2.30, and 2.40 c/dg) and values for % transduced cells (53%, 57%, 58%, and 61%) for these patients were below the median values for all patients treated in the Phase 3 studies (Table 7, Section 5.3.2), and the % transduced cells values were below the proposed threshold for commercial DP specification. A retrospective analysis showed that some process parameters used in the manufacture of these patients' DP lots were at the limits of the ranges characterized in process development. Based on this information, ranges for these parameters were narrowed to values had that yielded DPs with characteristics that correlated with positive clinical outcomes. (See Section 2).

Of the 5 patients treated in the Phase 3 studies who were not yet evaluable for TI (green shaded area in Figure 5), 3 patients had Month 6 data that could be used to predict the likelihood of achieving TI. Their Month 6 total Hb values were unsupported and ≥ 9g/dL (10.8, 10.9, and 11.1 g/dL) which has been demonstrated to be significantly associated with achieving TI ($p<0.0001$). Additionally, Month 6 HbA^{T87Q} values of 9.28 and 9.66 g/dL were available for 2 of these patients, giving them > 99% probability of achieving TI based on an exact logistic regression model. Thus, all 3 patients are predicted to be likely to achieve TI. The remaining 2 patients (both in HGB-212), who were not yet TI-evaluable, had not yet completed the Month 6 Visit and therefore did not yet have enough follow-up for a TI prediction.

In the Phase 1/2 studies, 15 of 22 patients (68.2%; 95% CI of 45.1% to 86.1%) met the definition of TI at any time, including during long term follow up in LTF-303. Patients in these studies received DP with lower median % transduced cells and VCN, resulting in lower median HbA^{T87Q}, than was observed in Phase 3 studies (Table 4). Phase 1/2 patients who achieved TI were on the higher end of the study ranges for VCN and HbA^{T87Q}, and were predominantly of non-β^0/β^0 genotype, who were all producing some endogenous HbA. In the Phase 1/2 studies, 12/14 patients with a non-β^0/β^0 genotype (85.7%) achieved TI at any time whereas 3/8 patients with a β^0/β^0 genotype (37.5%) achieved TI at any time. DP manufacturing improvements in transduction efficiency in the Phase 3 studies led to consistently higher HbA^{T87Q} production, which supported robust total Hb even in the absence of appreciable endogenous Hb.

Overall, across all studies, 47/58 (81.0%) of patients who were evaluable for TI achieved TI. For all patients who achieved TI, total Hb levels were maintained, and most had total Hb at normal or near-normal levels. In Phase 3, 35/39 (89.7%) patients have either achieved TI (N = 32) or are predicted to achieve TI (N = 3). Furthermore, achievement of TI appeared to be independent of age, sex, race, and *HBB* genotype in Phase 3. All patients who achieved TI maintain their TI status through last follow-up.

5.4.1. TI Characteristics

The results presented above in Figure 5 and below in Table 10 support stable and durable achievement of TI, indicating normal or near normal total Hb levels, in the majority of patients treated with beti-cel in the Phase 3 studies.

Table 10. Characteristics of the Patients Who Achieved TI in the Phase 3 Studies

Parameter	Statistics	HGB-207 N = 20	HGB-212 N = 12	Phase 3 N = 32
Duration of TI by Kaplan Meier[a], months	N	20	12	32
	Median	-	-	-
	Min, max	15.7+, 39.4+	12.5+, 32.8+	12.5+, 39.4+
Observed duration of TI, months	Median	26.99	20.65	25.66
	Min, max	15.7, 39.4	12.5, 32.8	12.5, 39.4
Weighted average Hb during TI, g/dL	Median	11.77	10.20	11.52
	Min, max	9.7, 13.0	9.3, 13.7	9.3, 13.7
Time from DPI to last RBC transfusion prior to TI, months	Median	0.87	0.82	0.84
	Min, max	0.5, 2.4	0.0, 1.9	0.0, 2.4
Time from DPI to achievement of TI, months	Median	15.39	15.67	15.66
	Min, max	14.8, 19.4	14.8, 24.5	14.8, 24.5

DPI, drug product infusion; Hb, hemoglobin; RBC, red blood cell; TI, transfusion independence.
a. If TI was maintained through all Hb assessments, the duration of TI will be censored at the last Hb assessment date. The + sign indicates a value is censored. The duration of TI begins with t_0 (the time when Hb is first ≥ 9 g/dL with no transfusions in the preceding 60 days) and ends at the time point when patient receives a chronic RBC transfusion or the weighted average Hb falls below 9 g/dL, whichever is earlier. When TI is censored, the true duration of TI is unobserved and is longer than the duration from t_0 to the last Hb assessment. The median duration of TI is not estimable using the Kaplan Meier method as all duration of TI are censored.

The time from beti-cel infusion to last RBC transfusion was relatively short, with most patients stopping RBC transfusions < 1 month after DP infusion. All 32 patients maintained TI through last follow-up, for up to 4 years in the Phase 3 studies.

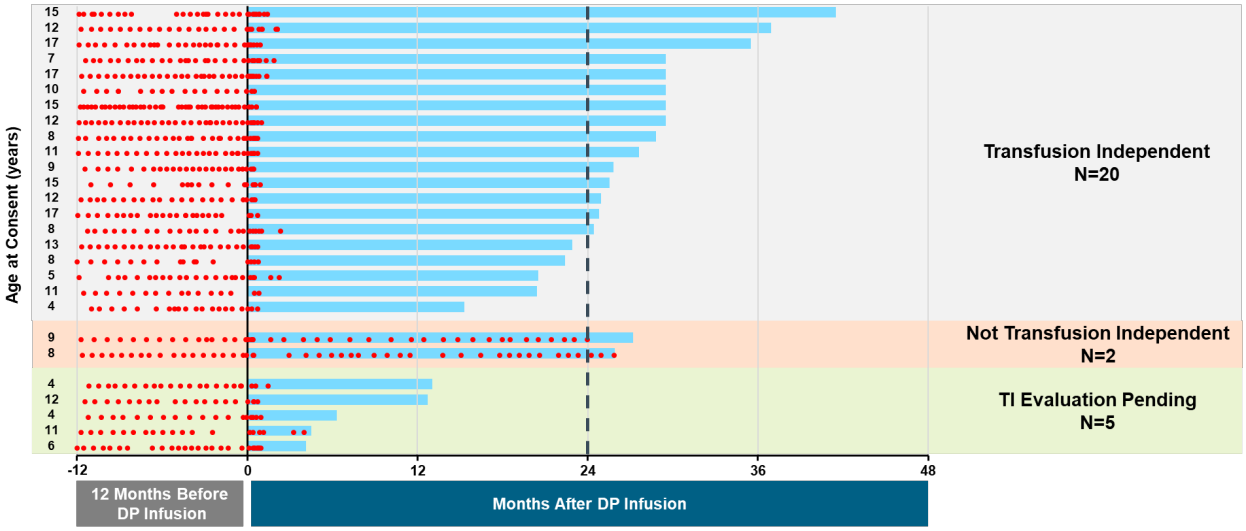
Fifteen patients achieved TI after treatment with beti-cel in the Phase 1/2 studies. The time from DP infusion to last RBC transfusion was also relatively short for most of these patients (median [min, max] was 1.81 [0.2, 27.7] months); however, the range was wider in the Phase 1/2 studies due to the 3 patients who achieved TI after completing 24 months in their parent study. All 15 patients in Phase 1/2 studies who achieved TI at any time maintained their TI status through all Hb assessments, with median (min, max) weighted average Hb during TI of 10.34 (9.1, 13.2) g/dL. The median (min, max) observed duration of TI for these 15 patients was 57.07 (15.8, 84.1) months.

These results overall indicate that TI status once achieved is durable (maximum length of TI maintenance to date is 7 years). The freedom from RBC transfusions is expected to be lifelong.

5.4.1.1. TI Characteristics for Pediatric Patients

Like Figure 5 above, Figure 6 demonstrates pediatric patient transfusion needs before and after treatment with beti-cel in the Phase 3 studies, with duration of follow-up presented with a blue bar and RBC transfusions presented with red dots. Figure 6 is specific for patients treated in the Phase 3 studies who were < 18 years of age at the time of consent/assent.

Figure 6. Transfusion Status in Phase 3 Pediatric Patients



Hb, hemoglobin; RBC, red blood cell; TI, transfusion independence.

In the Phase 3 studies, 20/22 (90.9%) TI-evaluable pediatric patients achieved TI and all maintained TI status through last follow-up. All 10 pediatric patients aged ≥ 12 to < 18 years were TI-evaluable and achieved TI. These 10 patients had a median (min, max) weighted average Hb during TI of 11.67 (9.6, 13.2) g/dL, median (min, max) time from DP infusion to last RBC transfusion of 0.95 (0.6, 2.2) months, and median (min, max) observed duration of ongoing TI of 26.15 (19.4, 37.9) months.

For pediatric patients < 12 years of age in the Phase 3 studies, 10/12 TI-evaluable patients achieved TI, with a median (min, max) weighted average Hb during TI of 10.0 (9.7, 11.5) g/dL,

median (min, max) time from DP infusion to last RBC transfusion of 0.80 (0.5, 2.4) months, and median (min, max) observed duration of ongoing TI of 20.34 (12.5, 26.1) months.

There were 5 pediatric patients overall not yet evaluable for TI; 3 of these patients are predicted to achieve TI and the remaining 2 patients did not have enough follow-up for a TI prediction.

As noted below (Section 5.4.3), total Hb levels for children < 12 years of age are normally lower than those for adults, so it is not unexpected that the patients < 12 years of age in the Phase 3 studies would have lower weighted average Hb levels during TI compared with the adult patients.

Overall, these data indicate that pediatric patients were able to achieve TI (with normal or near-normal levels of total Hb) at similar rates as patients ≥ 18 years of age after treatment with beti-cel in the Phase 3 studies, with similar times from DP infusion to last RBC transfusion: median (min, max) of 0.87 (0, 1.5) months for ≥ 18 years, N = 12; 0.84 (0.5, 2.4) months for <18 years, N = 20; 0.95 (0.6, 2.2) months for ≥ 12 years to < 18 years, N=10; and 0.80 (0.5, 2.4) months for < 12 years, N = 10.

5.4.2. HbA^{T87Q} by TI Status and Demographic Subgroups

To investigate whether *HBB* genotype, age, sex, and race impacted HbA^{T87Q} levels after beti-cel treatment, HbA^{T87Q} levels were summarized for TI patients and all patients treated in the Phase 3 studies at Month 24, separated into demographic subgroups (Table 11). Regardless of age, sex, race, and *HBB* genotype, patients with TDT treated with beti-cel were able to produce similar amounts of HbA^{T87Q}.

Table 11. HbA^{T87Q} by TI Status and Demographic Subgroups

Subgroup		Statistic	HbA^{T87Q} at Month 24, g/dL	
			TI Patients N = 32	Overall N = 41
Overall		N	26	30
		Median	9.137	8.752
		Min, Max	5.01, 12.43	0.34, 12.43
HBB genotype	β^0/β^0	N	5	6
		Median	9.441	9.087
		Min, Max	7.86, 12.43	0.71, 12.43
	Non-β^0/β^0	N	21	24
		Median	8.892	8.752
		Min, Max	5.01, 11.64	0.34, 11.64
Age, years[a]	≥ 18	N	11	13
		Median	9.381	8.892
		Min, Max	6.27, 12.43	0.89, 12.43
	< 18	N	15	17
		Median	8.739	8.732
		Min, Max	5.01, 10.83	0.34, 10.83
	≥ 12 to < 18	N	9	9
		Median	9.441	9.441
		Min, Max	8.42, 10.83	8.42, 10.83
	≥ 12	N	20	22

Subgroup		Statistic	HbA^{T87Q} at Month 24, g/dL	
			TI Patients N = 32	Overall N = 41
		Median	9.414	9.384
		Min, Max	6.27, 12.43	0.89, 12.43
	< 12	N	6	8
		Median	8.418	7.833
		Min, Max	5.01, 10.21	0.34, 10.21
Sex	Male	N	15	16
		Median	9.441	9.414
		Min, Max	5.01, 12.43	0.71, 12.43
	Female	N	11	14
		Median	8.739	8.728
		Min, Max	7.86, 11.40	0.34, 11.40
Race[b]	Asian	N	11	12
		Median	8.766	8.752
		Min, Max	6.27, 12.43	0.89, 12.43
	White	N	14	16
		Median	9.414	9.384
		Min, Max	7.41, 11.64	0.71, 11.64

TI, transfusion independence; TP, Transplant population.
HbA^{T87Q} = ratio of $\beta^{A\text{-}T87Q}$-globin to all β-like-globins multiplied by the total Hb.
a. Age at consent or date of assent applicable for patients < 18 years old.
b. The Other and Not reported race subgroups are excluded from this table because there are too few patients in those groups to support meaningful summary analyses.

5.4.3. Unsupported Total Hb by TI Status and Demographic Subgroup

Values for normal or near-normal levels of Hb are dependent on both age, and, in adults, sex. As presented in Table 9, there were no meaningful differences in achieving TI between these subgroups, or subgroups for *HBB* genotype or race. To investigate whether these demographic variables impacted total Hb levels after beti-cel treatment, unsupported total Hb levels were summarized for TI patients and all patients treated in the Phase 3 studies (Table 12). Note that the lower limit of normal total Hb for adult males (13.5 g/dL), females (12.0 g/dL), and children aged 2-12 years (11.5 g/dL) are defined separately based on published values from the literature (Kratz et al. 2004; Janus and Moerschel 2010).

As expected, median unsupported total Hb levels were lower in pediatric patients than adult patients, which aligns with the differences in total Hb levels observed for normal adults and children. Overall, however, although medians varied when patients were separated into subgroups based on *HBB* genotype, age, sex, and race, the unsupported total Hb ranges overlapped across these subgroups, indicating minimal differences in unsupported total Hb levels across these subgroups (Table 12).

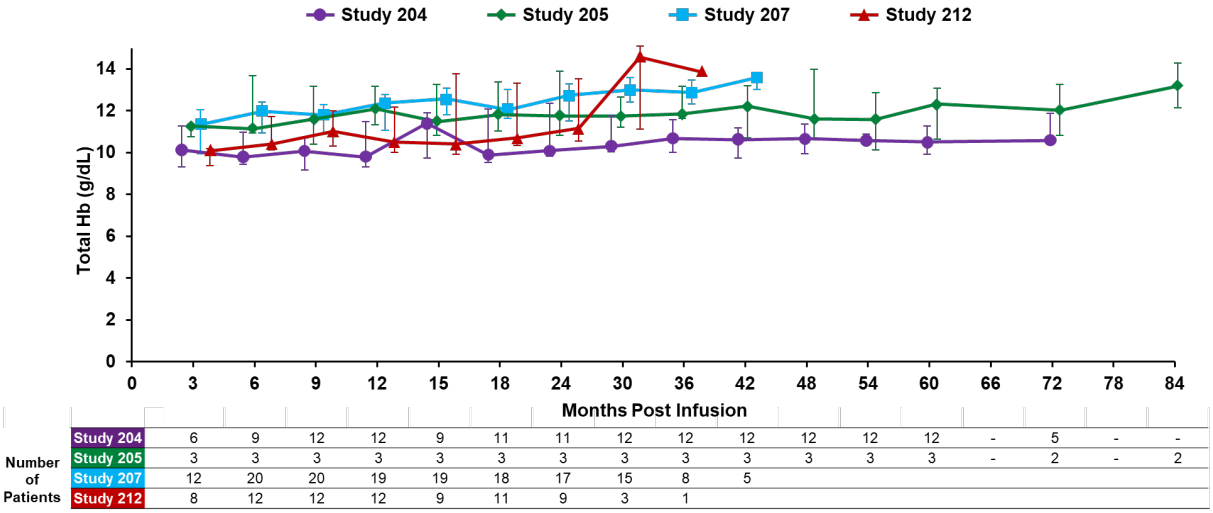
Table 12. Unsupported Total Hemoglobin at Month 24 in the Phase 3 Studies by TI Status (TP)

Subgroup		Statistic	Unsupported Total Hb at Month 24, g/dL	
			TI Patients N = 32	Overall N = 41
Overall		N	26	27
		Median	11.90	11.80
		Min, Max	9.5, 14.0	7.9, 14.0
HBB genotype	β^0/β^0	N	6	6
		Median	10.53	10.53
		Min, Max	9.7, 14.0	9.7, 14.0
	Non-β^0/β^0	N	20	21
		Median	12.48	12.45
		Min, Max	9.5, 13.5	7.9, 13.5
Age, years[a]	≥ 18	N	10	11
		Median	12.95	12.70
		Min, Max	9.7, 14.0	7.9, 14.0
	< 18	N	16	16
		Median	11.10	11.10
		Min, Max	9.5, 13.3	9.5, 13.3
	≥ 12 to < 18	N	10	10
		Median	11.75	11.75
		Min, Max	9.7, 13.3	9.7, 13.3
	≥ 12	N	20	21
		Median	12.55	12.50
		Min, Max	9.7, 14.0	7.9, 14.0
	< 12	N	6	6
		Median	10.55	10.55
		Min, Max	9.5, 11.8	9.5, 11.8
Sex	Male	N	16	16
		Median	11.90	11.90
		Min, Max	9.5, 14.0	9.5, 14.0
	Female	N	10	11
		Median	11.90	11.30
		Min, Max	9.7, 13.3	7.9, 13.3
Race[b]	Asian	N	11	11
		Median	12.00	12.00
		Min, Max	9.5, 14.0	9.5, 14.0
	White	N	14	15
		Median	12.13	11.80
		Min, Max	9.7, 13.5	7.9, 13.5

Hb, hemoglobin; RBC, packed red blood cells; TI, transfusion independence; TP, Transplant population.
Unsupported total Hb level is defined as the total Hb measurement level without any acute or chronic RBC transfusions within 60 days prior to the measurement date.
a. Age at consent or date of assent applicable for patients < 18 years old.
b. The Other and Not reported race subgroups are excluded from this table because there are too few patients in those groups to support meaningful summary analyses.

For the patients who achieved TI in any of the beti-cel studies, unsupported total Hb levels were stable and durable. Summary data showing unsupported total Hb over time for patients who achieved TI are shown by study in Figure 7.

Figure 7. Median Unsupported Total Hemoglobin Over Time in Patients Who Achieved TI by Parent Study



Hb, hemoglobin; RBC, red blood cells.
The markers represent the medians. The bars represent the interquartile ranges.
Unsupported total Hb level is defined as the total Hb measurement level without any acute or chronic RBC transfusions within 60 days prior to the measurement date.

The proportions of patients who had unsupported total Hb of < 9 g/dL, ≥ 9 g/dL, ≥ 10 g/dL, ≥ 11 g/dL, ≥ 12 g/dL, or ≥ 13 g/dL post-treatment were assessed for all patients who did not have any acute or chronic RBC transfusions within 60 days prior to the measurement date (i.e., unsupported). Table 13 presents these data at Month 6 by study phase for the patients who achieved TI.

Table 13. Proportion of TI Patients with Unsupported Total Hemoglobin Levels < 9 g/dL, ≥ 9 g/dL, ≥ 10 g/dL, ≥ 11 g/dL, ≥ 12 g/dL, or ≥ 13 g/dL g/dL at Month 6 (TP; All Studies)

		Phase 1/2	Phase 3
Parameter	Statistic	TI Patients N = 15	TI Patients N = 32
< 9 g/dL[a]	n/N (%)	2/12 (16.7)	1/32 (3.1)
≥ 9 g/dL	n/N (%)	10/12 (83.3)	31/32 (96.9)
≥ 10 g/dL	n/N (%)	6/12 (50.0)	24/32 (75.0)
≥ 11 g/dL	n/N (%)	2/12 (16.7)	18/32 (56.3)
≥ 12 g/dL	n/N (%)	2/12 (16.7)	10/32 (31.3)
≥ 13 g/dL	n/N (%)	2/12 (16.7)	2/32 (6.3)

Hb, hemoglobin; RBC, red blood cells.
Unsupported total Hb level is defined as the scheduled total Hb measurement level without any acute or chronic RBC transfusions within 60 days prior to the measurement date.
Denominator is number of patients who had unsupported total Hb at Month 6.
a. Although the Month 6 unsupported total Hb happen to be < 9 g/dL, the patients achieved TI with weighted average total Hb ≥9 g/dL with longer follow-up.

Maintaining an unsupported total Hb level ≥ 9 g/dL eliminates the need for regular transfusions and thereby limits the iron burden associated with transfusions. Higher Hb levels are expected to further reduce complications of thalassemia (Cappellini et al. 2021).

Of the 32 patients who achieved TI in a Phase 3 study, all had unsupported total Hb assessments at Month 6. Eighteen of 32 (56.3%) patients had an unsupported total Hb measurement ≥ 11 g/dL at Month 6, and therefore had near-normal or normal total Hb levels. Similar observations were made at Month 24, where 17 out of 26 (65.4%) TI patients with unsupported total Hb values at Month 24 had unsupported total Hb ≥ 11 g/dL at that time.

5.5. Secondary Efficacy Endpoints

Iron toxicity leading to organ damage in TDT results from both increased absorption of dietary iron due to dyserythropoiesis, as well as iron derived from RBC transfusions. A decrease in RBC transfusion requirements, if coupled with improved erythropoiesis, would be expected to slow or stop additional iron accumulation. A reduction in iron accumulation along with the use of iron removal therapy would be expected to result in decreases in iron burden over time, which would benefit patients by reducing iron toxicity. Many patients who stop receiving RBC transfusions after beti-cel treatment are eventually able to stop using iron removal therapy and have decreased iron burden over time.

5.5.1. Iron Removal Therapy

Patients with TDT require iron chelation therapy to prevent/mitigate iron accumulation caused by both increased iron absorption (iron avidity) as well as chronic RBC transfusions. All patients were therefore undergoing iron chelation therapy at screening in the parent study. Results from a recent report (Chaudhury et al. 2017) suggest that for patients with abnormal iron burden who are treated with allo-HSCT, liver iron levels may take multiple years to reach near-normal levels after transplant. Until this occurs, patients are recommended to remain on iron removal therapy.

After beti-cel infusion, patient iron removal therapy was managed at physician discretion. In the Phase 3 studies, 20/32 patients (62.5%) who achieved TI had stopped iron chelation therapy for at least 6 months as of last follow-up, with similar proportions in HGB-207 and HGB-212. The median stopped (min, max) time from DP infusion or last chelation to last follow-up for the 20 patients is 27.32 (6.0, 47.3) months. Twelve of these patients did not restart chelation after beti-cel infusion. The remaining 8 patients restarted and then stopped iron chelation, with a median time from last iron chelation use to last follow-up of 14.88 (6.0, 23.4) months. Of the 32 patients who achieved TI, 8 patients (7 patients in HGB-207 and 1 patient in HGB-212) received phlebotomy to remove iron.

Of the 4 patients treated in the Phase 3 studies who had not achieved TI, 1 patient had stopped iron chelation therapy. This patient did not restart iron chelation therapy after beti-cel treatment.

Of the 15 patients treated in the Phase 1/2 studies who achieved TI, all patients restarted iron chelation after beti-cel infusion. Ten (66.7%) of these patients subsequently discontinued chelation therapy, with a median time from last iron chelation use to last follow-up of 25.03 (12.0, 56.4) months. Of the 15 patients who achieved TI, 3 patients (20.0%) received phlebotomy to remove iron.

Across all the beti-cel clinical studies, a greater proportion of TI patients had stopped iron chelation therapy for at least 6 months (30/47; 63.8%) compared with non-TI patients (2/11; 18.2%). Overall, the 30 patients who achieved TI and stopped iron chelation therapy for at least 6 months, including 12 patients who did not restart iron chelation therapy after beti-cel infusion, were off chelation therapy for a median (min, max) of 27.06 (6.0, 56.4) months. These data overall indicate that many patients who achieved TI were also able to durably stop iron chelation therapy.

5.5.2. Iron Burden

For those patients who are no longer receiving RBC transfusions, their iron burden is expected to decrease over time, particularly for patients who use iron removal therapy, and may decrease to levels at which iron removal therapy is no longer needed.

Organ damage typically does not occur below a liver iron concentration (LIC) of 7 mg/g (Cappellini et al. 2021). Among Phase 3 patients who achieved TI and had measurable LIC, 21/32 patients (65.6%) had LIC values below 7 mg/g at baseline and 22/30 patients (73.3%) had LIC values below 7 mg/g at last follow-up. Thus, most patients who achieved TI in the Phase 3 studies had LIC < 7 mg/g at baseline and at last follow-up. Note that, for patients with abnormal iron burden who are treated with allo-HSCT, it can take multiple years after allo-HSCT for liver iron levels to reach near-normal levels (Chaudhury et al. 2017). The patients who achieved TI

after treatment with beti-cel in the Phase 1/2 studies had a longer duration of follow-up than those who achieved TI in the Phase 3 studies, so their data were also assessed for changes in iron burden over time. Across all beti-cel studies, of the patients who achieved TI and had available LIC data, 28/47 patients (59.6%) had LIC < 7 mg/g at baseline and 33/45 patients (73.3%) had LIC < 7 mg/g at last follow-up, showing that the percentage of TI patients who had LIC below 7 mg/g increased over time.

Among the patients who did not achieve TI at any time in the beti-cel clinical studies, 6/11 patients (54.5%) had LIC < 7 mg/g at baseline and 7/11 patients (63.6%) had LIC < 7 mg/g at last follow-up.

Most treated patients, regardless of TI status or study phase, had cardiac T2* values > 20 msec throughout the studies. The proportion of patients who had cardiac T2* > 20 msec was 59/63 (93.7%) at baseline, 45/51 (88.2%) at Month 12, 46/49 (93.9%) at Month 24, and 54/57 (94.7%) at last follow-up.

Serum ferritin, which carries iron in the blood and has traditionally been considered a marker of iron burden, was collected at every scheduled visit throughout these studies. However, because ferritin is an acute phase reactant and its levels can fluctuate due to infection, inflammation, and/or diet, serum ferritin levels do not always correlate with iron accumulation in organs and thus is not considered a robust measure of iron burden. However, ferritin is a good screening test and clinically significant iron overload (LIC > 7 mg/g) is uncommon in patients with serum ferritin levels of less than 1000 ng/mL (Majhail et al. 2008), which corresponds to the SI units of less than 2247 pmol/L. As expected, a transient increase in serum ferritin and LIC immediately after DP infusion was observed because of the disruption of normal iron homeostasis that is associated with HSCT procedures due to cell damage from the toxicity of the conditioning regimen (Majhail et al. 2008). Furthermore, patients were hypertransfused prior to conditioning, which releases marrow iron stores from accumulated dyserythropoietic cells. After an initial increase immediately following DP infusion, serum ferritin levels generally decreased over time in the clinical studies, regardless of TI status or study phase.

Few treated patients (10/62, 16.1%) had serum ferritin levels below 2247 pmol/L (1000 ng/mL) at Baseline, 6/41 patients in the Phase 3 studies and 4/21 patients in the Phase 1/2 studies. However, more than half (27/52, 51.9%) of patients were below the 2247 pmol/L threshold at Month 24, 16/30 patients in the Phase 3 studies and 11/22 patients in the Phase 1/2 studies. In addition, the majority of patients (15/23, 65.2%) were below the 2247 pmol/L threshold at Month 48, 15/22 patients in the Phase 1/2 studies and 0/1 patient in the Phase 3 studies.

5.5.3. Health-Related Quality of Life

In long-term follow-up study LTF-303, 12 patients (HGB-204, N = 7; HGB-205, N = 4; HGB-207, N = 1) who reached Month 36 completed a brief bespoke questionnaire to assess the impact of beti-cel treatment on their quality of life. Nine patients maintained TI status and 3 patients remained non-TI at Month 36. All 12 beti-cel–treated patients were employed or able to seek employment compared with 5 (56%) prior to beti-cel infusion at that timepoint. A majority of beti-cel treated patients reported substantial improvements in school participation and physical activity, and no longer required thalassemia symptom management at Month 36. In addition, all

patients answered "Yes" to feeling that they had benefited from having undergone beti-cel treatment.

5.5.4. Exploratory Endpoint: Dyserythropoiesis

Dyserythropoiesis underlies much of the pathology in β-thalassemia. Dyserythropoiesis leads to marked erythroid hyperplasia in the bone marrow, with accumulation of erythroid precursors, and thus patients with β-thalassemia have extremely cellular bone marrow with a low myeloid to erythroid ratio. Patients with TDT also typically have elevated levels of erythropoietin (EPO) (Huang et al. 2019; Paubelle and Thomas 2019). If patients produce sufficient Hb in RBCs, erythropoiesis is anticipated to improve over time. Measures of dyserythropoiesis in the beti-cel clinical studies included assessment of the bone marrow (myeloid to erythroid ratio and bone marrow cellularity) and EPO (Phase 3 studies only), and numbers and/or proportion of immature erythroid cells (reticulocytes and nucleated RBCs) in relation to mature red blood cells in peripheral blood in all studies.

Patients with TDT generally have a myeloid to erythroid ratio substantially less than 1 and normal bone marrow is expected to have a myeloid to erythroid ratio equal to or greater than 1 (up to 3 or 4; (Origa 1993)). Myeloid to erythroid ratios increased from baseline for most patients who achieved TI in the Phase 3 studies, from a median (min, max) ratio of 0.45 (0.1, 4.1) at baseline (N = 30) to 0.77 (0.2, 1.9) at Month 12 (N = 30) and 0.91 (0.3, 2.0) at Month 24 (N = 23) with corresponding increases in the percentage of patients having ratios ≥ 1 of 16.7%, 23.3%, and 39.1%, respectively, indicating improved erythropoiesis.

The number of patients with normocellularity of bone marrow increased over time for both TI and non-TI patients in the Phase 3 studies. For the patients who achieved TI, 7/28 (25.0%) had normocellular bone marrow at baseline, increasing to 11/24 (45.8%) at Month 12, and 13/20 (65.0%) at Month 24, also indicating improved erythropoiesis.

The majority of Phase 3 patients who achieved TI following DP infusion also had decreases in EPO, indicating improved erythropoiesis. At baseline, TI patients in the Phase 3 studies had median (min, max) EPO levels of 29.30 (8.9, 258.5) units per liter (U/L) (N = 27), with 11/27 patients (40.7%) having EPO in the normal range. At Month 24, TI patients in the Phase 3 studies had median (min, max) EPO levels of 16.50 (7.1, 107.9) U/L (N = 23), and 14/23 patients (60.9%) had EPO in the normal range.

Nucleated RBCs and reticulocytes (non-nucleated immature RBCs) are late-stage progenitors in RBC development; the nucleus of an erythroid cell is typically extruded before the cell is released into the bloodstream (Dzierzak and Philipsen 2013). The detection of nucleated RBCs and higher than normal numbers of reticulocytes in peripheral blood suggests that there is a very high demand for the bone marrow to produce RBCs, and immature RBCs are being released into circulation, often in response to anemia or less commonly when there is marrow dysplasia. The percentage of Phase 3 patients who achieved TI with no nucleated RBCs in their peripheral blood increased over time, from 70.0% at baseline to 91.3% at Month 24. The percentage of Phase 3 TI patients with absolute reticulocyte counts within the normal range also increased over time from 38.7% at baseline to 87.5% at Month 24.

Overall, the results suggest that achieving TI after treatment with beti-cel may allow for improvements in erythropoiesis.

5.6. Efficacy Conclusions

Of the 41 patients treated with beti-cel in the pooled Phase 3 studies, 32/36 (88.9%; 95% CI: 73.9%, 96.9%) TI-evaluable patients achieved the primary endpoint of TI, establishing a robust efficacy response. Another 3 patients not yet TI-evaluable are predicted to achieve TI by end of their study based on Month 6 HbA^{T87Q} and/or total Hb levels. Based on the patient-population studied, no differences were observed in the ability to achieve TI in the Phase 3 studies regardless of age, sex, race, *HBB* genotype, or the quantity of endogenous Hb produced before treatment. Across the Phase 3 studies, 4 of the 36 TI-evaluable patients did not achieve TI, 2 patients in HGB-207 and 2 patients in HGB-212.

Although the study designs required minimum thresholds to meet primary endpoint success criteria, the observed proportion achieving TI well exceeded those success criteria thresholds:

- HGB-207 Cohort 1: 14/15 (93.3%; 95% CI: 68.1, 99.5%)
- HGB-207 Cohort 2: 6/7 (85.7%; 95% CI: 42.1, 99.6%)
- HGB-212: 12/14 (85.7%; 95% CI: 57.2, 98.2%)

For all patients who achieved TI, Hb levels were maintained, and most had Hb at normal or near normal levels for their age.

Data from the earlier studies support the Phase 3 results. In the Phase 1/2 Studies HGB-204 and HGB-205, conducted before optimization of DP manufacturing, 15/22 (68.2%) patients achieved TI, all of whom have maintained TI during long-term follow-up in LTF-303.

Within 6 months of beti-cel treatment, patients had a stable and durable level of expression of HbA^{T87Q} in cells of the erythroid lineage. For the patients who achieved TI in the beti-cel studies, TI status and total Hb levels were stable and durable.

Results from long-term follow-up study LTF-303 suggest that TI status once achieved is durable, through a maximum follow-up of 7 years for patients treated in the Phase 1/2 studies and 4 years for patients treated in the Phase 3 studies. Following achievement of TI after beti-cel treatment, freedom from chronic RBC transfusions is expected to be lifelong.

In the Phase 3 studies, 20/32 patients (62.5%) who achieved TI have stopped iron chelation therapy for at least 6 months. Of these 20 TI patients who were no longer on chelation therapy, 12 patients did not restart chelation after beti-cel infusion, and the remaining 8 patients restarted and then stopped iron chelation. The median stopped (min, max) time from DP infusion or last chelation to last follow-up for the 20 patients is 27.32 (6.0, 47.3) months. These data indicate that many patients who achieved TI were also able to durably stop iron chelation therapy.

Iron burden, measured as LIC, was below 7 mg/g, the level below which organ damage typically does not occur (Cappellini et al. 2021) for the majority of TI patients in the Phase 3 studies at both baseline (65.6% of patients) and last follow-up (73.3% of patients). When including the patients who achieved TI in the Phase 1/2 studies, who have longer duration of follow-up, 59.6%

of patients had LIC < 7 mg/g at Baseline and 73.3% had LIC < 7 mg/g at last follow-up, showing that the percentage of TI patients who had LIC below 7 mg/g increased over time.

Increases in myeloid to erythroid ratios from baseline to Month 24 were observed for most patients who achieved TI in the Phase 3 studies, indicating improved erythropoiesis. A return to normal cellularity of bone marrow and EPO levels, along with decreases in nucleated immature RBCs in the peripheral blood, also indicate improved erythropoiesis in beti-cel-treated patients.

In summary, data from the beti-cel clinical studies, particularly the Phase 3 studies, demonstrate that in the majority of eligible patients, one-time treatment with beti-cel can lead to lasting TI, durable normal or near-normal total Hb levels, improvements in disease parameters, reductions in iron concentration, and reduced use of iron chelation therapy.

6. SAFETY

Treatment with beti-cel was preceded by 1) mobilization with G-CSF and plerixafor, 2) apheresis, and 3) busulfan myeloablative conditioning. Since beti-cel infusion and the preceding procedures carry their own risks, the overall safety analysis included evaluation of data collected from signing of the ICF, through mobilization/apheresis (M) and conditioning (C), beti-cel infusion, and up to last follow-up. In addition, safety specific to beti-cel was established based on investigator attribution and Sponsor review.

The overall safety profile of the beti-cel regimen was dominated by adverse events associated with conditioning, with the main risks attributed to beti-cel itself being prolong time to platelet engraftment (PE) and infusion reactions. No cases of insertional oncogenesis have been reported to date.

6.1. Disposition

Two populations were used for the safety analysis in the clinical development program: the ITT and TP. The ITT comprised 66 patients who initiated mobilization (Table 14). Three of these patients discontinued after 1 cycle of mobilization due to non-safety related reasons. The TP consisted of the remaining 63 patients who completed mobilization, underwent conditioning, and received beti-cel.

As of the BLA data cut-off date, 51 patients in the TP completed their 2-year parent study and had enrolled into the long-term follow-up study, LTF-303.

Table 14. Disposition of Patients with TDT in the beti-cel Clinical Program (ITT)

Parameter	Patients With TDT N = 66
Patients Mobilized (ITT), n (%)	66 (100)
Patients Infused with Drug Product (TP), n (%)	63 (95.5)
Patients discontinued, n (%)	3 (4.5)
Reasons for discontinuation, n (%)	
Withdrawal of consent	1 (1.5)
Investigator decision	1 (1.5)
Other[a]	1 (1.5)
Patients Enrolled in LTF-303, n (%)	51 (77.3)

ITT, intent-to-treat population; LTF, long-term follow-up study; TDT, transfusion-dependent thalassemia; TP, transplant population.
a. 1 patient in HGB-207 discontinued prior to infusion with drug product due to pregnancy.

6.2. Duration of Follow-Up

Patients in the Transplant Population (TP; patients in the ITT population who undergo beti-cel infusion) were followed for a median (min, max) of 35.48 (4.1, 86.5) months after transplantation. Overall exposure, which accounts for patient number and follow-up time, was 221.1 patient-years. Eighteen (28.6%) had at least 5 years of follow-up.

6.3. Neutrophil and Platelet Engraftment

Extensive depletion of the hematopoietic system and resulting cytopenias are the intended effects of pre-transplant conditioning. Bone marrow recovery from these effects begins during the initial post-transplant period when the transplanted cells enter the marrow and re-establish normal blood cell production. This process is called engraftment.

6.3.1. Neutrophil Engraftment

Patients undergoing HSCT, in general, remain hospitalized until neutrophil engraftment (NE) is achieved, as per standard-of-care. NE was defined as the presence of 3 consecutive absolute neutrophil count (ANC) laboratory values of $\geq 0.5 \times 10^9$/L obtained on different days after a post-transplant value $< 0.5 \times 10^9$/L. The first of these days was defined as the day of NE. Given the morbidity associated with failure of, or marked delay in, neutrophil engraftment, the Sponsor pre-defined NE failure as failure to achieve NE by Day 43. Treatment with back-up cells was recommended if any patient did not engraft within the 6-week period.

In the beti-cel clinical trials, all 63 patients successfully achieved and maintained NE; no reports of secondary engraftment failure were received; thus no rescue cells were required. The median (min, max) time to NE was 23 (13, 39) days.

6.3.2. Platelet Engraftment and Recovery

The day of platelet engraftment (PE) was defined as the first day corresponding to 3 consecutive platelet values $\geq 20 \times 10^9$/L obtained on different days after a post-transplant value of $< 20 \times 10^9$/L, with no platelet transfusions administered for 7 days immediately preceding and during the evaluation period.

In the beti-cel clinical studies, all 63 patients treated with beti-cel successfully achieved PE, with a median (min, max) time to PE of Day 45 (19, 191). Further recovery was as follows: platelets reached $\geq 50 \times 10^9$/L (n = 61) on median (min, max) Day 58.0 (20, 268) and $\geq 100 \times 10^9$/L (n = 57) on Day 80.0 (20, 891). On or after Day 100, 10 of these 63 (15.9%) patients had platelets $< 50 \times 10^9$/L.

6.3.3. Contextualization of the Engraftment Data

There is very limited data on expected time to successful neutrophil and platelet engraftment in β-thalassemia patients undergoing autologous transplantation. Therefore, in order to contextualize the data on engraftment, historic data on β-thalassemia patients receiving allogeneic transplants are provided. Table 15 shows median Day of NE and PE from four published articles (allogeneic transplantation in β-thalassemia) juxtaposed to the corresponding data noted with beti-cel.

Table 15. Day of Neutrophil and Platelet Engraftment (Allo-HSCT and beti-cel)

Group/Reference, N	Day of Neutrophil Engraftment, Median (min, max)	Day of Platelet Engraftment, Median (min, max)
Allo-HSCT[a]		
(Bernardo et al. 2012), N = 60	20 (11, 30)	20 (11,36)
(Sellathamby et al. 2012), N = 102	-	28 (13, 154)
(Anurathapan et al. 2016), N = 31	14 (11, 18)	30 (20, 45)
(Sun et al. 2019), N = 48	13 (8, 31)	12 (8, 31)
beti-cel, N = 63		
	23 (13, 39)	45 (19, 191)

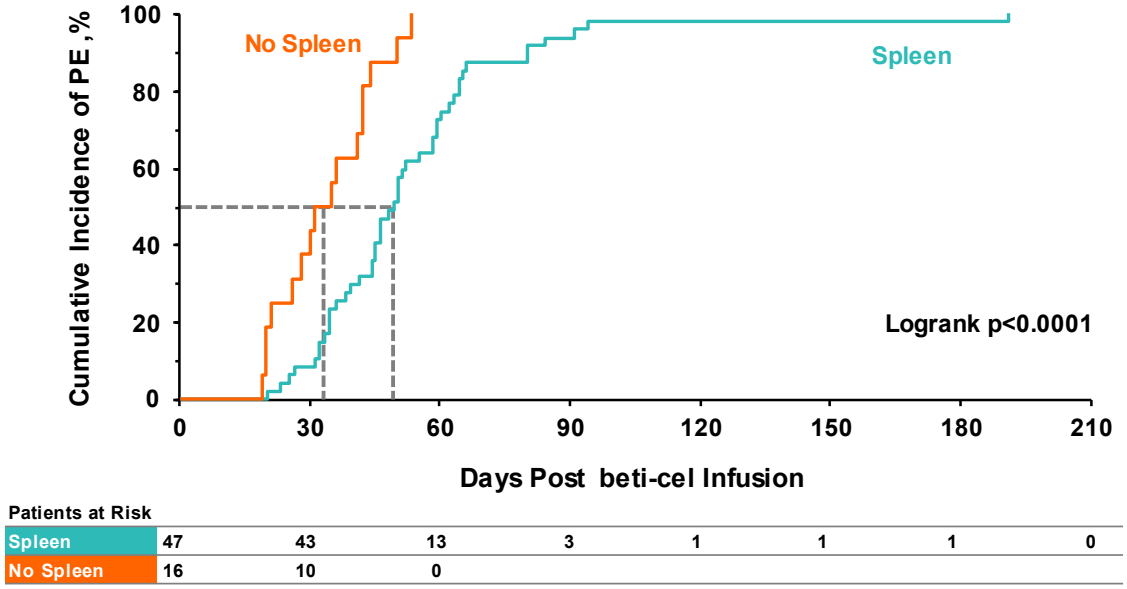
G-CSF, granulocyte-colony stimulating factor; HSCT, hematopoietic stem cell transplant.
a. Comparisons between allotransplant and beti-cel may be limited by factor including the donor source (bone marrow vs HSCTs, and use of G-CSF per institutional practices).

Overall engraftment times are longer with beti-cel compared to those seen with the allogeneic transplants (no formal statistical analyses were performed given the historical and retrospective nature of the allogenic data). This is particularly notable for platelet engraftment where the median time of 45 days was at least two weeks longer to that noted with allogeneic transplantation (12-30 days).

The specific mechanism of the slower platelet recovery is not clear. bluebird has conducted a number of analyses to identify potential intrinsic and extrinsic factors which may impact time to PE. A Cox proportional hazards model was utilized to identify covariates that impacted this endpoint. Of the myriad factors tested, the following showed a significant effect on time to PE: spleen status (splenectomy vs intact spleen), age, weight, baseline leukocyte count, and baseline platelet count. Of these, spleen status was determined to be the primary factor impacting platelet recovery as all of the other aforementioned covariates correlated with spleen status.

The effect of spleen status on PE is depicted graphically in Figure 8, a time to event plot which shows the cumulative incidence of PE along the y-axis vs time along the x-axis, stratified by spleen status. Patients without a spleen depicted in orange have a shorter median time to PE (33 days) versus those with an intact spleen shown in teal (49 days). Further, 20/21 (95%) patients with PE > 50 days, 12/12 (100%) patients with PE > 60 days and all 3 patients with PE > 90 days had an intact spleen.

Figure 8. Cumulative Incidence of Platelet Engraftment in Patients with Splenectomy vs Intact Spleen, %



PE, platelet engraftment.
Cumulative incidence of PE are obtained using the Kaplan-Meier method, where the event is PE. Patients who do not have PE are censored at their last contact date (all patients reached PE). The grey line represents median time to PE: for patients without a spleen = 33 days, for patients with spleen = 49 days.

Splenomegaly has been well described in patients with thalassemia, often seen in patients with advanced disease. In some cases, splenectomy is required to adequately control transfusion requirements and/or attendant clinical symptoms, such as abdominal pain (Mathews et al. 2009); in this citation, the authors specifically investigated outcomes of matched related allogeneic transplantation in patients with and without previous splenectomy (controlling for disease severity), and salient points from their observations include:

- Thalassemia patients with a previous splenectomy had a significantly shorter mean time to PE versus those with an intact spleen (22.5 vs 32.5 days, respectively).
- Previously splenectomized patients had worse overall survival. There was a significantly higher risk of peri-transplant infections associated with fatal outcomes in previously splenectomized patients (24% vs 5.3% fatal infections, splenectomy vs no splenectomy).

The findings of longer time to PE with an intact spleen corroborate the observations noted with beti-cel. However, the platelet engraftment times are longer with beti-cel in both splenectomized and non-splenectomized patients. It is unclear if this is reflective of platelet engraftment dynamics associated with autologous transplantation in thalassemia or is unique to beti-cel.

Therefore, as a conservative measure, bluebird has categorized delayed platelet engraftment as an important identified risk associated with beti-cel therapy.

While the main potential clinical consequence of slow platelet recovery is a propensity for bleeding and these AEs, as discussed in Section 6.4.3**,** there was 1 serious bleeding event in context of prolonged time to PE.

6.4. Adverse Events

6.4.1. Summary of Adverse Events

A high-level overview of the AE profile using the ITT population, which includes the three patients who never received DP, is shown in Table 16. Salient points include: 1) 98% of patients had at least one AE, with 11 out of 66 (16.7%) of patients having an AE deemed related to DP; 2) 53% of the patients (n=35) had at least one SAE; of these, 1 patient had an SAE deemed related to beti-cel; and 3) there were no AEs associated with fatal outcomes.

The overall AE profile associated with the beti-cel regimen needs to be interpreted in context of whole treatment paradigm which includes mobilization and apheresis, myeloablative conditioning, and DP infusion. Patients with AEs after DP infusion (Treatment Emergent Adverse Events; TEAEs) using a cutoff of 30% are shown in Table 17. Grade 3 or higher AEs noted in at least 30% of patients and treatment-emergent serious adverse events (2 or more patients) are shown in Table 18 respectively.

Adverse Event data with more granularity with regards to treatment time periods are provided for review in Section 11, Appendix C, Table 28. Time periods include (prior to mobilization, mobilization to conditioning, conditioning to neutrophil engraftment, and various cuts thereafter). Noteworthy observations include the following:

- AEs reported between mobilization and conditioning were typical of those noted with use of plerixafor and apheresis. These included, nausea, vomiting, catheter site pain, and thrombocytopenia.
- AEs following conditioning to NE were reflective of those noted with busulfan with greater than 50% of patients having cytopenias, febrile neutropenia, stomatitis, nausea and vomiting.
- The overall TEAE profile (Table 16) is dominated by the conditioning related events, as DP was administered shortly after myeloablation.
- The most frequently reported TESAE (Treatment Emergent Serious Adverse Events; TESAE) was (hepatic) veno-occlusive disease (noted in 7.9% of patients); further context is provided in Section 6.4.5. The only other TESAE noted in greater than 5% of patients was pyrexia.

Table 16. Adverse Events (ITT)

Patient Parameter	N = 66 n (%) E
≥ 1 AE	65 (98.5), 2255
≥ 1 Grade 3 AE	64 (97.0), 570
≥ 1 SAE	35 (53.0), 75
≥ 1 TEAE	63 (95.5), 1679
≥ 1 Grade 3 TEAE	63 (95.5), 530
≥ 1 TESAE	28 (42.4), 59
≥ 1 Grade 3 TESAE	20 (30.3), 44
≥ 1 AE related to beti-cel	11 (16.7), 20
≥ 1 Grade 3 AE related to beti-cel	3 (4.5), 3
≥ 1 SAE related to beti-cel	1 (1.5), 1
≥ 1 AE resulting in death	0 (0), 0

AE, adverse event; E, events; ITT, Intent-to-Treat; n, number of affected patients; N, total number of patients; SAE, serious adverse event; TE, treatment emergent.

Table 17. Treatment-Emergent Adverse Events in ≥ 30% of Patients, All Grades (TP)

Parameter Preferred Term	N = 63 n (%)
Patients with ≥ 1 TEAE	63 (100)
Thrombocytopenia	62 (98.4)
Anemia	51 (81.0)
Neutropenia	48 (76.2)
Stomatitis	45 (71.4)
Alopecia	39 (61.9)
Febrile neutropenia	32 (50.8)
Pyrexia	29 (46.0)
Vomiting	29 (46.0)
Epistaxis	27 (42.9)
Leukopenia	25 (39.7)
Abdominal pain	23 (36.5)
Diarrhea	21 (33.3)
Alanine aminotransferase increased	20 (31.7)

TEAE, treatment emergent adverse event; TP, Transplant Population.

Table 18. Treatment-Emergent Adverse Events in ≥ 30% of Patients, ≥ Grade 3 (TP)

Parameter Preferred Term	N = 63 n (%)
Patients with ≥ 1 ≥1 Grade 3 TEAE	63 (100)
Thrombocytopenia	61 (96.8)
Neutropenia	48 (76.2)
Anemia	47 (74.6)
Stomatitis	35 (55.6)
Febrile neutropenia	30 (47.6)
Leukopenia	24 (38.1)

TEAE, treatment emergent adverse event; TP, Transplant Population.

Table 19. Treatment-Emergent Serious Adverse Events in ≥ 2 Patients, Any Grade (TP)

Parameter Preferred Term	N = 63 n (%)
Patients with ≥ 1 treatment emergent SAE	28 (44.4)
Veno-occlusive liver disease	5 (7.9)
Pyrexia	4 (6.3)
Neutropenia	3 (4.8)
Thrombocytopenia	3 (4.8)
Appendicitis	2 (3.2)
Major depression	2 (3.2)
Stomatitis	2 (3.2)
Febrile neutropenia	2 (3.2)

SAE, serious adverse event; TP, Transplant Population.

The AEs and SAEs discussed above include all events regardless of attribution. Events deemed possibly related to beti-cel by the investigators are discussed below.

6.4.2. Drug Product-Related Adverse Events

AEs considered related to beti-cel by the investigator included 20 events that occurred in 11 of 63 patients in the TP (Table 20).

Table 20. Drug Product-Related Adverse Events by Grade (TP)

Parameter Preferred Term	N = 63 n (%)	Number of Events	Grade					
			1	2	3	4	5	SAE
Patients ≥ 1 DP-related event[a]	*11 (17.5)*	*20*	*12*	*5*	*3*	*0*	*0*	*1*
Thrombocytopenia	3 (4.8)	5	1	2	2	-	-	1
Leukopenia	1 (1.6)	1	-	1	-	-	-	-
Neutropenia	1 (1.6)	1	-	1	-	-	-	-
Abdominal pain[b]	5 (7.9)	5	4	1	-	-	-	-
Tachycardia[a]	1 (1.6)	1	1	-	-	-	-	-
Non-cardiac chest pain[b]	1 (1.6)	1	1	-	-	-	-	-
Dyspnea[b]	1 (1.6)	1	1	-	-	-	-	-
Hot flush[b]	1 (1.6)	2	2	-	-	-	-	-
Pain in extremity	1 (1.6)	1	1	-	-	-	-	-
Autoimmune disorder	1 (1.6)	1		-	1	-	-	-
Dysplasia	1 (1.6)	1	1	-	-	-	-	-

AE, adverse event; DP, drug product; SAE serious adverse event; TP, Transplant Population.
a. AEs or SAEs related to beti-cel per investigator assessment.
b. Infusion-related reactions.

One drug product-related SAE was reported:

- A serious event of Grade 3 Thrombocytopenia was reported in a 20-year-old female based on persistently low platelet counts (below 50×10^9/L) approximately 2 months after PE (Day 53). The event lasted a month and a half, and was considered resolved when her platelet counts reached 50×10^9/L. She had increased susceptibility to bruising, but no bleeding during the event.

The remaining 19 non-serious drug product-related events were mostly cytopenias and infusion-related events. Three patients had 6 non-serious events of thrombocytopenia, neutropenia, and leukopenia. Seven patients had 10 infusion-related events of abdominal pain, hot flush, dyspnea, tachycardia, and non-cardiac chest pain. These events occurred on the day of beti-cel infusion and were consistent with side effects of dimethylsulfoxide used for cryopreserving HSCs (Berz et al. 2007; Sanchez-Salinas et al. 2012; Shu et al. 2014; Brockbank 2016). Additionally, 1 event of pain in the extremity was recorded on Day 14 and thought to be coincident with engraftment and bone marrow recovery.

Two additional non-serious events were assessed by the investigator as possibly related to beti-cel and are described.

- A non-serious Grade 1 event of dysplasia was reported in a 16-year-old female in the HGB-204 study, based on a Month 24 bone marrow biopsy that documented mild dysplastic changes in the erythroid series. This patient did not achieve TI after treatment

with beti-cel and remained anemic. She had evidence of ongoing ineffective erythropoiesis based on findings of anemia despite normal marrow cellularity with a low myeloid: erythroid ratio. A follow-up bone marrow biopsy performed at Month 30 in this patient showed active trilineage hematopoiesis with maturation, no atypia and no blasts, and no dysplasia noted. A re-read of all these biopsy samples by an independent pathologist concluded that mild dysplastic-like changes in the erythroid series were present in both the Month 24 and Month 30 samples. The revised Month 30 report also concluded that the last sample (Month 30) is overall more cellular than the previous sample. ISA results up to Month 30 showed no clonal expansion, supporting the position that the erythroid dysplasia is a function of longstanding dyserythropoiesis.

Sponsor Assessment: Based on the review of the bone marrow findings in other patients, the dysplastic changes in the erythroid cells in this patient are consistent with dyserythropoiesis generally observed in other patients before and after beti-cel infusion. Details of bone marrow assessments in general are provided in Section 6.6.

- A non-serious Grade 3 event of autoimmune disorder (immune thrombocytopenia) was reported in a 21-year-old female in the HGB-212 study, based on a Month 24 laboratory finding of anti-platelet antibodies to glycoprotein IIb/IIIa in the context of ongoing Grade 1 thrombocytopenia (platelet counts 97 and 114 × 10^9 cells/L at the Month 24 visit). No immunosuppressive therapy was required for management. No bleeding events were reported.

  Sponsor Assessment: A clear causality between the anti-platelet antibodies and beti-cel therapy is not evident. Allo-antibodies and auto-antibodies to platelets have been described in heavily transfused patients (Lo Shyh-Chyi et al. 2005) *and immune cytopenia has been described following allogeneic transplantation* (Minchinton et al. 1984a, b; Minchinton and Waters 1985; Chapman et al. 1986; Wahid et al. 2002; Buxbaum and Pavletic 2020) *and rarely with autologous transplantation* (Matsuishi et al. 1990; Klumpp et al. 1992; Ashihara et al. 1993; Garcia Vela et al. 1994; Sivakumaran et al. 1995; Jillella et al. 2000).

6.4.3. Bleeding

As noted previously, the beti-cel treatment regimen was associated with a longer time to PE. To evaluate any attendant increasing bleeding tendency, all bleeding and serious bleeding events were evaluated in context of time to PE. Overall, bleeding events were noted in 40/63 (63.5%) patients in the transplant population. When segregated based on patients who achieved PE prior to and after the median time of 45 days, the proportion of patients with bleeding events was comparable (61.3% and 65.6%, respectively). Although these data are reassuring with regards to the bleeding risk associated with delays in PE, firm conclusions cannot be drawn given that bleeding was dominated by events proximate to conditioning in both groups.

Therefore, all serious bleeding events were further examined, and timing of such events assessed to note if there was any relation between delay in PE and the bleeding event. A total of 5 events were identified: 4 serious bleeding events and an SAE of hypotension which was a consequence of persistent nonserious epistaxis. Of these five:

- 2 events occurred prior to beti-cel therapy
- 3 events occurred after beti-cel therapy
  - An 18-year-old patient experienced bleeding secondary to trauma on Day 505 (PE on Day 44) and achievement of a normal platelet count on Day 99. The platelet count was normal at the time of this event.
  - A 20-year-old patient experienced serious hypotension on Day 11 to 12 (PE was on Day 45) and secondary hemorrhagic shock from ongoing non-serious (per investigator) Grade 3 epistaxis that started on Day 9. Heart rates were recorded as 150s beats/minute and blood pressures were around 70 – 100/40 – 50 mmHg. Estimated blood loss was approximately 1 liter. Laboratory test results included Hb 5.8 g/dL and platelets 20 × 109/L. The patient was transferred to the pediatric intensive care unit and treated with nasal packing, RBCs, platelet transfusions, IV fluids, fresh frozen plasma, vitamin K, and oxymetazoline. Following treatment, her condition rapidly improved. The bleeding occurred during the post-conditioning time period when thrombocytopenia is very common (Day 11).
  - The remaining patient had a serious bleed in context of delayed PE. A 5-year-old patient experienced a Grade 3 SAE of epistaxis on Day 69 in the setting of delayed PE (PE was Day 80). She was hospitalized, and the SAE resolved the next day following transfusions with RBCs and platelets. She achieved PE 10 days later (Day 80). Prior to the SAE, she had experienced Grade 1 (Day 23) and Grade 2 (Day 66) non-serious AEs of intermittent epistaxis, which were considered related to thrombocytopenia secondary to conditioning. Following PE, she has not experienced any further bleeding events. Her platelet count at last visit was 238 × 10^9/L (Day 617).

In summary, in the context of the clinical trials, major bleeding due to slow platelet recovery was only seen in 1 patient treated with beti-cel therapy. However, persistently low platelets do pose a potential risk for bleeding. Consequently, this information (prolonged time to PE) will be appropriately communicated to prescribers.

6.4.4. Cardiovascular Events

As noted in Table 28 cardiovascular events were not commonly reported in any of the treatment regimen periods. However, there was one serious report of congestive heart failure (CHF), which was deemed related to iron load and conditioning by the investigator. A brief summary is provided below.

- This was a 24-year-old patient who had 2 pretreatment assessments of cardiac T2* by MRI, both of which were below 20 msec, suggestive of iron overload (16.6 msec and 19 msec). Approximately 2 weeks after beti-cel treatment, she was noted to have pulmonary congestion on chest x-ray, and on Day 18 she developed dyspnea. An echocardiogram revealed an ejection fraction (EF) of 21% (no baseline available) and she was treated with supplemental oxygen, diuretics, angiotensin converting enzyme inhibition, and iron chelation, and was diagnosed with CHF. Over the next several weeks, the patient's clinical status improved, and her EF rose to 49%. A repeat

cardiac T2* was 15.4 msec. The event was downgraded to non-serious on Day 144 and was considered resolved approximately 1 year after treatment. The ejection fraction 9 months after onset of the CHF was 59%. The investigator attributed the event to conditioning.

6.4.5. Hepatobiliary Disorders

Adverse events of alanine transaminase (ALT) and/or bilirubin elevations were reported frequently in the ITT population (36.4% and 16.7% of patients, respectively [Table 28]). These were often reported post-conditioning and these observed frequencies are consistent with those in the busulfan label .

As noted previously in Section 6.4.1, the most commonly reported treatment emergent SAE was (hepatic) veno-occlusive disease (VOD), a well described complication of busulfan therapy. The US prescribing information notes a rate of 8% and further points out a relationship between busulfan AUC and risk of the event . In a prospective study of VOD rates in patients with β-thalassemia after allo-HSCT, VOD was observed in 54/521 (10.4%) patients (Lai et al. 2021).

In patients treated with beti-cel, serious VOD was seen in 5/63 (7.9%) patients and was assessed as Grade 3 or 4 in all patients. Onset dates ranged from Day 14 to Day 34. All events were assessed as related to conditioning with busulfan by the investigator. All affected patients were treated with defibrotide and recovered.

During the clinical development program, after 4/24 patients treated with beti-cel developed serious VOD, a protocol amendment was implemented to: 1) mandate VOD prophylaxis with ursodeoxycholic acid, 2) recommend a q6h conditioning regimen for children and adolescents to avoid higher peak concentrations of busulfan and 3) lower target AUC, based on publications linking busulfan AUC with VOD (Vassal et al. 1996; Dix et al. 1996). After these modifications, only 1 additional serious VOD event was reported (1/41), resulting in a total of 5/63 patients affected.

6.4.6. Infection Events

The infection related AEs were generally consistent with those seen with myeloablative conditioning. Notable points include the following:

- There were no infections with fatal outcomes.
- There were no reports of opportunistic infections.
- Seventeen patients (25.5%) had 23 serious infection events; six of the 23 were prior to drug administration. All 23 events resolved with the exception of the case of wild type HIV. This patient, who had 5 infection related SAEs is described below:
- A 20-year-old experienced a Grade 3 SAE of appendicitis from Day 9 to Day 21, and a Grade 3 SAE of diarrhea infectious from Day 396 to Day 399 attributed to food poisoning. At Month 24, he was diagnosed with an ongoing SAE of asymptomatic HIV infection that was confirmed by western blot assay to be a wild-type HIV-1 through detection of antibodies specific for HIV-1 proteins that are not encoded by BB305 LVV; a concurrent test for vector-derived replication competent lentivirus (RCL) using the

VSV-G assay did not detect RCL (Hongeng et al. 2021). This event is ongoing and has been updated to acquired immunodeficiency syndrome (AIDS). The patient experienced Grade 3 Salmonella bacteremia from Day 718 to Day 732 and Grade 3 Bacillus bacteremia from Day 829 to Day 849 attributed to food poisoning.

6.5. Potential Vector-Related Complications

6.5.1. Replication Competent Lentivirus

A concern for all lentiviral gene therapy products is the potential for the generation of RCL. Literature reviews show no published reports of vector-derived RCL detected in clinical studies using SIN LVVs (Cornetta et al. 2018).

No vector-derived RCL has been detected in any patient treated with beti-cel.

6.5.2. Integration Site Analysis

Upon transduction, LVVs integrate semi-randomly into the DNA of target cells; therefore, a potential risk exists for insertional oncogenesis with beti-cel treatment. After engraftment of transduced HSCs, a progenitor cell derived from a transduced HSC could undergo preferential expansion. This expansion may be without clinical consequences (benign clonal expansion) or result in malignancy (insertional oncogenesis, manifesting as myelodysplastic syndrome (MDS), leukemia, or lymphoma). The risk of insertional oncogenesis is limited to the hematopoietic cell compartment because the LVV proviral DNA only becomes integrated into the genomic DNA of hematopoietic stem and progenitor cells during DP manufacture.

ISA evaluates the polyclonality of the reconstituted hematopoietic system in those who receive beti-cel in clinical trials. ISA identifies the insertion sites (IS) present in a sample, and estimates their relative frequencies (RelFreq). In order to confirm >1 IS in the same cell, additional IS-specific analyses must be conducted on clonal populations like individual hematopoietic colonies. IS-specific analyses can also determine clonal contribution, i.e., the fraction of total cells that contain a specific IS, which can be helpful as part of a root cause investigation into clinical abnormalities, particularly when a clonal population is not available

Although ISA can be used to provide an indication of oligoclonality by assessing the relative frequency of individual IS, it cannot provide a determination of whether an IS that is present at high frequency is a representation of benign clonal expansion or is associated with malignancy. Hematologic assessments remain the standard means by which patients are evaluated for hematologic malignancy. Clinical data are required to inform treatment decisions. There are several reports of high frequency clones that have not resulted in adverse clinical consequences (De Ravin et al.; Negre et al. 2016; Thompson et al. 2018).

Patients are monitored routinely by ISA through 15 years post beti-cel treatment. There have been no reports of insertional oncogenesis in the beti-cel program.

After beti-cel treatment, ISA generally showed robust polyclonal reconstitution of the hematopoietic cell system, based on the identification of hundreds to thousands of unique mappable IS (UIS) in all patients, with the highest total number of UIS at any single time point ranging from 981 to 34857 per patient sample. Relative IS frequencies commonly fluctuate over time, and thus, individual IS may increase in frequency but then plateau or decline. This variance

likely reflects the dynamics of HSC clones and subsequent progenitors cycling through periods of expansion and quiescence in the bone marrow.

Oligoclonality: IS with a Relative Frequency ≥10%

bluebird bio has defined oligoclonality as having an IS with a RelFreq ≥10% and total VCN of ≥0.1 c/dg, with persistence met when this result is observed at 2 consecutive ISA evaluations. This definition determines the regulatory reporting criteria of persistent oligoclonality as required by FDA Guidance for Industry on Long Term Follow-Up After Administration of Human Gene Therapy Products (FDA 2020b) as described in Section 12, Appendix D.

Two patients treated with beti-cel met these criteria for persistent oligoclonality at their last timepoint. Details about these two patients are presented below and in Table 21. Additionally, another patient treated with beti-cel satisfied the criteria for oligoclonality for the first time at their last visit (Month 42), with a single IS having a RelFreq of ≥10% (see Figure 20 in Section 12, Appendix D).

Patient 204-14 showed expansion of a clone carrying at least 2 IS (one each in the CBFB and XPO7 genes) initially detected by non-quantitative [nr] LAM-PCR to peak at around 21% and 22% relative frequency at Month 36, and then detected at lower levels but still above 10% RelFreq when evaluated by S-EPTS/LM-PCR through Month 60 (see Figure 20 in Section 12, Appendix D).

Patient 204-13 showed several IS that were detected by non-quantitative [nr] LAM-PCR at relative frequencies of > 10% from Month 18 onwards, and one of these was also detected at >10% RelFreq by quantitative S-EPTS/LM-PCR through Month 60 (see Figure 20 in Section 12, Appendix D). The RelFreq of these IS appears to be stable over the past several visits. This pattern of expression is consistent with expansion of a clone that contains multiple IS, or with expansion of several clones to a similar extent.

At their recent follow up at Month 60 Visit, both patients continued to be transfusion independent, with unsupported total Hb levels of 10.9 g/dL and 10.1 g/dL respectively, and clinically well with no clinical or laboratory findings that suggest any developing or active malignancy. Both patients experienced delayed PE and continue to experience mild thrombocytopenia following treatment; however no other cytopenias are present that suggest a developing clonal malignant process. These patients will continue to be monitored per protocol, including continued ISA and CBC.

Table 21. Patients with Persistent Oligoclonality after beti-cel Treatment

Patient	Age at ICF (yrs)	Splenectomy	TI	NE (day)	PE (day)	Baseline Labs	Month 60 Labs
204-14	19	No	Yes	24	191	224 × 10^9/L	Hb: 10.9 g/L WBC: 4.9 × 10^9/L Plt: 138 × 10^9/L
204-13	31	No	Yes	18	91	159 × 10^9/L	Hb: 10.1 g/L WBC: 6.3 × 10^9/L Plt: 110 × 10^9/L

6.5.3. Malignancy

There have been no cases of malignancies (including myelodysplasia, leukemia, and lymphoma) in any patients treated with beti-cel (0/63, exact CI: 0 to 5.7%) across 221 patient-years of follow-up.

bluebird bio acknowledges that the risk window for malignancy after myeloablation and treatment with beti-cel may be longer than the current median follow-up of 3 years. However, with the 63 patients and adequate follow-up, there is 95% power to detect at least 1 malignancy if the underlying rate is 5% or greater.

Malignancies secondary to myeloablation and insertional oncogenesis as the result of beti-cel infusion remain potential risks that will require ongoing observation, to which bluebird bio is committed in the long-term follow-up Study LTF-303 and in the post-marketing registry REG-501.

Non-malignant Neoplasms

In order to retrieve any potential cases, all AEs and SAEs were reviewed to identify any other terms which might be suggestive of neoplasms. This search revealed the following: 1) case of lung nodule noted on Day 17 – this was in the presence of a lung infection and was noted to resolve; 2) a case of focal nodular hyperplasia in the liver; 3) a case of nonserious gall bladder polyp on Day 1294 which resolved on Day 1338 and 4) a case of an atypical placental nodule reported after the data cutoff. A brief narrative on the latter is provided below:

- A 33-year-old female patient experienced a serious, not related event of Placental polyp on Rel Day 2495; the patient had an early spontaneous miscarriage following in vitro fertilization where the patient had an unsuccessful embryo transfer. In anticipation of further *in vitro* fertilization, the patient underwent a hysteroscopy and curettage (b) (6) the pathology of which was consistent with an atypical placental site nodule. Per the pathologist report this carries a risk of 10-15% of malignant transformation. She underwent further imaging workup including an ultrasound and MRI and subsequently underwent a laparoscopy. However, at laparoscopy (b) (6) no visible lesion was noted in the uterus. Based on these findings the event has been deemed resolved/recovered by the Investigator and not related to beti-cel. The patient has been advised to undergo a hysterectomy procedure.

6.6. Bone Marrow Evaluations

Bone marrow samples were collected either routinely or based on investigator discretion across the TDT program as follows:

- Studies HGB-207 and HGB-212: Bone marrow biopsies at baseline, Month 12, and Month 24 were planned procedures for an exploratory objective (evaluation of dyserythropoieisis). The samples were read centrally and were not obtained for clinical decision making at the point of care. If required, additional assessment could be performed by the investigator locally.

- Studies HGB-204 and LTF-303: In study HGB-204, standard collections were not instituted, and bone marrow assessments were performed at Month 12 and Month 24 based on investigator judgment; these were read locally. LTF-303 is a long-term follow up study for patients enrolled in TDT studies and bone marrow biopsies are obtained at the discretion of the investigator.

It is important to interpret the morphologic abnormalities in these bone marrow samples in context of the physiological and pathological perturbations known to be associated with β-thalassemia. Normal red blood cell maturation – erythropoiesis – is a highly regulated process which results in the generation of approximately 200 billion red cells per day. The marked stress associated with abnormal hemoglobinization in β-thalassemia results in dyserythropoiesis manifested by expansion, and increased turnover, of erythroid precursors accompanied by enhanced apoptosis. The attendant morphological changes include (but are not limited to) morphologic dysplasia of cells in the erythroid lineage. (See Section 5.5.4 for additional information on dyserythropoiesis.)

Dysplasia was reported by central reviewers in several of the bone marrow samples from Studies HGB-207 and HGB-212. However due to certain limitations, such as variability amongst the pathologists in reporting dysplasia, bluebird bio arranged for all samples from the two pivotal Studies HGB-207, HGB-212, and available samples from Studies HGB-204 and LTF-303 to be read by either of two independent pathology experts. The overall conclusions were as follows:

- Erythroid lineage showed morphologic changes such as nuclear abnormalities and ring sideroblasts at baseline. The baseline bone marrow findings likely reflected stress erythropoiesis, the persistence of which after therapy at Month 12 and Month 24 was likely reflective of a heterogenous population of erythroid precursors owing to the fact that not all stem cells are transduced.
- There was no evidence of granulocyte dysplasia.
- Dysmegakaryopoiesis was seen at baseline in some samples, in particular in study HGB-212, and generally did not progress. Dysmeagarkyopoiesis was considered to be nonspecific.
- There was no evidence of MDS or emerging MDS.

6.7. Pregnancy

In the beti-cel clinical program, 5 pregnancies were reported of which 2 were reported in female partners of male patients. The pregnancies resulted in birth of 4 healthy babies (including 1 set of twins), 1 fetal death, and 1 ectopic pregnancy. Below are a few details about the 5 patients:

- A 22-year-old female patient discontinued from the study after mobilization due to pregnancy. She gave birth to a healthy baby.
- A 32-year-old female patient reported pregnancy post-in vitro fertilization in the LTF-303 study. The fetus was bradycardic and subsequently died. The fetal pole corresponded with a gestation of 6 weeks plus 1 day with no evidence of growth since the previous ultrasound. According to the investigator, the patient had experienced premature menopause as a consequence of conditioning and was required to undergo assisted

conception. This increased her chances of miscarriage when compared with an age-equivalent woman who can conceive naturally.

- A 35-year-old female reported pregnancy post-embryo transplant for in vitro fertilization. The outcome was an ectopic pregnancy.
- One male patient's female partner became pregnant from a banked sperm. The pregnancy resulted in birth of healthy twins.
- One male patient's female partner became pregnant although not as a result of banked sperm. The pregnancy resulted in a healthy baby at 37 weeks of gestation.

6.8. Subgroup Analyses

6.8.1. Safety by Genotype

As noted previously 43 patients in the program had a non-β^0/β^0 genotype, with remaining 20 being β^0/β^0 genotype. The former group had a median (min, max) follow-up of 36.86 (13.0, 86.5) months post-DP infusion and an overall exposure of 156.5 patient-years. Those with a β^0/β^0 genotype had a median (min, max) follow-up of 25.84 (4.1, 77.0) months and had an overall exposure of 64.6 patient-years.

Patients in the non-β^0/β^0 subgroup achieved NE 3 days earlier (median [min, max] Day 21.0 [13, 38]) than the those in the β^0/β^0 subgroup (median [min, max] Day 24.0 [14, 39]) and PE 5 days later (median [min, max] Day 46.0 [19, 191]) than their β^0/β^0 counterparts (median [min, max] Day 41.0 [25, 64]). These differences are not considered clinically significant.

The most common AEs by genotype are shown in Section 11, Appendix C, Table 29 and treatment emergent serious adverse event (TESAE) in ≥ 2 patients by genotype is shown in Section 11, Appendix C, Table 30. Overall, the adverse event profile of patients across the genotypes was comparable.

6.8.2. Safety by Age

Overall, no clinically significant differences by age were observed in patients treated with beti-cel. The safety data by age is stratified by those < 18 and ≥ 18 years.

Patients treated with beti-cel and ≥ 18 years of age (N = 31) had an overall follow-up of a median (min, max) of 59.17 (20.5, 86.5) months and exposure of 140.4 patient-years. Patients < 18 years of age (N = 32) had an overall follow-up of a median (min, max) of 26.55 (4.1, 83.5) months and an exposure of 80.7 patient-years.

Time to engraftment was longer in patients < 18 years of age (Table 22). Median times to NE and PE were 6 days and 12.5 days longer in the younger cohort, respectively. As shown in Table 22, the difference may be partially due to the fact that in the younger cohort, only 6.3% of the patients had a previous splenectomy, compared to 45.2% of the older counterparts. Otherwise the safety profiles between the two groups were comparable. The most common AEs by age are shown in Section 11, Appendix C, Table 31 and TESAEs in ≥ 2 patients by age is shown in Section 11, Appendix C, Table 32.

Table 22. Neutrophil and Platelet Engraftment by Age (TP)

Parameter	Statistic	< 18 N = 32	≥ 18 N = 31
Patients evaluable for NE Status	n	32	31
Success	n (%)	32 (100)	31 (100)
Failure	n (%)	0	0
Day of NE	n	32	31
	Median	26.0	20.0
	Min, max	16, 39	13, 30
Patients with PE	n (%)	32 (100)	31 (100)
Day of PE	n	32	31
	Median	50.5	38.0
	Min, max	20, 94	19, 191

NE, neutrophil engraftment; PE, platelet engraftment; TP, Transplant Population.

6.8.3. Long-Term Safety

Study LTF-303 is an ongoing multicenter, long-term safety and efficacy follow-up study for patients who have completed the parent studies in the beti-cel clinical program. These patients have follow-up assessments every 6 months through 5 years post-DP infusion and then at least annually through 15 years post-DP infusion.

As recommended in the 2020 FDA guidance for industry, "Long term follow-up after administration of human gene therapy products" (FDA 2020b), the following events are being captured:

- Any new malignancy
- Serious or non-serious immune-related AEs (e.g., autoimmune disorders, GVHD, opportunistic infections, HIV)
- New or worsening hematologic or neurologic disorders (as determined by the Investigator)
- All AEs assessed by the Investigator as at least possibly related to the drug product
- All SAEs regardless of relationship to the drug product (which are to be reported within 24 hours)

To date, a total of 51 TDT patients treated with beti-cel have been enrolled in Study LTF-303 and there have been no deaths, no drug product-related SAEs or AEs, and no AEs leading to study discontinuation. Eight patients experienced 12 SAEs through data cutoff; none were related to drug product (Table 20). Overall, the safety profile from this long-term follow-up did not have any safety concerns related to beti-cel.

Table 23. Serious Adverse Events in LTF-303

>M24 to M36 N = 5 (42%)	> M36 to M48 N = 2 (17%)	> M48 to M60 N = 2 (17%)	> M60 to 7 years N = 3 (25%)
Major depression	Gallbladder enlargement	Ectopic pregnancy	Diabetic ketoacidosis
Salmonella bacteremia	Gallbladder polyps	Gonadotropin deficiency	Pulmonary Embolism[a]
Bacillus bacteremia			Fetal death
Neutropenia			
Cholilethiasis			

a. Was ongoing at data cut-off, subsequently resolved.

Similar efforts to capture safety events post-marketing will be employed via a registry, the details of which are provided below along with bluebird bio's plan to only administer beti-cel at a limited number of highly trained Qualified Treatment Centers.

6.9. Pharmacovigilance Plan/Long-Term Safety and Post-Approval Monitoring

bluebird bio has developed post-marketing plans to enable continued characterization of the benefit/risk of beti-cel as well as monitoring for long-term efficacy and safety per FDA Guidance for Industry on Long Term Follow Up After Administration of Human Gene Therapy Products (FDA 2020b). The post-marketing monitoring will be multipronged. Patients treated with beti-cel in the clinical trials will continue to be followed in the ongoing clinical studies and in the long term follow up study LTF-303. In addition, a voluntary registry study, REG-501, is planned which will follow patients treated in the post-marketing setting for 15 years after receiving beti-cel. In the post marketing setting, beti-cel will only be distributed through a limited and targeted number of Qualified Treatment Centers to manage administration and ensure that the chain of identity is maintained.

6.9.1. LTF-303

Long-term Follow-up of Subjects with Transfusion Dependent β-thalassemia Who Were Treated with Betibeglogene Autotemcel

As described previously, patients treated in HGB-204, HGB-205, HGB-207 and HGB-212 are followed in the long-term follow-up study LTF-303 for a total of 15 years after beti-cel infusion, which includes 2 years in the parent study and an additional 13 years in LTF-303. Follow-up assessments are performed every 6 months through 5 years post-DP infusion and then annually from 5 years through 15 years post-DP infusion. Safety and efficacy evaluations are performed at each study visit including laboratory assessments (i.e., CBC and ISA) to monitor for hematopoietic reconstitution, clonal dynamics, and malignancy.

6.9.2. REG-501

A Prospective, Multicenter, Observational, Long-Term Safety and Effectiveness Registry Study of Patients with β-Thalassemia Treated with betibeglogene autotemcel

bluebird bio will initiate the REG-501 observational registry study in the US to characterize the safety and effectiveness of beti-cel in patients with β-thalassemia who require regular transfusions treated in the post-marketing setting. All patients treated with beti-cel in the 5-year enrollment period will be offered participation in REG-501. Clinical outcomes, gene-therapy specific data, and long-term safety outcomes (including malignancy) will be collected.

In REG-501, all enrolled patients will be followed for up to 15 years after infusion with beti-cel which will allow for collection of comprehensive and long-term safety and effectiveness data. REG-501 will collect baseline data, safety outcomes, and effectiveness outcomes as assessed during routine follow-up by healthcare providers (HCP).

Efforts will be made to support long term follow up in the registry. These efforts will include but are not limited to: education on participation in the registry study, outreach from the Qualified Treatment Center to patients and/or patient-identified follow-up care providers, patient-friendly registry study updates, and HCP-focused registry study updates.

Patients will also be given education on the importance of the registry, and Qualified Treatment Centers will be trained on registry education and enrollment.

The clinical management of each patient will be at the discretion of the healthcare provider; the registry study will record safety and effectiveness assessments in accordance with routine clinical care including at least annual CBCs. Gene therapy-specific laboratory assessments such as integration site analysis (ISA) will be offered at least annually in the context of routine blood draws and more often if requested in the context of relevant clinical workup. For all enrolled patients, the registry study will collect and report information on all SAEs, AEs of interest, and beti-cel related AEs. In addition, in the case of a newly diagnosed malignancy, bluebird bio will attempt to collect follow up samples including ISA as clinically feasible from patients regardless of enrollment in REG-501.

6.9.3. Qualified Treatment Centers

In the post-marketing setting, beti-cel will be made available only at Qualified Treatment Centers to manage administration and ensure that the chain of identity is maintained, given the complexity of autologous transplant. The Qualified Treatment Centers network includes clinical trial sites and sites with deep transplant and gene and cell therapy expertise. All planned Qualified Treatment Centers are Foundation for the Accreditation of Cellular Therapy (FACT) accredited.

A Qualified Treatment Center is a treatment center (including transplant center, apheresis collection center, and cell therapy lab) that has been qualified by bluebird bio to conduct specific activities related to the collection of cells, handling and administration of beti-cel. This will ensure chain of identity of the patient's cells and transfer of the patient's cells to the manufacturing site for DP manufacturing are conducted properly. Released DP will be shipped back to the treatment center for patient infusion. These Qualified Treatment Centers have been selected given their experience with disease management, stem cell transplant and, in most cases, have had experience with bluebird clinical trials. These Qualified Treatment Centers are well versed in follow-up post-transplant and will be further trained on the approved US prescribing

information and patient labeling, the reporting of AEs following treatment with beti-cel, and on participation in the post-marketing registry REG-501.

6.9.4. Labeling

Appropriate physician-directed labeling that includes warnings and precautions and FDA-approved patient labeling will be used to communicate safety concerns. In addition to FDA-approved labeling, HCP and patient educational material will also be employed. HCPs and patients will be educated on the benefits and risks of treatment with beti-cel, including efficacy outcomes and adverse reactions, warnings and precautions, and the importance of long-term follow-up, with a recommendation for at least annual blood work for 15 years post-treatment for monitoring of the potential development of malignancies.

HCP and patient-specific websites will be managed by the Sponsor to ensure beti-cel information is updated and readily available.

6.10. Safety Conclusions

beti-cel has been administered to 63 patients in the clinical trial setting and the totality of the data suggests the following:

- The peri-transplant adverse events were generally dominated by busulfan-based myeloablation, examples of which include hepatic veno-occlusive disease, and severe cytopenias. These will require careful patient monitoring. The deliberate roll out of beti-cel to Qualified Treatment Centers will help mitigate these risks as the prescribers will be well versed in identifying and managing these risks.
- beti-cel therapy was associated with prolonged time to platelet engraftment. It is unclear if this is specific to beti-cel or consistent with what would be expected for autologous transplantation in thalassemia, in general. bluebird bio will communicate this risk to the prescribers and facilitate appropriate education.
- Drug product related reactions were mostly nonserious and included cytopenias, and infusion related reactions that were mild and transient.
- To date no cases of insertional oncogenesis, or other malignancies, have been reported. bluebird bio remains committed to robust long term follow up of patients through the long-term follow-up study and the proposed post-marketing registry to capture any cases of malignancy which may develop during long-term follow-up.
- Besides malignancy, the aforementioned registry is designed to satisfy recommendations in the 2020 FDA guidance regarding capture of key safety events during long term follow up of gene therapy patients (FDA 2020b).

In conclusion, the safety profile of beti-cel supports a favorable benefit-risk for the product.

7. BENEFIT-RISK

7.1. Structured Benefit and Risks

Compelling and robust evidence for the clinical benefit of treatment with beti-cel in patients with β-thalassemia who require regular RBC transfusions was demonstrated in pivotal Studies HGB-207 and HGB-212 and supportive Studies HGB-204, HGB-205, and LTF-303. A structured benefit-risk assessment is presented in Table 24.

Table 24. beti-cel Structured Benefit and Risks

Dimension **Evidence and Uncertainties**	**Conclusions and Reasons**
Analysis of Condition • β-thalassemia requiring regular transfusions is a rare genetic disease. • Patients require lifelong, regular red blood cell transfusions for survival. • Without regular transfusions, patients typically experience significant morbidity and die from heart failure related to anemia in early childhood.	• β-thalassemia requiring regular transfusions is a rare and severe disease in both adult and pediatric patients.
• Unmet Medical Need • Standard of care is regular RBC transfusion with iron chelation therapy. • Transfusions are lifelong, time-consuming (required every 2 to 5 weeks), and associated with significant risks (iron overload and toxicity). • Iron overload results in cardiac, liver, and endocrine comorbidities (e.g., cardiac complications, osteoporosis, cirrhosis, hypothyroidism, diabetes) and shortened lifespan compared to the general population. • Iron chelation regimens are burdensome, may be associated with adverse reactions, and are not tolerated by all patients. Compliance is suboptimal. • Allo-HSCT is a potentially curative option usually limited to children with an HLA-matched donor (< 25% patents have an MSD).	• Even with optimal transfusion therapy, life expectancy and quality of life are reduced due to disease and treatment associated comorbidities. • β-thalassemia requiring regular transfusions has a poor prognosis and is incurable for the majority of patients. • There is significant unmet need for a curative treatment that is safe, effective, and not constrained by

Dimension Evidence and Uncertainties	Conclusions and Reasons
• Allo-HSCT is associated with significant risks of graft-versus-host disease, graft failure, and transplant-related mortality. Lymphodepletion during myeloablative conditioning and immunosuppression post-transplant are required even for those with matched donors. • Luspatercept-aamt is a chronic treatment option (administered by a healthcare professional once every 3 weeks) designed to reduce transfusion requirements. The burden of treatment is high, and most patients continue to require ongoing RBC transfusion therapy.	patient age, genotype, or donor availability. • Safe and effective new treatments are warranted.
Clinical Benefit • The primary endpoint was met statistically in 2 pivotal Phase 3 studies, with 88.9% (32/36) patients achieving TI, showing marked clinical benefit. • A majority of patients stopped receiving transfusions within one month of beti-cel infusion. • Durable weighted average hemoglobin levels during TI [median (min, max): 11.52 (9.3, 13.7) g/dL] over time and unsupported total Hb at Month 24 [median (min, max): 11.90 (9.5, 14.50) g/dL] were achieved for Phase 3 patients, with values approaching normal or near-normal levels in patients who achieved TI. • In a post-hoc analysis, all TI-evaluable Phase 3 patients treated with beti-cel that met the proposed commercial threshold for percentage of transduced cells achieved TI (N = 26). • All patients across all clinical studies with beti-cel who became TI have remained TI, with the longest duration of follow-up being 7 years. As HSCs are responsible for the production of RBCs for the lifespan of a patient, these genetically modified HSCs are expected to be durable and therefore TI is expected to be lifelong.	• beti-cel demonstrates compelling, consistent, and durable clinical efficacy in the majority patients of all genotypes and ages, across all studies (pivotal studies HGB-207 and HGB-212, and supportive studies HGB-204, HGB-205, and LTF-303). • beti-cel represents a new treatment paradigm and a major improvement in the treatment of these patients with high unmet medical need.

Dimension Evidence and Uncertainties	Conclusions and Reasons
• A majority of patients who achieved TI and were followed long-term had iron burden levels below the levels associated with clinically significant complications. • Over half of patients stopped iron chelation/phlebotomy therapy for at least 6 months. • Improvement in parameters of ineffective erythropoiesis was observed. • Patients who achieved TI reported improvements in health-related quality of life.	
Risks • 63 patients have been treated with beti-cel. Median follow-up is approximately 3 years, and at present, survival remains 100%, with no reports of GVHD. • All patients successfully engrafted, with median (min, max) times to neutrophil and platelet engraftment of 23 (13, 39) and 45 (19, 191) days, respectively. PE times are longer than those for patients with β-thalassemia undergoing allo-HSCT. The potential consequence of delayed platelet engraftment is bleeding. • Most of the safety events reported were dominated by the profiles of the agents commonly used for mobilization and myeloablation. These include: o Apheresis requires central vein cannulation with risks that are well described (bleeding, catheter sepsis, thrombosis related to indwelling catheters) o Common adverse events related to plerixafor include diarrhea, nausea, fatigue, injection site reactions, headache, arthralgias, dizziness, and vomiting (Mozobil 2021) o Myeloablation is associated with the risk of infection, especially in the short-term (within 6 weeks) until neutrophil engraftment is achieved. Overall, non-serious infections were reported in > 50% of patients, whereas serious infections were reported in 17.5%. However, there were no reports of infections due to	• The short-term safety profile of beti-cel is well characterized and consistent across clinical studies. • Safety events are manageable by appropriately trained health care providers. • Long-term risks such as insertional oncogenesis, RCL, or secondary malignancies will be further established in the 15-year long-term follow-up Study LTF-303 and post-marketing Registry (REG-501).

Dimension Evidence and Uncertainties	Conclusions and Reasons
opportunistic pathogens and no infections associated with fatal outcomes across all follow-ups o The treatment regimen may be associated with risks of bleeding due to myeloablation associated thrombocytopenia. Non-serious bleeding events were noted in > 50% of patients. There were 5 events (6.3%) of serious bleeds o Hepatic VOD is a risk associated with myeloablation with busulfan. The overall rate for VOD was 8%; the frequency decreased after VOD prophylaxis was implemented • Drug product-related AEs were mostly non-serious and included cytopenias and infusion-related events. One drug product-related SAE of thrombocytopenia was reported and has resolved. • beti-cel was generally well tolerated, with no deaths, hematologic malignancy or vector-related complications, including insertional oncogenesis, or RCL, observed. • Insertional oncogenesis is a safety concern associated with LVV gene therapy. BB305 LVV was designed to minimize any potential for insertional oncogenesis. No event of insertional oncogenesis was reported in patients treated with beti-cel. Characterizing the risk of insertional oncogenesis is limited to the available duration of exposure.	
Risk Management • Risk of delayed platelet engraftment will be managed by appropriate HCP/Prescriber education. • Routine pharmacovigilance practices at bluebird bio include a robust system for the collection, assessment, processing, and reporting of adverse reactions in the post-marketing setting. • Other routine activities planned for beti-cel include monthly signal detection, quarterly aggregate review of safety data, safety evaluation and updates to the product	• bluebird bio plans to minimize and manage the risks of treatment with beti-cel using internal processes, external relationships and restrictions, robust training and education.

Dimension Evidence and Uncertainties	Conclusions and Reasons
information using a dedicated cross-functional internal Safety Management Committee. • Risks in the commercial setting will be minimized by: 1) Restricting administration of beti-cel such that it is only administered at Qualified Treatment Centers 2) Rigorous training of Qualified Treatment Centers and health care providers 3) Education of health care providers, caregivers, and patients as detailed in the Prescribing Information and Education Material • bluebird bio is committed to robust long-term surveillance for patients treated with beti-cel through the ongoing follow-up of patients treated in the clinical trials and in a post-marketing registry. The collective combination of data from Study LTF-303, REG-501, and routine safety follow-up will enable further assessment of the potential risk of insertional oncogenesis and malignancy (from myeloablative conditioning and from beti-cel).	

7.2. Benefit-Risk Assessment

With a total of 221 patient-years of follow-up and up to 7 years of follow-up for individual patients, beti-cel treatment has demonstrated efficacy for children, adolescents, and adults with all genotypes of TDT, and an acceptable and manageable safety profile. The benefit:risk assessment is positive for the proposed indication.

TDT is a severe and burdensome disease. Despite major advances, with the current standard of care, consisting of regular pRBC transfusion and iron chelation therapy, most adult patients are still expected to have a shortened life expectancy (Chieco and Butler 2022). Allo-HSCT from a matched related donor is generally not recommended for patients > 15 years (Baronciani et al. 2016); therefore, most adolescents and adults with TDT have limited curative options. In these patients, while the current standard of care is designed to maintain hemoglobin levels adequate for a reasonable level of activity and quality of life, the regular transfusions exacerbate iron overload with attendant long-term complications, including end organ damage mainly in the heart and endocrine organs. beti-cel offers a curative treatment that is highly effective with approximately 90% of patients achieving transfusion independence, regardless of age or genotype. The near complete correction of ineffective erythropoiesis ameliorates the anemia and reduces excessive iron absorption resulting in cessation of transfusions and normalization of iron levels over time.

The safety profile of the beti-cel regimen largely reflects the known effects of autologous HSCT using the mobilization agents (G-CSF and plerixafor), and myeloablative conditioning (busulfan) necessary for the transplant. Overall, autologous HSCT has fewer and less severe toxicities than allogeneic HSCT, most notably GVHD. Veno-occlusive disease of the liver (sinusoidal obstructive syndrome) occurred in limited numbers of patients undergoing treatment with beti-cel and responded to treatment without sequelae. Some beti-cel treated patients experienced delayed platelet engraftment with limited bleeding complications. The long-term risk of insertional oncogenesis following treatment with beti-cel is unknown and will be monitored over time, but to date there have been no cases of insertional oncogenesis nor any other hematologic malignancy in 63 treated patients across studies in the beti-cel clinical development program, with a total of 221 patient-years of follow-up and up to 7 years of follow-up for individual patients. Given the increased risks associated with allo-HSCT in adolescents and adults with TDT, there is an unmet need for safe and effective curative therapies. The positive benefit:risk profile for adolescents and adults with TDT using the autologous approach of beti-cel is demonstrated by high rates of transfusion independence and acceptable rates of adverse events in adults and adolescents with TDT.

The benefit-risk profile of beti-cel treatment for children with TDT allows for expansion of curative options beyond HLA-matched sibling donor (MSD) allo-HSCT, which has a long and positive track record. However, a majority of children with TDT will lack a suitably matched related donor and outcomes using alternate donor stem cell sources have been inferior, with higher rates of graft failure, graft versus host disease, graft rejection, and transplant related mortality; therefore these transplants are not routinely performed. These children without a MSD would be good candidates for beti-cel therapy, which has comparable efficacy to allo-HSCT from a MSD, but without the risks of GVHD or TRM. Earlier intervention in children prior to excess transfusion-related iron deposition and organ dysfunction may be an additional benefit

and results in the Phase 3 beti-cel studies have demonstrated high rates of transfusion independence in children similar to adults. As with adults and adolescents, the beti cel safety profile is acceptable and manageable in children, and patients who become transfusion independent will stop loading iron as well.

Many families with younger children with TDT might prefer to wait for a transplant-based therapy for a variety of reasons, an important one being the option for fertility preservation once the child has achieved puberty. With the regular use of oral chelation, most young children with TDT who are compliant with chelation, currently do not have significant iron overload, and waiting to proceed with a transplant based curative treatment should not increase the risk for adverse events. For those without a matched-sibling donor, the long-term risks of delaying curative therapy must be balanced with the potential long-term risks of beti-cel therapy. All of these factors must be weighed in considering the optimal course of treatment for individual children with TDT.

In conclusion, given the high burden of disease and complications in TDT, and the limited availability of curative options, bluebird bio believes that the efficacy and safety demonstrated to date support the use of beti-cel as a curative treatment option for adults and adolescents, and for children. Treatment decisions should be made on an individual basis. The positive observed treatment benefits of beti-cel are considered to outweigh any identified and potential risks for patients with β-thalassemia who require regular RBC transfusions.

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9. APPENDIX A: MANUFACTURING AND PRODUCT UNDERSTANDING

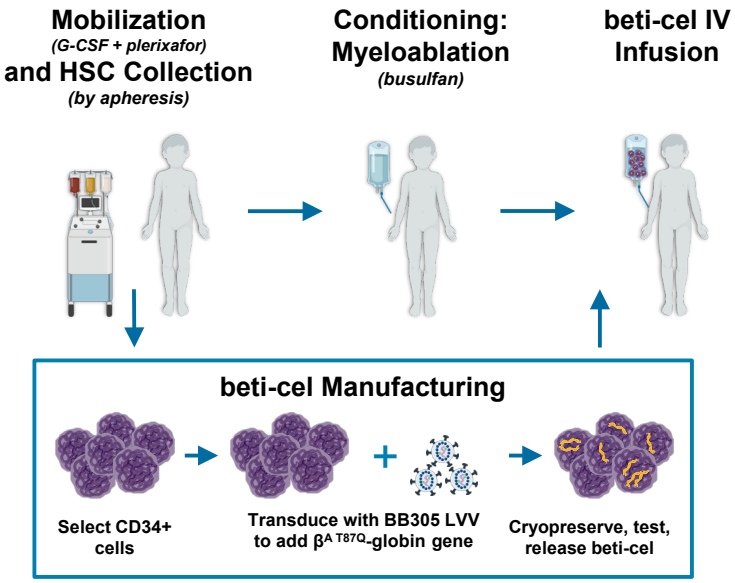
betibeglogene autotemcel (beti-cel) is a genetically modified autologous CD34+ cell-enriched population that contains hematopoietic stem cells (HSCs) transduced ex vivo with BB305 LVV encoding the $\beta^{A\text{-}T87Q}$-globin gene. It is a suspension for intravenous infusion; the cells are suspended in a cryopreservation solution.

Each lot of beti-cel is made from the autologous cells of a single patient collected in one mobilization cycle, and the resulting DP is administered to that same patient. The manufacture of beti-cel is based on (a) the enrichment of CD34+ cells from the cells collected from that patient by apheresis, (b) transduction of the enriched CD34+ cells with the critical component BB305 LVV, and (c) further processing of transduced cells to DP, including wash steps and re-suspension of the cell population in cryopreservation solution, filling into the final container, and cryopreservation.

Each patient undergoes HSC mobilization with G-CSF and plerixafor in combination, followed by apheresis to harvest the cells. The collected cells are shipped to the manufacturing site where CD34+ cells are selected and then transduced with BB305 LVV to manufacture beti-cel DP. The DP is tested to demonstrate that it meets all product quality standards after which it is released for patient administration. After myeloablative conditioning and beti-cel infusion, transduced HSCs engraft in the bone marrow and differentiate to reconstitute the hematopoietic system, including RBCs that contain HbA^{T87Q} to treat the patient's β-thalassemia (Figure 9).

More than 1 DP lot may be required to achieve the minimum beti-cel dose (5.0×10^6 CD34+ cells/kg patient weight). As such, a patient may undergo an additional mobilization with associated collection cycles to provide cellular material for use in the manufacture of an additional DP lot to provide a total CD34+ cell count that meets or exceeds the target dose.

Figure 9. Overview of beti-cel Treatment



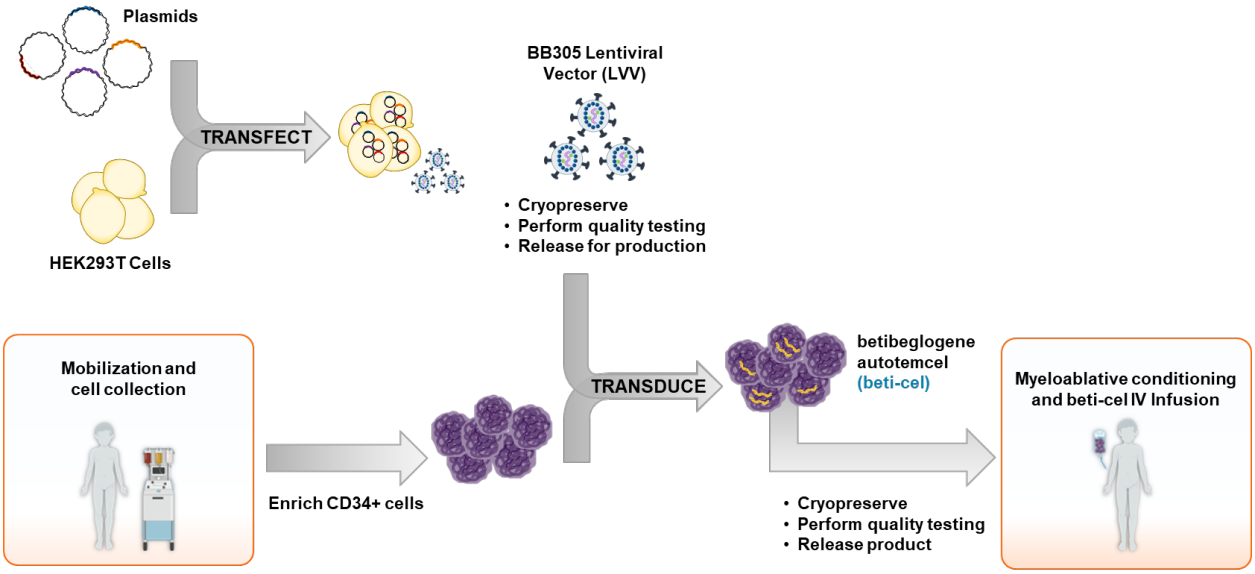
G-CSF, granulocyte-colony stimulating factor; HSC, hematopoietic stem cell; IV, intravenous; LVV, lentiviral vector

Information on the materials used in and key steps of the beti-cel manufacturing process is provided below.

9.1. Materials and Critical Components

The cellular starting material used to manufacture beti-cel drug substance is autologous hematopoietic progenitor cells collected by apheresis (HPC-A). The BB305 LVV critical component, used to transduce the CD34+ cells enriched from HPC-A, is produced using plasmids and HEK293T cells. A schematic overview of the manufacturing process illustrating how these materials are used to manufacture the LVV and DP is provided in Figure 10.

Figure 10. Overview of Materials and Use in beti-cel Manufacturing Process



9.1.1. Plasmids

A multi-plasmid system, consisting of a plasmid transfer vector (pBB305) containing the adult β^{A}-globin therapeutic gene with a substitution of glutamine (Q) for threonine (T) at position 87 (T87Q), referred to as $\beta^{A\text{-}T87Q}$-globin, and 3 packaging plasmids are used to produce BB305 LVV. pBB305 encodes the viral RNA genome and the other 3 plasmids encode viral packaging genes, including HIV-1-derived gag/pol and rev, and the vesicular stomatitis virus derived glycoprotein G (VSV-G) envelope. The multi-plasmid system was designed to prevent recombination and emergence of replication competent lentivirus (RCL).

Importantly, the viral packaging genes encoding these viral proteins are only present on plasmids. No viral packaging genes are included in the BB305 LVV and thus it is replication incompetent. In HEK293T cells the viral protein components produced from the plasmids lead to LVV particle formation and the packaging of the viral RNA genome, which is encoded by the pBB305 transfer vector. HIV-1 viral genes that are dispensable were removed from the plasmid system, and include those that encode HIV envelope, tat, vpr, vpu, and nef proteins. Notably, all these deleted genes are required for HIV pathogenesis.

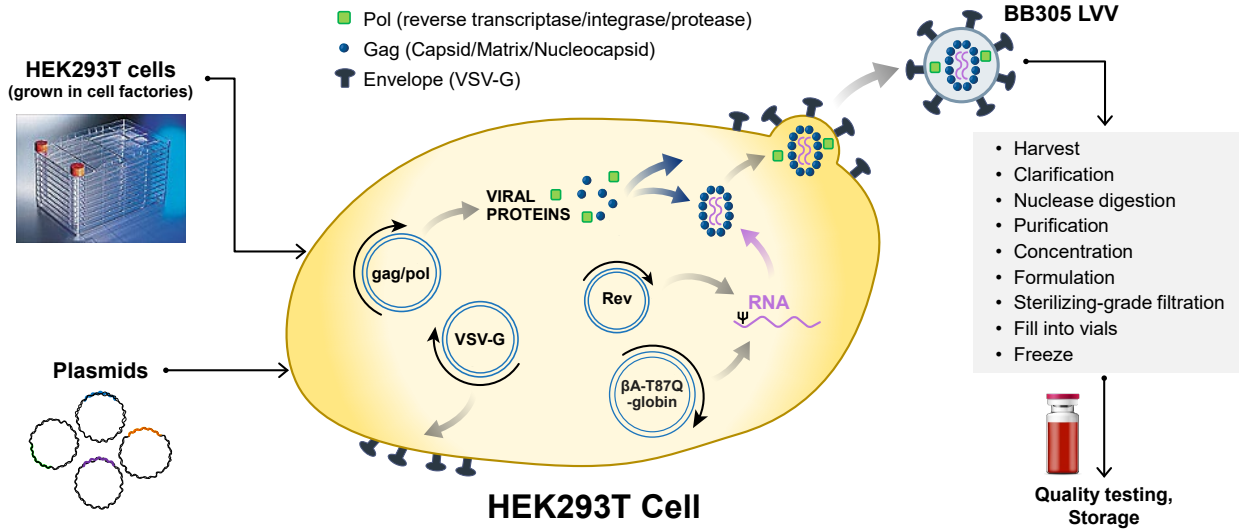
9.1.2. BB305 LVV

BB305 LVV is a replication incompetent, self-inactivating, third generation (LVV components are encoded on separate plasmids), HIV-1 based lentiviral vector with the VSV-G envelope protein, carrying the human $\beta^{A\text{-}T87Q}$-globin transgene under the transcriptional control of the erythroid specific human β-globin promoter and erythroid specific enhancer elements (DNase I hypersensitive sites HS2, HS3, and HS4) of the β-globin locus control region (see Section 12, Appendix D for more discussion about the human β-globin promoter). The self-inactivating feature of the BB305 LVV is due to the transcriptional enhancer and promoter having been deleted from the HIV-derived viral long terminal repeat; this greatly limits the potential for generating new viral RNA genomes in transduced cells, also limiting the ability of the integrated provirus to influence the transcription of nearby genes. The $\beta^{A\text{-}T87Q}$-globin variant can be distinguished from the wild-type β^{A}-globin by HPLC, based on the 1 amino acid difference in their sequences.

BB305 LVV is produced using a split-plasmid system that is common to all third-generation lentiviral vectors and is generated by transient transfection of HEK293T cells with the plasmid transfer vector pBB305 and the 3 packaging plasmids. BB305 LVV produced in the HEK293T cells (as shown in Figure 11) is harvested, purified via chromatography, formulated, and filled into vials prior to storage at ≤ - 65°C. HEK293T is a modified human embryonic kidney cell line. A HEK293T Master Cell Bank and Working Cell Bank have been established and tested in accordance with ICH and FDA guidance documents. The HEK293T cell banks have been demonstrated to be free of adventitious contaminants, including HIV-1 and HIV-2.

BB305 LVV is not directly administered to patients; it is used to transduce the patient's own CD34+ cells ex vivo.

Figure 11. Production of BB305 LVV by Transfection of HEK293T Cells



BB305 LVV manufacturing process consistency is mainly controlled by (1) raw material and reagent qualification programs, (2) in-process monitoring, (3) in-process control testing (4) lot

release and stability tests, and (5) validation of the manufacturing process and continuous process verification.

BB305 LVV lot release tests include assays for quality, identity, safety (including a test to detect RCL), purity, and potency. One potency assay is used to quantify the concentration of LVV infectious particles (transducing units/mL), which informs the amount of BB305 LVV used in the beti-cel manufacturing process. The other potency assay measures the ability of the transgene delivered by the LVV to encode functional $\beta^{A\text{-}T87Q}$-globin protein that can assemble with alpha-globin and heme to form the intact hemoglobin heterotetramer.

9.1.3. Autologous Cells

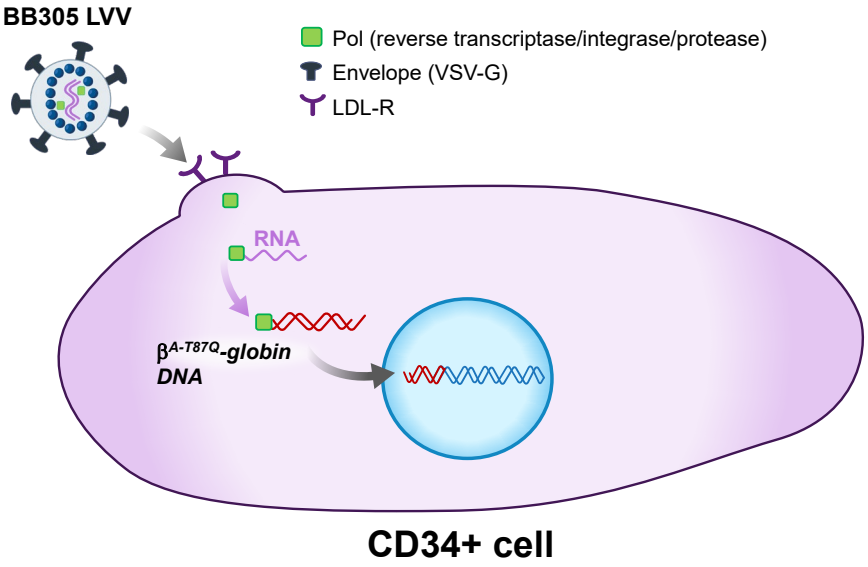
beti-cel manufacture starts with HPC-A collected from the patient by apheresis after mobilization with G-CSF and plerixafor. The mobilized cells contain mature blood-derived mononuclear cells, such as macrophages, B-cells, T-cells, and neutrophils. Approximately 1% of the mobilized cells express high levels of the surface transmembrane protein CD34, which is considered a marker for hematopoietic stem and progenitor cells.

9.2. beti-cel (Drug Product)

9.2.1. Manufacturing Process

The autologous cells are shipped from the apheresis collection center to the DP manufacturing facility where they are processed using a device approved by FDA for separation of hematopoietic stem cells to enrich for cells expressing CD34, the cell surface marker common to hematopoietic stem and progenitor cells. The CD34+ enriched cell population is stimulated ex vivo with a mixture of recombinant human cytokines and transduced ex vivo with BB305 LVV as shown in Figure 12. Transduced cells will carry at least 1 copy of the $\beta^{A\text{-}T87Q}$-globin gene.

Figure 12. Transduction of Autologous Cells



LDL-R, low-density lipoprotein receptor; LVV, lentiviral vector; VSV-G, vesicular stomatitis virus-G protein.

The transduced cells are washed to remove impurities, counted, and formulated in cryopreservation solution before being frozen and stored in the vapor phase of liquid nitrogen. beti-cel is tested for identity, potency, purity, and safety using validated assays. The DP must meet release criteria prior to the patient undergoing the conditioning regimen for beti-cel infusion. The cells are maintained at -140°C or below through storage and shipping until the day of infusion, when they are thawed and administered intravenously.

The beti-cel manufacturing process is designed to maximize and maintain the quantity of CD34+ cells recovered from the collected autologous cells while maintaining their stem cell characteristics. Culture time following transduction cannot be extensive as it is necessary to minimize the potential for any HSC differentiation. In this respect, the beti-cel manufacturing approach differs from that of chimeric antigen receptor T-cell (CAR-T cell) products that frequently use a culture step following genetic modification to increase the number of cells to achieve clinical dosing requirements. The beti-cel manufacturing process has been developed with the focus of maximizing the quantity of CD34+ cells at the end of the process. However, if there are not enough CD34+ cells to achieve a minimum dose, the patient will repeat the mobilization and apheresis cycles, and a second DP lot will be produced. Both DPs will be sequentially administered to the patient on the day of infusion.

9.2.2. Process Development

Following the HGB-204 clinical trial, based on increased process and product understanding, bluebird bio implemented DP manufacturing process changes to improve transduction efficiency for clinical studies HGB-207, HGB-212, and future commercial manufacturing. The intended outcome of these process changes was to increase the percentage of CD34+ cells in beti-cel that are transduced with BB305 LVV (% transduced cells). Correlations between DP attributes and clinical parameters were assessed and the beti-cel attribute of % transduced cells was found to be most predictive of PB VCN PD parameter levels linked with positive clinical outcomes (i.e., transfusion independence).

9.2.3. Manufacturing Control Strategy

Manufacturing process consistency is assured through (1) raw material and reagent qualification programs, (2) in-process monitoring, (3) in-process control testing (4) lot release and stability testing, (5) manufacturing process validation and continuous process verification, and (6) traceability by using a chain-of-identity system.

The DP release testing includes assays for identity, safety, purity, and potency and strength. The suite of potency assays includes those that quantify DNA insertions, confirm the ability of the transduced CD34+ cells to form diverse hematopoietic colonies, quantify $\beta^{A\text{-}T87Q}$-globin expression, and confirm the ability of the DP cells to terminally differentiate into red blood cells, thereby confirming the ability of the expressed $\beta^{A\text{-}T87Q}$-globin to form functional HbA^{T87Q}. Further details of the specifications are not disclosed here as this information is considered to be proprietary (note: this information was shared with FDA as part of the BLA).

9.2.4. Chain of Identity

The Sponsor has implemented a DP chain of identity (COI) strategy based on the requirements of donor-to-recipient bi-directional product tracking. In this strategy, all operations from patient

enrollment to delivery of DP to the Qualified Treatment Center for administration are controlled, assuring each individual patient is infused with DP manufactured with their own HSCs. Policies and procedures govern practices at the Qualified Treatment Center, DP manufacturer, and bluebird bio to assure the bluebird bio COI system functionality and expectations. Applicable training exists and is required for appropriate personnel at the Qualified Treatment Center, DP Manufacturer, and at bluebird bio.

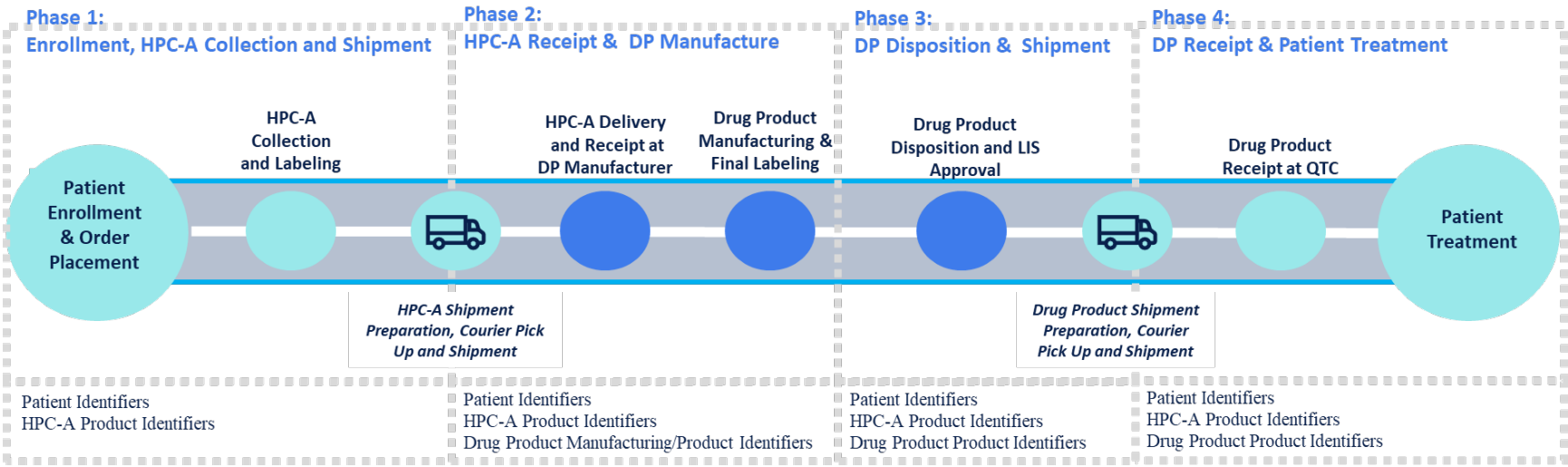
bluebird bio's COI system leverages a combination of physical labeling and procedural controls based on three COI unique traceability identifier categories, each subsequently comprised of specific COI data elements. The three categories of COI unique traceability identifiers are: Patient Identifier(s), HPC-A Product Identifier(s), and DP Manufacturing/Product Identifier(s). The combination of these identifiers enables donor-to-recipient tracking and traceability and assures that the HPC-A collected from a patient, that is used to make DP, is returned to that same patient.

The COI process contains 4 distinct phases (Figure 13) in which defined COI data elements are assigned and/or verified by the Sponsor, the Qualified Treatment Centers, and the DP Manufacturer. The 4 phases include:

- Enrollment, HPC-A Collection and Shipment
- HPC-A Receipt and DP Manufacturing
- DP Disposition and Shipment
- DP Receipt at Qualified Treatment Centers and Patient Treatment

Throughout the process phases, defined COI data elements are checked and verified before proceeding with the next step to ensure tracking and tracing, and to assure each patient only receives DP produced from their own HPC-A.

Figure 13. Chain of Identity Summary



DP, drug product; HPC-A, hematopoietic progenitor cells obtained by apheresis; QTC, qualified treatment center.

10. APPENDIX B: HGB-207 AND HGB-212 STUDY ENDPOINTS AND SCHEDULE OF EVENTS

Only Study HGB-212 is provided below. Study HGB-212 (version 5.0, dated 08 October 2020) is representative of the schedule of events and study endpoints in Study HGB-207.

10.1. Study Endpoints

10.1.1. Efficacy Endpoints

Primary Endpoint

- The proportion of subjects who meet the definition of "transfusion independence" (TI). TI is defined as a weighted average Hb ≥9 g/dL without any pRBC transfusions for a continuous period of ≥12 months at any time during the study after drug product infusion.

Secondary Endpoints

- Characterization of subjects achieving TI:
  - Proportion of subjects who meet the definition of TI at the Month 24 Visit
  - Duration of TI
  - Time from drug product infusion to achievement of TI
  - Weighted average Hb during TI
- Characterization of transfusion reduction (TR):
  - The proportion of subjects who meet the definition of TR, defined as demonstration of a ≥60% reduction in the annualized volume of pRBC transfusion requirements (in mL/kg) in the post-treatment time period from 12 months post-drug product infusion through Month 24 (approximately a 12-month period), compared to the annualized mL/kg pRBC transfusion requirement during the 2 years prior to study enrollment.
  - Proportion of subjects with a reduction in the annualized mL/kg pRBCs transfused from 12 months post-drug product infusion through Month 24 (approximately a 12-month period) of at least 50%, 60%, 75%, 90% or 100% compared to the annualized mL/kg pRBC transfusion requirement during the 2 years prior to enrollment
  - Annualized number and volume of pRBC transfusions from 12 months post-drug product infusion through Month 24 compared to the annualized number and volume of transfusions during the 2 years prior to enrollment
  - Time from drug product infusion to last pRBC transfusion
  - Time from last pRBC transfusion to the Month 24 Visit
- Weighted average nadir Hb during the 2 years prior to enrollment compared to weighted average nadir Hb from 12 months post-drug product infusion through the Month 24 Visit

- Unsupported total Hb levels over time, including Month 6, Month 9, Month 12, Month 18, and Month 24
- Unsupported total Hb levels ≥10 g/dL, ≥11 g/dL, ≥12 g/dL, ≥13 g/dL, ≥14 g/dL at Month 6, Month 12, Month 18, and Month 24
- Characterization of use of iron chelation and/or therapeutic phlebotomy among all subjects:
  - Proportion of subjects who have not received chelation therapy for at least 6 months following drug product infusion
  - Time from last iron chelation use to last follow-up
  - Proportion of subjects using therapeutic phlebotomy and annualized frequency of phlebotomy use per subject following drug product infusion
- Evaluation of the change in iron burden over time, as measured by:
  - Change in liver iron content by magnetic resonance imaging (MRI) at baseline to Month 12 and Month 24 Visits
  - Change in cardiac T2* on MRI at baseline to Month 12 and Month 24 Visits
  - Change in serum ferritin at baseline to Month 12 and Month 24 Visits
- Evaluation of health-related quality of life (HRQoL) over time including Month 12 and Month 24 as compared to baseline, using the following validated tools:
  - Pediatrics: Pediatric Quality of Life Inventory (PedsQL; parent general core and general core)
  - Adolescents: PedsQL (parent general core and general core) and EuroQol-5D (Youth version) (EQ-5D-Y)
  - Adults: EuroQol-5D (EQ-5D), Functional Assessment of Cancer Therapy-Bone Marrow Transplant (FACT-BMT), and Short Form-36 (SF-36) v2

Exploratory Endpoints

- Assessment of growth and puberty parameters (age appropriate), bone density, diabetes, endocrine evaluations, and neurocognitive development (pediatric subjects <18 years of age)
- Assessment of change in dyserythropoiesis
- Correlations of pre-treatment variables (e.g., drug product vector copy number [VCN]) with response (e.g., peripheral blood VCN, HbA^{T87Q})
- Measures of health resource utilization (including comparing annualized number of transfusions, number of hospitalizations, and number of days hospitalized, from 12 months post-drug product infusion through Month 24 Visit with the annualized corresponding parameters during the 2 years prior to enrollment)
- Length of in-patient hospital stay from initiation of conditioning to discharge

10.1.2. Safety Endpoints

Secondary Endpoints

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

Exploratory Endpoint

- The number of subjects with clonal predominance

10.1.3. Pharmacodynamic Endpoints

Secondary Endpoints

- $\beta^{A\text{-}T87Q}$-globin expression over time, including Month 6, Month 9, Month 12, Month 18, and Month 24, as measured by assessing the ratio of $\beta^{A\text{-}T87Q}$-globin to all β-like-globins, and α-globin to all β-like-globins, in whole blood
  - Correlation of $\beta^{A\text{-}T87Q}$-globin expression at early time points post drug product infusion to $\beta^{A\text{-}T87Q}$-globin expression at later time points, as well as clinical outcomes.
- VCN in peripheral blood over time, including Month 6, Month 9, Month 12, Month 18, and Month 24

Exploratory Endpoint

- Relationship between measures of myeloablation and pharmacodynamics and clinical outcomes

Additionally, exploratory methods may be used to evaluate pharmacodynamic endpoints.

10.2. Schedule of Events for HGB-212

Table 25. Schedule of Events: Screening and CD34+ Cell Harvest

Procedure	Screening (Up to 90 Days Before Mobilization)	Pre-mobilization (Up to 60 Days Before Mobilization)	Mobilization[1]	Days of Harvest
Signing of informed consent form (ICF)/assent	X			
Demographics and medical history[2]	X			

Procedure	Screening (Up to 90 Days Before Mobilization)	Pre-mobilization (Up to 60 Days Before Mobilization)	Mobilization[1]	Days of Harvest
Physical examination	X[3]		X[3]	X[3]
Vital signs	X		X[4]	X[4]
Hypertransfusion to pre-transfusion hemoglobin (Hb) ≥ 11 g/dL		X[5]		
Blood for clinical laboratory tests	X[6]		X[7]	X[7]
Estimated glomerular filtration rate (GFR)	X			
Urinalysis	X			
Blood for serology	X[8]			
Blood for immunological testing	X[9]			
Blood for serum β-human chorionic gonadotropin for women of child-bearing potential (serum pregnancy test)	X		X[10]	
Blood for hormonal and dyserythropoiesis testing	X[11]			
Blood for replication competent lentivirus (RCL)[12], vector copy number (VCN), globin high-performance liquid chromatography (HPLC), and globin in autologous cells	X			
Blood for thalassemia genotyping[13]	X			
Blood for storage: potential biomarker analysis (optional)	X			
Peripheral blood CD34+ cell count				X[14]
Sperm/testicular tissue or oocyte banking, if requested	X[15]			
Liver biopsy (if required)	X			
Bone marrow (morphology studies)	**X**[16]			
12-lead electrocardiogram (ECG)	X			
Imaging: Chest X-ray, cardiac Doppler echocardiology (incl. left ventricular ejection fraction [LVEF]), cardiac and liver T2* magnetic resonance imaging (MRI), standard-of-care) liver and spleen MRI, bone imaging (X-ray and/ or DEXA scan)[17]	X			
Pulmonary function tests	X[18]			
Transfusion regimen			X[19]	
Health-Related Quality of Life (HRQoL) assessment	X			
Adverse event collection	Continuous from ICF signing			
Prior and concomitant medications (incl. iron chelators for the prior 2 years)	Continuous from ICF signing			

[1] Up to 2 cycles of mobilization are permitted.
[2] Includes records for the prior 2 years transfusion history and in-patient hospitalization; includes genotype (mutations at *HBB* gene).
[3] Includes weight, height, neurocognitive development evaluation (for patients <18 years of age), performance status, and Tanner staging (if relevant) at Screening. A complete physical examination (including weight) should be performed within 5 days prior to or on the first day of every mobilization cycle, and on every day of apheresis prior to apheresis. An abbreviated physical examination is required every day after completion of apheresis.
[4] Vital signs should be performed on the first day of every mobilization cycle, and on each apheresis day, prior to the apheresis procedure then again after the apheresis procedure is completed. If the patient undergoes a bone marrow

harvest, vital signs should be performed on the day of the bone marrow harvest prior to the harvest and then again prior to discharge.

[5] Pre-mobilization hypertransfusion is recommended, but not required. Iron chelation should be managed appropriately to minimize additional iron overload. Pre-transfusion hemoglobin, and if available, serum ferritin and serum transferrin receptor should be recorded as unscheduled labs

[6] Includes hematology (complete blood count [CBC], platelets, reticulocytes, nucleated red blood cells [RBC]s), iron studies (iron, ferritin, transferrin, serum transferrin receptor), serum chemistry (including fasting glucose/insulin and Homeostasis Model Assessment index), liver function tests, prothrombin and partial thromboplastin time. Oral glucose tolerance test is required for any abnormal fasting glucose.

[7] CBC should be performed during mobilization and every day of apheresis.

[8] Including testing for presence of human immunodeficiency virus (HIV) 1, HIV 2, hepatitis B virus (HBV), hepatitis C virus (HCV), and rapid plasma regain. Blood may also be drawn for additional serology testing if patient has risk factors or clinical evidence of infection with other communicable disease agents or disease. Tests should be done according to country-specific and institutional guidelines

[9] T cell subsets (CD4, CD8), B cells (CD19), natural killer cells (CD16 and/or CD56); immunoglobulins (IgG, IgM, and IgA)

[10] Should be confirmed negative prior to mobilization

[11] Hormonal testing includes thyroid function (free T4, thyroid stimulating hormone), parathyroid hormone. For patients after puberty, also includes luteinizing hormone (LH), follicle-stimulating hormone (FSH) and estradiol (females only); testosterone (males only). For patients < 18 years of age, includes growth hormone (insulin-like growth factor [IGF-1] and insulin-like growth factor binding protein [IGFBP-3]). Dyserythropoiesis testing includes erythropoietin (EPO) and hepcidin.

[12] Two samples required to be archived for potential replication-competent lentivirus (RCL) testing: one for the RCL screening test, another for RCL peripheral blood leukocytes (PBL) coculture test

[13] Confirm *HBB* genotype. Also archive a blood sample for determining the presence of potential thalassemia phenotype-modifying mutations at other genetic loci at a central laboratory, as well as archive a blood sample for potential comparison of genotyping methods for regulatory purposes.

[14] Peripheral blood CD34+ count should be performed on the day prior to apheresis, as well as on the day of apheresis.

[15] May occur any time before conditioning; hormonal treatment, if applicable as part of banking, should stop at least 7 days prior to conditioning.

[16] Bone marrow collection for morphology, cellularity, cell count and iron stains. For patients undergoing re-screening, bone marrow collection does not need to be performed.

[17] Age appropriate bone imaging (bone age/ mineral density) to be done based on investigator's judgment.

[18] Including oxygen saturation; corrected % predicted forced vital capacity (FVC), % predicted Forced expiratory volume in 1 second (FEV1); % predicted residual volume (RV); and % predicted diffusing capacity for carbon monoxide (DLco; corrected for Hb and/or alveolar volume, as clinically indicated). If patient cannot perform these pulmonary function tests due to age or cognition-related restrictions, respiratory exam, chest radiograph, and pulse oximetry will substitute for these assessments.

[19] To maintain Hb ≥ 11 g/dL prior to mobilization.

Table 26. Schedule of Events: Conditioning and Drug Product Infusion in HGB-212

	Preconditioning		Conditioning	Infusion	Post-Infusion Until Engraftment
	Approximately 30 Days Before Busulfan	Up to 7 days Before Busulfan	Day -6 to -3 Inclusive[1]	Day 1	Day 1 Until Discharge
Physical examination		X[2]	X	X[3]	X[4]
Vital Signs		X	X	X[5]	X[6]
Stop iron chelation		X[7]			

	Preconditioning		Conditioning	Infusion	Post-Infusion Until Engraftment
	Approximately 30 Days Before Busulfan	Up to 7 days Before Busulfan	Day -6 to -3 Inclusive[1]	Day 1	Day 1 Until Discharge
Hypertransfusion to pre-transfusion hemoglobin (Hb) ≥ 11 g/dL	X[8]				
Blood for clinical laboratory tests[9]		X	X	X	X[4,10]
Blood for serum β-human chorionic gonadotropin for female patients of child-bearing potential (serum pregnancy test)		X			
Blood for serum transferrin receptor		X		X	X[11]
Blood for reticulocyte count		X		X	X[11]
Re-confirmation of eligibility		X[12]			
Anti-seizure and veno-occlusive disease (VOD)/ sinusoidal obstruction syndrome (SOS) prophylaxis			X[13]		
Busulfan chemotherapy			X		
Blood for busulfan pharmacokinetics[14]		X	X		
Infusion of LentiGlobin BB305 Drug Product				X	
Adverse event collection	Continuous from ICF signing				
Concomitant medication collection	Continuous from ICF signing				

[1] Planned Days relative to Day 1, but may vary depending on length of wash-out (48 hour minimum) before drug product infusion.

[2] Includes performance status at preconditioning visit.

[3] To be performed on Day 1 before drug product administration.

[4] At least twice a week during hospitalization.

[5] Vital sign monitoring including electrocardiogram (ECG), pulse oximetry, and blood pressure measurements, should be employed during drug-product infusion. Additionally, vital signs (excluding ECG) should be measured for 2 hours after infusion (e.g., every 30 to 60 minutes).

[6] To be performed daily until discharge.

[7] Iron chelation must be stopped at least 7 days before initiating conditioning.

[8] Pre-conditioning hypertransfusion is recommended, but not required. Iron chelation should be managed appropriately to minimize additional iron overload. Pre-transfusion hemoglobin, and if available, serum ferritin and serum transferrin receptor should be recorded as unscheduled labs.

[9] Complete blood count (CBC); serum chemistry; liver function tests; and any other tests that are clinically indicated or required by institutional guidelines, including for example testing for cytomegalovirus (CMV), Epstein Barr virus, Herpes simplex virus (HSV), and vesicular stomatitis virus (VZV) immunoglobulin (IgG). On Day 1 labs are to be collected prior to infusion. Clinical laboratory tests should be obtained on a more frequent basis if clinically indicated (e.g., monitoring & evaluation of adverse events).

[,10] CBCs are performed daily during hospitalization until absolute neutrophil count (ANC) engraftment is demonstrated.

[11] Reticulocytes and serum transferrin receptor should be monitored daily from Day 1 through Day 7.

[12] Review CBC, serum chemistry, liver function tests, physical examination, performance status, and adverse event history; serum β-human chorionic gonadotropin for women of child-bearing potential (serum pregnancy test); verification that LentiGlobin BB305 Drug Product has been clinically dispositioned, is available on site and that rescue cells are available. Note that if Screening Visit and Preconditioning Visits are > 1 year apart, the following tests must be repeated: pulmonary function tests (PFTs), estimated glomerular filtration rate (GFR), and cardiac Doppler echocardiology (incl. left ventricular ejection fraction [LVEF]).

[13] Anti-seizure prophylaxis to start at least 12 hours before initiating busulfan and to continue at least 24 hours after completion of 4-day busulfan course; veno-occlusive disease (VOD)/sinusoidal obstruction syndrome (SOS) prophylaxis as mandated in protocol.
[14] A test dose of busulfan several days before beginning myeloablation to pre-determine busulfan dose is also permitted. PK analysis of first dose of busulfan on first and third day of dosing is required.

Table 27. Schedule of Events: Follow-Up in HGB-212

	Follow-Up: Day (D), Month (M) (Visit Window, days) Post-Drug Product Infusion																	
	D30	D60	D90	D120	D150	D180	D210	D240	D270	D300	D330	D360	D420	D450	D480	D540	D600	D660	D720
Procedure	M1 (±7)	M2 (±7)	M3 (±7)	M4 (±14)	M5 (±14)	M6 (±14)	M7 (±14)	M8 (±14)	M9 (±14)	M10 (±14)	M11 (±14)	M12 (±30)	M14 (±30)	M15 (±30)	M16 (±30)	M18 (±30)	M20 (±30)	M22 (±30)	M24 (±30)
Physical examination[1]	X	X	X	X	X	X			X			X		X		X			X
Vital signs	X	X	X	X	X	X			X			X		X		X			X
Blood for serum β-human chorionic gonadotropin for women of child-bearing potential (serum pregnancy test)[2]			X			X													
Blood for clinical laboratory tests[3]	X	X	X	X	X	X			X			X		X		X			X
Blood for CBC only							X	X		X	X		X		X		X	X	X
Blood for fasting Glucose/Insulin levels[4]						X						X				X			X
Estimated glomerular filtration rate (GFR)			X			X						X							X
Blood for iron studies[5]			X			X						X		X		X			X
Blood for immunology[6]			X			X						X							X
Blood for hormonal and dyserythropoiesis testing[7]												X							X
Blood for globin in autologous cells												X							X
Blood for globin HPLC & VCN		X	X			X			X			X				X			X
Blood for RCL analyses[8]			X			X						X							X[9]
Blood for ISA						X						X				X			X
Blood for storage: potential biomarker analysis (optional)						X						X							X
Bone marrow[10]												X							X
Liver and spleen standard-of-care MRI												X							X
Pulmonary function tests[11]												X							X

	Follow-Up: Day (D), Month (M) (Visit Window, days) Post-Drug Product Infusion																		
	D30	D60	D90	D120	D150	D180	D210	D240	D270	D300	D330	D360	D420	D450	D480	D540	D600	D660	D720
Procedure	M1 (±7)	M2 (±7)	M3 (±7)	M4 (±14)	M5 (±14)	M6 (±14)	M7 (±14)	M8 (±14)	M9 (±14)	M10 (±14)	M11 (±14)	M12 (±30)	M14 (±30)	M15 (±30)	M16 (±30)	M18 (±30)	M20 (±30)	M22 (±30)	M24 (±30)
Cardiac and liver T2*MRI & echocardiology[12]												X							X
12-lead electrocardiogram												X							X
Bone imaging (X-ray and/or Dexa Scan)[13]																			X
HRQoL Assessment			X[14]			X						X				X			X
Record transfusions	Continuous from ICF signing																		
Record hospitalizations	Continuous from post-drug product infusion discharge																		
Adverse event collection	Continuous from ICF signing																		
Concomitant medication (incl. iron chelators & phlebotomy)	Continuous from ICF signing																		

[1] Includes weight at every visit, height, and performance status every 6 months after drug product infusion. Tanner staging should be performed every 6 months during puberty, if relevant. For patients <18 years of age, neurocognitive development will be evaluated every 6 months.

[2] Should be confirmed prior to mobilization

[3] Hematology (complete blood count [CBC], platelets, reticulocytes, nucleated red blood cells [RBCs]); serum chemistry and liver function tests, and additional clinical laboratory tests as clinically indicated. Clinical laboratory tests should be obtained on a more frequent basis if clinically indicated (e.g., monitoring & evaluation of adverse events). If the results from blood tests are not as expected, additional testing may need to be performed and may include a physical exam, blood tests, imaging tests, or a bone marrow biopsy to allow for further investigation of stem cells.

[4] Fasting glucose and insulin levels (homeostatic Model Assessment (HOMA) for Insulin Resistance index) at least every 6 months. An oral glucose tolerance test should be performed for an abnormal fasting glucose.

[5] Iron studies (iron, ferritin, serum transferrin receptor, transferrin) should also be performed prior to restarting iron chelation/phlebotomy.

[6] T cell subsets [CD4, CD8], B cells (CD19), and natural killer cells (CD16 and/or CD56); immunoglobulins (IgG, IgM, and IgA)

[7] Hormonal testing includes thyroid function (free T4, thyroid stimulating hormone); parathyroid hormone. For patients after puberty, also includes LH, FSH and estradiol (females only); testosterone (males only). For patients < 18 years of age, includes growth hormone (insulin-like growth factor (IGF)-1 and insulin-like growth factor binding protein (IGFBP)-3). Dyserythropoiesis testing includes erythropoietin (EPO) and hepcidin.

[8] Two samples required, one for RCL screening test, another for potential coculture of peripheral blood leukocytes (PBLs) if replication-competent lentivirus (RCL) screening test is positive

[9] If a patient's previous RCL tests were all negative, the 24 Month sample will be archived.

[10] Bone marrow for dyserythropoiesis studies (reticulocytes, nucleated RBC, serum transferrin receptor, hepcidin, and erythropoietin), as well as morphology, cellularity, cell count, and iron content; other research tests (e.g., vector copy number [VCN], integration site analysis [ISA], high performance liquid chromatography [HPLC]) may be performed if sufficient sample is available.

[11] Including oxygen saturation; corrected % predicted forced vital capacity (FVC), % predicted forced expiratory volume in 1 second (FEV1) % predicted residual volume (RV); and % predicted diffusing capacity for carbon monoxide (DLco; corrected for Hb and/or alveolar volume, as clinically indicated). If patient cannot perform these pulmonary function tests due to age or cognition-related restrictions, then respiratory exam, chest radiograph, and pulse oximetry will substitute for these assessments. If patient becomes able to perform spirometry and lung diffusion capacity test, these pulmonary function tests should be performed per schedule of events and/or at an unscheduled timepoint.
[12] Annual echocardiography is only required if clinically significant abnormality is observed on the Screening echocardiogram, or any subsequent echocardiogram, or if there is evidence of iron overload (cardiac T2* ≤ 20 ms) or other clinically significant abnormality on cardiac T2* magnetic resonance imaging (MRI).
[13] Age appropriate bone imaging (bone age/ mineral density) to be done based on investigator judgment.
[14] At the Day 90 Visit, only the European Quality of Life-5 dimension (EQ-5D) and Functional Assessment of Cancer Therapy - Bone Marrow Transplantation (FACT-BMT) tools are to be completed.

11. APPENDIX C: ADDITIONAL SAFETY INFORMATION

Table 28. Adverse Events Occurring in ≥ 10% of Patients With TDT by Time Period (ITT)

System Organ Class Preferred Term	Time Period						
	ICF to < M n (%), E	M to < C n (%), E	C to < NE n (%), E	NE to M24 n (%), E	>M24 to M36 n (%), E	Day 1 to Last Follow-Up n (%), E	Total (ICF to Last Follow-Up) n (%), E
Number of patients at risk	**66**	**66**	**63**	**63**	**52**	**63**	**66**
Number of patients with ≥ 1 AE, Events	**37 (56.1), 83**	**61 (92.4), 277**	**63 (100), 1283**	**60 (95.2), 593**	**7 (13.5), 10**	**63 (100), 1679**	**65 (98.5), 2255**
Blood and lymphatic system disorders	**0, 0**	**19 (28.8), 25**	**63 (100), 467**	**28 (44.4), 105**	**1 (1.9), 1**	**63 (100), 535**	**64 (97.0), 598**
Thrombocytopenia	0, 0	13 (19.7), 15	62 (98.4), 158	7 (11.1), 13	0, 0	62 (98.4), 168	63 (95.5), 186
Anemia	0, 0	3 (4.5), 3	52 (82.5), 93	19 (30.2), 69	0, 0	51 (81.0), 138	53 (80.3), 165
Neutropenia	0, 0	1 (1.5), 1	48 (76.2), 102	9 (14.3), 14	1 (1.9), 1	48 (76.2), 114	48 (72.7), 118
Febrile neutropenia	0, 0	0, 0	32 (50.8), 32	0, 0	0, 0	32 (50.8), 32	32 (48.5), 32
Leukopenia	0, 0	0, 0	24 (38.1), 70	4 (6.3), 6	0, 0	25 (39.7), 70	25 (37.9), 76
Gastrointestinal disorders	**8 (12.1), 10**	**21 (31.8), 41**	**60 (95.2), 279**	**33 (52.4), 65**	**0, 0**	**61 (96.8), 276**	**64 (97.0), 395**
Stomatitis	0, 0	0, 0	46 (73.0), 94	0, 0	0, 0	45 (71.4), 92	46 (69.7), 94
Vomiting	3 (4.5), 4	8 (12.1), 10	34 (54.0), 47	12 (19.0), 13	0, 0	29 (46.0), 38	41 (62.1), 74
Nausea	2 (3.0), 2	11 (16.7), 13	34 (54.0), 35	5 (7.9), 7	0, 0	12 (19.0), 16	38 (57.6), 57
Abdominal pain	2 (3.0), 2	4 (6.1), 5	20 (31.7), 25	7 (11.1), 8	0, 0	23 (36.5), 29	27 (40.9), 40
Diarrhea	0, 0	1 (1.5), 1	20 (31.7), 24	6 (9.5), 6	0, 0	21 (33.3), 25	24 (36.4), 31
Constipation	0, 0	0, 0	18 (28.6), 20	4 (6.3), 5	0, 0	18 (28.6), 20	21 (31.8), 25
Dyspepsia	0, 0	2 (3.0), 2	6 (9.5), 9	5 (7.9), 7	0, 0	10 (15.9), 14	12 (18.2), 18
Abdominal pain upper	0, 0	2 (3.0), 2	4 (6.3), 4	1 (1.6), 1	0, 0	3 (4.8), 3	7 (10.6), 7
Gingival bleeding	0, 0	1 (1.5), 1	1 (1.6), 1	5 (7.9), 5	0, 0	6 (9.5), 6	7 (10.6), 7
General disorders and administration site conditions	**5 (7.6), 5**	**29 (43.9), 46**	**37 (58.7), 73**	**27 (42.9), 42**	**1 (1.9), 1**	**44 (69.8), 101**	**51 (77.3), 167**
Pyrexia	4 (6.1), 4	2 (3.0), 2	20 (31.7), 32	13 (20.6), 16	0, 0	29 (46.0), 48	31 (47.0), 54
Catheter site pain	0, 0	13 (19.7), 17	4 (6.3), 5	1 (1.6), 1	0, 0	3 (4.8), 3	16 (24.2), 23
Fatigue	0, 0	3 (4.5), 3	5 (7.9), 7	7 (11.1), 7	0, 0	10 (15.9), 10	14 (21.2), 17
Mucosal inflammation	0, 0	0, 0	10 (15.9), 12	0, 0	0, 0	9 (14.3), 11	10 (15.2), 12
Pain	1 (1.5), 1	5 (7.6), 6	3 (4.8), 3	2 (3.2), 2	1 (1.9), 1	4 (6.3), 4	10 (15.2), 13
Non-cardiac chest pain	0, 0	1 (1.5), 1	2 (3.2), 2	5 (7.9), 5	0, 0	6 (9.5), 6	7 (10.6), 8

System Organ Class Preferred Term	Time Period						
	ICF to < M n (%), E	M to < C n (%), E	C to < NE n (%), E	NE to M24 n (%), E	>M24 to M36 n (%), E	Day 1 to Last Follow-Up n (%), E	Total (ICF to Last Follow-Up) n (%), E
Hepatobiliary disorders	**2 (3.0), 3**	**1 (1.5), 1**	**5 (7.9), 7**	**8 (12.7), 11**	**1 (1.9), 1**	**14 (22.2), 21**	**16 (24.2), 25**
Venoocclusive liver disease	0, 0	0, 0	3 (4.8), 3	4 (6.3), 5	0, 0	7 (11.1), 8	7 (10.6), 8
Infections and infestations	**15 (22.7), 18**	**15 (22.7), 17**	**20 (31.7), 27**	**43 (68.3), 81**	**1 (1.9), 1**	**45 (71.4), 106**	**54 (81.8), 145**
Upper respiratory tract infection	1 (1.5), 1	2 (3.0), 2	0, 0	10 (15.9), 11	0, 0	10 (15.9), 11	11 (16.7), 14
Nasopharyngitis	2 (3.0), 2	2 (3.0), 2	0, 0	6 (9.5), 7	0, 0	6 (9.5), 7	8 (12.1), 11
Injury, poisoning and procedural complications	**16 (24.2), 17**	**23 (34.8), 34**	**18 (28.6), 27**	**20 (31.7), 27**	**2 (3.8), 2**	**31 (49.2), 56**	**43 (65.2), 107**
Procedural pain	13 (19.7), 14	19 (28.8), 26	4 (6.3), 5	4 (6.3), 4	1 (1.9), 1	9 (14.3), 10	30 (45.5), 50
Transfusion reaction	0, 0	1 (1.5), 1	12 (19.0), 15	2 (3.2), 2	0, 0	14 (22.2), 17	15 (22.7), 18
Investigations	**5 (7.6), 7**	**4 (6.1), 7**	**25 (39.7), 66**	**29 (46.0), 69**	**0, 0**	**35 (55.6), 112**	**39 (59.1), 149**
Alanine aminotransferase increased	1 (1.5), 1	1 (1.5), 1	12 (19.0), 17	16 (25.4), 21	0, 0	20 (31.7), 29	24 (36.4), 40
Aspartate aminotransferase increased	1 (1.5), 2	1 (1.5), 1	10 (15.9), 12	11 (17.5), 13	0, 0	15 (23.8), 20	19 (28.8), 28
Blood bilirubin increased	1 (1.5), 1	0, 0	7 (11.1), 8	4 (6.3), 4	0, 0	8 (12.7), 10	11 (16.7), 13
Gamma-glutamyltransferase increased	0, 0	0, 0	4 (6.3), 4	6 (9.5), 7	0, 0	9 (14.3), 11	9 (13.6), 11
Metabolism and nutrition disorders	**2 (3.0), 2**	**18 (27.3), 23**	**27 (42.9), 55**	**8 (12.7), 15**	**0, 0**	**28 (44.4), 62**	**41 (62.1), 96**
Hypocalcemia	0, 0	13 (19.7), 15	3 (4.8), 3	0, 0	0, 0	2 (3.2), 2	15 (22.7), 18
Decreased appetite	0, 0	1 (1.5), 1	13 (20.6), 18	1 (1.6), 1	0, 0	13 (20.6), 16	14 (21.2), 20
Hypokalemia	1 (1.5), 1	3 (4.5), 4	8 (12.7), 11	3 (4.8), 5	0, 0	9 (14.3), 16	13 (19.7), 21
Hypophosphatemia	1 (1.5), 1	0, 0	5 (7.9), 6	1 (1.6), 1	0, 0	6 (9.5), 7	7 (10.6), 8
Musculoskeletal and connective tissue disorders	**2 (3.0), 2**	**22 (33.3), 25**	**13 (20.6), 17**	**17 (27.0), 20**	**1 (1.9), 2**	**23 (36.5), 32**	**37 (56.1), 66**
Bone pain	0, 0	10 (15.2), 11	2 (3.2), 2	3 (4.8), 3	0, 0	3 (4.8), 3	14 (21.2), 16
Pain in extremity	0, 0	1 (1.5), 1	6 (9.5), 6	6 (9.5), 6	1 (1.9), 1	10 (15.9), 12	11 (16.7), 14
Back pain	0, 0	6 (9.1), 6	2 (3.2), 2	2 (3.2), 2	0, 0	2 (3.2), 2	9 (13.6), 10
Nervous system disorders	**3 (4.5), 3**	**17 (25.8), 25**	**22 (34.9), 33**	**9 (14.3), 18**	**0, 0**	**19 (30.2), 34**	**32 (48.5), 79**
Headache	2 (3.0), 2	11 (16.7), 12	15 (23.8), 19	7 (11.1), 13	0, 0	14 (22.2), 21	28 (42.4), 46
Dizziness	0, 0	4 (6.1), 4	4 (6.3), 7	2 (3.2), 3	0, 0	5 (7.9), 7	7 (10.6), 14
Psychiatric disorders	**0, 0**	**5 (7.6), 5**	**15 (23.8), 20**	**6 (9.5), 6**	**1 (1.9), 1**	**13 (20.6), 16**	**20 (30.3), 32**
Insomnia	0, 0	1 (1.5), 1	9 (14.3), 9	4 (6.3), 4	0, 0	9 (14.3), 9	12 (18.2), 14
Anxiety	0, 0	2 (3.0), 2	7 (11.1), 8	0, 0	0, 0	4 (6.3), 4	9 (13.6), 10

System Organ Class Preferred Term	Time Period						
	ICF to < M n (%), E	M to < C n (%), E	C to < NE n (%), E	NE to M24 n (%), E	>M24 to M36 n (%), E	Day 1 to Last Follow-Up n (%), E	Total (ICF to Last Follow-Up) n (%), E
Renal and urinary disorders	**1 (1.5), 1**	**0, 0**	**10 (15.9), 13**	**2 (3.2), 2**	**0, 0**	**10 (15.9), 12**	**13 (19.7), 16**
Hematuria	0, 0	0, 0	6 (9.5), 6	1 (1.6), 1	0, 0	5 (7.9), 5	7 (10.6), 7
Reproductive system and breast disorders	**2 (3.0), 2**	**2 (3.0), 2**	**9 (14.3), 12**	**9 (14.3), 14**	**0, 0**	**14 (22.2), 27**	**15 (22.7), 31**
Vaginal hemorrhage	0, 0	0, 0	6 (9.5), 8	2 (3.2), 3	0, 0	7 (11.1), 11	7 (10.6), 11
Respiratory, thoracic and mediastinal disorders	**4 (6.1), 7**	**12 (18.2), 13**	**36 (57.1), 64**	**29 (46.0), 55**	**0, 0**	**47 (74.6), 114**	**52 (78.8), 140**
Epistaxis	1 (1.5), 1	3 (4.5), 3	21 (33.3), 27	11 (17.5), 16	0, 0	27 (42.9), 41	27 (40.9), 47
Cough	1 (1.5), 1	1 (1.5), 1	5 (7.9), 5	14 (22.2), 15	0, 0	16 (25.4), 19	17 (25.8), 22
Pharyngeal inflammation	0, 0	0, 0	13 (20.6), 17	0, 0	0, 0	13 (20.6), 17	13 (19.7), 17
Dyspnea	0, 0	0, 0	4 (6.3), 4	5 (7.9), 5	0, 0	6 (9.5), 7	8 (12.1), 9
Hypoxia	0, 0	2 (3.0), 2	3 (4.8), 3	2 (3.2), 3	0, 0	5 (7.9), 6	7 (10.6), 8
Skin and subcutaneous tissue disorders	**4 (6.1), 4**	**5 (7.6), 5**	**46 (73.0), 105**	**26 (41.3), 32**	**0, 0**	**52 (82.5), 125**	**54 (81.8), 146**
Alopecia	0, 0	0, 0	36 (57.1), 39	5 (7.9), 5	0, 0	39 (61.9), 42	41 (62.1), 44
Pruritus	0, 0	1 (1.5), 1	15 (23.8), 19	2 (3.2), 2	0, 0	13 (20.6), 15	18 (27.3), 22
Skin hyperpigmentation	0, 0	0, 0	10 (15.9), 10	1 (1.6), 1	0, 0	11 (17.5), 11	11 (16.7), 11
Rash	0, 0	2 (3.0), 2	4 (6.3), 4	3 (4.8), 4	0, 0	6 (9.5), 8	8 (12.1), 10

C, Initiation of conditioning; E, number of events; ICF, informed consent form (date of signature); ITT, Intent-to-Treat; M, initiation of mobilization; M24, Month 24; M36, Month 36; NE, neutrophil engraftment; TDT, transfusion-dependent thalassemia.

Table 29. Treatment-Emergent Adverse Events Occurring in ≥ 10% of Patients With TDT by Genotype (TP)

System Organ Class Preferred Term	Non-β^0/β^0 n (%)	β^0/β^0 n (%)	Overall n (%)
Patients at Risk, n	**43**	**20**	**63**
Patients with ≥ 1 Treatment-Emergent AE	**43 (100)**	**20 (100)**	**63 (100)**
Blood and lymphatic system disorders	**43 (100)**	**20 (100)**	**63 (100)**
Thrombocytopenia	43 (100)	19 (95.0)	62 (98.4)
Anemia	34 (79.1)	17 (85.0)	51 (81.0)
Neutropenia	34 (79.1)	14 (70.0)	48 (76.2)
Febrile neutropenia	19 (44.2)	13 (65.0)	32 (50.8)
Leukopenia	22 (51.2)	3 (15.0)	25 (39.7)
Gastrointestinal disorders	**42 (97.7)**	**19 (95.0)**	**61 (96.8)**
Stomatitis	33 (76.7)	12 (60.0)	45 (71.4)
Vomiting	16 (37.2)	13 (65.0)	29 (46.0)
Abdominal pain	15 (34.9)	8 (40.0)	23 (36.5)
Diarrhea	15 (34.9)	6 (30.0)	21 (33.3)
Constipation	10 (23.3)	8 (40.0)	18 (28.6)
Nausea	9 (20.9)	3 (15.0)	12 (19.0)
Dyspepsia	7 (16.3)	3 (15.0)	10 (15.9)
General disorders and administration site conditions	**30 (69.8)**	**14 (70.0)**	**44 (69.8)**
Pyrexia	22 (51.2)	7 (35.0)	29 (46.0)
Fatigue	8 (18.6)	2 (10.0)	10 (15.9)
Mucosal inflammation	4 (9.3)	5 (25.0)	9 (14.3)
Hepatobiliary disorders	**10 (23.3)**	**4 (20.0)**	**14 (22.2)**
Venoocclusive liver disease	6 (14.0)	1 (5.0)	7 (11.1)
Infections and infestations	**32 (74.4)**	**13 (65.0)**	**45 (71.4)**
Upper respiratory tract infection	5 (11.6)	5 (25.0)	10 (15.9)
Injury, poisoning and procedural complications	**22 (51.2)**	**9 (45.0)**	**31 (49.2)**
Transfusion reaction	10 (23.3)	4 (20.0)	14 (22.2)
Procedural pain	3 (7.0)	6 (30.0)	9 (14.3)
Investigations	**25 (58.1)**	**10 (50.0)**	**35 (55.6)**
Alanine aminotransferase increased	17 (39.5)	3 (15.0)	20 (31.7)
Aspartate aminotransferase increased	13 (30.2)	2 (10.0)	15 (23.8)
Gamma-glutamyltransferase increased	7 (16.3)	2 (10.0)	9 (14.3)
Blood bilirubin increased	7 (16.3)	1 (5.0)	8 (12.7)
Metabolism and nutrition disorders	**19 (44.2)**	**9 (45.0)**	**28 (44.4)**

System Organ Class Preferred Term	Non-β^0/β^0 n (%)	β^0/β^0 n (%)	Overall n (%)
Decreased appetite	6 (14.0)	7 (35.0)	13 (20.6)
Hypokalemia	7 (16.3)	2 (10.0)	9 (14.3)
Musculoskeletal and connective tissue disorders	**14 (32.6)**	**9 (45.0)**	**23 (36.5)**
Pain in extremity	6 (14.0)	4 (20.0)	10 (15.9)
Nervous system disorders	**12 (27.9)**	**7 (35.0)**	**19 (30.2)**
Headache	9 (20.9)	5 (25.0)	14 (22.2)
Psychiatric disorders	**10 (23.3)**	**3 (15.0)**	**13 (20.6)**
Insomnia	7 (16.3)	2 (10.0)	9 (14.3)
Reproductive system and breast disorders	**8 (18.6)**	**6 (30.0)**	**14 (22.2)**
Vaginal hemorrhage	2 (4.7)	5 (25.0)	7 (11.1)
Respiratory, thoracic, and mediastinal disorders	**31 (72.1)**	**16 (80.0)**	**47 (74.6)**
Epistaxis	19 (44.2)	8 (40.0)	27 (42.9)
Cough	11 (25.6)	5 (25.0)	16 (25.4)
Pharyngeal inflammation	4 (9.3)	9 (45.0)	13 (20.6)
Skin and subcutaneous tissue disorders	**35 (81.4)**	**17 (85.0)**	**52 (82.5)**
Alopecia	24 (55.8)	15 (75.0)	39 (61.9)
Pruritus	10 (23.3)	3 (15.0)	13 (20.6)
Skin hyperpigmentation	5 (11.6)	6 (30.0)	11 (17.5)

AE, adverse event; TDT, transfusion-dependent thalassemia; TP, transplant Population.

Table 30. Treatment-Emergent Serious Adverse Events in ≥ 2 Patients by Genotype (TP)

Parameter	Non-β^0/β^0 N = 43 n (%)	Parameter	β^0/β^0 N = 20 n (%)
≥ 1 TESAE	20 (46.5)	≥ 1 TESAE	8 (40.0)
Venoocclusive liver disease	4 (9.3)	Pyrexia	2 (10.0)
Thrombocytopenia	2 (4.7)		
Neutropenia	2 (4.7)		
Appendicitis	2 (4.7)		
Pyrexia	2 (4.7)		

TESAE, treatment-emergent serious adverse event; TP, Transplant Population.

Table 31. Treatment-Emergent Adverse Events Occurring in ≥ 10% of Patients With TDT by Age (TP)

System Organ Class Preferred Term	< 18 Years n (%)	≥ 18 Years n (%)	Overall n (%)
Number of patients at risk	**32**	**31**	**63**
Number of patients with ≥ 1 treatment-emergent AE	**32 (100)**	**31 (100)**	**63 (100)**
Blood and lymphatic system disorders	**32 (100)**	**31 (100)**	**63 (100)**
Thrombocytopenia	31 (96.9)	31 (100.0)	62 (98.4)
Anemia	28 (87.5)	23 (74.2)	51 (81.0)
Neutropenia	28 (87.5)	20 (64.5)	48 (76.2)
Febrile neutropenia	16 (50.0)	16 (51.6)	32 (50.8)
Leukopenia	13 (40.6)	12 (38.7)	25 (39.7)
Gastrointestinal disorders	**30 (93.8)**	**31 (100)**	**61 (96.8)**
Stomatitis	23 (71.9)	22 (71.0)	45 (71.4)
Vomiting	16 (50.0)	13 (41.9)	29 (46.0)
Abdominal pain	7 (21.9)	16 (51.6)	23 (36.5)
Diarrhea	8 (25.0)	13 (41.9)	21 (33.3)
Constipation	8 (25.0)	10 (32.3)	18 (28.6)
Nausea	6 (18.8)	6 (19.4)	12 (19.0)
Dyspepsia	3 (9.4)	7 (22.6)	10 (15.9)
General disorders and administration site conditions	**24 (75.0)**	**20 (64.5)**	**44 (69.8)**
Pyrexia	17 (53.1)	12 (38.7)	29 (46.0)
Fatigue	3 (9.4)	7 (22.6)	10 (15.9)
Mucosal inflammation	6 (18.8)	3 (9.7)	9 (14.3)
Hepatobiliary disorders	**9 (28.1)**	**5 (16.1)**	**14 (22.2)**
Venoocclusive liver disease	4 (12.5)	3 (9.7)	7 (11.1)
Infections and infestations	**19 (59.4)**	**26 (83.9)**	**45 (71.4)**
Upper respiratory tract infection	3 (9.4)	7 (22.6)	10 (15.9)
Injury, poisoning and procedural complications	**13 (40.6)**	**18 (58.1)**	**31 (49.2)**
Transfusion reaction	5 (15.6)	9 (29.0)	14 (22.2)
Procedural Pain	5 (15.6)	4 (12.9)	9 (14.3)
Investigations	**16 (50.0)**	**19 (61.3)**	**35 (55.6)**
Alanine aminotransferase increased	9 (28.1)	11 (35.5)	20 (31.7)
Aspartate aminotransferase increased	8 (25.0)	7 (22.6)	15 (23.8)
Gamma-glutamyltransferase increased	3 (9.4)	6 (19.4)	9 (14.3)
Blood bilirubin increased	2 (6.3)	6 (19.4)	8 (12.7)
Metabolism and nutrition disorders	**13 (40.6)**	**15 (48.4)**	**28 (44.4)**

System Organ Class Preferred Term	**< 18 Years** **n (%)**	**≥ 18 Years** **n (%)**	**Overall** **n (%)**
Decreased appetite	7 (21.9)	6 (19.4)	13 (20.6)
Hypokalemia	4 (12.5)	5 (16.1)	9 (14.3)
Musculoskeletal and connective tissue disorders	**9 (28.1)**	**14 (45.2)**	**23 (36.5)**
Pain in extremity	2 (6.3)	8 (25.8)	10 (15.9)
Nervous system disorders	**9 (28.1)**	**10 (32.3)**	**19 (30.2)**
Headache	9 (28.1)	5 (16.1)	14 (22.2)
Psychiatric disorders	**6 (18.8)**	**7 (22.6)**	**13 (20.6)**
Insomnia	4 (12.5)	5 (16.1)	9 (14.3)
Reproductive system and breast disorders	**2 (6.3)**	**12 (38.7)**	**14 (22.2)**
Vaginal hemorrhage	0	7 (22.6)	7 (11.1)
Respiratory, thoracic, and mediastinal disorders	**23 (71.9)**	**24 (77.4)**	**47 (74.6)**
Epistaxis	14 (43.8)	13 (41.9)	27 (42.9)
Cough	8 (25.0)	8 (25.8)	16 (25.4)
Pharyngeal inflammation	5 (15.6)	8 (25.8)	13 (20.6)
Skin and subcutaneous tissue disorders	**25 (78.1)**	**27 (87.1)**	**52 (82.5)**
Alopecia	15 (46.9)	24 (77.4)	39 (61.9)
Pruritus	8 (25.0)	5 (16.1)	13 (20.6)
Skin hyperpigmentation	5 (15.6)	6 (19.4)	11 (17.5)

AE, adverse event; TDT, transfusion-dependent thalassemia; TP, transplant Population.

Table 32. Treatment-Emergent Serious Adverse Events in ≥ 2 Patients by Age (TP)

Parameter	< 18 Years N = 32 n (%)	Parameter	≥ 18 Years N = 31 n (%)
≥ 1 TESAE	12 (37.5)	≥ 1 TESAE	16 (51.6)
Pyrexia	4 (12.5)	Venoocclusive liver disease	2 (6.5)
Venoocclusive liver disease	3 (9.4)		
Neutropenia	2 (6.3)		
Thrombocytopenia	2 (6.3)		
Febrile neutropenia	2 (6.3)		
Stomatitis	2 (6.3)		

TESAE, treatment-emergent serious adverse event; TP, Transplant Population.

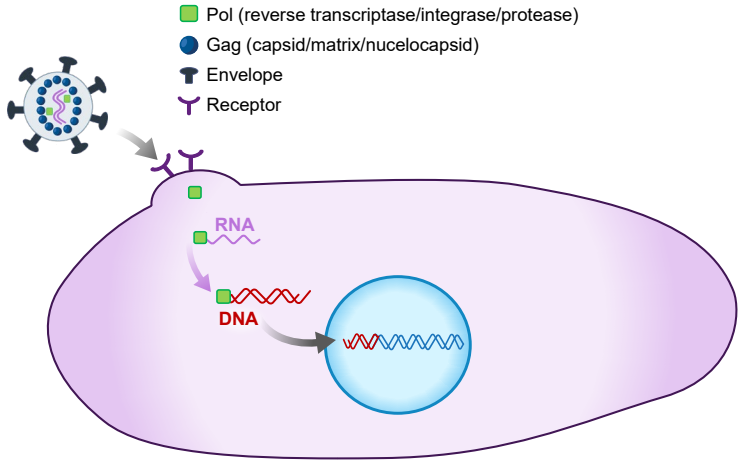
12. APPENDIX D. LENTIVIRAL VECTOR SAFETY

12.1. Introduction to Retroviral Vector Integration

Retroviruses, including lentiviruses, are RNA viruses that, upon infection of a host cell, reverse transcribe their viral RNA into DNA, which is then integrated semi-randomly into host cell genomic DNA, a process called transduction. The integrated retroviral sequence is called the provirus.

Retroviral vectors are modified retroviruses in which the viral genes that encode viral proteins are replaced with a therapeutic transgene. Due to the absence of viral genes, the integrated vector provirus is incapable of replication and further propagation; hence retroviral vectors are replication incompetent. Integration of the transgene into the host genome is permanent and expression of the transgene depends on the presence of regulatory elements that control production of the therapeutic protein. A diagrammatic representation of transduction is shown in Figure 14.

Figure 14. Diagrammatic Representation of a Retroviral Vector Transducing a Cell



Gene therapy using retroviral vectors to insert the transgene semi-randomly into the genome of patient's cells has an inherent risk of disrupting normal gene expression, including that of genes involved in the control of cell replication, which could increase the risk of vector-mediated malignancy (termed insertional oncogenesis).

Gamma retroviral vectors (GRVs) and lentiviral vectors (LVVs) are 2 distinct classes of retroviral vectors that have been used in gene therapy. Although both result in permanent integration of transgenes into the patient genome, they have different biases for where they insert, which influences the inherent safety profile and risk of insertional oncogenesis (Poletti and Mavilio 2021).

Each human gene has a promoter which serves as the "on" switch for the gene, typically adjacent to the transcriptional start site (TSS). When the gene is "turned on", the gene sequences that encode the protein (exons) are copied, or transcribed, into RNA along with the non-coding

regions between each exon (introns). The introns are intervening sequences that are frequently larger than the exons and are removed during RNA processing (called "splicing") before export from the nucleus and translation into proteins.

When GRVs transduce a cell, their proviruses preferentially integrate in transcriptionally active genes near the TSS (Wu et al. 2003). In contrast, LVVs tend to integrate away from the TSS in transcriptionally active genes, in introns (Schroder et al. 2002; Wu et al. 2003; Mitchell et al. 2004; Hematti et al. 2004).

The first GRVs used in gene therapy retained the viral promoters and enhancers that are present in each of the long terminal repeats (LTRs) of retroviruses. Because the provirus integrated preferentially near the TSS, these viral gene regulatory elements were in close proximity to the endogenous gene promoter, and thus there was a high risk of the GRV insertion increasing expression of the nearby endogenous gene. In fact, insertional oncogenesis was observed clinically with the use of these types of GRVs in several genetic diseases and was associated with insertion of the GRV provirus increasing the expression of a nearby endogenous proto-oncogene (Hacein-Bey-Abina et al. 2003b, a; Ott et al. 2006). Specifically, insertional oncogenesis was observed with the use of GRVs across 4 different disease indications resulting in incidences of insertional oncogenesis ranging from approximately 3% to 90% (Tucci et al. 2022), as follows:

– 1 case of lymphoid T-cell leukemia out of 33 patients with adenosine deaminase-severe combined immunodeficiency

– 5 cases of T-cell acute lymphoblastic leukemia out of 20 patients with X-linked severe combined immunodeficiency

– 4 cases of myeloblastic syndromes out of 5 patients with chronic granulomatous disease

– 9 cases of acute leukemia out of 10 patients with Wiskott-Aldrich syndrome

The frequent severe adverse event of insertional oncogenesis necessitated the development of a safer vector design.

Recent modifications of retroviral vectors have reduced the likelihood of insertional oncogenesis by removing the promoters and enhancers from the LTRs in both GRVs and LVVs (called "self-inactivating (SIN) vectors"), and instead rely on an internal promoter to control transgene expression (Miyoshi et al. 1998; Kraunus et al. 2004). The self-inactivation design removes the ability of the LTRs to have enhancer and promoter effects on nearby endogenous genes. Additionally, when designing a GRV or LVV, the internal promoter may be selected based on its ability to restrict transgene expression to a subset of cell types. However, depending upon where integration occurs, there is still the possibility that the internal promoter used for transgene expression could have an enhancer-like effect on nearby endogenous genes.

12.2. Lentiviral Vectors in bluebird bio Programs

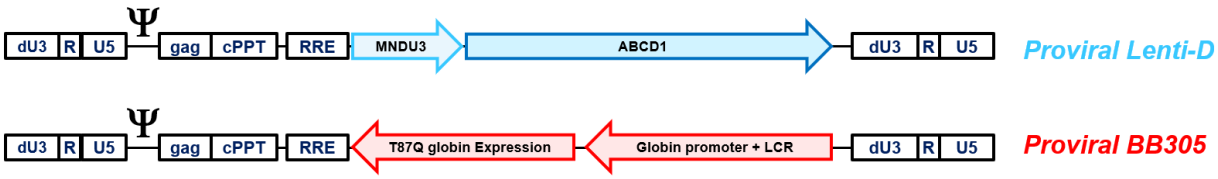
bluebird bio has 3 unique products that contain ex vivo LVV-transduced hematopoietic stem cells (HSC) that are currently being used in clinical trials, which use 2 different LVVs and 3 different sources of HSCs.

- **beti-cel**
  - **BB305 LVV**
  - CD34+ HSCs from patients with transfusion-dependent **β-thalassemia (TDT)**
- **lovo-cel**
  - **BB305 LVV**
  - CD34+ HSCs from patients with **sickle cell disease (SCD)**
- **eli-cel**
  - **Lenti-D LVV**
  - CD34+ HSCs from patients with **cerebral adrenoleukodystrophy (CALD)**

There are key differences between these 3 drug products: the LVV, the cells collected for transduction, and the manufacturing conditions.

BB305 LVV and Lenti-D LVV are illustrated in Figure 15.

Figure 15. LVV Proviral Structures



Key structural differences between BB305 LVV and Lenti-D LVV are summarized as follows:

- **Transgenes differ, tailored to disease-specific genetic defect.**
  - TDT and SCD are both caused by defects in the β-globin gene, and so the BB305 LVV encodes a functional β-globin ($\beta^{A\text{-}T87Q}$-globin).
  - CALD is caused by lack of the peroxisomal transmembrane adrenoleukodystrophy protein (ALDP), and so Lenti-D encodes a functional ALDP (encoded by an *ABCD1* cDNA).
- **Transgene structure**
  - $\beta^{A\text{-}T87Q}$-globin is expressed using the natural intron/exon configuration since intron 2 is known to be required for maximal β-globin production (Collis et al. 1990).
  - ALDP is expressed from a cDNA derived from the *ABCD1* gene, without introns, as adequate protein production is not dependent on splicing.

- **Transcriptional controls differ, tailored to cell-specific expression needed**
  - BB305 uses the human β-globin promoter and enhancer which drives high levels of gene expression, but only in the erythroid lineage (Grosveld et al. 1987). Thus, human β-globin production is restricted to this lineage.
  - Lenti-D uses the synthetic MNDU3 promoter which drives high levels of gene expression in multiple cell lineages (Challita et al. 1995; Haas et al. 2003). ALDP is thought to be produced in cerebral macrophages and microglial cells to stop progression of CALD, and so a ubiquitous promoter was chosen to ensure appropriate expression of ALDP in all hematopoietic cells, including those engrafting in the central nervous system.

The sourcing of cells used for transduction in each drug product differs. All programs currently transduce hematopoietic stem cells (HSCs) present in the CD34+ cell population. However, the CD34+ cells are obtained by apheresis after mobilization by plerixafor and G-CSF in combination for beti-cel and eli-cel, but by plerixafor alone for lovo-cel, because G-CSF is not well-tolerated by patients with SCD. The genetic mutations present in CD34+ cells from these 3 patient populations are different. The properties of the CD34+ cells may be influenced not only by their method of mobilization, but also by the different bone marrow environments generated by the disease states.

Furthermore, methods of manufacturing are not identical. For example, cells from SCD patients require additional precautions to prevent clotting during early manufacturing steps for lovo-cel, and different components are used in the manufacturing processes for eli-cel, beti-cel and lovo-cel.

Thus, differences in LVV structure, inherent cell properties, and manufacturing may impact LVV integration profiles, as well as expansion of transduced cells after engraftment in treated patients.

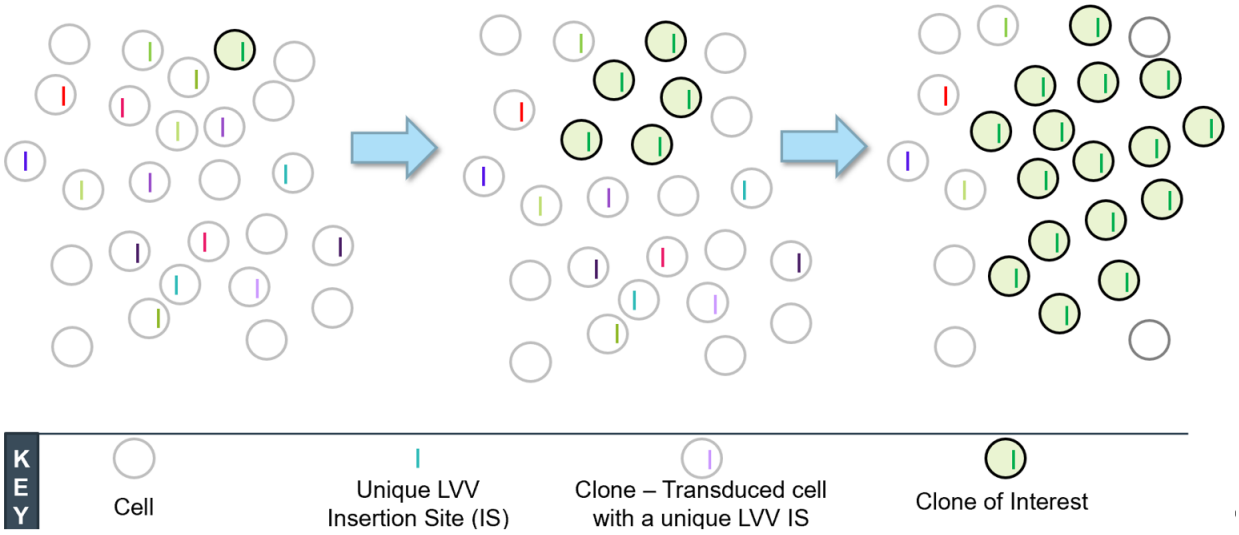
12.3. Determining Integration Profiles by Integration Site Analysis (ISA)

During transduction, each proviral integration into the cellular DNA results in a unique integration site (IS). Even though it is formally possible to have identical (at the same genomic base pair) IS in different cells, this occurrence is exceedingly rare. After gene therapy, each transduced HSC in a patient will have a unique integration profile, with some cells having multiple IS; additionally, some HSCs will not have any proviral integrations at all. A high throughput sequencing method that allows for identification of unique mappable IS is called integration site analysis (ISA). Patients generally have between hundreds to tens of thousands of unique IS detected by ISA at each timepoint analyzed.

Tracking the relative frequency over time of an IS allows for inferences into clonal dynamics of the cells that contain that IS, i.e., clones derived from the same progenitor cell, like an HSC. Due to normal clonal dynamics, relative contribution of clones can fluctuate over time; however, sometimes expansion of one or a few clones can occur to such a degree that one or a few clones dominate the peripheral blood cell population. Figure 16 is a diagrammatic representation of nucleated blood cells present in a patient after hematopoietic reconstitution, where one clone

containing a unique IS preferentially expands compared to all other clones that contain different unique IS. ISA allows for the quantitation of the relative frequency of unique IS over time providing insight into clonal dynamics.

Figure 16. Diagrammatic Representation of Clonal Expansion



Although not all clonal expansions are associated with malignancy, hematologic malignancies are always associated with expansion of a single clone. If that clone contains an IS, ISA can identify the IS in that clone and allow it to be tracked over time. This enables root cause investigations into any potential role of that IS in perturbing local gene expression that could have contributed to the development of the malignancy. The ability to track genome modifications in such a quantitative manner is a powerful tool unique to gene therapy using integrating viral vectors.

Importantly, while ISA is useful in detecting clonal expansions, it is not predictive. It cannot predict which, if any, clones will be preferentially expanded in a population. It cannot predict if, or how, oligoclonality will change over time. It cannot predict clinical outcomes or disease onset. As ISA is only able to detect transduced cells, it cannot detect the expansion of clones that do not contain a proviral sequence. Clinical signs/symptoms are still required for a diagnosis of hematological disease. Thus, frequent complete blood count (CBC) analyses for patients treated with gene therapy products are recommended as part of long-term follow-up and care.

bluebird bio recommends regular ISA monitoring for all patients in our clinical studies. This approach has been modified over time as our understanding of clonal dynamics has matured, along with the improved methodology of ISA that has increased the accuracy of relative frequency (RelFreq) estimates of insertion sites (IS). ISA is currently performed using the quantitative S-EPTS/LM-PCR method (Schmidt et al. 2001). Patients in bluebird bio clinical studies receive routine ISA every 6 months for the first 5 years post-treatment and then annually through year 15 after treatment, coupled with CBC analysis every 6 months for the entirety of a 15-year follow-up period. Depending on RelFreq results, enhanced monitoring is recommended for patients whose results suggest oligoclonality, which bluebird bio defines as any IS with a

RelFreq ≥ 10% with a VCN of ≥ 0.1 c/dg. Should there be any increased concern for malignancy, based on clinical signs and symptoms as well as the location of IS of interest and its rate of expansion, additional clinical and molecular work-ups are undertaken to further investigate the potential presence of malignancy. bluebird bio has been in an ongoing dialogue with the FDA on ISA monitoring for several years.

The majority of patients treated with bluebird bio investigational drug products do not have an IS that is persistently above 10% RelFreq. However, some patients do, and are being more closely monitored for clinical signs and symptoms of hematological changes that could be associated with a clonal process.

An earlier approach used the [nr]LAM-PCR ISA methodology (Schmidt et al. 2007), after which additional analyses of IS of interest were performed by qPCR with IS-specific primers, which simultaneously provided both an accurate RelFreq (normalizing against results using universal LVV primers) as well as an estimate of clonal contribution (normalizing against results of an endogenous gene, providing a percentage contribution of the clone containing the IS to all cells in a sample; IS-specific vector copy number (VCN)). The optimized S-EPTS/LM-PCR ISA method provides a more accurate RelFreq in agreement with IS-specific VCN.

12.4. Role of LVV Integration in Malignancies in bluebird bio Programs

There have been a total of 5 malignancies across all bluebird bio LVV clinical studies to-date.

- **beti-cel**: There have been no malignancies in any patient treated with beti-cel.
- **lovo-cel**: There have been 2 malignancies, MDS (converting to AML; MDS/AML) and AML, in 2 patients treated with an early version of lovo-cel. Both cases were determined to not have BB305 LVV involvement and therefore were not insertional oncogenesis((Hsieh et al. 2020; Goyal et al. 2022).
- **eli-cel**: There have been 3 malignancies, all MDS, in 3 patients treated with eli-cel. These cases of MDS were likely mediated by Lenti-D LVV insertion and thus represent insertional oncogenesis.

No cases of insertional oncogenesis have been seen in patients treated with beti-cel or lovo-cel, both products made using BB305 LVV. Root cause investigations of the malignancies in eli-cel suggest that the specific properties of the Lenti-D LVV contribute to the higher risk for insertional oncogenesis in patients treated with eli-cel when compared with beti-cel or lovo-cel.

12.4.1. MDS in patients treated with eli-cel: likely insertional oncogenesis

All 3 cases of MDS in patients treated with eli-cel are associated with expansion of a clone that contains at least one IS in a known proto-oncogene: MECOM in 2 patients and PRDM16 in 1 patient. Changes in expression of these genes have been observed in these patients' cells, indicating that the IS is having an impact on the expression of the nearby gene. There are several mechanisms by which an LVV insertion could increase gene expression. The Lenti-D LVV is a SIN LVV with an internal MNDU3 promoter, which could act as an enhancer to increase gene expression. Alternatively, since both the MECOM and PRDM16 genes are normally active in HSCs and silenced during differentiation, the presence of an actively transcribed LVV may

interfere with gene silencing. Notably, MNDU3 is an engineered, virally derived synthetic promoter, that is active in multiple cell types.

It should be noted that IS are frequently detected in proto-oncogenes, including MECOM, without any observation of oligoclonality or malignancy (e.g. (Reinhardt et al. 2021)). Thus, additional factors play a role in the development of malignancy in treated patients. A summary of results of other genetic tests in these patients is presented in Table 33. Some other mutations have been identified in 2 of the 3 patients, but their significance in contributing to the development of MDS is not clear.

Table 33. Genetic Findings in Patients with MDS after Treatment with eli-cel

Time Period	Patient 104-18	Patient 104-08	Patient 102-03
First observation of ≥ 10% RelFreq of relevant IS	Month 6	Month 6	Month 92; not observed at previous visit at Month 60[b]
Location of IS currently at ≥ 10% RelFreq [a]	MECOM, SLC6A16	MECOM, ACTR3, RAP2C-AS1, ST3GAL6-AS1	PRDM16, GAB3, CAMK2A, TYK2, SNX12, MIR106A
Mutation analyses at or after diagnosis			
Karyotype	46XY, Chr.14 aberration; germline	46XY normal	46XY normal
NGS	CDKN2A c.168C>G, germline	None detected	KRAS c.35G>C, 13.6%VAF NRAS c.35G>C, 2.5% VAF
IS associated with increased expression of adjacent gene?	Yes, MECOM. Other IS not tested	Yes, MECOM Other IS not tested	Yes

Abbrev.: IS, integration site; NGS, next generation sequencing according to Rapid Heme Panel; VAF, variant allele frequency; VUS, variant of unknown significance.

[a] Location of all IS mentioned that are at currently ≥10% RelFreq in each patient are demonstrated to be in a single clone for Pts 104-18 and 104-08; not yet determined for Pt 102-03.

[b] Patient missed in-person visits in the approximately 32 months prior to MDS diagnosis due to COVID concerns.

In the eli-cel program, several patients currently satisfy the criteria for oligoclonality (i.e., have an IS present at ≥ 10% RelFreq and VCN of ≥ 0.1 c/dg), with the levels stable for several years in some patients, without evidence of malignancy. This observation is consistent with other published reports of oligoclonality without malignancy, (e.g. ((Negre et al. 2016; Reinhardt et al. 2021; Magrin et al. 2022)). Patients with oligoclonality are subject to enhanced monitoring via more frequent complete blood counts for the presence of any hematological abnormalities. Additionally, as mentioned above, should there be any increased concern for malignancy, based on clinical signs and symptoms as well as the location of IS of interest and its rate of expansion,

additional clinical and molecular work-ups are undertaken to further investigate the potential presence of malignancy.

Table 34 summarizes the number of patients that currently satisfy the criteria for oligoclonality and persistent oligoclonality (i.e., those meeting the criteria for oligoclonality at two consecutive determinations) and Figure 17 provides ISA profiles for the IS of interest in those patients treated with beti-cel.

Table 34. Summary of Patients Currently with an IS ≥10% RelFreq

Study in which Patient was Treated with Gene Therapy	Current Persistent Oligoclonality, n	Current Oligoclonality (last visit was first oligoclonal result), n
eli-cel (n = 64)		
ALD-102 (n = 32)	5 [a]	1
ALD-104 (n = 32)	3	2
lovo-cel (n =49)		
HGB-205 (n=3)	0	0
HGB-206, Group A (n = 7)	2 [b]	0
HGB-206, Group B (n = 2)	0	0
HGB-206, Group C (n = 35)	1*	0
HGB-210 (n = 2)	0	0
beti-cel (n =63)		
HGB-205 (n=4)	0	0
HGB-204 (n = 18)	2	0
HGB-207 (n = 23)	0	1*
HGB-212 (n = 18)	0	0

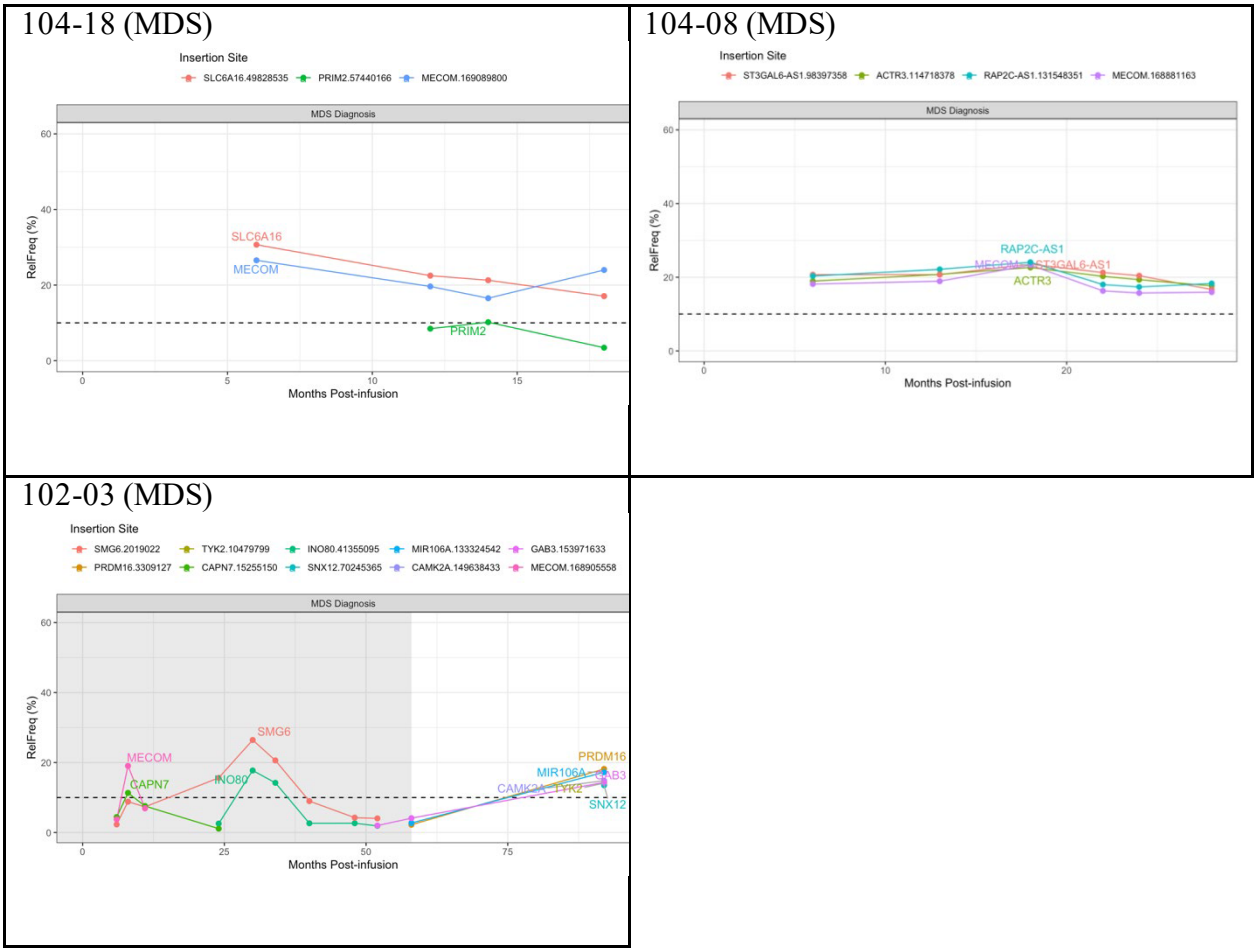
Data as of 29Apr2022 for all patients, except for 2 patients indicated by an asterisk, which included late breaking data. n defined as patients treated with gene therapy with available ISA data.

[a] one patient withdrew from study

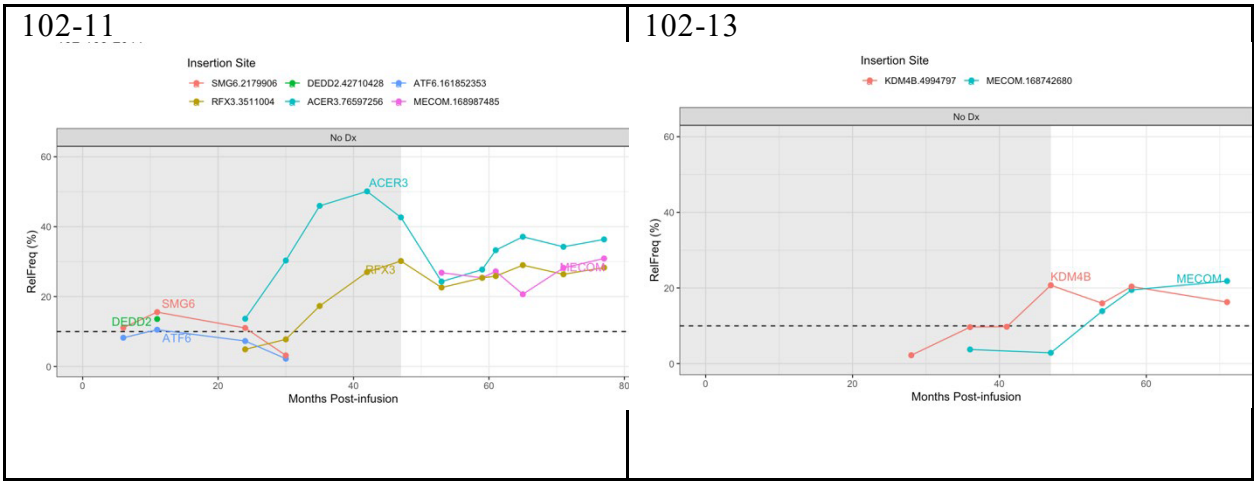
[b] one patient deceased

Figure 17. Oligoclonality in Patients Treated with eli-cel

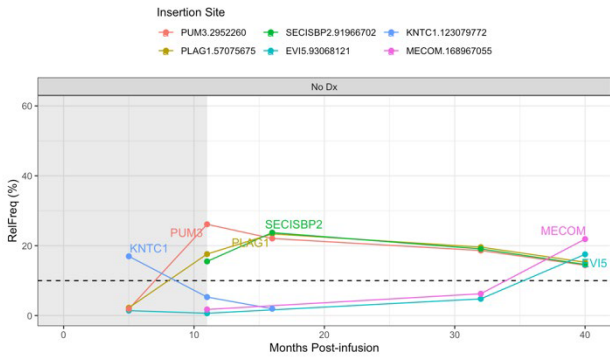
A. Persistent Oligoclonality in Patients with a Malignancy



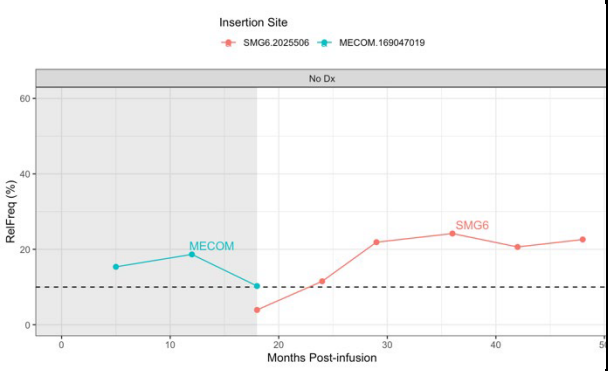
B. Ongoing Persistent Oligoclonality in Patients Without Evidence of a Malignancy



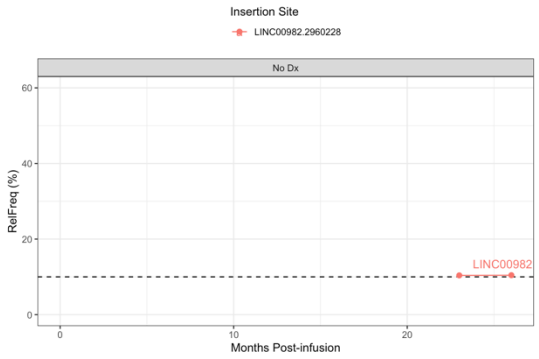
102-31



102-23

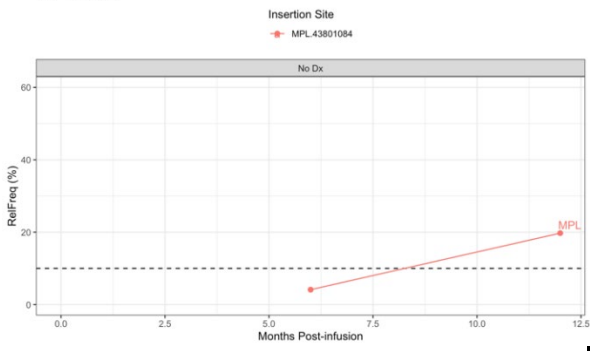


104-09

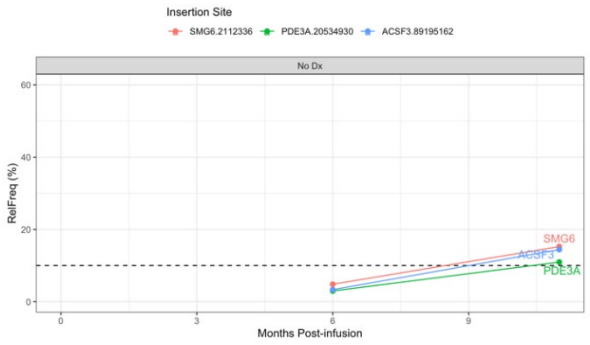


C. Other Patients with Oligoclonality at Latest Visit

104-22



104-27



Data as of 29Apr2022.
Notes: ISA methods: gray shading = [nr]LAM-PCR; no shading = S-EPTS/LM-PCR
All IS shown had a RelFreq of ≥10% at least once.
X-axis and Y-axis scales may differ between patients.

12.4.2. MDS/AML in patients treated with lovo-cel: not due to insertional oncogenesis

Two patients treated with lovo-cel, manufactured with an early process that is no longer in use, were diagnosed with MDS/AML and AML. These 2 malignancies were determined to not be due to insertional oncogenesis (Hsieh et al. 2020; Goyal et al. 2022).

The first case had blasts that did not contain the provirus, and thus could not have been mediated by LVV integration. Notably, the blasts had numerous hallmark AML mutations.

The second case also had blasts with similar hallmark AML mutations, but in addition contained an LVV IS in a gene called VAMP4. ISA showed that the relative frequency of this IS increased markedly during the period in which clinical signs and symptoms indicated malignancy (Figure 19A). Molecular analyses indicated that the LVV IS was a passenger mutation that played no role in the development of AML (see below).

A summary of these key findings is presented in Table 35.

Table 35. Genetic Findings in Patients with MDS/AML after Treatment with lovo-cel

Time Period	Patient 206-A-02	Patient 206-A-01
Baseline, before lovo-cel administration	No mutations or cytogenetic abnormalities detected (microarray, NGS)	No mutations or cytogenetic abnormalities detected (microarray, NGS)
After lovo-cel administration, prior to AML diagnosis treatment		No mutations detected at Visits M3, M6, M18, M24 (microarray, NGS)
At or after AML diagnosis:	Monosomy 7 Abnormal 19p RUNX1 Missense mutation (p.Asp198Gly) PTPN11 Exon 3 missense (p.Phe71Leu) KRAS Missense mutation (p.Gly12Ala)	Monosomy 7 Partial loss of 11p RUNX1 Exon 5 stop gained (p.Ala149*fs) PTPN11 Exon 3 missense (p.Ala72Val)
Persistent oligoclonality of IS observed?	No; malignant cells do not contain an IS	Yes, IS in VAMP4 gene
IS associated with dysregulation of adjacent gene?	Not applicable	No dysregulation detected

Note: Mutations in red font have been previously associated with cases of AML in the literature.

The role of the IS in VAMP4 was robustly evaluated after bluebird bio sought expert guidance and alignment with numerous key opinion leaders in the field of cell and gene therapy. After evaluating all established criteria for determining LVV involvement in development of AML,

which are summarized in Figure 18, the totality of evidence supported that the IS in VAMP4 is a passenger, non-causative insertion.

Figure 18. Evidence Supporting a Benign Passenger Role for IS in VAMP4 Gene

LVV Exoneration Criteria	Findings
1. **Classical driver alterations consistent with MDS/AML**	Monosomy 7, partial loss of 11p, *RUNX1, PTPN11*
2. **No substantial change in gene expression around IS**	No remarkable expression changes in 10 MB region around *VAMP4* IS
3. **Vector is NOT transcriptionally active in tumor cells**	Very low level HBB detected in CD34+ cells
4. **Transcriptional profile consistent with properties of known MDS/AML driver alterations**	RNAseq data consistent with monosomy 7 and contains *PTPN11* and *RUNX1* mutations
5. **Insertion site(s) found in other patients without sequelae**	*VAMP4* IS common and this patient is the only one with *VAMP4* IS >0.05% at any point
6. **Insertion site(s) unremarkable with respect to cancer-associated genes**	*VAMP4* has no known association with cellular proliferation or oncogenesis
7. **Insertion site(s) does not disrupt genomic elements**	*VAMP4* insertion does not disrupt mapped genomic features

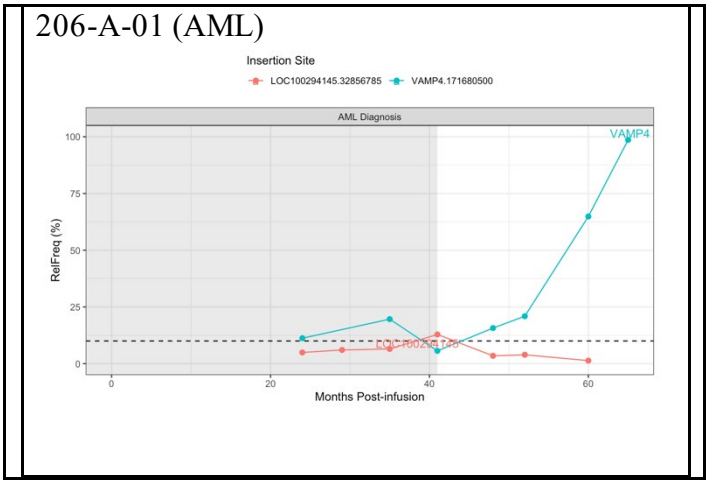
A parallel root cause investigation identified both lovo-cel and disease-specific risk factors for development of malignancy. Patients with sickle cell disease have been determined to be at an increased risk of hematological malignancy (Seminog et al. 2016; Brunson et al. 2017). To address this risk, genetic screening has been implemented as well as additional informed consent conversations between patients and physicians so that patients are aware of this elevated baseline risk. Additionally, monitoring in both the parent clinical study and in the long-term follow-up study has been increased.

In lovo-cel treated patients in Group A of Study HGB-206, an early manufacturing process was used in which CD34+ cells were collected from bone marrow harvest, resulting in both reduced numbers and quality of CD34+ cells (Tisdale et al. 2020). This likely contributed to an increased proliferative burden on engrafting CD34+ cells and hematopoietic stress following lovo-cel administration. Protocol changes implemented during Study HGB-206, including use of plerixafor-mobilized apheresis for collection of CD34+ cells, have increased both total numbers and quality of enriched CD34+ cells in the apheresis collection. Additionally, lovo-cel administered to Group A patients in Study HGB-206 was manufactured using an early process that had generally low transduction efficiencies, that led to low expression of the transgenic $\beta^{A\text{-}T87Q}$-globin and incomplete resolution of disease. Protocol changes implemented throughout the evolution of Study HGB-206 have addressed this and improved transduction efficiency has led to higher $\beta^{A\text{-}T87Q}$-globin expression, reduction of RBC sickling, and reduced vaso-occlusive events. Thus, resolution of disease in patients treated with lovo-cel manufactured under current protocols has resulted in less erythropoietic stress after treatment.

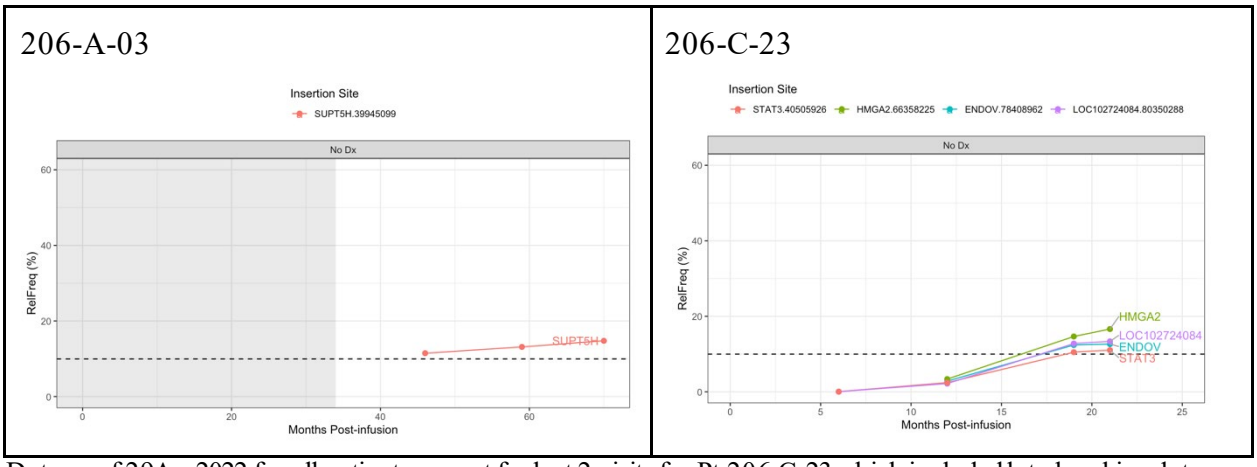
In addition to the patient who had AML, 2 additional patients have an IS present persistently at ≥ 10% RelFreq, without evidence of malignancy (Figure 19).

Figure 19. Oligoclonality Observed in Patients Treated with lovo-cel

A. Persistent Oligoclonality in Patient with a Malignancy



B. Ongoing Persistent Oligoclonality in Patients Without Evidence of a Malignancy



Data as of 29Apr2022 for all patients, except for last 2 visits for Pt.206-C-23 which included late-breaking data.
Notes: ISA methods: gray shading = [nr]LAM-PCR; no shading= S-EPTS/LM-PCR
All IS shown had a RelFreq of ≥10% at least once.
X-axis and Y-axis scales may differ between patients.

12.4.3. No malignancies in patients treated with beti-cel

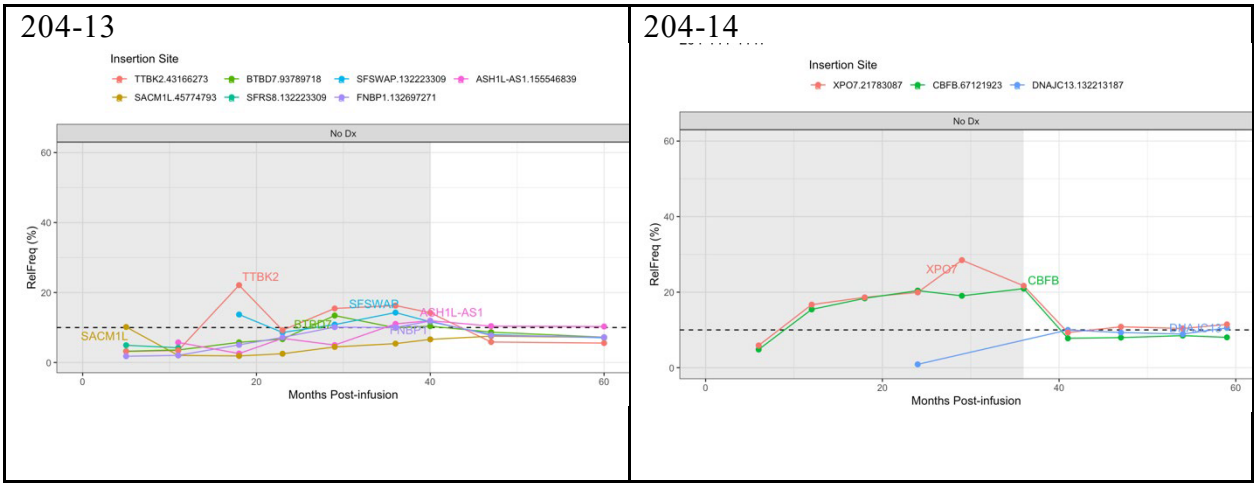
There have been no cases of malignancy, and thus no insertional oncogenesis, in patients treated with beti-cel to date. The SIN LVV design coupled with an erythroid-specific internal promoter restricts the activity of the promoter and enhancer to only those cells involved in the production of hemoglobin, and thus limits the potential for gene dysregulation. Unlike in sickle cell disease, there is no evidence in the published literature to suggest that patients with β-thalassemia have an elevated risk of hematologic malignancy.

Two patients treated with beti-cel have an IS present persistently ≥ 10% RelFreq, without evidence of malignancy. Thus, the presence of persistent oligoclonality is not predictive of

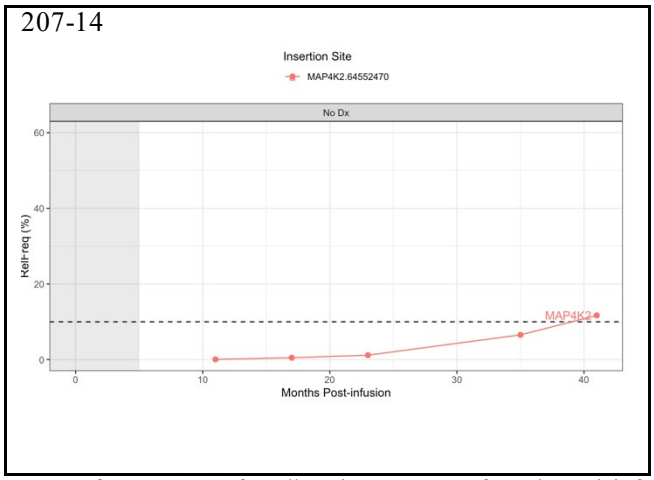
malignancy and can be present stably over several years (Figure 20A). One additional patient has an IS at >10% RelFreq at the latest ISA evaluation (Figure 20B).

Figure 20. Oligoclonality in Patients Treated with beti-cel

A. **Ongoing Persistent Oligoclonality Without Evidence of Malignancy**



B. **Other Patients with Oligoclonality at Latest Visit**



Data as of 29Apr2022 for all patients, except from last visit for Pt.207-14 which included from late-breaking data.
Notes: ISA methods: gray shading = [nr]LAM-PCR; no shading= S-EPTS/LM-PCR
All IS shown had a RelFreq of ≥10% at least once.

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12.5. Summary of Differences Between Drug Products and Patient Populations that Could Explain Differences in Frequency of Occurrence of Malignancy

Oncogenesis is a known hazard for all myeloablative therapies that involve the transplantation of hemopoietic stem cells, even in the absence of gene therapy, due to the need for toxic myeloablative agents such as busulfan. All bluebird bio gene therapy programs have this in

common. However, there appear to be differences in the rates of malignancy that occur in the different programs. These are potentially due to:

Different vector design. The ubiquitous promoter present in the Lenti-D LVV compared to the cell-lineage restricted BB305 LVV is likely to increase the risk of dysregulation of nearby genes in multiple cell types, including HSCs. The demonstration of MECOM dysregulation in 2 patients with MDS who were previously treated with eli-cel is consistent with this.

Different disease states influencing general risk for malignancy due to somatic mutations. SCD is known to be associated with an increased risk of malignancy, often associated with hallmark somatic mutations, and 2 cases of MDS/AML in the lovo-cel program support a role for this.

Differences in manufacturing processes. Manufacturing conditions differ between drug products, including differences in the source of HSCs (which may have different properties depending on the disease and the way the cells were mobilized or collected), as well as transduction conditions, and can influence the transduction frequency. Optimization of manufacturing conditions during development was performed in each program, and processes differ between programs.

12.6. Conclusions

In summary, retroviral vector understanding and design has improved substantially since the original GRVs utilized in gene therapy trials in the 1990s and early 2000s. LVV properties, both inherent and engineered, limit the risk of any one insertion to cause gene dysregulation in nearby endogenous genes. IS can be tracked with a high-throughput ISA method that can provide insight into clonal dynamics but, importantly, is not predictive of clinical sequelae. Therefore, regular CBC analyses for all patients are recommended, and are implemented for 15 years after treatment by bluebird bio in clinical studies.

Oncogenesis is a known hazard for transplantation of hemopoietic stem cells even in the absence of gene therapy due to the need for toxic myeloablative agents, such as busulfan, and this risk can be exacerbated by underlying disease characteristics.

Insertional oncogenesis is an acknowledged hazard associated with gene therapy products and different bluebird bio products appear to have different risk profiles. Factors that affect the risk of insertional oncogenesis likely include the internal transgene promoter in the LVV.

The risk of oncogenesis with each product must be weighed against the severity of the disease, the availability of other treatments and their risks, and the probability and magnitude of benefit that gene therapy could offer in each disease.