Cellular, Tissue, and Gene Therapies Advisory Committee Meeting

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FDA Briefing Document

Advisory Committee Meeting

June 9-10, 2022

BLA 125755
Elivaldogene Autotemcel
bluebird bio, Inc.

DISCLAIMER STATEMENT

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

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ABBREVIATIONS

ABCD1	Adenosine triphosphate (ATP) binding cassette, subfamily D, member 1
ALDP	Adrenoleukodystrophy Protein
Allo-HSCT	Allogeneic hematopoietic stem cell transplant
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
BLA	Biologics License Application
BM	Bone marrow
c/dg	Copies per diploid genome
CALD	Cerebral adrenoleukodystrophy
CBC	Combined blood count
CD15+	Cluster of differentiation 15 positive
CD34+	Cluster of differentiation 34 positive
cDNA	Complementary deoxyribonucleic acid
Chr	Chromosome
CI	Confidence interval
DAT	Direct antiglobulin test
DNA	Deoxyribonucleic acid
DP	Drug product
Eli-cel	Elivaldogene autotemcel
FDA	Food and Drug Administration
FISH	Fluorescence in situ hybridization
G-CSF	Granulocyte colony stimulating factor
GdE	Gadolinium enhancement
GT	Gene therapy
GVHD	Graft versus host disease
Hgb	Hemoglobin
HLA	Human leukocyte antigen
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplant
ISA	Integration site analysis
ISS	Integrated Summary of Safety
ITT	Intent-to-treat
KM	Kaplan-Meier
LTFU	Long-term follow-up
LTR	Long terminal repeat
LVCF	Last visit carried forward
LVV	Lentiviral vector
Mbp	Megabase pair
MD	Matched donor
MDS	Myelodysplastic syndrome
MFD	Major functional disability
MPSV	Myeloproliferative sarcoma virus
MRD	Matched related donor (other than sibling)

MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MSD	Matched sibling donor
MURD	Matched unrelated donor
NE	Neutrophil engraftment
NFS	Neurologic function score
NGS	Next generation sequencing
NMSD	No matched sibling donor
(NR)LAM-PCR	Linear amplification polymerase chain reaction plus non-restricted linear amplification polymerase chain reaction
Nt	Nucleotide
OS	Overall survival
PB	Peripheral blood
PCR	Polymerase chain reaction
polyA	Polyadenylation
PE	Platelet engraftment
PLT	Platelet
PS	Propensity Score
RCL	Replication-competent lentivirus
rUTES-101	Re-coded untreated population strictly eligible for ALD-102
S-EPTS/LM-PCR	Shearing extension primer tag selection ligation-mediated polymerase chain reaction
SAE	Serious adverse event
SCD	Sickle cell disease
SCID-X1	X-linked severe combined immunodeficiency
TDT	Transfusion dependent thalassemia
TEAE	Treatment-emergent adverse event
TP	Transplant population
TPES	Transplant population strictly eligible for ALD-102
TTE	Time-to-event
µL	Microliter
UMD	Unmatched donor
URD	Unmatched related donor
UT	Untreated population
UTES	Strictly eligible for ALD-102 untreated population
UURD	Unmatched unrelated donor
VAF	Variant allele frequency
VCN	Vector copy number
VLCFA	Very long chain fatty acids
WBC	White blood cell
X-ALD	X-linked adrenoleukodystrophy

1 CLINICAL INDICATION

Bluebird bio, Inc. (the Applicant) has proposed the indication for elivaldogene autotemcel (eli-cel) of "treatment of patients less than 18 years of age with early cerebral adrenoleukodystrophy (CALD) who do not have an available and willing human leukocyte antigen (HLA)-matched sibling hematopoietic stem cell (HSC) donor."

2 EXECUTIVE OVERVIEW

The Cellular, Tissue, and Gene Therapies Advisory Committee is convened to discuss the BLA submitted by bluebird bio, Inc. Applicant for elivaldogene autotemcel (eli-cel). Eli-cel is a drug product containing genetically modified autologous CD34+ HSCs transduced with Lenti-D lentiviral vector (LVV) encoding *adenosine triphosphate-binding cassette, sub-family D, member 1 (ABCD1)* cDNA for human adrenoleukodystrophy protein (ALDP).

Background

Childhood CALD is a rare neurodegenerative X-linked metabolic disease that affects the brain and causes progressive neurodegeneration followed by death usually during the second decade of childhood if left untreated. CALD is caused by mutations in the *ABCD1* gene, which encodes the adrenoleukodystrophy protein (ALDP). Deficiency of ALDP impairs transport and metabolism of very long-chain fatty acids (VLCFAs). The accumulating VLCFAs initiate a neuroinflammatory cascade thought to cause the neurologic manifestations of CALD. CALD is a heterogeneous disease, and the time course of clinical progression is highly variable. Boys typically present initially with inattention, hyperactivity or academic challenges between age 4-10 years. The disease leads to vision and hearing impairment, gait difficulties, seizures, cognitive impairment, weakness and stiffness of limbs with eventual loss of voluntary movement, loss of communication, incontinence, deafness, and cortical blindness, and death.

There are no FDA-approved treatments for CALD in the United States (US). Currently, allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the standard of care, typically performed when patients have early active disease as diagnosed by cerebral lesions and gadolinium enhancement on MRI with no or minimal neurologic dysfunction. If the graft takes, clinical and radiographic disease stabilization usually occurs within 12-24 months. Unaffected HLA-matched siblings are the preferred donors due to lower rates of HSCT complications, including potentially life-threatening graft rejection, graft versus host disease (GVHD) and infection. Unfortunately, only approximately 30% of boys with CALD have matched sibling donors. Because of the significant morbidity and mortality associated with allo-HSCT in patients without matched sibling donors, eli-cel, a novel one-time autologous gene therapy product, was developed with the intention of generating a safer treatment.

Issues

The primary evidence of safety and effectiveness are generated from 2 multi-national, multi-center, single-arm, open-label, single-dose studies: Study ALD-102 (started in August 2013 and completed in March 2021), and Study ALD-104 (an ongoing study started in January 2019). There is also ongoing long-term follow-up of these subjects in Study LTF-304. Data from the interventional studies were compared to 2 external control data sources: (1) Study ALD-101, a retrospective natural history study in boys with CALD who received either no treatment (diagnosed between 1988 and 2010) or allo-HSCT (treated between 1997 and 2010), and (2) Study ALD-103, a hybrid prospective-retrospective observational study in boys who were treated more recently with allo-HSCT (between 2013 and 2019). Study populations commonly referred to in the BLA are detailed in Table 1.

Table 1: BLA Study Populations

Study or Studies	Description	Population
ALD-102	Subjects treated with eli-cel ("transplant population")	TP-102
ALD-104	Subjects treated with eli-cel ("transplant population")	TP-104
ALD-101	Untreated (UT) subjects with gadolinium enhancement (GdE+) on brain MRI	UTG-101
ALD-101	Untreated (UT) subject similar ("strictly eligible")[1] to TP-102	UTES-101
ALD-101, ALD-103	Subjects treated with allo-HSCT ("transplant population") similar ("strictly eligible")[1] to TP-102	TPES-101, TPES-103
ALD-101, ALD-103	TPES subjects with HLA- matched sibling HSC donors	TPES-101/ TPES-103 MSD
ALD-101, ALD-103	TPES subjects with no HLA- matched sibling HSC donors	TPES-101/ TPES-103 NMSD
ALD-101, ALD-103	TPES subjects with HLA- matched HSC donors	TPES-101/ TPES-103 MD
ALD-101, ALD-103	TPES subjects with HLA- unmatched HSC donors	TPES-101/ TPES-103 UMD

Abbrev: TP, transplant population; UT, untreated population; GdE, gadolinium enhancement; UTG, untreated with gadolinium enhancement; UTES, untreated population strictly ALD-102 eligible; allo-HSCT, allogeneic hematopoietic stem cell transplant; TPES, strictly ALD-102 eligible transplant population; HSC, hematopoietic stem cell; MSD, matched sibling donor; NMSD, no matched sibling donor; MD, matched donor; UMD, unmatched donor.

[1] Strictly eligible includes matched to the following eligibility criteria for Study ALD-102: Neurologic function score (NFS) ≤1, Loes score 0.5 to 9, Gadolinium enhancement (GdE+) on brain MRI

The primary efficacy endpoint for Study ALD-102 was number and proportion of subjects remaining alive and without any of the 6 pre-defined Major Functional Disabilities (MFDs) at the Month 24 visit following treatment with eli-cel (i.e., Month 24 MFD-free survival). The 6 MFDs are loss of communication, cortical blindness, tube feeding, wheelchair dependence, complete loss of voluntary movement, and total incontinence.

For success, the lower bound of the 2-sided 95% exact confidence interval (CI) of Month 24 MFD-free survival for the cohort had to exceed a clinical benchmark of 50%.

This clinical benchmark was derived from two populations in the natural history study (ALD-101). The two ALD-101 sub-populations used for calculating the benchmark were:

> Population #1: The untreated population with presence of gadolinium enhancement (GdE+) on brain MRI, for whom MFD-free survival at 24 months following the first GdE+ MRI was 21% (exact 95% CI of 6.1% to 45.6%). The benchmark is thus above the upper bound of the 95% CI for MFD-free survival in the untreated GdE+ population.
>
> Population #2: The "strictly ALD-102-eligible[1]" HSCT-treated group ("TPES-101 population") who were treated with HSCTs from an alternative donor (no matched sibling donor, NMSD) for whom the lower bound of the 95% exact CI of MFD-free survival at 24 months following HSCT was 50.1% (mean 76% with exact 95% CIs of 50.1% to 93.2%). The lower bound of the 95% CI for MFD-free survival in the TPES-101 NMSD population exceeded the upper bound for untreated Population #1. Therefore, 50.0%, separating the outcomes of untreated and treated patients, was selected as the proposed benchmark.

FDA concerns regarding the Applicant's benchmark analysis include:

1. Study ALD-101 Populations #1 and #2 had very different baseline characteristics, making it unclear whether HSCT is better than no treatment in the early active disease population over a 2-year period (the population enrolled in Study ALD-102).

2. Neither Population #1 nor Population #2 was comparable at baseline to subjects treated with eli-cel in Study ALD-102 (i.e., Populations #1 and #2 had higher-risk baseline characteristics).

3. Repeat HSCT was imputed as a failure of MFD-free survival (which FDA does not believe is equivalent to death or an MFD and should not be counted as such. In ALD-101, HSCT was repeated for graft failure rather than progression of disease).

4. Based on FDA re-examination of the ALD-101- derived natural history data, 24-months is insufficient for observing the development of MFDs in boys who are very early in their disease course (the enrolled population).

5. Bias may have been introduced because MFD assessments were done by unblinded clinical investigators. Some MFDs (such as wheelchair dependence and tube feeding) may be over- or under-called, based on knowledge of treatment assignment. For example, if the subject was "dependent" on a wheelchair only after 10 blocks of walking, the subject may

[1] "Strictly ALD-102- Eligible" is the Applicant's terminology for subjects in Studies ALD-101 and ALD-103 who would have been eligible for enrollment in ALD-102 based on the following key eligibility criteria at Baseline: (1) NFS ≤1, (2) Loes score between 0.5 and 9, (3) GdE+.

have been identified as “wheelchair” dependent in ALD-101, but not in ALD-102. The absence of central assessors may limit the validity and reliability of the MFD assessments.

Additional comparator data were unavailable because Study ALD-103 had been terminated once the Applicant’s goal number of subjects were enrolled (n= 59), and subjects were not followed after study termination. FDA requested additional data-cuts from Studies ALD-102 and ALD-104 to have 24-month data from 13 subjects from Study ALD-104 and more longitudinal data from Study ALD-102. FDA focused analyses on one of the Applicant’s secondary analyses, MFD-free survival over time, that was represented as a time-to-event (TTE) Kaplan-Meier (KM) analysis and that compared MFD-free survival between Study ALD-102 and Study ALD-103. The overall analyses showed little difference between eli-cel and HSCT but as might have been expected given the natural history of CALD, there were few clinical endpoint events within the timeframe of the study. The paucity of endpoint events across all study populations made it difficult to assess the treatment effect. Comparability issues between ALD-102 and ALD-103 populations, and uncertainty about whether these subjects would have experienced MFDs or death in the absence of HSCT over the follow-up period also challenge our ability to make conclusions about the relative efficacy of HSCT and eli-cel. This is especially true for long-term efficacy regarding neurologic manifestations. Additional data including a longer observation time would facilitate a better understanding of the comparative efficacy and comparative durability of efficacy between HSCT and eli-cel.

FDA identified a sub-population who had better outcomes with eli-cel. The TPES ALD-101/ALD-103 subjects who received HSCT from HLA-unmatched donors (TPES-UMD) did poorly compared to subjects who received HSCT from HLA-matched[2] donors (TPES-MD), with a mortality rate of 3/17 (17.6%), compared to 0%, respectively, in the first 6 months following HSCT. There was also 0% mortality in the eli-cel-treated subjects in the first 6 months. Of the 3 deaths in TPES-UMD, 2 were transplant-related, and 1 was related to disease progression. Small numbers of subjects and uncertainties regarding baseline comparability between the TPES-UMD and TPES-MD subjects challenge our ability to make firm conclusions about the benefit of eli-cel compared to HSCT in patients who do not have a matched donor.

The uncertainty regarding efficacy at 24 months following treatment is particularly problematic in the context of the recent discovery of a serious safety concern, the development of myelodysplastic syndrome (MDS), a life-threatening malignancy which occurred in 3 subjects. The FDA thinks two of the events are definitely related to the product and the third is highly likely to be related, given integration site analyses that showed increased relative frequencies of integration and clonal expansion with genes known to be associated with malignancy. Also, the rarity of the condition in the absence of a provoking event, and the lack of known association between MDS and CALD are other factors that have influenced our concern regarding a causal relationship. As of the

[2] HLA-matched refers to matching on 100% of assessed alleles

August 18, 2021, data cut, excluding the three subjects who have now been diagnosed with MDS, 50 of 51 (98%) of the subjects treated with eli-cel had evidence of lentiviral integration into a single important proto-oncogene. In addition, an integration site with a relative frequency of 10% or greater had been detected in 20 of 51 (39%) subjects. Although our understanding of the clinical significance of the integrations is limited, our observation of the growth of clones with proto-oncogene integration sites suggests that these clones may have a selective advantage and may evolve into cancer. The cases of Acute Myeloid Leukemia (AML) following treatment with a related LVV-based product in patients with sickle cell disease also add to our concern. The 3 cases of MDS in the eli-cel-treated subjects occurred at 14 months, 22 months, and 7.5 years. Because more than 50% of subjects have less than two years of follow-up data (follow-up duration median 23.5 months, mean 32 months, range 1.5 months to 7 years and 4 months), FDA expects that longer follow-up will uncover additional cases of this potentially life-threatening complication of treatment.

The FDA seeks the opinion of the Committee regarding the following issues:

(This section is provided early in draft for the Committee)

Topics for Discussion

- Which population, if any, has clinically meaningful benefit with eli-cel
- Risk of insertional oncogenesis from LVV integration in eli-cel-treated subjects
- The relevance of safety data from lovo-cel and beti-cel to eli-cel
- Recommendations on post-treatment monitoring for risk of insertional oncogenesis and risk mitigation

Discussion Questions

1. We have several main efficacy concerns:
    a. The benchmark calculation that was used for the primary efficacy analysis was based on data from populations that were not comparable to the eli-cel population at baseline (i.e., “the early active disease population”). There were a multitude of problems with the benchmark calculation that made the primary efficacy analysis uninterpretable.
    b. Because the studies were open-label, the identification of an MFD primary endpoint event may have been susceptible to the introduction of bias.
    c. The principal comparator allo-HSCT data in Study ALD-103 were partially collected retrospectively. Retrospective data collection can introduce bias.
    d. The subjects in ALD-103 were somewhat older and had higher Loes scores (a prognostic biomarker discussed in Appendix 1) than the eli-cel population, raising concerns about comparability.
    e. The repeat HSCT events in ALD-103 were counted toward efficacy in the Applicant’s original K-M analyses, biasing the results in favor of eli-cel.
    f. Subjects in Studies ALD-102 and ALD-103 had a relatively stable course (few endpoint events). This stability might be expected in a population of patients

with CALD during an early or preclinical stage of their disease (even in the absence of any treatment). This, combined with paucity of data beyond 2 years of observation led to insufficient data for robust analysis of the efficacy of eli-cel.

g. Although the efficacy of eli-cel looked similar to the efficacy of HSCT in the K-M analysis, it has not been demonstrated that HSCT is more effective than no treatment in the early active disease population. Therefore, comparability to HSCT may not translate to superiority to no treatment in this early active disease population.
h. When pooling subjects in ALD-101 and ALD-103, the HLA-unmatched HSCT population appears to have a worse prognosis compared to HLA-matched HSCT and eli-cel, with a high early death rate. The biological plausibility of a "real" difference in prognosis between an unmatched and a matched HSCT population must be weighed against the uncertainty related to having few subjects in the unmatched HSCT subgroup (n=17).

Please discuss whether the efficacy data support the presence of a clinically meaningful benefit of eli-cel. If so, in what population?

2. In addition to the occurrence of MDS in eli-cel-treated subjects, there have been diagnoses of myeloid malignancies after administration of a related product, lovo-cel, to subjects with sickle cell disease. Please discuss whether the diagnosis of myeloid malignancy in subjects receiving lovo-cel increases concern for malignancy with eli-cel.

3. Eli-cel has a risk of hematologic malignancy, a potentially fatal adverse event. The number of cases of malignancy (currently 3/67, or 4.4%) seems likely to increase over time. There are at least three cases with concern for impending MDS in addition to the three recognized cases of MDS. In the MDS cases, there is recurrent viral integration into the *MECOM* locus with *EVI1* overexpression, and persistent cytopenias and/or clonal expansion in other subjects. Please discuss the acceptability of this risk in the proposed patient population.

Voting Questions

1. Is the lovo-cel safety data relevant to the safety assessment of eli-cel?

2. Do the benefits of elivaldogene autotemcel for the treatment of subjects with early CALD outweigh the risks, including the potential for insertional oncogenesis?
   a. If you voted "yes," please discuss any recommendations for post-approval risk monitoring and mitigation.
   b. If you voted "no", please discuss what additional information you would consider necessary to support a favorable benefit-risk profile.

3 BACKGROUND

3.1 Regulatory Background

Table 2 is a tabular summary of the main interactions between the FDA and the Applicant.

Table 2: Regulatory Milestones

Date	Milestones
19 Apr 2012	Orphan Drug Designation of Lenti-D Drug Product for treatment of adrenoleukodystrophy (#12-3682)
27 Mar 2013	IND submission by bluebird bio, Inc.
09 Aug 2017	Rare Pediatric Disease Designation (#RPD 2016-79)
21 May 2018	Breakthrough Therapy Designation of Lenti-D Drug Product for treatment of cerebral adrenoleukodystrophy
22 Jul 2021	BLA rolling submission part 1: Nonclinical section
23 Sep 2021	BLA rolling submission part 2: CMC section
18 Oct 2021	BLA rolling submission part 3: Clinical section
17 Dec 2021	BLA accepted for filing
14 Jan 2022	Major amendment

Abbrev: IND, Investigational New Drug; BLA, Biologics License Application; CMC, Chemistry, Manufacturing and Control.

On July 16, 2021, elivaldogene autotemcel was approved for the treatment of patients less than 18 years of age with early CALD without an available matched sibling donor (MSD) by the European Commission. However, it was withdrawn from the European market prior to any patients being treated due to financial considerations and inability to reach agreement with European payers on reimbursement. The approval occurred prior to any case of MDS being reported.

3.2 Product Description

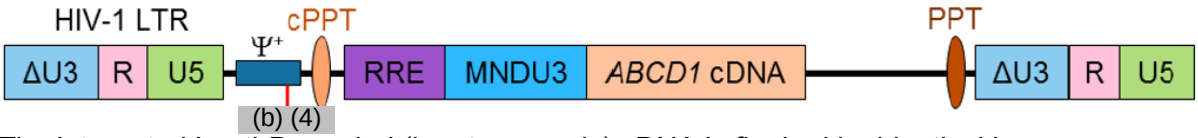
Eli-cel (elivaldogene autotemcel) is a genetically modified autologous CD34+ cell-enriched population that contains hematopoietic stem cells (HSCs) transduced with a lentiviral vector (LVV) containing *adenosine triphosphate-binding cassette, sub-family D, member 1 (ABCD1)* complementary deoxyribonucleic acid (cDNA) encoding human adrenoleukodystrophy protein (ALDP).

3.2.1 The Lentiviral Vector

The Lenti-D LVV is a third-generation replication-incompetent, self-inactivating, human immunodeficiency virus (HIV)-1-based, LVV pseudotyped with the vesicular stomatitis virus envelope glycoprotein G. The genomic organization is illustrated in Figure 1. An internal enhancer/promoter from the U3 region of mouse myeloproliferative sarcoma

virus with a negative control region deletion is utilized to control expression of the human *ABCD1* transgene. Upon transduction, the LVV integrates into the genomic DNA of the hematopoietic stem cell. The transgene is inherited by all the cell's progeny.

Figure 1: Genomic Organization of Integrated Lenti-D Proviral (Transgenic) DNA



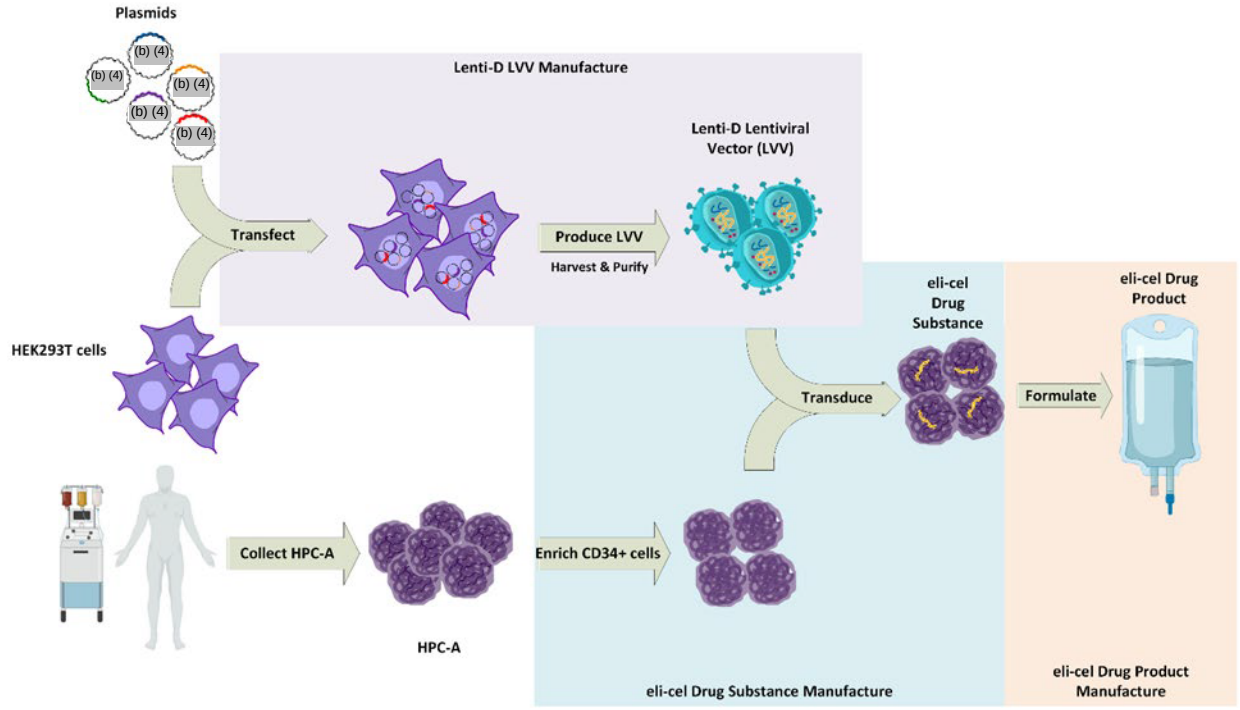
The integrated Lenti-D proviral (i.e., transgenic) cDNA is flanked by identical human immunodeficiency virus-1 (HIV-1)-based 5' and 3' long terminal repeats (LTRs) each containing wildtype repeat (R) and unique 5 (U5) regions, but with modified unique 3 (U3) regions (ΔU3). The ΔU3 regions contain a deletion of the U3 enhancer/promoter that confers the self-inactivating property that prevents LTR-driven transcription, reduces the possibility of replication-competent lentivirus (RCL) formation, and limits the potential for insertional oncogenesis. The extended packaging signal (Ψ+), central polypurine tract (cPPT) and Rev-response element (RRE) are incorporated to facilitate retroviral packaging, reverse transcription, and nuclear export of the viral RNA genome, respectively. (b) (4) . Expression of the *ABCD1* transgene, encoding the adrenoleukodystrophy protein (ALDP), is under local control of the internal MNDU3 promoter ((b) (4) Plasmid No. (b) (4)). The internal MNDU3 promoter consists of only the U3 enhancer/promoter region from the murine myeloproliferative sarcoma virus (MPSV) LTR, modified by deletion of the negative control region (NCR). The Lenti-D LVV production system uses a third-generation-like, 5-plasmid, split-packaging system to further reduce the possibility of RCL formation.

Source: bluebird bio BLA, 2.6.6 Toxicology Written Summary p. 89

3.2.2 The Elivaldogene Autotemcel Drug Product

The eli-cel drug product (DP) consists of an autologous CD34+ cell-enriched population containing HSCs transduced with Lenti-D LVV, suspended in CryoStor® CS5 cryopreservation solution. The process of generating eli-cel for a patient starts with collection of peripheral blood mononuclear cells by apheresis from the patient and shipment to the DP manufacturing facility. There, the cells are enriched for those expressing CD34 using the (b) (4) cell selection system. The CD34+ cell-enriched population is stimulated ex vivo with a mixture of growth factors. Next, the cells are transduced with Lenti-D LVV in the cytokine mixture. After transduction, the cells are washed, resuspended in the cryopreservation solution, and filled into bags before controlled freezing to -140℃. The DP is maintained at that temperature during shipping to the administration site and until the day of infusion, when it is thawed, and infused intravenously as a single dose without additional processing steps. The major steps in the manufacturing process are demonstrated in Figure 2 below.

Figure 2: Eli-cel Manufacturing Process Overview



Source: bluebird bio BLA, 2.3.a.2 p. 3

3.3 *ABCD1* Mutation-Associated Cerebral Adrenoleukodystrophy

Cerebral adrenoleukodystrophy (CALD) is a rare (35-40% of the 1:20,000 males affected with X-ALD) neurodegenerative metabolic disorder caused by X-linked mutations in *ABCD1* that lead to impaired peroxisomal expression of ALDP needed to transport very long chain fatty acids (VLCFAs) into the peroxisome for degradation.[11,12] This primarily affects the adrenal cortex through direct toxicity and brain white matter where perivascular infiltrates result in progressive inflammatory demyelination.[13,14] The most concerning symptoms of CALD are neurologic disability and premature death. The disease is heterogeneous, and the time course of clinical progression is highly variable. Boys typically present with inattention, hyperactivity or academic challenges by 4-10 (median 7) years of age.[18-20] The disease progresses to neurologic dysfunction, disability and ultimately to death by the second decade of life from complications of the disease without treatment. Many patients have primary adrenal insufficiency, which may manifest prior to neurologic symptoms or afterwards, or may not occur. Adrenal insufficiency can cause fatigue and muscle weakness and lead to life-threatening adrenal crisis in the setting of illness/injury without treatment; however, there is approved and available therapy to treat adrenal insufficiency.

There are no FDA-approved treatments for CALD in the United States (US). Currently, allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only therapy that is considered by experts to be able to stabilize disease progression. HSCT became standard of care treatment for CALD around 2001.[1] At that time, nearly all boys were

treated following evidence of brain involvement. Retrospective studies have documented more favorable neurologic outcomes when allo-HSCT is performed early in the course of disease, prior to onset of significant neurologic dysfunction or radiographic disease burden.[2,3] Allo-HSCT may increase rapidity of disease progression in those with advanced cerebral disease (Loes score >9, discussed in Appendix 1), and thus is no longer recommended for patients who meet this criterion. Disease progression may still occur for up to 12-24 months following allo-HSCT, after which clinical and radiographic disease appear to stabilize or progress more slowly. Some patients have experienced resolution of lesions on MRI following allo-HSCT. Because the disease course is progressive and progression may occur within the 2 years following HSCT, the usual medical practice is to treat soon after CALD diagnosis irrespective of available donor and despite uncertainty about the time course of disease progression. HSCT is done early in an effort to prevent disability and death.

The preferred HSC donor is a human leukocyte antigen (HLA)-matched unaffected sibling, but these are available for only ~30% of patients.[3] It has been traditionally believed that HSCT outcomes are inferior and potentially life-threatening risks such as graft rejection, graft versus host disease and infection are increased with alternative donors. Morbidity and mortality following HSCT are significant, with 5-year survival varying between 50-70%, depending on donor type, with percentages reflecting death from disease progression and transplant-related causes.[24] Allo-HSCT does not treat or prevent adrenal insufficiency (AI), which is treated with steroids. Eli-cel is a novel one-time autologous gene therapy product intended to treat CALD and mitigate risks associated with HSCT.

3.4 Mechanism of Action of Elivaldogene Autotemcel

Eli-cel is designed to provide CD34+ cells a normal copy of the *ABCD1* gene encoding ALDP. The patient's genetically altered cells containing a normal *ABCD1* gene are infused intravenously. The intent is to have them engraft in the bone marrow and differentiate into various cell types, including monocytes. According to the Applicant, after engraftment and differentiation, some of the monocytes migrate to the brain where they further differentiate into cerebral microglia that can produce ALDP and replace ALDP-deficient microglial cells. Also, according to the Applicant, the functional ALDP enables local degradation of very long-chain fatty acids in the brain, preventing further inflammation and demyelination, thus putatively stabilizing the disease. ALDP deficiency is only corrected in CD34+-derived cell types. Therefore, eli-cel will not correct adrenal insufficiency and some other manifestations of CALD.

3.5 Concern of LVV Oncogenicity

Lentiviral vectors (LVVs) have potential oncogenicity due to the resulting permanent alteration of the host genome that occurs during transduction. LVV integration into the DNA of target cells has the potential to affect the expression of nearby genes. After

engraftment of transduced HSCs, a progenitor derived from transduced HSCs can undergo preferential expansion due to altered expression of nearby genes, resulting in the presence of a predominant clone and subsequent malignancy (i.e., insertional oncogenesis).

Insertional oncogenesis is a major concern when using integrating vectors including lentiviral vectors for permanent cell modification. Four genetic mechanisms for clonal expansion and/or insertional oncogenesis related to γ-retroviral and lentiviral vectors that have been described are 1) gene activation by integration of an enhancer sequence present in a vector (enhancer insertion), 2) gene activation by promoter insertion, 3) gene inactivation by insertional disruption, and 4) gene activation by mRNA 3' end substitution. In each example, vector integration in patients' cells is associated with clonal expansion.

The potential of gene activation by integration of an enhancer sequence has been highlighted in infants undergoing gene therapy for X-linked severe combined immunodeficiency (SCID-X1) with γ-retroviral vectors. Several SCID-X1 patients developed a T-cell leukemia that was caused by the inserted Moloney murine leukemia virus vector switching on an adjacent oncogene. Similar insertional oncogenesis events were observed in patients who were treated for chronic granulomatous disease.

To mitigate the potential for oncogenicity, Lenti-D has been rendered self-inactivating through deletion of a portion of the 3' LTR. This deletion removes the LTR promoter sequence as well as transcription factor binding sites. Following reverse transcription of the vector, this deletion is transferred to the 5′ LTR so that neither LTR maintains promoter function following pro-viral integration.

4 CLINICAL DEVELOPMENT

The BLA includes 2 interventional single-arm, open-label trials: Study ALD-102, the completed Phase 2/3 clinical trial and Study ALD-104, an ongoing Phase 3 clinical trial. All eli-cel-treated subjects are followed in Study LTF-304 (detailed in Appendix 2), an ongoing long-term follow-up study, to help ensure 15 years of follow-up.

In addition to the eli-cel interventional studies, the clinical development program included two external studies: Study ALD-101, a retrospective natural history study in subjects who either received no treatment or were treated with allo-HSCT, and Study ALD-103, a more contemporaneously conducted hybrid retrospective/ prospective study in subjects who were all treated with allo-HSCT. Study ALD-101 was used to inform endpoint selection for CALD clinical trials and to establish the threshold for benchmark analysis. The data collected in Study ALD-101 were from a time (1988-2010) when delayed diagnosis was more common due to decreased availability of genetic testing, lack of newborn screening, and HSCT not having yet been optimized. Subjects were therefore generally older and had more advanced disease at baseline compared to the Study ALD-102 population. Also, because it was retrospective, there may have been selection bias and missing data.

Compared to Study ALD-101, Study ALD-103 was a mostly contemporaneous (2013-2019) external control study in children with CALD treated with allo-HSCT. Objectives were to evaluate safety and efficacy of allo-HSCT in the treatment of CALD and act as a comparator for Study ALD-102. Study ALD-103 was terminated after the Applicant's goal number (n=59) of subjects had enrolled in the study [so that only 18/27 (67%) of the TPES-103 subpopulation was followed for at least 24 months], limiting the utility of the dataset for long-term comparisons.

With changing diagnostic modalities and disease scoring systems over time, CALD is now diagnosed earlier through brain MRI screening, often prior to onset of clinical symptoms. While early diagnosis allows for early intervention, the natural history of disease progression in this pre-symptomatic population is not well understood; there is some evidence that symptom onset often occurs more than 2 years after diagnosis even in the absence of interventions.

The 5 clinical studies are summarized in Table 3. The table reflects data through August 2021. Additional efficacy data were obtained for 7 subjects in Study ALD-104 through January 2022, which are not reflected in the table but are discussed in Section 7. Additionally, BLA safety updates have been received on the first of each month for established cases of hematologic malignancy and ad hoc for new cases, and these data are not reflected in the table but are discussed in Section 8.

Table 3: Summary of Clinical Data and Number of Subjects in the Marketing Application, by Study

Study (Status)	Study Dates	Data Cut[a]	Study Objectives	Number of Subjects Enrolled	Number Treated with eli-cel	Number Treated with allo-HSCT	Number Untreated	Follow-Up (months), median (min,max)[b]
ALD-102 (complete)	21 Aug 2013 to 26 Mar 2021	Last Data Cut: 18 Aug 2021	Evaluate efficacy/ safety for 2 years following eli-cel treatment in CALD	32	32	NA	NA	49.0 (13.4, 88.1)[b]
ALD-104 (ongoing)	24 Jan 2019 to ongoing	Last Data Cut: 18 Aug 2021	Evaluate efficacy/ safety for 2 years following eli-cel treatment in CALD	35	35	NA	NA	6.3 (1.4, 26.9)[b]
LTF-304 (ongoing)	22 Jan 2016 to ongoing	Last Data Cut: 18 Aug 2021	Evaluate efficacy/ safety of eli-cel treatment for total 15 years	28[c]	28[c]	NA	NA	As noted above for Studies ALD-102 and ALD-104
ALD-101 (complete)	Apr 2011 to May 2012[d]	Data Cut: 27 Mar 2012	• Evaluate the natural history of disease in untreated CALD • Evaluate efficacy/ safety of allo-HSCT in CALD	137	NA	65	72	39.2 (0.4, 117.5)
ALD-103 (complete)	10 Apr 2015 to 6 Dec 2019[e]	Data Cut: 06 Dec 2019	Evaluate efficacy/ safety for 4 years following allo-HSCT in CALD	59	NA	59	0	23.00 (0.9, 49.5)

Source: adapted from bluebird bio, Inc. original BLA submission, Clinical Overview 2.5, Table 1, pp. 15-16
Abbrev.: allo-HSCT, allogeneic stem cell transplantation; CALD, cerebral adrenoleukodystrophy; NA, not applicable
[a] Data cut dates for original BLA submission, with the exception of additional data cut for safety and efficacy data in Studies ALD-104 and ALD-102 subjects in LTF-304 through August 2021; data on an additional 7 subjects in Study ALD-104 through January 2022 are included in the BLA efficacy review but are not reflected in the table.
[b] Follow-up durations for Studies ALD-102 and ALD-104 include time in LTF-304
[c] As of August 18, 2021, 28 subjects from Study ALD-102 were being followed in LTF-304. An additional subject had originally enrolled but was lost to

follow-up after the Month 36 visit. Seven (7) subjects in Study ALD-104 had recently completed 24 months of follow-up and were in various stages of enrollment in LTF-304 and are not included in the table for this reason.

[d] Data collection dates for untreated subjects diagnosed with CALD between June 27, 1988 and January 14, 2010, and subjects treated with allo-HSCT between March 12, 1997 and September 21, 2010.

[e] Study dates for partial retrospective and prospective study where subjects were treated with allo-HSCT between 2013 and 2019

4.1 Phase 2-3 Study - Study ALD-102 (August 21, 2013 to March 26, 2021)

4.1.1 Study Design

Study ALD-102 was a Phase 2/3 international multi-center, non-randomized, open-label single-arm study that enrolled and treated 32 boys with CALD with a single intravenous (IV) dose of eli-cel. Subjects were followed for 24 months following drug product infusion, after which time they were to enroll in the separate long-term follow-up study (LTF-304, detailed in Appendix 2) for a total of 15 years follow-up after drug product infusion.

4.1.2 Study Objectives

The primary objectives of Study ALD-102 were to evaluate the safety and efficacy of eli-cel administered as a single intravenous dose in the treatment of subjects with CALD.

4.1.3 Key Enrollment Criteria

Inclusion Criteria

- Males 17 years of age or younger
- Active CALD defined by elevated VLCFA levels, brain magnetic resonance imaging (MRI) demonstrating Loes scores between 0.5 and 9 and gadolinium enhancement (GdE+)
- Asymptomatic or minimally symptomatic neurologic clinical course as defined by Neurologic Function Score (NFS) of $\leq$ 1

Exclusion Criteria

- Recipient of an allogeneic transplant or previous gene therapy
- Available and willing 10/10 HLA-matched sibling donor

These eligibility criteria reflect the childhood CALD population with early active cerebral disease felt most likely to benefit from treatment with allo-HSCT, the primary comparator population for eli-cel.

4.1.4 Treatment Plan

Dose Regimen

Following apheresis and cell transduction, subjects underwent myeloablative and lymphodepleting conditioning with a 4-day course of busulfan followed by a rest day and then a 4-day course of cyclophosphamide. After an additional rest day, eli-cel drug product infusion occurred on Relative (Rel) Day 1, with thawed eli-cel administered via intravenous infusion as a single dose of $\geq 5.0\times10^6$ CD34+ cells/kg.

Concomitant Medications
Concomitant use of medications to lower VLCFA levels (e.g., Lorenzo's oil, statins) and other investigational agents were disallowed during study participation. Information about medications related to mobilization and conditioning is presented in Section 5.

4.1.5 Study Assessments

The efficacy assessments were primarily assessment of functional status using methods and metrics that are described in detail in this section below: the Neurologic Function Scores (NFS) for overall score and determination of Major Functional Disabilities (MFDs). Prognostic evaluations were also done periodically, including the Loes score and gadolinium enhancement on brain magnetic resonance imaging (MRI), discussed in detail below. Additional efficacy assessments included neurologic examinations, neurodevelopmental and intelligence clinical outcome assessments (COAs), and peripheral blood assessments for ALDP and VLCFA levels.

Safety assessments included routine physical exams, vital signs, hematology and chemistry laboratories, and adverse event monitoring. In addition, subjects had periodic peripheral blood assessments for integration sites, vector copy number, and the presence of recombinant lentivirus. Details regarding integration site analysis are provided in Appendix 3.

Design of Neurologic Function Score (NFS) and Major Functional Disability (MFD) Scoring Systems
The Neurologic Function Score (NFS) is a 25-point composite scale that assesses functional disabilities in 15 domains.[14] It is the most commonly used clinical evaluation tool for CALD patient evaluation.[14 3] A score of 0 indicates absence of clinical signs of cerebral disease, and higher scores correspond to increasing severity of functional deficits. The scoring system and definitions used for the clinical studies are provided in Appendix 1, Table 1.

The Major Functional Disabilities (MFDs) are a subset of the NFS that are considered largely irreversible clinical neurologic changes in CALD. Data from the retrospective natural history study (ALD-101) helped to identify the MFDs, which were chosen by the Applicant based on impact on independent functioning. The 6 MFDs are loss of communication, cortical blindness, tube feeding, wheelchair dependence, complete loss of voluntary movement, and total incontinence, defined in Table 4.

Table 4: Major Functional Disabilities (MFDs) for CALD

Symptom / Neurologic Exam Finding	**Definition**
Loss of communication	Individual should meet one of the following criteria (psychogenic syndromes, such as catatonia, should be ruled out): (1) With normal consciousness and ability to perform movements, individual does not follow command and/or permanently fails to perform verbal or nonverbal simple task on neurologic evaluation, or (2) Individual is permanently mute and unable to communicate by verbal or non-verbal ways.
Cortical blindness	Individual fails to visually track, find objects, or count fingers. Individual has permanent and complete vision loss affecting bilateral vision. Pupils may react to light.
Tube feeding	Individual is not able to swallow safely by mouth to maintain nutrition and hydration. Alternative method of feeding required.
Wheelchair dependence	Individual is unable to take more than a few steps, restricted to wheelchair; may need aid to transfer; wheels himself, but may require motorized chair for full day's activities.
Complete loss of voluntary movement	Individual is unable to effectively use his upper and lower extremities to perform simple or one-step activities. The criteria may still be met if there are singular apparently random movements of the arms.
Total incontinence	In an individual who was previously continent, the permanent and continuous loss of urinary and/or fecal control.

Source: Adapted from bluebird bio Protocol ALD-102 Version 10.0, Section 10.3, Table 7, originally from Moser et al. 2000.
Abbrev: CALD, cerebral adrenoleukodystrophy

Assessment of NFS score and determination of MFD events occurred at baseline and at each study visit after treatment with eli-cel. All NFS and MFD assessments were performed by a pediatric neurologist or other appropriately trained and qualified physician.

On their face, the 6 MFDs appear to capture valid and clinically meaningful events which impact CALD patient functioning. There are some potentially subjective elements of the definitions, specifically regarding tube feeding and wheelchair dependence, which can increase the likelihood of biased or inaccurate scoring for the clinical assessments conducted in Study ALD-102 and retrospective chart review for Studies ALD-101 and ALD-103. Given the open-label design of all studies in the sponsor's development program, the absence of central raters masked to treatment assignment and/or time is a significant limitation for the interpretability of the available NFS/MFD evidence.

Brain Magnetic Resonance Imaging (MRI) Evaluations

Cerebral lesions (Loes score and pattern) and gadolinium enhancement (GdE) were evaluated on brain magnetic resonance imaging (MRI) at baseline and at Months 1, 6, 12, 18 and 24 following eli-cel treatment.

The Loes score (detailed in Appendix 1) is a commonly used MRI assessment of extent of cerebral lesions in patients with CALD.[22] A severity score (0 to 34) is assigned based on extent of demyelinating lesions on MRI and presence of focal and/or global atrophy. A score of 0 indicates a normal MRI, and higher scores indicate increased severity of cerebral lesions.

Patterns of cerebral involvement on MRI have also been described (detailed in Appendix 1),[26] and were documented for each subject.
Gadolinium enhancement (GdE) was documented as present (GdE+) or absent (GdE-) for each MRI during the study. Significance of GdE is discussed in Appendix 1.

A central blinded reviewer ((b) (4)) assessed all MRIs for Studies ALD-102, ALD-103, and ALD-104.

4.1.6 Endpoints and Success Criteria

The primary efficacy endpoint was the proportion of subjects who were alive and had none of the six defined MFDs at the Month 24 visit (i.e., Month 24 MFD-free survival).

To be considered a success on the primary efficacy endpoint (i.e., achieve Month 24 MFD-free survival), subjects must have met the following criteria at the Month 24 visit:

- Be alive
- Be MFD-free
- Not received rescue cell administration or an allo-HSCT
- Not withdrawn from study or been lost to follow-up

The success criterion required that the lower bound of the 2-sided 95% exact confidence interval (CI) of Month 24 MFD-free survival for the cohort exceed 50% (the clinical benchmark derived from 2 populations in Study ALD 101):

> Population #1: The untreated population with presence of gadolinium enhancement (GdE+) on brain MRI, for whom MFD-free survival at 24 months following the first GdE+ MRI was 21% (exact 95% CI of 6.1% to 45.6%). The 50% benchmark is thus above the upper bound of the 95% CI for MFD-free survival in the untreated GdE+ population.
>
> Population #2: The “strictly ALD-102-eligible” HSCT-treated group (“TPES-101 population”) who were treated with HSCTs from an alternative donor (no matched sibling donor, NMSD) for whom the lower bound of the 95% exact CI of MFD-free survival at 24 months following HSCT was 50.1% (mean 76% with exact 95% CIs of 50.1% to 93.2%). The lower bound of the 95% CI for MFD-free survival in the TPES-101 NMSD population is thus the same as the 50% benchmark.

Although FDA agreed with the primary efficacy endpoint and clinical benchmark for success, FDA emphasized that comparability of external control groups to support the benchmark would need to be demonstrated. Upon review of BLA data, FDA has concerns with the clinical benchmark for the primary efficacy endpoint due to the following concerns with Study ALD-101:

- Lack of comparability between Populations #1 and #2,
- Lack of comparability between ALD-101 and ALD-102 populations,
- retrospective data collection of ALD-101,
- imputation methods used in the calculation of the benchmark, and potential bias in the assessment of MFDs.

How these limitations impact the efficacy analysis is further discussed in Section 7.

The secondary efficacy endpoints were pre-specified, but not hierarchically ordered, and included:

- MFD-free survival over time
- Overall survival (OS)
- Proportion of subjects who demonstrated resolution of gadolinium positivity on MRI (i.e, GdE-) at the Month 24 Visit
- Time to sustained resolution of gadolinium positivity on MRI (i.e., GdE-), with sustained defined as GdE- without subsequent MRI with gadolinium positivity
- Change in total NFS from Baseline to Month 24

The primary safety endpoint was the proportion of subjects who experienced either acute (≥ Grade II) or chronic graft versus host disease (GVHD) by Month 24. Success on the primary safety endpoint was defined as a statistically significant reduction in the proportion of subjects who either experienced ≥ Grade II acute GVHD or chronic GVHD in Study ALD-102 compared to the Study ALD-103 transplant population.

4.2 Phase 3 Study – Study ALD-104 (January 24, 2019 to ongoing)

4.2.1 Study Design

Study ALD-104 is an international, multicenter, non-randomized, open-label, single-arm Phase 3 study in which boys with CALD receive a single intravenous dose of eli-cel.

Study ALD-104 is very similar to Study ALD-102, with the same study duration, assessments and primary efficacy endpoint. The primary differences are that study ALD-104 uses a different conditioning regimen prior to eli-cel administration, the drug product contains more LVV provirus, and the primary safety endpoint is the proportion of subjects with neutrophil engraftment (NE) after drug product infusion. Key differences from Study ALD-102 are noted below.

4.2.2 Study Objectives

The objectives of the study were to assess the safety and efficacy of eli-cel after myeloablative conditioning with busulfan and fludarabine in subjects with CALD.

4.2.3 Key Enrollment Criteria

The key enrollment criteria for Study ALD-104 are the same as for Study ALD-102, except subjects are not excluded for having an available and willing matched sibling donor.

4.2.4 Treatment Plan for Subjects in the Treatment Group

As shown in Appendix 4, Study ALD-104 differed from Study ALD-102 in the conditioning regimen and in growth factor therapy. In ALD-104, subjects were administered fludarabine for lymphodepletion (instead of cyclophosphamide used in Study ALD-102).

As demonstrated in Appendix 5, Study ALD-104 differed from Study ALD-102 in that the dose of drug product was higher in some respects, including the percent of LVV positive cells was higher. Lastly, in Study ALD-104, G-CSF administration was mandated after eli-cel, whereas in Study ALD-102, G-CSF was optional.

4.2.5 Study Assessments

The key efficacy and safety assessments are the same as those in Study ALD-102.

4.2.6 Endpoints

The primary efficacy endpoint of number and proportion of subjects achieving Month 24 MFD-free survival is the same as in Study ALD-102.

The secondary efficacy endpoints are similar to those in Study ALD-102, and include:

- Proportion of subjects without gadolinium enhancement on MRI (i.e., GdE-) at Month 24
- Value and change in total NFS from Baseline to protocol scheduled visits
- MFD-free survival over time
- Overall survival (OS)
- Detectable vector copy number (VCN) on peripheral blood cells by Month 6

The primary safety endpoint is the proportion of subjects with neutrophil engraftment after drug product infusion.

4.3 Historical External Control – Untreated and allo-HSCT – Study ALD-101

(Dates of Study: April 2011 – May 2012, Data used in analyses: Untreated subjects diagnosed between June 27,1988 and January 14, 2010 and subjects treated with allo-HSCT between March 12, 1997 and September 21, 2010)

4.3.1 Study Design

Study ALD-101 was a retrospective, non-interventional natural history study for boys with CALD who were either untreated or treated with allo-HSCT.

4.3.2 Study Objectives

Study objectives were to characterize the natural history of disease in untreated children with CALD, and to evaluate efficacy and safety of allogeneic HSCT in subjects with CALD to inform endpoints for CALD clinical trials.

4.3.3 Key Enrollment Criteria

Datasets from subjects meeting the following criteria were eligible for inclusion in the study:

- Males between the ages of 3 and 15 years of age
- Confirmed diagnosis of CALD, by elevated VLCFA levels or genetic mutation and baseline cerebral lesion(s) on brain MRI
- Had data available for at least 2 years or until death following:
    - Allo-HSCT with either bone marrow or cord blood (Allo-HSCT Cohort, n=65), or
    - Diagnosis (Untreated Cohort, n=72)
- Baseline Loes score of >0 and <15

4.3.4 Treatment Plan

No investigational treatment was administered in this retrospective natural history study.

4.3.5 Study Assessments

Assessments were primarily to characterize:

- Demographics
- Diagnosis and characterization of disease
- Interventions, including concomitant medications and, in the allo-HSCT cohort, characterization of HSC donor, preparative regimens, and GVHD prophylaxis
- Efficacy outcome assessments including survival status, neurologic and neuropsychologic function, disability, neuroradiologic assessment (Loes score), ambulation, nutrition, schooling, and laboratory assessments of VLCFA levels
- For the allo-HSCT cohort, additional efficacy and safety outcome assessments to include engraftment, acute and chronic GVHD, infections, and SAEs

4.4 Contemporaneous External Control- allo-HSCT – Study ALD-103 (April 10, 2015 to December 6, 2019)

4.4.1 Study Design

Study ALD-103 was an international multicenter mixed retrospective/prospective natural history study of boys with CALD who had undergone allo-HST. This study intended to be a contemporaneous external control to Study ALD-102. International study sites were similar to those for Studies ALD-102 and ALD-104.

Subjects were to be enrolled in one of three cohorts:

1. Allo-HSCT prospective: subjects who would receive allo-HSCT on study and be followed for 48 months after most recent allo-HSCT

2. Allo-HSCT partial prospective/retrospective: subjects who previously received allo-HSCT and would consent in time to complete a Month 24 visit on study, to be followed for 48 months after most recent allo-HSCT

3. Allo-HSCT retrospective: subjects who received allo-HSCT on or after January 1, 2013 and died before study data collection, with duration of follow-up depending on when subject died.

4.4.2 Study Objectives

Study objectives were to evaluate the safety and efficacy of allo-HSCT in boys with CALD.

4.4.3 Key Enrollment Criteria

Inclusion:

- Males aged 17 years and younger at time of parent/guardian consent
- Confirmed diagnosis of CALD as defined by abnormal VLCFA profile and cerebral lesions on brain MRI

- Depending on the cohort, subject must:
  - Prospective: be scheduled for allo-HSCT evaluation/procedure at a study site
  - Partial prospective/retrospective: have received allo-HSCT and be consented in time to complete the Month 24 visit on study
  - Retrospective: have received most recent allo-HSCT on or after January 1, 2013

Exclusion:

- Previous treatment with gene therapy
- Receipt of experimental transplant procedure

4.4.4 Treatment Plan

Subjects received allo-HSCT, inclusive of preparative regimens, per institutional standards. Only subjects in the prospective cohort received allo-HSCT on study.

4.4.5 Study Assessments

Efficacy assessments were primarily assessment of functional status using NFS for overall score and determination of MFDs. Additional efficacy assessments included neurologic examinations, brain MRI for Loes score and gadolinium enhancement, global and neuropsychological assessments, and peripheral blood assessments for VLCFA levels and exploratory biomarkers.

Safety assessments included routine physical exams, vital signs, hematology and chemistry laboratories, and adverse event and graft versus host disease (GVHD) monitoring.

For consistency and accuracy of results, all neurologic examinations and NFS and MFD assessments were to be performed by a pediatric neurologist or other appropriately trained and qualified physician. All MRI interpretations were performed by a central radiologist, (b) (4) .

Analysis Plan

Data from subjects who were similar to the enrolled population receiving eli-cel in Study ALD-102 (TPES-103 population) were to provide comparator data which would be analyzed using Kaplan-Meier estimates of MFD-free survival and overall survival over time.

5 STUDY MEDICATIONS

The eli-cel product is described in detail in **3.2.2 THE ELIVALDOGENE AUTOTEMCEL DRUG PRODUCT.** The prescribed dose was ≥ 5.0 x10^6 CD34+ cells/kg. The minimum drug product vector copy number (VCN) was 0.5 copies per diploid genome (c/dg) for the initial 17 subjects in Study ALD-102. The minimum VCN for all other subjects was increased to 0.7 c/dg. Summary statistics for some characteristics of the drug product by study are presented in Appendix 5.

Prior to eli-cel administration, subjects underwent stem cell mobilization, apheresis, and conditioning. Studies ALD-102 and ALD-104 had several differences in pre-treatment regimen. Both used busulfan for conditioning, however the dosing differed. Study ALD-102 used cyclophosphamide for lymphodepletion, whereas Study ALD-104 used fludarabine. Also different were the requirements for plerixafor for mobilization and G-CSF for post-DP bone marrow stimulation. These differences are presented in Appendix 4.

6 STUDY POPULATION

6.1 Overview of Study Populations

Analysis Populations

The **Transplant Population (TP)** consists of subjects who receive(d) a HSC infusion, including eli-cel in Studies ALD-102 and ALD-104 or allo-HSCT in Studies ALD-101 and ALD-103, abbreviated as TP-102, TP-104, TP-101 and TP-103, respectively. For data from the long-term follow-up study, LTF-304, eli-cel populations are referred to by the parent study population (i.e., TP-102 or TP-104).

The **Integrated Summary of Safety population (ISS population)** consists of subjects who receive eli-cel in Studies ALD-102 and ALD-104 and includes data obtained in those studies and LTF-304.

The **Untreated Population (UT)**, abbreviated UT-101, consists of subjects in Study ALD-101 who did not receive treatment with allo-HSCT and for whom retrospective data was collected for the natural history of disease.

Loes score, NFS and gadolinium enhancement (GdE) status on brain MRI are important prognostic factors for disease progression in CALD patients. Subjects in Studies ALD-101 and ALD-103 were characterized as “similar” on these baseline characteristics to the subjects treated with eli-cel in Studies ALD-102 and ALD-104.

1. Similar allo-HSCT- treated subjects in Studies ALD-101 and ALD-103:

a. **The Strictly ALD-102-Eligible Transplant Population (TPES):** Subjects in TP-101 or TP-103 who have the same baseline characteristics for NFS, Loes, and GdE status to be eligible for enrollment in Study ALD-102:
   i. NFS ≤ 1 at Baseline
   ii. Loes score ≥0.5 to ≤ 9 at Baseline
   iii. GdE+ at Baseline

2. Similar untreated subjects in Study ALD-101:

   a. **The Strictly ALD-102-Eligible Untreated Population (UTES-101):** untreated subjects from Study ALD-101 meeting the following criteria:
      i. GdE+ at some point during the study
      ii. NFS ≤ 1 at the time of the first GdE+ assessment during the study
      iii. Loes score ≥0.5 to ≤ 9 at the time of the first GdE+ assessment during the study

      The timepoint of the first GdE+ assessment was identified as the Baseline visit for inter-study analyses. There was 1 subject in the UTES-101 population.

   b. **The GdE+ Untreated Population (UTG-101):** untreated subjects from Study ALD-101 who are GdE+ at some point during the study. The time of first GdE+ assessment in UTG-101 is identified as the Baseline visit for the inter-study analysis.

Gadolinium was not routinely used or documented on MRI readings at the time many subjects in Study ALD-101 were diagnosed and followed for their disease. Of 72 untreated subjects in Study ALD-101, 21 had a GdE+ MRI at some time during follow-up in the study (the UTG-101 population), 9 had GdE- MRIs only, and 42 were unknown due to never having had GdE evaluation (or documentation). To reduce lead-time bias, FDA re-coded the GdE+ untreated (UTG-101) population to make the Baseline visit the time of CALD diagnosis, rather than the time of first GdE+ MRI. Because GdE+ status reflects more advanced disease, to be conservative, brain MRIs performed without contrast at time of diagnosis for UTG-101 were imputed as being GdE+. Values for Loes or NFS missing at time of diagnosis were imputed as last visit carried forward (LVCF) when possible, or as first value after diagnosis if no prior values were available to establish Baseline values. This re-coding resulted in a re-coded Strictly ALD-102-Eligible Untreated (termed rUTES-101) population of 7 subjects, used for relative efficacy comparisons in analysis, discussed in Section 7.

Allo-HSCT Subgroups

Matched sibling donors (MSD) are the preferred HSCT donors, and therefore the Applicant focused their comparative analyses on MSD and NMSD (no matched sibling donor) subgroups. NMSD includes matched unrelated donors (MURD), unmatched related donors (URD), and unmatched unrelated donors (UURD). In this analysis, "matched" refers to any full HLA-matching of all evaluated alleles (e.g., 6 out of 6, 10 out

of 10). “Unmatched” includes mismatch on 1 or more alleles (e.g., 4 out of 6, 9 out of 10). Because HSCT outcomes differ between matched and unmatched donors, FDA included these populations in the sub-group analysis. Table 5 describes the donor characteristics for the allo-HSCT comparator populations.

Table 5: Donor Characteristics for allo-HSCT Populations

Subgroup or Subpopulation	TPES-101 (n=26)	TPES-103 (n=27)	TPES-101 and TPES-103 Pooled (n=53)
Matched Donor	14 (54)	20 (74)	34 (64)
Unmatched Donor	10 (38)	7 (26)	17(32)
Unknown Matching of Donor	2 (8)	0	2 (4)
Matched Sibling Donor	5 (19)	10 (37)	15 (28)
No Matched Sibling Donor	21 (81)	17 (63)	38 (72)
Matched Unrelated Donor (MURD)	9 (35)	10 (37)	19 (36)
Unmatched Related Donor (URD)[1]	1 (4)	0 (0)	1 (2)
Unmatched Unrelated Donor (UURD)	9 (35)	7 (26)	16 (30)

Source: Reviewer’s analysis of ADHSCT dataset
Abbrev.: TP, Transplant Population; TPES, Strictly ALD-102-eligible Transplant Population; HLA, human leukocyte antigen; MSD, Matched Sibling Donor; NMSD, No Matched Sibling Donor; MRD, Matched Related Donor; MURD, Matched Unrelated Donor; URD, Unmatched Related Donor[1], UURD, Unmatched Unrelated Donor.
[1]All unmatched related donors, including unmatched sibling donors

6.2 Subject Disposition

Disposition for subjects treated with eli-cel (TP-102 and TP-104) and allo-HSCT in the strictly ALD-102-eligible transplant populations (TPES-101 and TPES-103) are presented in Table 6. It is notable that Study ALD-103 was terminated early, resulting in a significant amount of missing allo-HSCT data. Median duration of follow-up following allo-HSCT was 24.3 months for TPES-103 subjects (approximately half the 51.8-month follow-up time achieved in the TP-102 subjects). In the TPES-103 NMSD population of interest specifically, median duration of follow-up was 11.1 months, and only 9 of 17 (53%) subjects had at least 24 months of data for analysis. The majority of reasons for study discontinuation in ALD-103 were early termination of study (48.1%) and repeat HSCT (18.5%).

Table 6: Study Subject Disposition: TP-102, TP-104, TPES-101 and TPES-103

Parameter	TP-102	TP-104	TPES-101	TPES-103
Received eli-cel or HSCT (TP), n (%)[1]	**32**	**35**	**26**	**27**
Median Duration of Follow-Up (months)[2]	51.8	11.8	52.8	24.3
Study Status:	--	--	--	--
Ongoing, n (%)[3]	28 (87.5)	35 (100.0)	0	0
Completed Study, n (%)	0	0	22 (84.6)[5]	4 (14.8)
Discontinued Study, n (%)	4 (12.5)[6]	0[6]	4 (15.3)[5]	23 (85.2)
Reason for Study Discontinuation:	--	--	--	--
Rescue/ Repeat HSCT, n (%)	2 (6.3)	0	2 (7.7)	5 (18.5)
Death, n (%)[4]	1 (3.1)	0	2 (7.7)	3 (11.1)
Lost to/Refuses Follow-Up, n (%)	1 (3.1)	0	0	1 (3.7)
Termination of Study by Sponsor, n (%)	0	0	0	13 (48.1)
Protocol Deviation, n (%)	0	0	0	1 (3.7)

Source: Adapted from bluebird bio, Inc. original BLA submission, interstudy TLFs Table 1.1.2; updated with data through January 2022 data cut
Abbrev.: TP, Transplant Population; TPES, Strictly ALD-102-eligible Transplant Population; HSCI, hematopoeitic stem cell infusion.
Note: For ALD-101 and ALD-103 subjects who had multiple allo-HSCTs, the discontinuation reason for the initial allo-HSCT is presented. For ALD-102 and ALD-104 subjects, the discontinuation reason from ALD-102 or ALD-104 is presented if the subject discontinued in that study; otherwise, the discontinuation from LTF-304 is presented. In addition, a subject is considered as having completed the study if he completes LTF-304.
[1]The TP consists of subjects who received eli-cel in studies ALD-102 and ALD-104 (TP-102 and TP-104, respectively), and subjects who received allo-HSCT in studies ALD-101 and ALD-103 (TP-101 and TP-103, respectively).
[2]For TP-102 and TP-104, median duration of follow-up is updated for most recent data cut through January 2022.
[3]LTF-304 is the long-term follow-up study to support eli-cel studies (ALD-102 and ALD-104). Subjects still being followed in LTF-304 are listed as “ongoing” for study status.
[4]For all studies, death is only counted as reason for study discontinuation if subject was not already withdrawn for another reason (e.g., to receive rescue allo-HSCT)
[5]For TPES-101, all subjects were considered discontinued per the Applicant. This was adjusted to be consistent with dispositions listed for the other studies in this table.
[6]Subjects who have received allo-HSCT for treatment of MDS are not discontinued.

7 EFFICACY

The primary efficacy analysis submitted by the Applicant in the BLA included 32 subjects treated with eli-cel in Study ALD-102 (population TP-102). FDA conducted analyses that included an additional 13 subjects who completed Month 24 follow-up in Study ALD-104.

Success on the primary efficacy endpoint was defined as >50% of subjects achieving Month-24 MFD-free survival. This benchmark definition is discussed in Section 4.1.6 .

The efficacy comparator for secondary and exploratory endpoints of MFD-free survival and overall survival over time and change from Baseline in NFS and Loes score at Month 24 is the TPES subgroup of 27 subjects who underwent allo-HSCT in Study ALD-103 (TPES-103), of which 10 subjects had matched sibling donors (MSDs), and 17 had no matched sibling donors (NMSDs). The NMSD population received HSCT from unmatched unrelated donors (UURD, n=7) or matched unrelated donors (MURD, n=10). Additional analysis was performed combining data from the TPES subgroup of 26 subjects who were treated with allo-HSCT in Study ALD-101 (TPES-101) with data from TPES-103.

7.1 Study Population

Study populations evaluated in the efficacy analysis are outlined in Section 6.1.

7.1.1 Subject Disposition

Disposition of subjects in the study populations are discussed in Section 6.2 and presented in Table 6 above.

7.1.2 Demographics and Baseline Characteristics

Key demographics and baseline characteristics for populations used in the determination of the clinical benchmark and for the analysis of the primary efficacy endpoint are summarized in Table 7. It is notable that the untreated GdE+ ALD-101 population, UTG-101 (Population #1 of the benchmark), was not similar to the Study ALD-102 population (TP-102) with regard to the following covariates that are considered prognostic factors: age at diagnosis, baseline Loes and baseline NFS scores. UTG-101 had more advanced baseline disease and risk factors for rapid progression of disease. Most notably, their median baseline Loes and NFS scores were 11 and 3.5, respectively (compared to TP-102 with baseline Loes and NFS of 2 and 0, respectively).

Also, the matched allo-HSCT ALD-101 population (TPES-101) with no matched sibling donor (NMSD), (Population #2 of the benchmark), was older with higher baseline Loes scores. These differences complicate interpretation of success on the primary efficacy endpoint of Month 24 MFD-free survival, discussed further in Section 7.2.

Key demographics and baseline characteristics for study subjects considered efficacy evaluable at Month 24 are summarized in Table 8. The eli-cel treatment group may have been treated at an earlier stage of disease than the allo-HSCT TPES population; such a discrepancy would favor eli-cel in the comparative analysis.

Table 7: Baseline Demographics and Disease Characteristics, Clinical Benchmark Populations and TP-102

Parameter	Statistic	UTG-101 (n=21)	TPES-101 NMSD (n=21)	TP-102 (n=32)
Age (Years)[1]	**Median (Min,Max)**	8 (4,15)	8 (4,14)	6 (4,14)
Age at Diagnosis (Years)	**Median (Min,Max)**	8 (4,15)	7 (3,12)	6 (1,13)
Baseline Loes	**Median (Min,Max)**	11 (2.0,15.0)	4.5 (0.5, 9.0)	2 (1.0, 9.0)
Baseline NFS	**Median (Min,Max)**	3.5[2] (0, 25)	0 (0,1)	0 (0,1)

Source: Reviewer's analysis of ADSL datasets
Abbrev: UTG, GdE+ Untreated population; TP, Transplant Population; TPES, Strictly ALD-102-eligible Transplant Population; NMSD, No Matched Sibling Donor subgroup; NFS, Neurologic Function Score
[1]Age reflects age at diagnosis for UTG-101 and age at time of treatment for TPES-101 NMSD, TP-102.
[2]NFS at baseline only available for 14 of the 21 UTG-101 population.

Table 8: Baseline Demographics and Disease Characteristics, 24-Month Efficacy Evaluable TPES and eli-cel Populations

Parameter	Statistic	rUTES-101 (n=7)	Pooled TPES-101 and TPES -103 (n=38)	Pooled TPES- 101 and TPES-103 NMSD (n=26)	TP-102 (n=32)	TP-104 (n=13)	Pooled TP-102 and TP-104 (n=45)
Age (Years)[1]	**Median (Min, Max)**	9 (5,15)	8 (5,14)	8 (5,14)	6 (4,14)	9 (5,13)	6 (4,14)
Age at Diagnosis (Years)	**Median (Min, Max)**	9 (5,15)	7 (4,12)	7 (4,12)	6 (1,13)	8 (4,10)	6 (1,13)
Baseline Loes	**Median (Min, Max)**	4.5 (2,9)	3.5 (0.5, 9.0)	3.5 (0.5, 9.0)	2.0 (1.0, 9.0)	3.0 (1.0, 7.0)	2.0 (1.0, 9.0)
Baseline NFS	**Median (Min, Max)**	0 (0,1)	0 (0,1)	0 (0,1)	0 (0,1)	0 (0,1)	0 (0,1)

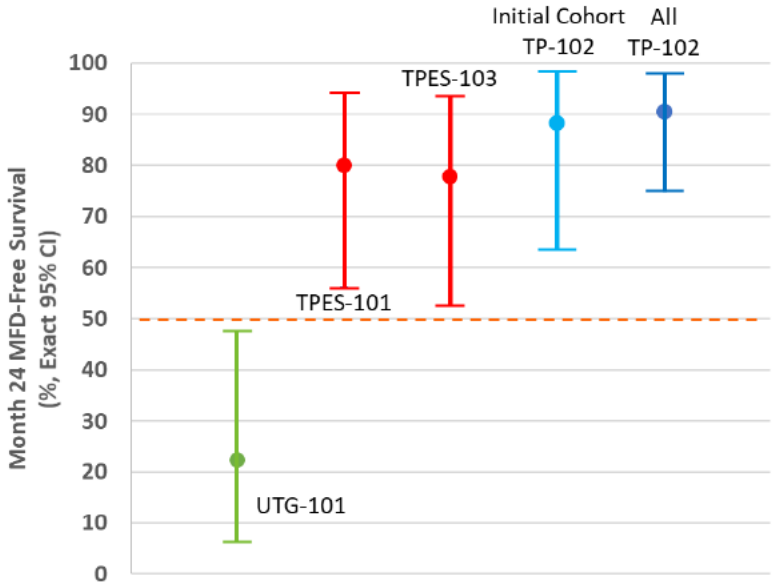
Source: Reviewer's analysis of ADSL datasets
Abbrev: TP, Transplant Population; TPES, Strictly ALD-102-eligible Transplant Population; NMSD, No Matched Sibling Donor subgroup; NFS, Neurologic Function Score.
[1] Age reflects age at time of treatment with allo-HSCT or eli-cel, or at time of diagnosis for the rUTES-101 population

7.2 Primary Efficacy Endpoint: Month 24 MFD-Free Survival

The primary efficacy endpoint was number and proportion of subjects achieving Month 24 MFD-free survival with success defined as >50% (lower bound of a 2-sided 95% CI). The clinical benchmark of 50% is described in Section 4.1.6.

Eli-cel was successful on the primary efficacy endpoint with a point estimate of 90.6% (exact 95% CI of 75.0% to 98.0%) MFD-free survival at Month-24. There were 3 failures of MFD-free survival in TP-102 by Month 24 in the primary analysis: 1 subject developed total incontinence (MFD) at Month 9 and subsequently died at Month 22, and 2 subjects withdrew to receive rescue allo-HSCT at the investigator's discretion due to progressive disease on brain MRI (at Months 13 and 17). Figure 3 compares eli-cel on the primary efficacy endpoint of Month-24 MFD-Free Survival to the clinical benchmark Population #1 and to the TPES-101 and TPES-103 populations. Of note, both the Initial Cohort TP-102 (n=17) and the Overall TP-102 cohort (n=32) are presented in the figure for comparison.

Figure 3: Month 24 MFD-Free Survival: Comparison to Benchmark



Source: bluebird bio BLA 2.5 Clinical Overview, Figure 5
Dots indicate the point estimate for MFD-free survival at Month 24, and the bars are the exact 95% CI. The orange dotted line indicates the clinical benchmark of 50%. The green bar indicates patients enrolled in study ALD-101 who were GdE+ at any time and did not have an allo-HSCT (UTG-101); the red bars indicate subset populations from studies ALD-101 and ALD-103, who received allo-HSCT and most closely matched the entry criteria for study ALD-102 (TPES-101, TPES-103). The blue bars indicate the patient populations who were treated with eli-cel in Study ALD-102 (N = 17, Initial Cohort and N = 32, Overall Cohort).

Success on the primary efficacy endpoint, as defined, was intended to show eli-cel was better than no treatment (as the upper bound of 95% CI for UTG-101 was less than 50%) and of similar efficacy to allo-HSCT (as the lower bound of the 95% CI for TPES-101 NMSD was 50.1%). FDA has six primary concerns with the benchmark populations and derivation of the benchmark which cast doubt on the conclusion that eli-cel is effective even though Study ALD-102 was successful on its primary endpoint:

1. FDA believes that the nonoverlapping confidence intervals between Population #1 and Population #2 do not show that HSCT is better than no treatment over the 2 years following diagnosis in the early active disease population (the population enrolled in ALD-102) because the UTG-101 population (Population #1) and the TPES-101 NMSD (transplanted population) (Population #2) were dissimilar at baseline. In fact, only one subject in the UTG-101 population would have met the UTES criteria. The UTG-101 population (n=21) had significantly more advanced disease at baseline with median age at diagnosis, Loes and NFS scores of 8 years, 11 and 3.5, respectively, than the TPES-101 NMSD (transplanted) population (n=21) who had medians of 8 years, 4.5, and 0, respectively at time of transplant. As HSCT is standard of care, we do not have an appropriate untreated control for comparison, and we do not know what would have happened to the TPES-101 NMSD population over the 2-year follow-up period had they not been treated.

2. The overall populations from ALD-101 were not comparable to the eli-cel population. This may be partly due to changing diagnostic and disease characterization modalities over time that contributed to older age and more advanced disease at time of diagnosis for Study ALD-101 populations compared to TP-102. Timing of the study “visits” varied between the studies by as much as 10-20 years (or more in a few cases). Eli-cel-treated subjects in TP-102 (n=32) had median age at treatment, Loes and NFS scores of 6 years, 2, and 0, respectively).

3. MFD is a partly subjective endpoint event and can be affected by knowledge of treatment assignment. Ideally in an open-label study, the MFD scores would have been provided by a team of central raters to mitigate the potential for clinician rating bias. Reliable measurement is particularly critical in the study of rare, heterogeneous diseases like CALD due to variability between and within individuals. The absence of central raters in all studies calls into question the interpretability of the NFS/MFD scores.

4. Imputation of repeat allo-HSCT in the TPES-101 population drove the benchmark calculation (i.e., many failures of Month 24 MFD-free survival were due to repeat HSCT due to graft failure) for the TPES-101 NMSD population. Repeat allo-HSCT was imputed as failure of MFD-free survival for the TPES-101 population, which favored eli-cel. FDA does not agree that repeat HSCT is commensurate

with disease progression, development of MFDs or death. Without this imputation, the point estimate for MFD-free survival by KM estimate for the TPES-101 NMSD population would have been 88.8% (95% CI of 62.1% to 97.1%).

5. Exploratory analysis* of Study ALD-101 suggests that 24 months of follow-up is insufficient time to assess efficacy based on MFD-free survival in a population with early active cerebral disease (as defined by Loes score between 0.5 and 9) who are asymptomatic or with mild functional limitations (NFS score of ≤1) and high risk of progression (GdE+). Few MFDs or deaths occurred by 24 months across appropriately matched comparator groups (including the untreated group) in all studies.

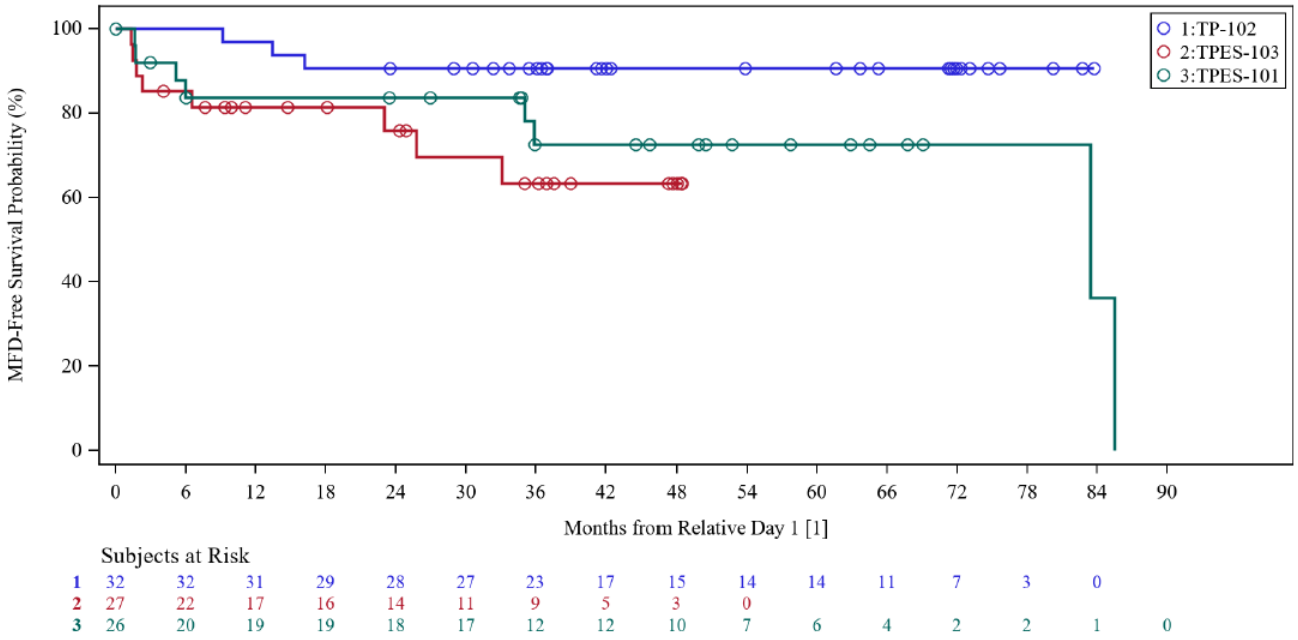
   * Of note, in a reviewer-initiated exploratory analysis, FDA re-coded the UTG-101 subjects so that baseline values for Loes and NFS were the values that were present at time of diagnosis rather than time of first GdE+ MRI (many UTG-101 MRIs did not utilize gadolinium at time of diagnosis as it was not yet routine). This re-code resulted in 7 untreated subjects in Study ALD-101 (rUTES-101) who would be considered similar to the eli-cel population at baseline on the MRI findings and NFS. Five (71%) of these 7 subjects ultimately developed MFDs, with mean time to first MFD or death from time of diagnosis of 46 months (median 20.4 months). Two subjects maintained MFD-free survival at time of last contact (70.1 and 186.6 months from date of diagnosis, respectively). The subject followed for 186.6 months remained asymptomatic. It is worth noting these subjects had older age at diagnosis compared to the eli-cel population. The protracted time-course for decline of these untreated subjects provides evidence that 24 months may be an insufficient time after treatment for assessing efficacy of eli-cel.

7.3 Secondary Efficacy Endpoint: Kaplan-Meier Estimated MFD-Free Survival Over Time

The analysis of the secondary efficacy endpoint of MFD-free survival over time was presented as Kaplan-Meier (KM) estimates of time to event for the TP-102 eli-cel population, and relative efficacy was demonstrated with KM estimates comparing TP-102 to the TPES-101 and TPES-103 allo-HSCT populations. The TPES-103 population had similar comparability issues to the TPES-101 population, namely older age at treatment and higher baseline Loes score compared to the TP-102 population, as shown in Table 8. The Applicant provided propensity score (PS) adjustments to account for such differences, but FDA does not believe PS adjustments are sufficient to account for the known and unknown baseline differences between groups. Because PS adjustments were minimal, FDA presents the primary analysis KM estimates (Figure 4

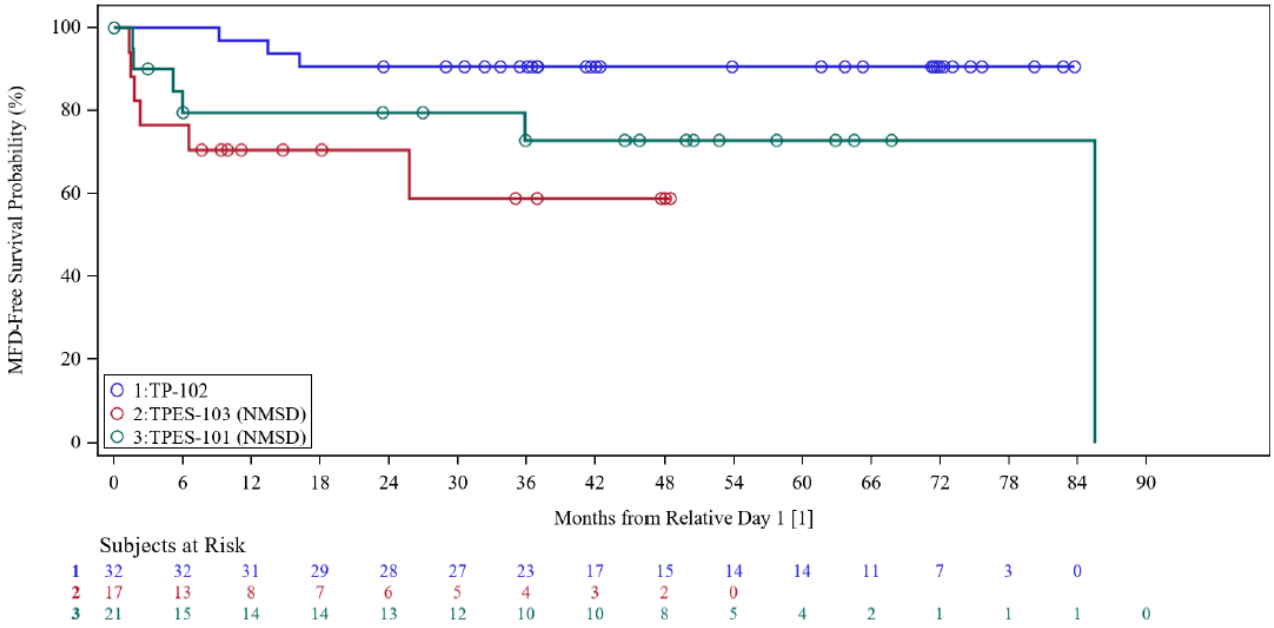
and Figure 5). In the Applicant's analysis, repeat HSCT was imputed as failure of MFD-free survival for the TPES populations. Comparison of MFD-free survival over time in TP-102 to TPES-101 and TPES-103 with this imputation is shown in Figure 4. Comparison of TP-102 to the TPES-101 and TPES-103 populations for whom no matched sibling donor (NMSD) was available and thus alternative donors were used is shown in Figure 5. In both figures, eli-cel appears superior to matched allo-HSCT populations. As previously stated, FDA does not agree that repeat HSCT is an outcome equivalent to MFD or death, and therefore does not agree that repeat HSCT should be imputed as failure of MFD-free survival. Taking this and other previously discussed data limitations into account (bias influencing MFD identification, retrospective data collection for part of Study ALD-103, few MFDs and deaths in the overall populations), the KM comparisons between TPES-103 populations and TP-102 as performed by the Applicant are difficult to interpret.

Figure 4: Major Functional Disability (MFD)-Free Survival Over Time, TP-102, TPES-103 and TPES-101



Source: bluebird bio, Inc., Original BLA submission, Figure 2.1.1.1
Abbrev.: TP, Transplant Population; TPES, Strictly ALD-102-eligible Transplant Population.
Note: Estimates of MFD-free survival and restricted mean survival time are obtained using the Kaplan-Meier method, where events include deaths, MFDs, and rescue cell administration or second allo-HSCT. For all studies except ALD-101, subjects who did not experience any event are censored at their date of last contact. Subjects who do not experience any event in ALD-101 are censored at their last NFS assessment.
[1] For TP-102, Rel Day 1 is the day of eli-cel infusion; for TPES, Rel Day 1 is the day of the allo-HSC infusion

Figure 5: Major Functional Disability (MFD)-Free Survival, TP-102, TPES-103 (NMSD) and TPES-101 (NMSD)



Source: bluebird bio, Inc., Original BLA submission, Figure 2.1.1.1
Abbrev.: TP, Transplant Population; TPES, Strictly ALD-102-eligible Transplant Population; NMSD, No Matched Sibling Donor subgroup.
Note: Estimates of MFD-free survival and restricted mean survival time are obtained using the Kaplan-Meier method, where events include deaths, MFDs, and rescue cell administration or second allo-HSCT. For all studies except ALD-101, subjects who did not experience any event are censored at their date of last contact. Subjects who do not experience any event in ALD-101 are censored at their last NFS assessment.
[1] For TP-102, Rel Day 1 is the day of eli-cel infusion; for TPES, Rel Day 1 is the day of the allo-HSC infusion

Of 67 total subjects treated with eli-cel, 14 (20.9%) have completed at least 5 years of follow-up, 13 of whom have maintained MFD-free survival (1 subject developed MDS at ~7.5 years). Of 53 subjects in the TPES (HSCT) populations, 14 (26.4%) were followed for at least 5 years after allo-HSCT (all in the TPES-101 population), 10 of whom have maintained MFD-free survival. The 4 subjects in the TPES-HSCT population who did not maintain MFD-free survival all remained alive at end of study but developed MFDs – 1 prior to 24 months (14.6 months) and the remaining 3 at 35.9 months, 83.4 months, and 85.5 months. Although this is encouraging for long-term efficacy with eli-cel, the comparability issues between the 2 populations reduce confidence in this comparison.

7.3.1 Exploratory Analyses Pooling TP-102 and TP-104 and Using Revised Imputation Schemes

To increase the robustness of the FDA analysis of the data, additional analyses were performed after pooling eli-cel-treated subjects in Study ALD-102 with the 35 enrolled subjects in Study ALD-104 at the time 13 (37%) ALD-104 subjects had reached 24 months of follow-up. In doing so, it became important to address the cases of myelodysplastic syndrome (MDS) that had developed in eli-cel-treated subjects. These MDS cases were not imputed as failure of MFD-free survival in the primary analysis because they were diagnosed after the March 2021 data cut for the BLA submission. FDA believes that MDS should be imputed as failure of MFD-free survival because of the associated morbidity and mortality. Sensitivity analysis was performed with the following imputation scheme in the following populations, as seen in Figure 6:

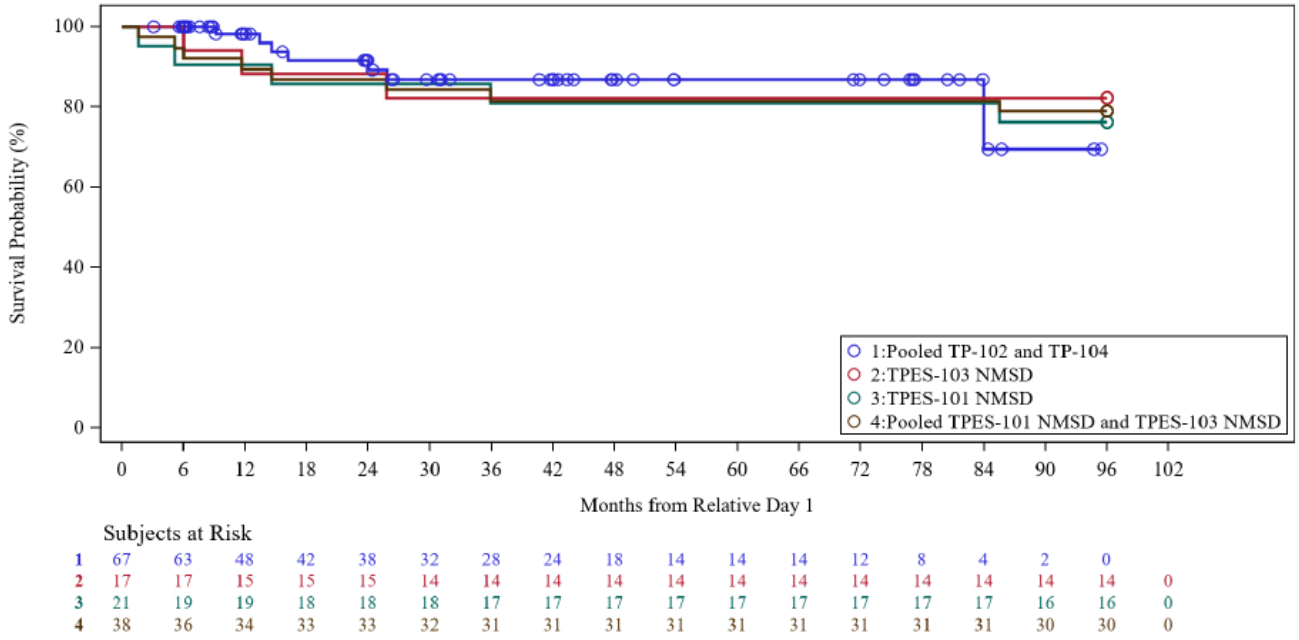
Imputation scheme used:

1. Failures of MFD-free survival for allo-HSCT cohorts include MFD and death only. Due to missing data from the early termination of Study ALD-103, to be conservative, “success” was imputed to Month 96 for subjects who did not experience an event by the date of last contact, rather than censoring at date of last contact.
2. Failures of MFD-free survival for eli-cel cohorts include MFD, rescue allo-HSCT, death, and MDS. Subjects who did not experience an event were censored at date of last contact.

Populations used:

1. Pooled TP-102 and TP-104 eli-cel treated subjects
2. TPES-101 and TPES-103 allo-HSCT-treated subjects without a matched sibling donor (NMSD subpopulation)

Figure 6: Major Functional Disability (MFD)-Free Survival Over Time Sensitivity Analysis, Pooled TP-102 and TP-104, TPES-103 NMSD, TPES-101 NMSD



Source:bluebird bio, Inc., BLA ad hoc Figure 80.2.6
Abbrev: TP, Transplant Population; TPES, Strictly ALD-102-eligible Transplant Population; NMSD, No Matched Sibling Donor Subgroup; MDS, Myelodysplastic syndrome.
Note: Estimates of Event-free survival and restricted mean survival time are obtained using the Kaplan-Meier method, where events include deaths, MFDs, MDS, and rescue cell administration or second allo-HSCT. Subsequent allo-HSCT is not considered as failure for treated subjects in ALD-101 and ALD-103. Subjects who did not experience any event are censored at their date of last contact for eli-cel treated subjects, and censored at imputed 96 month post infusion for ALD-101 and ALD-103 subjects. For ALD-101 and ALD-103 subjects, all imputed 96 month visits were counted as "successes." For eli-cel treated subjects, event date was carried backward to the past visit(s) if that visit(s) was missed.

KM estimates of MFD-free survival over time appear similar for eli-cel and allo-HSCT in the TPES-101 and -103 NMSD populations when MDS is imputed as failure of MFD-free survival and repeat HSCT in TPES populations is not imputed as failure. However, the results are difficult to interpret for the following reasons:

1. The impact of the incomparability between the TPES-101 and TPES-103 NMSD and eli-cel populations on the results is not estimable and cannot be accounted for simply by pooling data, adjusting the imputation scheme, or utilizing propensity score adjustments.
2. The small number of events constituting failure of MFD-free survival across all populations in 24 months of follow-up suggests that 24 months may not be sufficient to establish efficacy; many TP-104 and TPES-103 subjects did not have 24 months of data. Paucity of data beyond 24 months for both populations further complicates comparison of relative efficacy and assessment of durability of effect.

3. The MFD assessments may have been biased by knowledge of treatment assignment.
4. The TPES comparator data were collected retrospectively or partly retrospectively, which could have introduced selection bias.

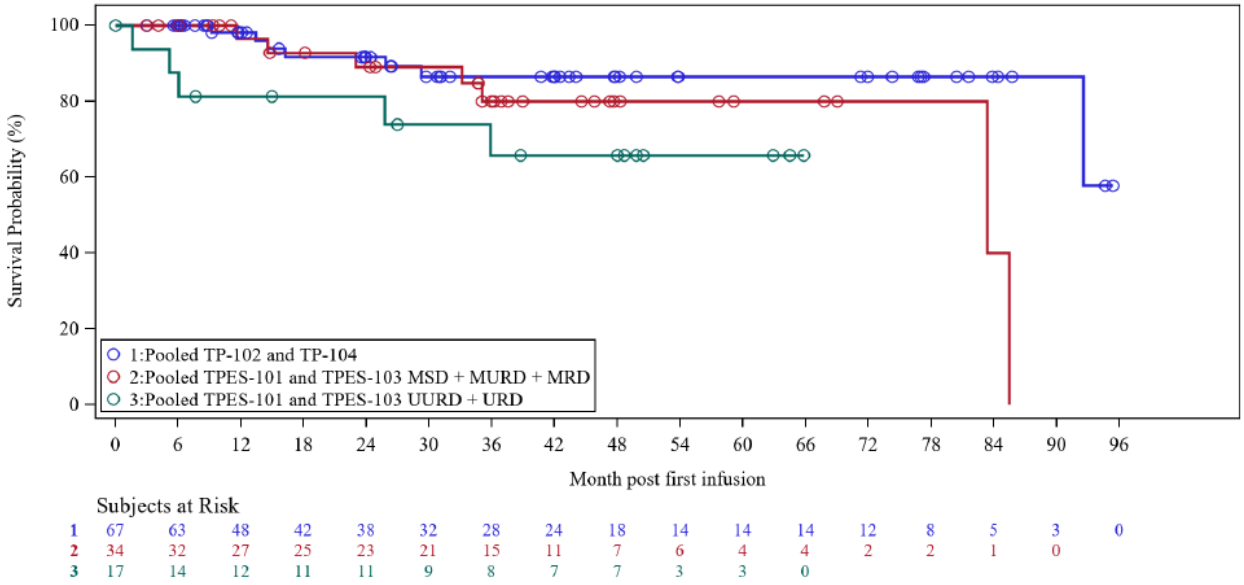
7.3.2 Exploratory Subgroup Analyses

Additional analysis of the allo-HSCT study population subgroups identified a population for which risks of allo-HSCT appear to be greater and for whom events (including death) appear to occur sooner. While it has been traditionally understood that a matched sibling donor (MSD) is superior to all other donor types for allo-HSCT, it may be more important to make a distinction between patients with matched donors (MDs) and patients with HLA-unmatched donors (UMDs). Analyses of events in Studies ALD-101 and ALD-103 demonstrate trends toward worse outcomes for those with UMD compared to those with MDs, regardless of relatedness of donor to the subject. UMD includes unmatched related donors (URD) and unmatched unrelated donor (UURD). MD includes MSD and matched unrelated donors (MURD). No matched non-sibling related donors (MRD) were used for HSCT in the TPES populations.

KM curves comparing MFD-free survival over time for eli-cel (pooled TP-102 and TP-104), allo-HSCT (pooled TPES-101 and TPES-103) from subjects with MDs and UMDs are shown in Figure 7 and also shown in Table 9. Imputation schemes used in this analysis were as follows:

1. Failures of MFD-free survival for allo-HSCT cohorts include MFD and death only. Subjects who did not experience an event in Study ALD-103 were censored at date of last contact. Subjects who did not experience an event in Study ALD-101 were censored at date of last NFS assessment.
2. Failures of MFD-free survival for eli-cel cohorts include MFD, rescue allo-HSCT, death, and MDS. Subjects who did not experience an event were censored at date of last contact.

Figure 7: Major Functional Disability (MFD)-Free Survival Over Time, Pooled TP-102 and TP-104, Pooled TPES-101 and TPES-103 HLA-Matched Donors and Pooled TPES-101 and TPES-103 HLA-Unmatched Donors



Source: bluebird bio, Inc., BLA ad hoc Figure 80.2.34
Abbrev: TP, Transplant Population; TPES, Strictly ALD-102-eligible Transplant Population; HSCT, Hematopoietic stem cell transplantation; MDS, Myelodysplastic syndrome; MSD, Matched Sibling Donor Subgroup; MURD, Matched Unrelated Donor Subgroup; MRD, Matched Related Donor Subgroup; UURD, Unmatched Unrelated Donor Subgroup; URD, Unmatched Related Donor Subgroup. Note: Estimates of Event-free survival and restricted mean survival time are obtained using the Kaplan-Meier method, where events include deaths, MFDs, MDS, and rescue cell administration or allo-HSCT. Allo-HSCT (including second or subsequent allo-HSCT) is not considered as failure for ALD-101 and ALD-103 subjects. For all studies except ALD-101, subjects who did not experience any event are censored at their date of last contact. Subjects who do not experience any event in ALD-101 are censored at their last NFS assessment. Note: Two subjects from ALD-101 were not included in the figure due to missing donor HLA typing information.

Table 9: Time to MFD and Death from Time of HSCT for Pooled TPES-101 and TPES-103 Populations Based on HLA-Matching of HSC Donor

Parameter	Statistic	HLA-Unmatched Donor (n=17)	HLA-Matched Donor (n=34)
Subjects with at least one MFD	**n (%)**	2 (11.8)	5 (14.7)
Time to first MFD (months)	**Median** **Min, Max**	18.7 1.6, 35.9	35.1 11.6, 85.5
Deaths	**n (%)**	4 (23.5)	3 (8.8)
Time to death (months)	**Median** **Min, Max**	6.1 5.2, 25.8	23.0 12.8, 33.1
Duration of follow-up from HSCT (months)	**Median** **Min, Max**	48.0 5.2, 109.0	38.2 4.1, 108.1

Source: Reviewer's analysis of ADSL, ADBASE, and ADHSCT datasets
Abbrev: MFD, Major Functional Disability; HSCT, Hematopoietic Stem Cell Transplant; TPES, Strictly-Eligible for ALD-102 Transplant Population; HLA, Human Leukocyte Antigen; HSC, hematopoietic stem cell.

Time to MFD or death was prolonged by approximately double (or more) in subjects with HLA-matched donors. As seen in Figure 7 and Table 9, there is steep drop off for the UMDs during the first 6 months after which the curves are similar. This is primarily due to deaths, discussed further in Section 7.4. More deaths occurred in the TPES population with UMDs (23.5%, compared to 8.8% for MDs). Small numbers of subjects decrease confidence in these results. However, there is biological plausibility (one would predict poorer prognosis in UMDs compared to MDs because of HLA-mismatch and increase risk for rejection and GVHD.)

To support the biological plausibility of a difference in prognosis between subjects with UMDs, further analysis on GVHD and repeat HSCT was done. Subjects who were treated with allo-HSCT from UMDs experienced more transplant-related events compared to those with MDs, as seen in Table 10. Time to repeat HSCT was shorter for those with UMDs than those with MDs (median 1.7 months and 6.5 months, respectively). Incidence of primary or secondary graft failure, repeat HSCT, and acute GVHD by Month 24 was at least double in those with UMDs compared to those with MDs. Incidence of acute GVHD >Grade 2 and/or chronic GVHD by Month 24 was also increased in those with UMDs.

Table 10:Graft Failure, Repeat HSCT, and Acute or Chronic GVHD by Donor HLA Matching

Parameter	HLA-Unmatched Donor	HLA-Matched Donor
Number of subjects, n	17	34
HSC graft failure, n (%)	6 (35.3)	4 (11.8)
Repeat HSCT, n (%)	5 (29.4)	3 (8.8)
Acute GVHD by Month 24, n (%)	8 (47.1)	6 (17.6)
Acute Grade ≥ 2 or Chronic GVHD by Month 24, n (%)	9 (52.9)	12 (35.3)

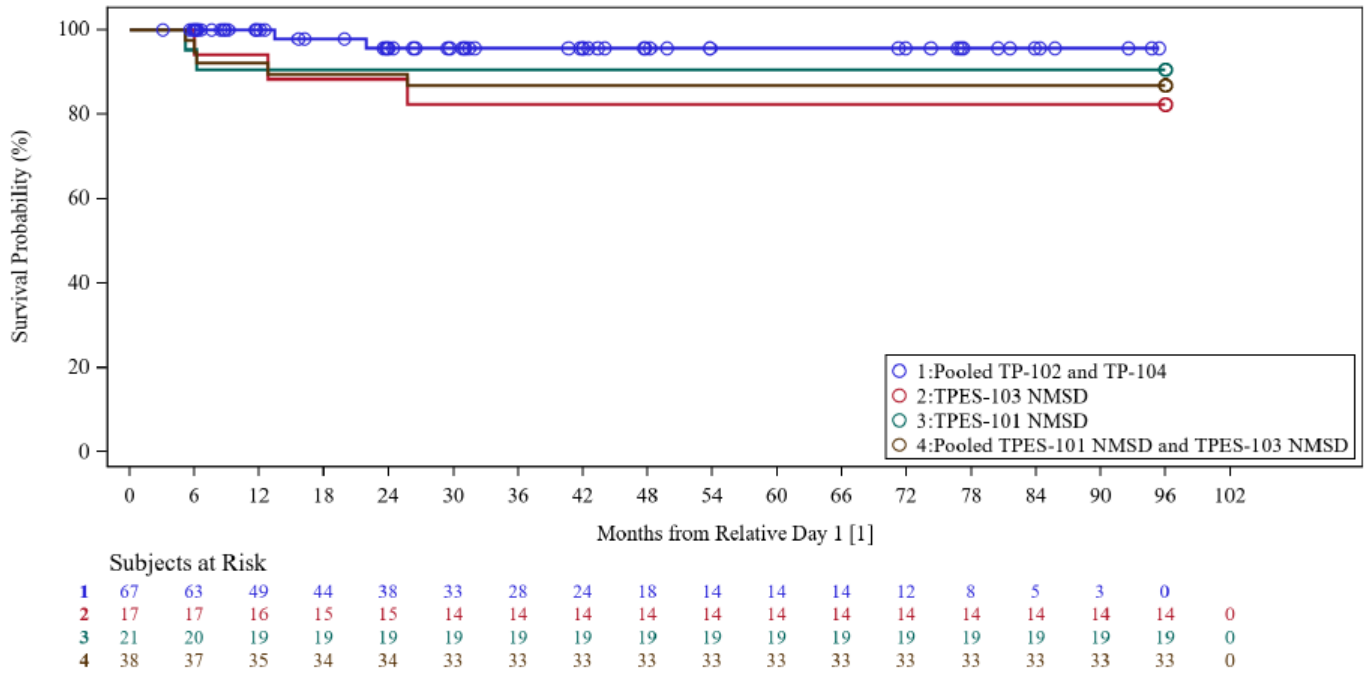
Source: Reviewer's analysis of ADSL and ADHSCT datasets
Abbrev: HLA, Human Leukocyte Antigen; HSC, hematopoietic stem cell; GVHD, graft versus host disease

7.4 Secondary Endpoint: Overall Survival

KM curves for overall survival (OS), particularly over the initial 24 months for which TP-102 was followed, appeared similar for eli-cel and TPES groups (KM curve not shown).

Figure 8 makes it appear that OS is similar between eli-cel and HSCT in CALD patients who have early active cerebral disease and no matched sibling donor. However, as previously stated, comparability between groups, paucity of 24-month data in TP-104 and TPES-103 populations, and paucity of long-term data beyond 24 months decrease our confidence in these assessments. For TPES-101 and TPES-103 NMSD, if subjects were alive at time of last contact, survival to 96 months was imputed. Subjects treated with eli-cel in TP-102 and TP-104 were censored at date of last contact. Additionally, there were two deaths in subjects treated with eli-cel in Study ALD-102. One subject died on study, and one died following withdrawal from the study for rescue allo-HSCT. Both deaths are captured in this analysis.

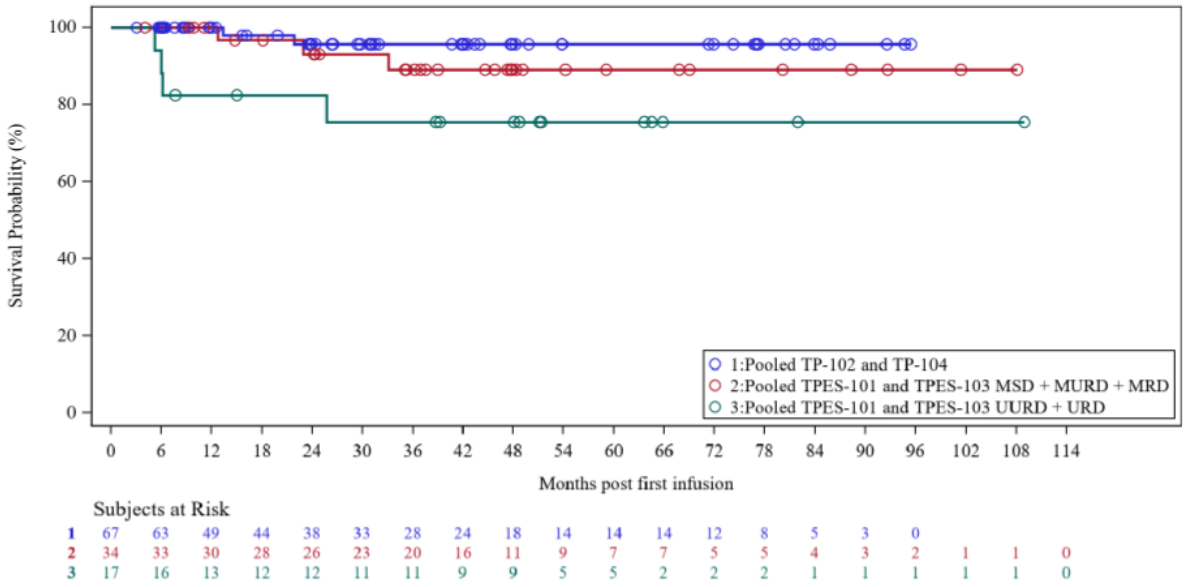
Figure 8: Overall Survival, Pooled TP-102 and TP-104, TPES-101 NMSD and TPES-103 NMSD



Source: bluebird bio, Inc.,BLA ad hoc Figure 80.2.2.1.1.2
Abbrev.: TP, Transplant Population; TPES, Strictly ALD-102-eligible Transplant Population; NMSD, No Matched Sibling Donor Subgroup.
Note: Estimates of overall survival rates and restricted mean survival time are obtained using the Kaplan-Meier method, where the event is death of any cause. Subjects who are alive are censored at their last contact date, and censored at imputed 96 month post infusion for ALD-101 and ALD-103 subjects. No eli-cel subject died after a missed visit.
Note: Subject 102-16, who withdrew from the study to undergo allo-HSCT, is hard coded as a death event at the last contact date before withdrawal.
[1] For TP-102 and TP-104, Rel Day 1 is the day of eli-cel infusion; for TPES, Rel Day 1 is the day of the allo-HSC infusion.

Shown in Figure 9, an initial decline in OS is seen in the HLA-unmatched TPES population due to deaths by 6 months that is not seen in the eli-cel or HLA-matched (related or unrelated) allo-HSCT populations. In this analysis, all subjects are censored at date of last contact. Both deaths in TP-102 are captured in this analysis.

Figure 9: Overall Survival, TP-102 and TP-104 Pooled, TPES-101 and TPES-103 Pooled Donor HLA Typing Subgroups (matched vs. unmatched)



Source: bluebird bio, Inc., BLA ad hoc Figure 80.14.4
Abbrev: TP, Transplant Population; TPES, Strictly ALD-102-eligible Transplant Population; MSD, Matched Sibling Donor Subgroup; MURD, Matched Unrelated Donor Subgroup; MRD, Matched Related Donor Subgroup; UURD, Unmatched Unrelated Donor Subgroup; URD, Unmatched Related Donor Subgroup. Note: Estimates of overall survival rates and restricted mean survival time are obtained using the Kaplan-Meier method, where the event is death from any cause. Subjects who are alive are censored at their last contact date. Note: Subject 102-16, who withdrew from the study to undergo allo-HSCT, is hard coded as a death event at the last contact date before withdrawal. Note: Two subjects from ALD-101 were not included in the summary due to missing donor HLA typing information.

Table 11 details deaths by subject, including treatment arm, study population, study population subgroup, HLA match, time to death and cause of death. Deaths in the re-coded strictly ALD-102- eligible untreated population (rUTES-101) are provided for comparison. All deaths before or during the sixth month following treatment were in subjects treated with allo-HSCT from unmatched donors (UMD), also seen in Figure 9. Of 17 pooled TPES-UMD subjects, 3 died before or during the sixth month (17.6%), compared to no deaths in eli-cel, allo-HSCT TPES with matched donor (MD), and untreated populations. One unmatched allele (e.g., 9 out of 10) was associated with death, suggesting that any degree of mismatch is a risk factor for early death. The earliest death in a TPES subject with a matched donor (MD) was 12.8 months, in a subject treated with eli-cel was 16.3 months, and in a re-coded strictly ALD-102- eligible untreated (rUTES-101) subject was 27.6 months. Deaths for all subjects are detailed in Appendix 6. The rUTES-101 statistic suggests that early deaths may be more likely to occur due to treatment-related events rather than disease progression.

Table 11: Subject-Specific Time-to-Death for Eli-Cel, TPES, and rUTES-101 Populations

Treatment Arm	Population	Subgroup	HLA Match	Subject ID	Time of Death[1] (months)	Cause of Death
Allo-HSCT	TPES-101	UMD (UURD)	9 out of 10	101-09	5.2	Septicemia and GVHD
Allo-HSCT	TPES-103	UMD (UURD)	8 out of 10	103-35	6.0	Transplant-Related
Allo-HSCT	TPES-101	UMD (UURD)	9 out of 10	101-10	6.2	Progressive Disease
Allo-HSCT	TPES-103	MD (MURD)	10 out of 10	103-58	12.8	Progressive Disease
Eli-cel	TP-102	n/a	n/a	102-16	16.3	Transplant-Related (following rescue allo-HSCT)
Eli-cel	TP-102	n/a	n/a	102-18	21.9	Viral Infection
Allo-HSCT	TPES-103	MD (MSD)	10 out of 10	103-32	23.0	Transplant-Related
Allo-HSCT	TPES-103	UMD (UURD)	9 out of 10	103-22	25.8	Cardiac Arrest[2]
Untreated	rUTES-101	n/a	n/a	101-72	27.6	Progressive Disease
Allo-HSCT	TPES-103	MD (MSD)	6 out of 6	103-44	33.1	Septic Shock
Untreated	rUTES-101	n/a	n/a	101-67	51.9	Progressive Disease
Untreated	rUTES-101	n/a	n/a	101-87	72.5	Death[3]
Untreated	rUTES-101	n/a	n/a	101-56	91.8	Progressive Disease

Source: Reviewer's analysis of ADSL, ADBASE, and ADHSCT datasets
Abbrev: HLA, Human Leukocyte Antigen; Allo-HSCT, allogeneic hematopoietic stem cell transplant; TP, Transplant Population; TPES, Strictly ALD-102-eligible Transplant Population; rUTES, re-coded Strictly ALD-102-eligible Untreated Population; MD, HLA-Matched Donor; UMD, HLA-Unmatched Donor; UURD, Unmatched Unrelated Donor subgroup; MURD, Matched Unrelated Donor subgroup; MSD, Matched Sibling Donor subgroup.
[1]Time of death is measured in months from time of treatment for the eli-cel and allo-HSCT populations, and from time of diagnosis for untreated populations.
[2] Presumed transplant-related from GVHD and infection
[3] Listed death without further clarification of etiology

When evaluated in the entire transplant populations (TP-101 and TP-103, detailed in Appendix 6), death rates were nearly double in the total UMD population compared to the total MD population (33% and 17%, respectively). Of 124 total subjects, there were 31 deaths (25%). Of these 31 deaths, 21 (68%) were in subjects treated with HSCT

from UMDs. Regardless of cause, deaths in subjects with UMDs largely occurred within the first 6 months following treatment. Median time to death from transplant-related causes, progressive disease, and unknown cause were 5.6, 6.2, and 9.3 months, respectively (compared to 3.5, 31.4, and 33.1 months, respectively, in the subjects who had MDs). Mortality by 6 months and 12 months were 19% and 28%, respectively, in the unmatched group, compared to 9% and 10%, respectively, in the matched donor group. The subjects with UMDs and with MDs received their first transplants between 1997-2019 and had a similar age distribution at time of first transplant (median 8 years). This is further detailed in Appendix 6. Early mortality differences between UMD and MD in the entire transplant population (TP) supports the possibility that there is a true increase in early mortality in CALD patients treated with HSCT from unmatched donors.

Early mortality in subjects treated with allo-HSCT from HLA-unmatched donors potentially identifies a population for whom the benefit/ risk of eli-cel may be favorable. However, the benefit/ risk profile of eli-cel compared to allo-HSCT on overall/ long-term survival is still unknown. This is largely due to lack of long-term data, supported by the observation that deaths in a similar untreated population (rUTES-101) did not occur until after 24 months.

We note that this exploratory analysis is limited by the relatively small number of subjects and events and group comparability concerns. Although FDA recognizes this limitation, the biological plausibility of death related to complications of unmatched allo-HSCT is high.

7.5 Additional Efficacy Endpoints: Change in NFS, Loes and Gadolinium Enhancement (GdE)

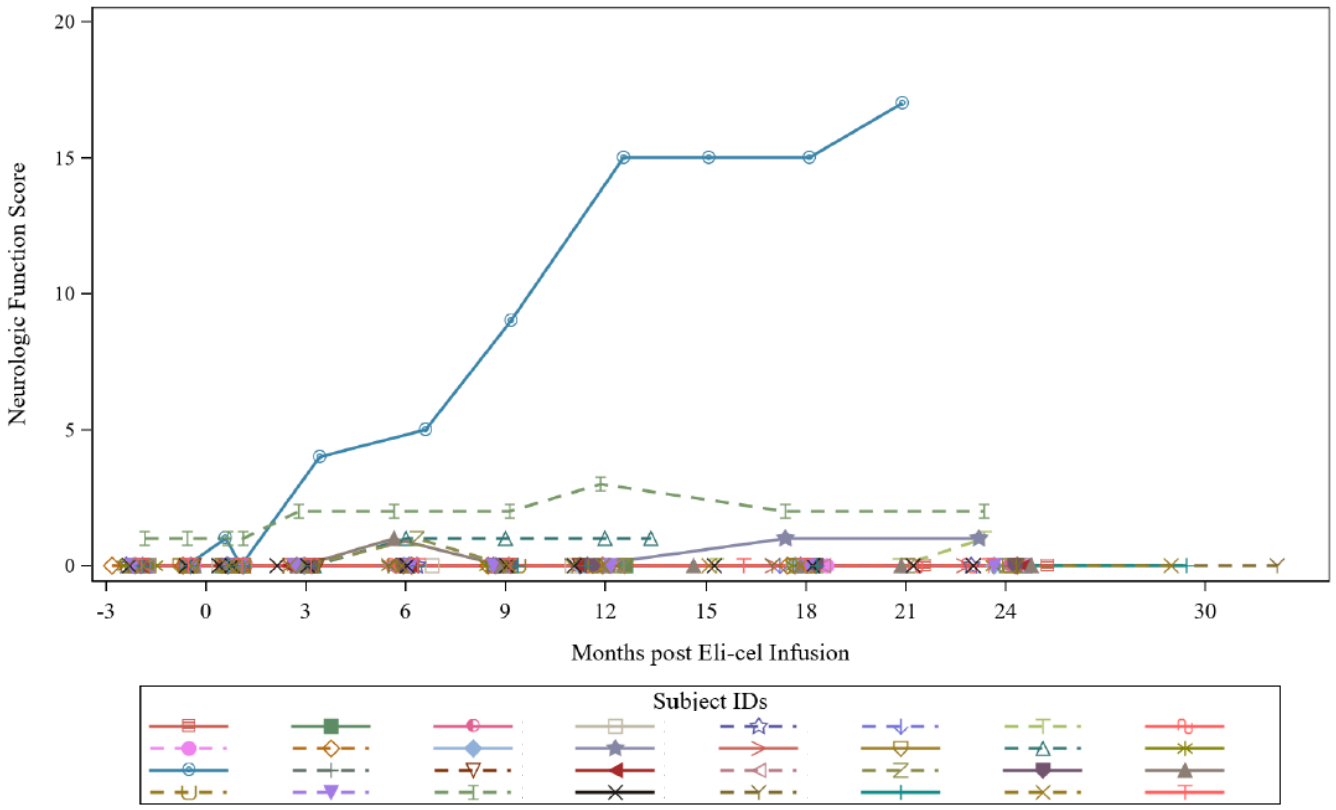
Results of analyses of other secondary and exploratory efficacy endpoints are detailed in this section. In the primary analysis, 30 of 32 subjects in Study ALD-102 were evaluable for NFS and GdE at the Month 24 visit. Two (2) subjects withdrew from the study to receive allo-HSCT prior to the Month 24 visit. The subject who developed MFDs and subsequently died was considered evaluable at Month 24 for these endpoint analyses.

Change in total NFS from Baseline to Month 24

NFS over time for each subject in Study ALD-102 through Month 24 is shown in Figure 10. A stable NFS at Month 24 was defined as maintaining an NFS ≤4 without an increase >3 from Baseline. By this definition, 29 subjects (96.7%) in TP-102 had a stable NFS at Month 24. Summary statistics of change in total NFS from Baseline at Month 24 for the Month 24- evaluable TP-102 subjects, TP-104 subjects and TPES-HSCT subjects in Studies ALD-101 and ALD-103 are shown in Appendix 7, Table 7. TPES-HSCT subjects had similar changes in NFS at Month 24 to eli-cel treated subjects.

While FDA agrees that NFS at Month 24 is stable for most subjects in Study ALD-102 by the provided definition, it is not clear that the definition for stability is appropriate. Any increase in NFS confers an increase in neurologic or functional symptoms, and thus any increase in NFS could be clinically significant. As with other efficacy assessments, FDA is also not confident that 24 months is sufficient time to assess stability of NFS.

Figure 10: Neurologic Function Score (NFS) Over Time, By Subject in Study ALD-102



Source: bluebird bio, Inc. Original BLA submission, Figure 14.2.4

Change in Loes score from Baseline to Month 24

As seen in Appendix 7, Table 7, subjects treated with eli-cel (TP-102 and TP-104) were less likely to have a decrease in Loes score at Month 24 (2.9%) compared to the allo-HSCT TPES populations (13.3%), and nearly half (48.6%) of the subjects treated with eli-cel had a change in Loes score from Baseline of 4 or more (compared to 20% of the TPES populations). TP-102 subjects treated with eli-cel had higher change in Loes score from Baseline to Month 24 than TPES subjects treated with allo-HSCT. The clinical significance of this is unknown. While disease progression may be expected in the 2 years following allo-HSCT, followed by stabilization of disease, it is not clear that this stabilization occurs after eli-cel administration, at or following Month 24. It is also not clear how the greater change from Baseline in Loes score affects relative efficacy of eli-cel compared to allo-HSCT. Additionally, while a stable Loes score at Month 24 was

defined as either maintaining a Loes score ≤9 or not increasing by ≥6 from Baseline, FDA is not sure that this is an appropriate definition of stability. Only longer duration of follow-up for observation of clinical change associated with MRI changes would help to understand the implications of these differences.

Proportion of subjects who demonstrated resolution of gadolinium positivity on MRI (i.e, GdE-) at the Month 24 Visit

Of the 30 Month 24-evaluable subjects in TP-102, 26 (86.7%) had a GdE- MRI at the Month 24 visit, compared to 100% of TPES-103 subjects evaluable at Month 24.

8 SAFETY

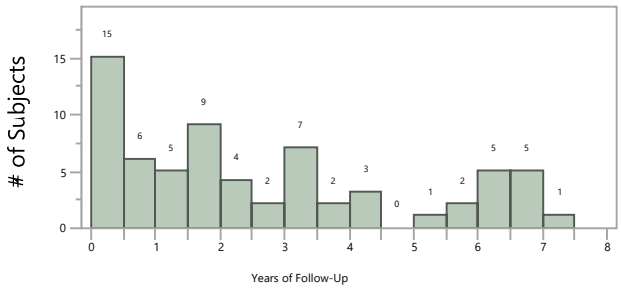
8.1 Sources of Data for Safety

The safety review focuses on 29 subjects with CALD that were treated in the ALD-102 Phase 2/3 study and subsequently enrolled in the long-term follow-up study, LTF-304. Subjects treated in Study ALD-102 were the primary source of safety data because they had the longest duration of follow-up. Subjects enrolled in Study ALD-102 were followed for a median duration of approximately 4 years. Excluding three subjects who were discontinued from Study ALD-102 (one who died and two who underwent rescue allo-HSCT), follow-up ranged between 2.3 and 7.3 years.

Secondary safety data were provided from ALD-104, an ongoing Phase 3 study that enrolled 35 boys with CALD. These data are limited because the median duration of follow-up was six months (range 23 days to 2 years).

Figure 12 demonstrates the years of follow-up for the ISS population. The overall short duration of follow-up for this product that has a serious risk of myelodysplastic syndrome that may take years to develop is a limitation of the safety data.

Figure 11: Years of Follow-Up, ISS Population



Source: Reviewer's analysis, derived from ADSL dataset

The safety data have several additional important deficiencies. First is the open-label study design. It is difficult to avoid bias in open-label studies, and bias may have influenced the identification and assessment of adverse events.

A second important deficiency in the safety data is the absence of comparable safety data in subjects treated with HSCT. The natural history study and external control study performed by the Applicant to provide comparator data (Studies ALD-101 and ALD-103, respectively) largely involved retrospective data collection. Data that are collected retrospectively are often incomplete and inconsistent, due to inconsistencies around follow-up assessments and due to differences in documentation in different institutions and by different personnel. Therefore, Studies ALD-101 and ALD-103 do not provide comparable safety data for side-by-side comparison to the systematically collected eli-

cel adverse event data.

8.2 Safety Summary

Myelodysplastic syndrome (MDS), acute myeloid leukemia (AML) and insertional oncogenesis are the major safety concerns with eli-cel. MDS was diagnosed in three subjects. However, one of those three children appears to have AML, with 15-20% CD34+ blasts overall and 20-30% in discrete foci, read by the pathologist as “worrisome for evolving AML.” Furthermore, 15-20% CD34+ blasts will be classified as AML after forthcoming updates to AML diagnostic criteria.

MDS is a group of bone marrow malignancies characterized by some combination of low blood counts, abnormal cells in the bone marrow, and/or abnormality in the bone marrow cellular genetics. MDS is often a precursor to AML, and it is very rare in pediatric patients, with an incidence of 1.8 per million children per year in the 0-to-14-year age group. Review of the published literature did not reveal any cases of MDS or other hematologic malignancies in patients with CALD. The only curative treatment for MDS in children is hematopoietic stem cell transplant (HSCT). Prognosis for pediatric MDS varies by subtype, but overall three-year survival for children with secondary MDS after treatment with HSCT is 20-30%.

Treatment with eli-cel involved not only administration of the eli-cel product, but the administration of chemotherapy. Myeloablative chemotherapy serves to destroy the existing bone marrow, so that the eli-cel can more effectively engraft and replace the bone marrow cells harboring the CALD-causing mutation. The myeloablative chemotherapy required for administration of eli-cel caused many serious and severe adverse events, such as cytopenias and mucositis, and thereby a risk of serious infections. Specifically, in the ISS population, 262 hematologic adverse events occurred within the first 30 days after eli-cel.

Not all adverse events that occurred can be reasonably attributed to chemotherapy. Excluding the 262 early hematologic adverse events, a total of 938 treatment-emergent adverse events (TEAEs) occurred through the August 18, 2021, data cut, including 71 serious adverse events (SAEs) and 280 severe adverse events. Refer to Appendix 8 for additional information about SAEs in the ISS population.

Also important for consideration is the possibility that the hematopoietic stem cell processing or the presence of vector within the cells interferes with their resumption of function after they are administered to the child. The possibility that eli-cel interferes with hematopoietic and immune reconstitution is based on neutrophil and platelet engraftment that are delayed relative to what would be expected for autologous HSCT, the failure of blood counts to return to baseline levels, and on the occurrence of numerous serious opportunistic infections in eli-cel-treated subjects.

8.3 Safety Issues

8.3.1 Insertional oncogenesis

CALD is not associated with an increased risk of hematologic malignancy. In September 2021, the Applicant searched the published literature and did not identify any cases of hematologic malignancies in this population. However, the Applicant found a single reported case of chronic myelogenous leukemia reported in a patient with adult adrenomyeloneuropathy, a phenotype of adrenoleukodystrophy that is distinct from childhood CALD,

Insertional oncogenesis is a serious risk that has been observed with eli-cel. Malignancy has always been a theoretical risk, due to the product's permanent alteration of the host genome, as described in **SECTION 3.5 CONCERN OF LVV ONCOGENICITY**. In addition to the several hematologic malignancies after eli-cel administration, MDS and AML have occurred after administration of a related LVV-based product (lovo-cel) for sickle cell disease. There are also concerns regarding evolving malignancy with the LVV-based product (beti-cel) for beta--thalassemia manufactured with an identical LVV to that used in lovo-cel. The lovo-cel and beti-cel products and their relationship to eli-cel are described in Appendix 9, and the integration site data for both lovo-cel and beti-cel and the cases of malignancy for lovo-cel are described in in Appendix 10.

Unlike lovo-cel, where the available data are not sufficient to attribute the development of malignancy to the gene therapy, eli-cel appears to have caused malignancy in three children. In addition to the diagnosed cases of malignancy, eli-cel has a concerning pattern of frequent integration into proto-oncogenes across the rest of the study population.

Three children in the eli-cel development program have been diagnosed with MDS. Two of these cases occurred within two years of eli-cel administration and are unequivocally the result of expansion of a clone that has LVV integration into a proto-oncogene. Both subjects had a predominant clone[3] with integration into the *MDS1* and *EVI1* complex locus, also referred to as *MECOM*, and overexpression of *EVI1*. Inversion or translocation in *MECOM* is a recognized cause of MDS and AML, and overexpression of *EVI1* is associated with poor prognosis.

The third subject also had integration into *MECOM*, although based on relative frequency of integration site, it appears his MDS may have developed due to integration into another proto-oncogene and a paralog of *MECOM*, *PRDM16*. Selected information about these three subjects is presented in the following table.

3 In this application, a clone was considered predominant when IS-specific VCN measured by qPCR was >0.5 c/dg. Refer to Appendix 3 for more details regarding integration site analysis.

Table 12: Characteristics Eli-cel-Treated Subjects with Malignancy

Subject #	104-08	104-18	102-03
Age at eli-cel administration	13 years	11 years	5 years
Age at malignancy	14 years	12 years	12 years
Time of malignancy relative to eli-cel administration	22 months	14 months	7.5 years
Eli-cel CD34+ cells administered	12.1 x 10^6/kg	5.7 x 10^6/kg	6 x 10^6/kg
Eli-cel % LVV+ cells	70%	Not reported	62%
Eli-cel vector copies per transduced cell	2.6	Not reported	2.6
Eli-cel VCN (c/dg)	1.8	Not reported	1.8
Neutrophil engraftment day*	188	27	37
Platelet engraftment day*	Not Engrafted	106	37
Key integration sites	*MECOM, ACTR RAP2C, STGAL6*	*MECOM, SLC6A16*	*PRDM16, GAB3, SNX12*
Gene expression studies	Increased *EVI1*	Increased *EVI1*	RNA sequencing analysis pending
Bone marrow at malignancy diagnosis	MDS with single lineage dysplasia	MDS with single lineage dysplasia	MDS with excess blasts 2 (MDS-EB-2)
Cellularity	80%	10-20%	60-70%
Other features	Dysmegakaryopoiesis	Dysmegakaryopoiesis	15% blasts
Flow cytometry	Negative	Negative	15% myeloblasts
FISH	Normal	Normal	Normal
Karyotype	Normal	del(14)(q11.2q13) versus inv(14)(p11.2q11.2)	Normal
Rapid Heme Panel next generation sequencing	Normal	*CDKN2A* SNV** – 41% VAF	*KRAS* SNV – 14% VAF *NRAS* SNV – 3% VAF *JAK* SNV** – 48%
PB WBC / Hgb / Plt	2.2 / 10.7 / 19	2.6 / 13 / 123	14.9 / 10 / 17

Abbrev: % LVV+, percent of cells transduced with lentivirus; c/dg, copies per diploid genome; VCN, vector copy number; FISH, fluorescence in situ hybridization; kg, kilogram; MDS, myelodysplastic syndrome; del, deletion; inv, inversion; SNV, single nucleotide variant; VAF, variant allele frequency; PB WBC / Hgb / Plt, peripheral blood white blood cells (x 10^9), hemoglobin (g/dL), platelets (x 10^9)
*Based on FDA definitions for engraftment that did not permit concomitant G-CSF or eltrombopag
**Variant of unknown significance
Source: Reviewer's analysis

In addition to the three children who have been diagnosed with MDS, FDA is concerned about the possible development of MDS in four additional children where integration

sites in proto-oncogenes are increasing in relative frequency. Selected information about these four subjects is presented in the following table.

Table 13: Characteristics of Subjects with Concern for Evolving Malignancy

Subject #	102-11	102-31	104-09	104-22
Age at eli-cel administration	7 years	4 years	9 years	13 years
Date of eli-cel Administration	Feb 2015	Apr 2018	Aug 2019	Sep 2020
Cells administered	10.5 x 10^6/kg	5 x 10^6/kg	14.5 x 10^6/kg	14.4 x 10^6/kg
% LVV+	59%	62%	67%	47%
Vector copies per transduced cell	2.7	3.4	2.7	3.2
Eli-cel VCN (c/dg)	1.6	2.1	1.8	1.5
Neutrophil engraftment day*	27	32	167	13
Platelet engraftment day*	41	60	356	29
Key integration sites	*MECOM, ACER3, RFX3*	*MECOM, EVI5, SECISBP2, PLAG1, PUM3*	*LINC00982, SMG6, MECOM, MPL*	*MECOM, MPL*
Gene expression studies	Increased *EVI1*	Increased *EVI1*	Not reported	Not reported
Bone marrow	Maturing trilineage hematopoiesis	Maturing trilineage hematopoiesis	Trilineage hematopoiesis, atypical megakaryo-poiesis, 2% blasts	n/a
Cellularity	30-40%	40-50%	30-40%	n/a
Other features	Megakaryocytes with overall normal morphology and include some small forms	Unremarkable megakaryocytes	Small megakaryocytes with monolobated nuclei and very rare forms with widely spaced nuclei	n/a
Flow cytometry	Negative	Negative	No definitive abnormal myeloid blast population	n/a
FISH	Normal	Normal	Not reported	n/a
Karyotype	Normal	Normal	Normal	n/a
Next Generation Sequencing	Rapid Heme Panel Normal	Rapid Heme Panel Normal	MDS Panel – *MPL* SNV – 47% VAF *CALR* SNV** – 47% VAF	n/a
PB WBC / Hgb / Plt	6.4 / 14.9 / 307	5.1 / 11.2 / 184	5.1 / 14.6 / 100	4.9 / 14.5 / 118

Abbrev: % LVV+, percent of cells transduced with lentivirus; c/dg, copies per diploid genome; VCN, vector copy number; FISH, fluorescence in situ hybridization; kg, kilogram; MDS, myelodysplastic syndrome; SNV, single nucleotide variant; VAF, variant allele frequency; PB WBC / Hgb / Plt, peripheral blood white blood cells (x 10^9), hemoglobin (g/dL), platelets (x10^9)
* Based on FDA definitions for engraftment that did not permit concomitant G-CSF or eltrombopag
**Variant of unknown significance

Source: Reviewer's analysis

FDA also has broadly applicable concerns about the risk of malignancy because 53 of 54 subjects (98%) in the ISS population with integration site analysis data had at least one integration into the proto-oncogene *MECOM* that could possibly cause hematologic malignancy in those subjects. No integration site data for the last 13 of the 67 treated subjects (19%) were included in the BLA due to the data cut occurring prior to ISA results being available.

A brief summary of the eli-cel treated children who have been diagnosed with MDS follows. Additional detail about these subjects is provided in Appendix 11.

Subject 104-18 was treated with eli-cel on (b) (6), at the age of 11, and was diagnosed with MDS with unilineage dysplasia 14 months later. MDS is attributable to eli-cel because the subject had a predominant clone with integration into *MECOM*, a known proto-oncogene, and increased *EVI1* expression in the *MECOM* locus in whole blood. His MDS has been treated with HSCT and was last reported on February 10, 2022, to be in remission.

Subject 104-08 (S104-08) was treated with eli-cel on (b) (6), at the age of 13, and met criteria for MDS with single lineage dysplasia (megakaryocytic) approximately two years later. MDS is attributable to eli-cel because the subject had a predominant clone with integration into *MECOM* and the specific *MECOM* integration was found in the megakaryocytes. Also supporting the causality of eli-cel is the identification of increased *EVI1* expression in the *MECOM* locus in whole blood. His MDS has been treated with HSCT and was last reported on February 11, 2022, to be in remission.

Subject 102-03 was treated with eli-cel on (b) (6), at the age of 5, and he was diagnosed with MDS vs. AML approximately 7.5 years later. The Applicant has attributed his case of malignancy as likely caused by eli-cel but has not provided the data to support their conclusion. Preliminary information suggested that the clone contained an integration site in the proto-oncogene, *PRDM16.*

A brief summary of eli-cel treated children where there is grave concern for the development of MDS follows. Additional detail about these subjects is provided in Appendix 12.

Subject 102-31 was treated with eli-cel on (b) (6), at the age of 4, and has a concerning integration site in the *MECOM* proto-oncogene. This integration site is increasing in relative frequency, currently represents 40% of CD15+ cells in the peripheral blood, and is accompanied by increased *EVI1* expression in peripheral blood.

Subject 102-11 (S102-11) was treated with eli-cel on (b) (6), at the age of 7, and he has a concerning integration site in the *MECOM* proto-oncogene of a predominant clone. Nearly 100% of S102-11's CD15+ cells are derived from this clone

with integration in *MECOM.* Additionally, *EVI1* expression is elevated in this subject's peripherally-derived CD15+, CD15-, and CD3- cells.

Subject 104-09 was treated with eli-cel on (b) (6), at the age of 9. He had prolonged, profound, post-transplant pancytopenia which was initially attributed by the investigator to parvovirus infection. However, parvovirus is unlikely to fully explain his hematologic abnormalities because parvovirus typically causes anemia and has characteristic bone marrow findings that were absent in this case. Conversely, S104-09's long-lasting thrombocytopenia, hypocellular bone marrow with atypical platelet progenitor cells, and integration into proto-oncogenes *MECOM and MPL* are highly concerning factors that point to evolving malignancy.

Subject 104-22 was treated with eli-cel on (b) (6), at the age of 13. He has concerning integration site patterns because of a rising relative frequency of integration into the proto-oncogenes *MECOM* and *MPL*. He has mildly low platelet counts but blood counts are otherwise normal.

Neither the FDA nor the Applicant has identified any baseline or clinical factors following eli-cel that are able to sufficiently predict who will develop malignancy following eli-cel. Three cases of malignancy make it difficult to determine who is likely to develop malignancy. None the less, FDA has observed the following for further consideration as early potential signals or risk factors:

- Age: All three subjects with malignancy were early adolescents at the time their malignancy developed.

- Thrombocytopenia at 100 days: After 100 days, four subjects had platelets < 50 x 10^9. This included the three subjects who developed malignancy and (S104-09) who may be developing malignancy. Of the three who developed malignancy, (S104-08) and (S104-18) had platelets <50 x10^9 through at least day 180 and (S102-03) platelets were persistently <50 x 10^9 through day 65 and then again transiently at day 132.

- VCN in the eli-cel drug product (DP) vs. peripheral blood (PB) VCN at 6 months: For the 49 subjects who had peripheral blood VCN data at 6 months (the point in time when PB VCN has roughly stabilized after DP administration) the median decrease was 0.5 c/dg. Six subjects have had an increase in PB VCN at 6 months compared to DP VCN, and include the following:

    - All three subjects who developed malignancy
    - One subject (S104-09) who may be developing malignancy also had a higher PB VCN at 6 months than in the DP
    - Two subjects who have not been identified as at highest risk of malignancy (S102-06 and S104-29)

Also complicating the assessment of risk factors is the absence of baseline screening for elevated risk of malignancy. The protocols did not incorporate baseline bone marrow biopsies or cytogenetic studies. Baseline assessment may have aided in identifying subject factors that contributed to the development of malignancy.

8.3.2 Cytopenias

Subjects are expected to experience profound cytopenias after myeloablation for HSCT, and time to resolution of cytopenias is affected by many variables. However, after eli-cel administration, blood counts recovered slowly and, in many cases, incompletely. Prolonged cytopenias introduce risks of bleeding, transfusion complications, and serious infections. They also can be a signal of problems in the bone marrow where the blood cells are generated and a harbinger of hematologic malignancy.

Neutrophil and platelet recovery after autologous peripheral blood-derived HSCT generally occur within two weeks after transplant. However, many of the eli-cel-treated subjects did not have evidence of restored hematopoiesis as early as would be expected after autologous transplant of peripherally derived hematopoietic stem cells. The incidence of engraftment failure was significant. Excluding three subjects who were treated shortly before the August 18, 2021, data cut and did not have confirmed engraftment, 15 of 64 (23%) had neutrophil engraftment failure, platelet engraftment failure, or both.

The definition of neutrophil engraftment in the Study ALD-102 and ALD-104 protocols was three consecutive absolute neutrophil counts ≥ 0.5 x 10^9/L on three different days within 42 days of eli-cel administration. All subjects fulfilled these criteria for engraftment. However, the protocol definition did not account for use of G-CSF to stimulate the production of neutrophils. While G-CSF is routinely administered as a prophylactic measure after myeloablation, its concurrent use precludes meeting neutrophil engraftment criteria. Therefore, FDA calculated time of neutrophil engraftment as three consecutive absolute neutrophil counts ≥ 0.5 x 10^9/L on three different days within 42 days of eli-cel administration while not supported by G-CSF administration.

Excluding the three most recently treated subjects who have insufficient follow-up data to confirm engraftment, 6 of 64 subjects (9%) did not meet criteria for neutrophil engraftment by Day 42. The median day of neutrophil engraftment day was Day 27, the range from 13 to 189 days, and the interquartile range from 21 to 34 days.

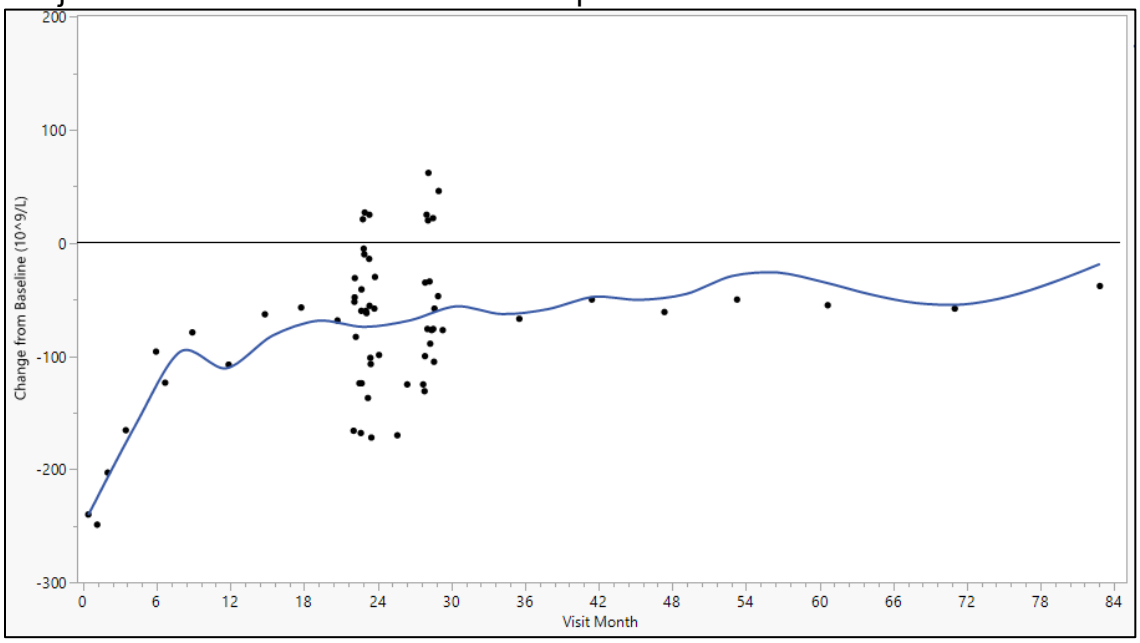
Platelet engraftment was defined in the Study ALD-102 and ALD-104 protocols as the first of three consecutive days with a platelet count of 20 x 10^9/L or higher in the absence of platelet transfusion for seven consecutive days. Because the protocols did not define the timeframe for primary platelet engraftment failure, the FDA used the

duration of 42 days that also was used to define neutrophil engraftment failure. Similar to G-CSF administration precluding neutrophil engraftment, a subject would not meet platelet engraftment criteria while supported with thrombopoietin mimetics. Excluding the three most recently treated subjects who have insufficient follow-up data to confirm engraftment, 14 of 64 subjects (22%) did not meet criteria for platelet engraftment by Day 42. One of the 14 subjects (S104-08) seems to have developed MDS without ever achieving platelet engraftment. Excluding S104-08, the median day of platelet engraftment was Day 29, range from 14 to 356 days, and interquartile range from 22 to 37 days.

Blood counts that were abnormal or were decreased from baseline for prolonged periods were very common among eli-cel-treated subjects. In the ISS population, 15 of 52 (28.8%) subjects had a severe (Grade 3 or higher) cytopenia on or after Day 60, including decreased platelet count in 15.4% and decreased neutrophil count in 21.1%. On or after Day 100, 14.9% of subjects in Studies ALD-102 and ALD-104 had a severe cytopenia (decreased platelet count in 8.5% and decreased neutrophil count in 10.6%). In addition, the large majority of subjects had normal baseline blood counts, and many did not return to normal after treatment. These prolonged cytopenias increase the risk of subjects developing bleeding complications, transfusion complications, and serious infections.

With regard to platelets, even after engraftment, most subjects' platelet counts did not return to baseline during the course of the study. The figure below plots the median value for change in platelet count from baseline at each time point for the ISS population excluding the two subjects whose baseline platelet counts were outside of the normal range of 150 - 450 x $10^{\wedge 9}$/L. The figure demonstrates that platelet counts were significantly depressed for a prolonged period of time, being 100 x $10^{\wedge 9}$/L below baseline at six months and very slow to improve thereafter, coming to within 50 x $10^{\wedge 9}$/L of baseline at 3.5 years post-eli-cel.

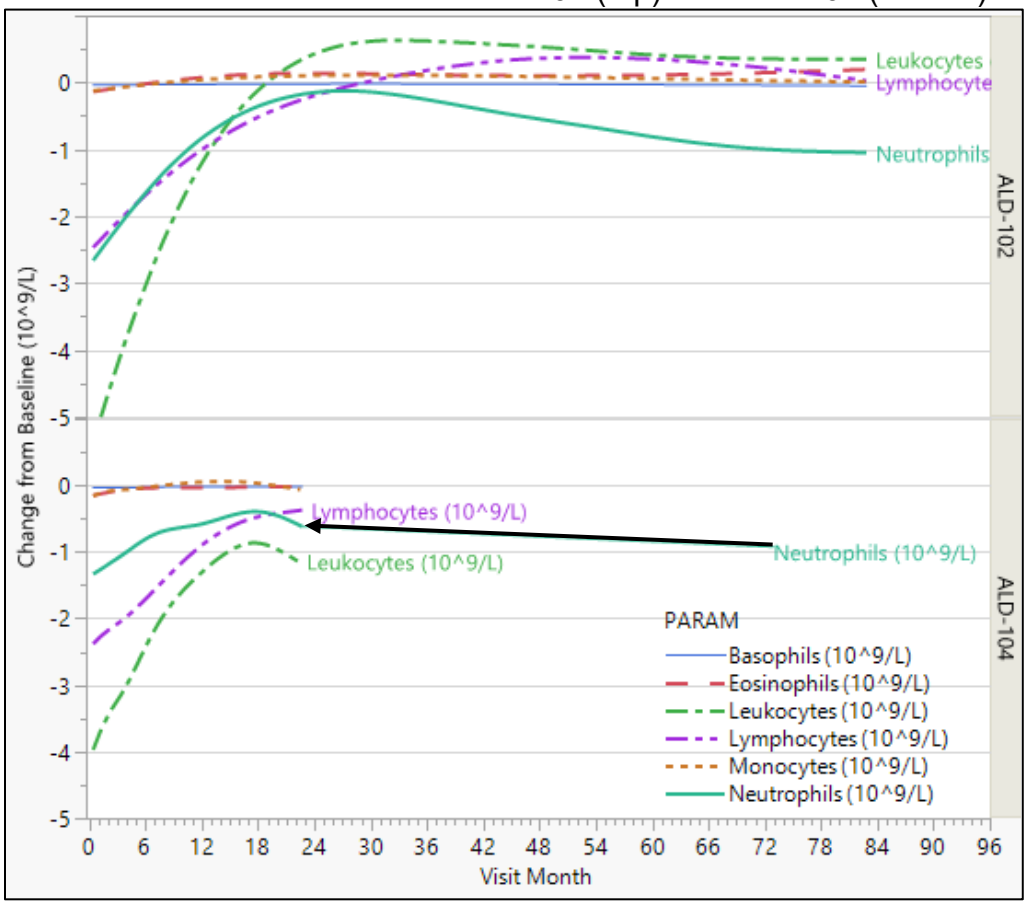
Figure 12: Platelet Count Change from Baseline Over Time: Median Value for All Subjects with Normal Baseline in ISS Population



Note: the horizontal line represents zero change from baseline and points below represent a decline from baseline.
Source: Reviewer's analysis, derived from ADLB dataset

Certain critical types of infection-fighting cells also failed to return to baseline after eli-cel administration. The following figure demonstrates the change from baseline in white blood cells (WBC, depicted as leukocytes) among subjects who had normal baseline values for each respective WBC subtype. Studies ALD-102 and LTF-304 data are presented in the top portion of the figure. The data show that total WBC counts in Study ALD-102 subjects were below baseline for 18 months after treatment with eli-cel. Additionally, key white blood cell subtypes fared worse than overall white blood cells. For example, lymphocytes did not recover to baseline until 2.5 years after eli-cel administration, and neutrophils never recovered to baseline values during the observation period.

Figure 13: WBC and WBC Subtypes Change from Baseline Over Time for All Subjects with Normal Baseline in Studies ALD-102 (top) and ALD-104 (bottom)

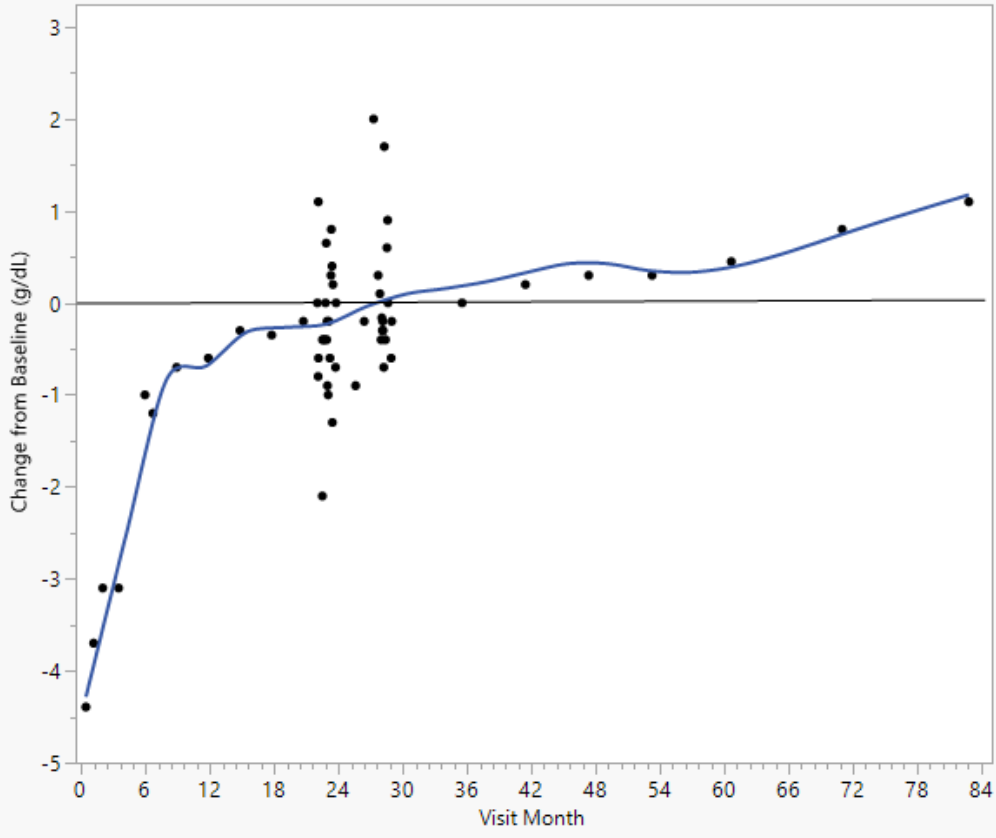


Source: Reviewer's analysis, derived from ADLB dataset

The bottom portion of the figure demonstrates that during the Study ALD-104 follow-up period of up to 24 months, neutrophil, lymphocyte, and overall WBC values (depicted as leukocytes) remained well below baseline.

Anemia was also persistent among eli-cel treated subjects. While the hemoglobin levels were overall not as severely abnormal as the abnormalities of platelets, neutrophils, and lymphocytes, the figure below demonstrates that the average time point at which hemoglobin returned to baseline levels was more than two years after eli-cel administration.

Figure 14: Hemoglobin Change from Baseline Over Time: Median Value for All Subjects with Normal Baseline in ISS Population



Source: Reviewer's analysis, derived from ADLB dataset

Platelet, neutrophil, lymphocyte, and hemoglobin recovery was prolonged after eli-cel administration compared to what would be expected after autologous peripheral blood HSCT. The underlying pathology responsible for the prolonged cytopenias is not clear. Nonetheless, cytopenias are an important safety consideration because they increase the risk of complications related to bleeding, infection, and transfusions.

8.3.3 Infections, Including Opportunistic Infections

Infections are a well-recognized risk of hematopoietic stem cell transplant. The types of infections observed are affected by many factors, such as type of transplant (autologous or allogeneic), stem cell source (bone marrow, peripheral blood, or umbilical), pre-transplant conditioning regimen, concomitant medications, and time since transplantation. Early infections after HSCT are often serious and attributable to neutropenia and/or mucosal injury. After neutrophil engraftment, the risk of infection declines dramatically. With autologous HSCT, central venous catheter infections predominate, and the risk of opportunistic infection beyond three months is small. With allogeneic HSCT, serious infections with a relatively delayed onset are more likely

because immune reconstitution takes longer and may be complicated by the development of graft vs host disease.

Opportunistic infection appears to be an important risk of eli-cel. As an autologous transplant of peripherally-derived HSCs, immune constitution would be expected to occur rapidly compared to other types of HSCT. However, there were several serious infections that demonstrate infection to be an important risk. It is unclear whether these infections were related to the eli-cel drug product, the conditioning and transplant, or patient factors such as adrenal insufficiency.

Eighty-six infections were reported in 34 of 67 (51%) eli-cel treated subjects. The most significant opportunistic pathogens are categorized by time of onset and summarized below.

During the first month after eli-cel administration, corresponding to the period of the most profound neutropenia, there were seven severe infections (e.g., requiring intravenous antibiotics) in seven (9%) subjects. These included three central venous catheter infections, a soft tissue infection, pneumonia, and bacteremia. There were also several less severe infections that may be clinically important in the immunocompromised patient. These included cases of candidiasis, enterocolitis, and skin infection.

Between Day 30 and 100, four subjects had five infections that were serious adverse events. They were BK cystitis, pseudomonal and stenotrophomonas maltophilia bacteremia, otitis media, and another central venous catheter infection. There were also several viral infections that were probably related to the subjects' ongoing immune compromise. These infections were Epstein-Barr virus viremia (Day 81 to 271), human herpesvirus 6 viremia (starting Day 77 and unresolved), and cytomegalovirus reactivation (Day 90 to 116).

Several serious bacterial infections occurred in the last post-engraftment period, which is not typical after autologous HSCT. They were the following:

- Streptococcal bacteremia (S104-05) – Days 127 to 133
- Mycobacterium central venous catheter infection (S102-26) – Days 167 to 194
- Pseudomonal bacteremia (S104-09) – Days 240 to 251

There was also an adverse event of Epstein-Barr virus infection reactivation on Day 547 in S102-17 that may have been related to impaired immunity, and there were several central venous catheter infections in addition to those already presented above.

Given the numerous bacteremias, viremias, and central venous catheter infections that occurred in eli-cel-treated subjects, opportunistic infection is clearly an important risk. However, there are not sufficient data to determine whether the infection risk with eli-cel

is comparable in number, severity, and timing of the infectious risk associated with other autologous hematopoietic stem cell transplants.

8.3.4 Other Important Risks

Two additional important potential risks of eli-cel are the development of replication-competent lentivirus and the development of graft versus host disease. Neither of these complications has occurred in eli-cel clinical studies.

Replication-competent lentivirus would be capable of infecting cells *in vivo* and replicating to produce additional infectious virus. Replication-competent lentivirus could multiply and spread to many cell types in addition to a patient's HSCs. The Applicant performed regular assessments for replication-competent lentivirus and found no evidence that eli-cel had transformed into a replicating virus.

Also important for consideration is the risk of graft versus host disease (GVHD), as its incidence was the primary safety endpoint in Study ALD-102. Because eli-cel is an autologous product, it was not expected to cause GVHD. The data in the BLA confirm that GVHD is not a significant risk of eli-cel, with no subject in the ISS population having been diagnosed with Grade 2 or higher GVHD.

9 BENEFIT - RISK ANALYSIS

9.1 Efficacy Summary

Although the primary endpoint was met, multiple issues with how the benchmark was derived make the results difficult to interpret. These issues include lack of comparability between the two populations within the natural history study, ALD-101, that were used to determine the benchmarks, lack of comparability of the overall ALD-101 population to the single-arm study ALD-102 population, an imputation scheme that favored eli-cel, and retrospective data collection of ALD-101. In addition, although the data provided by the Applicant suggest that there is similar efficacy on MFD-free survival and overall survival between eli-cel and allo-HSCT, the results are difficult to interpret primarily because of insufficient duration of follow-up in the studies. Other significant issues that affect interpretability include lack of comparability between the subjects in the observational HSCT treatment study (ALD-103) and ALD-102, small number of events, and the retrospective nature of some of the ALD-103 data. Considering these issues, it is unclear whether eli-cel's efficacy is non-inferior to allo-HSCT. FDA is not confident that 24 months is sufficient time to demonstrate efficacy of eli-cel, and paucity of data beyond 24 months limits the ability to assess disease stability and durability of effect compared to allo-HSCT.

FDA is concerned that there may not be sufficient information in the TPES NMSD population to establish the efficacy of eli-cel. However, there are preliminary data showing a trend toward improved overall survival with eli-cel compared to the TPES subpopulation with HLA-unmatched donors (UMD). Most events constituting failure of MFD-free survival happened by 6 months in the TPES-UMD group. Additional HSCT risks, including engraftment failure, repeat HSCT and GVHD, appear to occur more frequently in this population as well. Trends toward higher rates of early mortality were mirrored in the greater allo-HSCT populations (TP-101 and TP-103) with UMD compared to MD, as well. Considering the risks of HSCT, the benefit-risk profile of eli-cel might be favorable when compared to allo-HSCT from an HLA-unmatched donor in patients with early active CALD.

Aside from donor factors, it is unclear if there is a CALD population for whom the benefit of treatment with eli-cel outweighs the significant and unknown long-term risk of MDS. Based on review of untreated subjects in Study ALD-101, FDA is concerned that the early active CALD population, as defined, has an unpredictable course, and some untreated subjects had disease stability or were asymptomatic for years. It does not appear there are any clear predictive factors for rapid disease progression in this early active CALD population, particularly with such small numbers of events across studies. The potential risk of MDS is concerning in a patient who might not experience disease progression, regardless of treatment (or lack there-of), for a considerable amount of time.

9.2 Safety Summary

Hematologic malignancy is the primary safety concern with eli-cel. Three malignancies have been diagnosed among the 67 subjects treated with eli-cel, and it seems likely additional cases will emerge over time, driven by LVV integration into proto-oncogenes. The significant yet uncertain risk of this life-threatening complication must be considered in the context of the product's benefit to patients.

These uncertainties might be addressed with more time in follow-up, better understanding of the long-term outcomes in similar patients treated with allo-HSCT and eli-cel over time, and the addition of a blinded clinical adjudication committee for MFD events. The overall benefit-risk profile of eli-cel is difficult to characterize because of the uncertain benefit and the uncertain magnitude of the risk of MDS.

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Appendices: Table of Contents

Appendix 1: CALD Scoring Systems and Disease Characterization

The Neurologic Function Score (NFS) is a 25-point composite scale designed by Dr. Gerald Raymond and colleagues that assesses functional disabilities in 15 domains and is the most commonly used clinical evaluation tool in CALD patients (H. W. Moser et al., 2000) (Miller et al., 2011). A score of 0 indicates absence of clinical signs of cerebral disease, and higher scores correspond to increasing severity of functional deficits. The scoring system and definitions used for the clinical studies is provided in Table 1. Major functional disabilities (MFDs) are indicated by asterisks.

Table 14: Neurologic Function Score (NFS) for CALD

Symptom / Neurologic Exam Finding	**Definition**	**Score**
Hearing / auditory processing problems	Individual with previously normal hearing develops permanent auditory processing difficulties and impairment of comprehension to verbal sounds on neurologic evaluation.	1
Aphasia / apraxia	Individual should meet one of the following two criteria: (1) Individual with previously age-appropriate speech and language development has impaired fluency or naming or repetition or content or comprehension or motor speech on the clinical examination; patient may have partial or incomplete aphasia or motor speech disorder of the speech, or (2) Individual with newly developed apraxia. Apraxia can be defined as 'loss of the ability to execute or carry out any complicated learned and purposeful movements, despite having the desire and the physical ability to perform the movement. Examples of apraxia include, but are not limited to, limb-kinetic apraxia, ideomotor apraxia, conceptual apraxia, speech apraxia, etc.	1
Loss of communication*	Individual should meet one of the following criteria (psychogenic syndromes, such as catatonia, should be ruled out): (1) With normal consciousness and ability to perform movements, individual does not follow command and/or permanently fails to perform verbal or nonverbal simple task on neurologic evaluation, or (2) Individual is permanently mute and unable to communicate by verbal or non-verbal ways.	3
Vision impairment / field cut	An individual with previously normal (corrected) vision develops visual field defect affecting one or both eyes, and/or maximal visual acuity (corrected) worse than 20/30 using bedside pocket vision screening card.	1

Symptom / Neurologic Exam Finding	Definition	Score
Cortical blindness*	Individual fails to visually track, find objects, or count fingers. Individual has permanent and complete vision loss affecting bilateral vision. Pupils may react to light.	2
Swallowing / other CNS dysfunction	Swallowing is safe; however individual requires minimal cueing to use compensatory strategies. The individual may occasionally self-cue. All nutrition and hydration needs are met by mouth at mealtime.	2
Tube feeding*	Individual is not able to swallow safely by mouth to maintain nutrition and hydration. Alternative method of feeding required.	2
Running difficulties / hyperreflexia	An individual with previously normal gait develops minimal but permanent difficulties during running. He may be fully ambulatory without aid or may have some limitation of full activity or requires minimal assistance.	1
Walking difficulties/ spasticity / spastic gait (no assistance)	Individual develops walking difficulties but is ambulatory without aid; disability severe enough to preclude full daily activities.	1
Spastic gait (needs assistance)	Individual requires constant bilateral assistance (canes, crutches, braces).	2
Wheelchair dependence*	Individual is unable to take more than a few steps, restricted to wheelchair; may need aid to transfer; wheels himself, but may require motorized chair for full day's activities.	2
Complete loss of voluntary movement*	Individual is unable to effectively use his upper and lower extremities to perform simple or one-step activities. The criteria may still be met if there are singular apparently random movements of the arms.	3
Episodes of incontinence	Individual who was previously continent for at least 6 months develops permanent and frequent episodes of hesitance, urgency, retention of bowel or bladder, or urinary incontinence during daytime and nighttime (diurnal and nocturnal enuresis).	1
Total incontinence*	In an individual who was previously continent, the permanent and continuous loss of urinary and/or fecal control.	2
Nonfebrile seizures	Individual who develops non-febrile seizure.	1

Source: Adapted from bluebird bio Protocol ALD-102 Version 10.0, Section 10.3, Table 7, originally from Moser et al. 2000.
Abbrev: NFS, neurologic function score; CALD, cerebral adrenoleukodystrophy; CNS, central nervous system.
*Indicates a major functional disability (MFD)

The Loes score is a commonly used MRI assessment of extent of cerebral lesions in patients with CALD.[22] A severity score (0 to 34) is assigned based on extent of demyelinating lesions on MRI and presence of atrophy. Points are assigned based on unilateral or bilateral brain involvement, areas of brain involvement, and presence of focal and/or global atrophy. A score of 0 indicates a normal MRI, and higher scores indicate increased severity of cerebral lesions. Early disease is defined by Loes scores between 0.5 and 9 in the BLA studies. Loes scores >9 indicative of advanced disease prior to allo-HSCT have been associated with increased rapidity of disease progression following transplant, and thus allo-HSCT is no longer recommended as treatment in this population.

Patterns of cerebral involvement on MRI have also been described,[26] and were documented for all MRIs in all BLA studies:
Pattern 1: parieto-occipital white matter
Pattern 2: frontal white matter
Pattern 3: corticospinal tract
Pattern 4: cerebellar white matter
Pattern 5: concomitant parieto-occipital and frontal white matter
Other: any pattern other than those listed above

These patterns were identified by Loes and colleagues in 2003, and patterns appeared to trend with age and gadolinium enhancement (discussed below) to attempt to predict rapidity of disease progression. Patients with pattern 1 and 2 disease with contrast enhancement on MRI (GdE+) experienced rapid disease progression if MRI findings were present at an early age (Loes et al., 2003). Disease progression was much slower for patients with pattern 3 or 4 disease. Pattern 5 disease was uncommon but was associated with much more rapid progression than other patterns. This study also demonstrated that patterns 1 and 5 occurred primarily in childhood, patterns 2 and 4 in adolescence, and pattern 3 in adults. Patterns were evenly distributed across TPES allo-HSCT and eli-cel treatment groups in the BLA studies, and events constituting progression (failure of MFD-free survival) were too infrequent to conduct analysis on relatedness of patterns to disease progression. Patterns are included in death narratives in Appendix 6 for reference.

Gadolinium enhancement (GdE) was documented as present (GdE+) or absent (GdE-) for each brain MRI in Studies ALD-102, ALD-103, and ALD-104. GdE was not routinely assessed at the time of study ALD-101, and for many MRIs was not documented. In Study ALD-101, GdE was documented as present (GdE+), absent (GdE-) or not documented if not assessed or unknown. Presence of GdE has been associated with breakdown of the blood-brain barrier and is thought to represent progressive inflammatory demyelination. It is associated with increased risk of rapid disease progression. GdE+ MRI was required at the Baseline visit for Studies ALD-102 and ALD-104 to indicate high risk of disease progression prior to treatment with eli-cel, and was required for the untreated and allo-HSCT populations in Studies ALD-101 and

ALD-103 to be considered “strictly ALD-102-eligible” (termed the UTES and TPES for the untreated and allo-HSCT populations, respectively).

Appendix 2: Study Design for Long-Term Follow- Up Study, LTF-304 (January 22, 2016 to ongoing)

Study Design
Study LTF-304 is the non-interventional long-term follow-up (LTFU) study that enrolls all subjects treated with eli-cel after completion of the parent studies (ALD-102 and ALD-104). Subjects are to be followed every 6 months through 5 years after eli-cel infusion, and then annually through 15 years after eli-cel infusion.

Study Objectives
The study objectives are to monitor long-term safety and continued efficacy following treatment with eli-cel.

Key Enrollment Criteria
Any subject who received eli-cel drug product in studies ALD-102 and ALD-104 and is able and willing to comply with study procedures is eligible.

Treatment Plan
No investigational treatment is administered as a part of this follow-up study.

Study Assessments
The 13-year follow-up study primarily focuses on long-term safety. Note the following assessment timepoints are relative to the time of eli-cel administration and not to the start date of the long-term follow-up study.

- Adverse events: Every six months until Year 5 and yearly from Year 6 to 15
- Integration site analysis and vector copy number:
  - Every six months through Year 5, and once subsequently at Year 7
  - Added yearly assessments from Year 6 to 15 starting in June 2021
- Replication-competent lentivirus: Year 3, 4, and 5

Long-term efficacy assessments evaluated in the parent studies are to be followed long-term to evaluate durability of effect at the following intervals:

- NFS and MFD assessments: Every six months until Year 5, yearly from Year 6 to 10 and then at Year 15

MRI for Loes score and gadolinium enhancement annually until Year 5, then at Years 7, 10 and 15

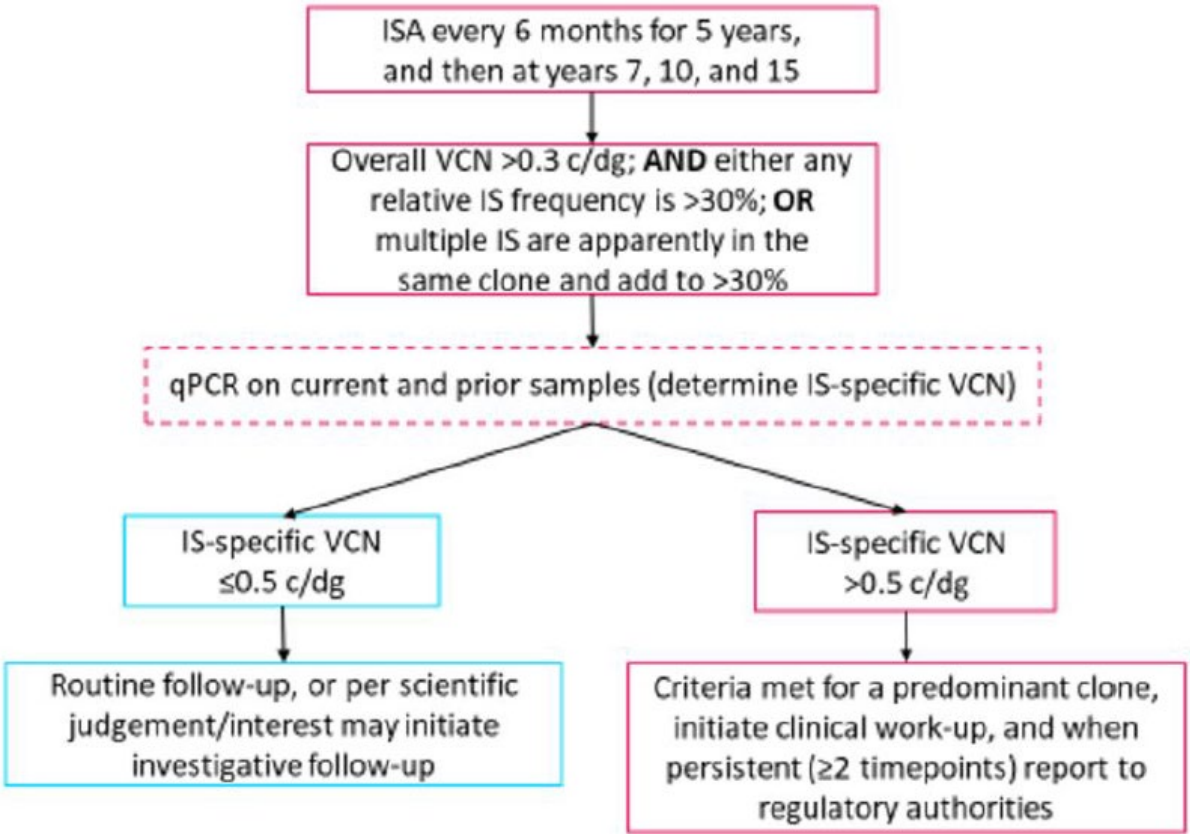
Appendix 3: Integration Site Analysis

Integration site data include integration site relative frequency, integration site-specific vector copy number (VCN), overall VCN. These data were obtained for the evaluation of clonal dynamics and for monitoring for clonal expansion after eli-cel administration. In Studies ALD-102, ALD-104, and LTF-304, integration site analysis (ISA) was performed in peripheral blood on a scheduled basis. ISA identifies the location of integration sites within the cell population and their relative frequency of integration. At scheduled timepoints in conjunction with ISA, vector copy number (VCN), which is total number of vector copies or integrations within a population of cells, was also measured.

The method for ISA changed during the course of Study ALD-102. The original method was standard linear amplification PCR (LAM-PCR) and non-restricted LAM-PCR (nrLAM-PCR), together referred to as (NR)LAM-PCR. However, the use of restriction enzymes in (NR)LAM-PCR can cause amplification bias and inaccurate estimations of relative frequencies. The use of more accurate method, shearing extension primer tag selection ligation-mediated polymerase chain reaction (S-EPTS/LM-PCR), was implemented in June 2019. Thereafter in Study ALD-102 and LTF-304, and for all of Study ALD-104, S-EPTS/LM-PCR was used for measuring overall integration site relative frequency.

The schematic for assessment of clonal predominance also changed multiple times during the eli-cel development program and is still under discussion. The following figure demonstrates the schematic that was in place from September 2020 to June 2021.

Figure 15: Schematic for Assessment of Clonal Predominance



Abbrev: ISA, integration site analysis; VCN, vector copy number; c/dg, copies per diploid genome; IS, integration site(s); qPCR, quantitative polymerase chain reaction
Source: Original BLA 125755/003, Protocol ALD-102 Version 10.0, p.70

Changes to the schematic that were implemented in June 2021 were the following:

- Annual ISA from Years 5 to 15 instead of only at Years 7, 10, and 15
- New requirement for repeating ISA with a new sample as soon as possible and no later than within three months if criteria were met for a predominant clone

The schematic outlines how overall VCN and integration site relative frequency can trigger determination of integration-specific vector copy number via qPCR. QPCR results demonstrating an integration-site-specific VCN of > 0.5 c/dg would determine a clone to be predominant.

Not included in the schematic is the means for determining when a clone might have multiple integration sites. The Applicant defines multiple integration sites apparently in the same clone as more than one relative frequency where values are within 20% of each other (e.g., 5% ± 1%, 10% ± 2%, 15% ± 3%, etc.).

Appendix 4: Study ALD-102 and ALD-104 Differences in Conditioning Regimen and Growth Factor Therapy

Prior to eli-cel administration, subjects underwent stem cell mobilization, apheresis, and conditioning. However, Studies ALD-102 and ALD-104 had several differences in pre-treatment regimen and in the use of post-DP growth factor therapy, as demonstrated below.

Table 15: Mobilization, Conditioning Regimen, and Growth Factor Therapy By Study

Purpose	ALD-102	ALD-104
Mobilization	G-CSF Plerixafor optional (34% received plerixafor)	G-CSF Plerixafor
Conditioning agent AUC (μM*min/L) median (min, max)	Busulfan 4729 (4039, 5041)	Busulfan 5359 (4873, 5640)
Lymphodepleting agent	Cyclophosphamide 4d of 50 mg/kg/day	Fludarabine Initial subjects: 6d of 30 mg/m^2 Subsequent: 4d of 40 mg/m^2
Growth factor therapy	G-CSF optional (~66% received G-CSF)	G-CSF post infusion Day 6 through at least NE

Abbreviations: G-CSF, granulocyte colony-stimulating factor; AUC, area under the curve; NE, neutrophil engraftment
Source: Reviewer's analysis, derived from ADPP and ADCM datasets

Appendix 5: Study ALD-102 and ALD-104 Product Differences

Summary statistics for some characteristics of the drug product by study follow.

Table 16: Eli-cel Vector Copy Number, Percent of Cells Containing Lentiviral Vector, and Total Cell Count by Study

Characteristics	ALD-102	ALD-104
Drug substance (b) (4)	(b) (4)	(b) (4)
DP VCN (c/dg) Median (min, max)	1.2 (0.5, 2.7)	1.6 (1.2, 3.1)
%LVV+ Median (min, max)	45 ((b) (4))	63.5 ((b) (4))
Cell count (x 10^6 CD34+ cells/kg) Median (min, max)	11.4 (5.0, 20.1)	12.5 (5.7, 38.2)

Abbreviations: TU, transducing unit; DP, drug product; VCN, vector copy number; c/dg, copies per diploid genome; %LVV, percent of cells transduced with lentivirus
Source: Reviewer's analysis, derived from ADSL dataset and 2.3.S Drug Substance p.18

Appendix 6: Details of Deaths in BLA Study Populations

Details of Subject Deaths in rUTES-101, TPES-101 and TPES-103, and TP-102 Populations

Subject deaths in rUTES-101, TPES-101 and TPES-103, and TP-102 are detailed in narrative form below and summarized in Table 4.

Subject Deaths – Untreated Population (rUTES-101)

-**Subject 101-72,** a white male from Argentina without specified ethnicity, was diagnosed at age 11 years. Baseline NFS, Loes score and pattern were 0, 5 and 2, respectively. He developed MFD(s) starting at 18.8 months following diagnosis and died at 27.6 months of progressive disease.

-**Subject 101-67,** a white male from US without specified ethnicity, was diagnosed at age 15 years. Baseline NFS, Loes score and pattern were 0, 9, and 5, respectively. He developed MFD(s) starting at 20.4 months following diagnosis and died at 51.9 months of progressive disease.

-**Subject 101-87**, white male from US without specified ethnicity, was diagnosed at age 9 years. Baseline NFS, Loes score and pattern were 1, 8.5, and 1, respectively. He developed MFD(s) starting at 55.5 months following diagnosis and died at 72.5 months but cause of death is not reported.

-**Subject 101-56,** a white, Hispanic/Latinx male from US, was diagnosed at age 5 years. Baseline NFS, Loes score, and pattern were 1,4.5 and 1, respectively. He developed MFD(s) starting at 13.8 months following diagnosis and died at 91.8 months of progressive disease.

Summary: Untreated subjects died of progressive disease, as expected. Subjects were generally older (minimum 5 years old but otherwise 9-15 years) at time of diagnosis, and with baseline Loes scores all 4.5 or above. Despite generally more severe baseline disease (older age, higher baseline Loes score), even in those with symptoms (NFS 1) at baseline, progression occurred relatively slowly, with first MFD documented 13.8 months after diagnosis and all deaths occurring after 24 months (first at 27.6 months).

Subject Deaths – allo-HSCT Population – HLA-Unmatched Donors (TPES-101 and TPES-103 UMD)

-**Subject 101-09**, a male without specified country of origin, race or ethnicity, was transplanted in France at age 6 years with an unmatched (9 out of 10) unrelated bone marrow donor. Baseline NFS, Loes score and pattern prior to transplant were 0, 3.5, and a mix of patterns 1 and 3, respectively He died 5.2 months after HSCT (7 months after diagnosis) of transplant-related causes (GVHD and septicemia).

-**Subject 103-35**, a white male without specified ethnicity, was diagnosed and transplanted in the Netherlands at age 7 years with an unmatched (8 out of 10) unrelated cord blood donor. Baseline NFS, Loes score and pattern prior to transplant were 0, 2, and 1, respectively. He received repeat HSCT 1.3 months after the initial transplant due to engraftment failure and died 6 months after initial HSCT (8 months after diagnosis and shortly after repeat HSCT) of transplant-related causes.

-**Subject 101-10**, a male without specified country of origin, race or ethnicity, was diagnosed at age 11 years and transplanted in France at age 12 years with an unmatched (9 out of 10) unrelated bone marrow donor. Baseline NFS, Loes score and pattern prior to transplant were 1, 5.5, and a mix of pattern 3 and “other”, respectively. He developed a MFD 1.6 months following transplant and died 6.2 months after HSCT (9.9 months after diagnosis) of progressive disease.

-**Subject 103-22**, a white, non-Hispanic male was diagnosed and transplanted in the US at age 8 years. with an unmatched (9 out of 10) unrelated bone marrow donor. Baseline NFS, Loes score and pattern prior to transplant were 0, 1.5, and 3, respectively. He died 25.8 months after HSCT (33.2 months after diagnosis) of cardiac arrest, presumed due to transplant-related causes. Throughout his post-transplant course, he suffered from GVHD and infections, ultimately developing multi-organ system failure, thrombosis, and myocardial infection.

Summary: Subjects were of “average” baseline risk based on age, NFS and MRI findings. Three of four deaths occurred by approximately 6 months following HSCT. Three of four deaths in subjects with HLA-unmatched donors were due to transplant-related causes, and the one death due to progressive disease was in Subject 101-10, who was symptomatic at baseline (NFS 1), had a higher baseline Loes (5.5), multiple areas of brain involvement (as per mixed pattern 3 and “other”) and began developing MFD(s) 1.6 months after transplant, which is too soon for the transplant to be effective (i.e., he likely would have experienced disease progression regardless of treatment).

Subject Deaths – allo-HSCT Population – HLA-Matched Donors (TPES-101 and TPES-103 MD)

-**Subject 103-58**, a white, Spanish, Hispanic/Latinx male, was diagnosed at age 2 years and transplanted in Spain at age 6 years with cells from a 10 out of 10 matched unrelated bone marrow donor. Baseline NFS, Loes score and pattern prior to transplant were 0, 1, and 1, respectively. He received repeat HSCT at 6.5 months due to graft failure and developed MFD(s) starting at 11.6 months following the first transplant. He died 12.8 months following the initial transplant (69.1 months following diagnosis) of progressive disease.

-Subject 103-32, a white, German male without specified ethnicity was diagnosed at age 7 years and transplanted in Germany at age 8 years with cells from a 10 out of 10 matched sibling bone marrow donor. Baseline NFS, Loes score and pattern prior to transplant were 0, 9, and 1, respectively. He died of transplant related causes 23 months following transplant (26.3 months following diagnosis).

-**Subject 103-44**, a white, Hispanic/Latinx, Argentinian male, was diagnosed and transplanted in Argentina at age 6 years with cells from a 6 out of 6 matched sibling peripheral blood donor. Baseline NFS, Loes score and pattern prior to transplant were 0, 7, and 1, respectively. He died at 33.1 months following transplant (36.6 months following diagnosis) of septic shock to an abdominal focus. He had intestinal obstruction of unknown cause, and thus it is not clear if death was transplant-related.

Summary: Subjects with HLA-matched donors were of “average” baseline risk based on age, NFS and MRI findings. Causes of death were mixed, and interestingly the subject with the most advanced baseline disease on MRI (Subject 103-44) did not die of progressive disease, and the subject with earliest baseline disease (Subject 103-58) died of progressive disease despite requiring repeat HSCT for graft failure. One subject died of transplant related causes (Subject 103-32) and Subject 103-44’s underlying cause of death is unclear. No deaths occurred in the first year, and the first death was at 12.8 months from progressive disease.

Subject Deaths – eli-cel Population (TP-102)

-**Subject 102-16**, a white, Hispanic/Latinx male, was diagnosed and treated in the US with eli-cel at age 7 years.Baseline NFS, Loes score and pattern prior to treatment were 0, 2 and 3, respectively. He had progression of disease with change of NFS from 0 to 1 (episodes of incontinence), MRI was GdE+ and Loes score increased from 2 to 9 at Month 13 following eli-cel therapy. He was therefore withdrawn from Study ALD-102 at

the investigator's discretion to receive rescue allo-HSCT for progressive disease. He died 3 months later (16 months following eli-cel therapy) of transplant-related causes.

-**Subject 102-18**, a white, non-Hispanic French male was diagnosed at age 6 years and treated in the US with eli-cel at age 6 years. Baseline NFS, Loes score and pattern prior to treatment were 0, 6.5 and 5, respectively. His Loes score increased rapidly, with change from 6.5 to 13.5 by relative day 14 following treatment, at which time NFS had increased from 0 to 1 (running difficulties/hyperreflexia). He had resolution of GdE positivity by MRI by Month 6 with no re-emergence of GdE positivity despite clinical deterioration. At Month 3, NFS increased to 4 (running difficulties/hyperreflexia, aphasia/apraxia, vision impairment/field cut, walking difficulties/spasticity). By Month 6, NFS was 5 with addition of episodes of incontinence. Loes score at Month 6 was 14. He developed his first MFD at Month 9 (total incontinence), and NFS was 9 with the same findings at Month 6 and the addition of the MFD and spastic gait requiring assistance. Loes score at that time was 20. By Month 12, he developed hearing difficulties and new MFDs of cortical blindness and loss of communication (NFS 15, Loes score 23). At Month 21 he developed a new MFD, wheelchair dependence (NFS 17, Loes score 20). At that time he had respiratory distress and viral infection (adenovirus and coronavirus), which resulted in acute hepatic failure, acute kidney injury, rhadbomyolysis, and ultimately cardiorespiratory arrest and death (21.9 months following eli-cel infusion). The cause of death was viral infection, presumably adenovirus infection leading to multi-system organ failure.

Summary: Despite Subject 102-18 dying of viral infection, he experienced significant disease progression before his death. Subject 102-16 also had evidence of disease progression, for which he received allo-HSCT but died of transplant-related causes. Too few events limit conclusions about deaths following eli-cel treatment.

Table 17: Summary of Deaths in rUTES-101, TPES-101, TPES-103, and TP-102

Treatment Arm	Subgroup	HLA Match	Subj ID	Age [1]	Race/ COO/ Eth	Base-line NFS	Base-line Loes	Base-line Pattern	Time of MFD or HSCT (months)[2]	Time of Death[2] (months)	Cause of Death
Allo-HSCT	UMD (UURD)	9/10	101-09	6	NR/ FRA/ Unk	0	3.5	1+3	--	5	Septicemia and GVHD
Allo-HSCT	UMD (UURD)	8/10	103-35	7	W/ NLD/ Unk	0	2	1	HSCT 1	6	Transplant-Related
Allo-HSCT	UMD (UURD)	9/10	101-10	12	NR/ FRA/ Unk	1	5.5	3+O	MFD 2	6	Progressive Disease
Allo-HSCT	MD (MURD)	10/10	103-58	6	W/ SPA/ HIS	0	1	1	HSCT 7, MFD 12	13	Progressive Disease
Eli-cel	n/a	n/a	102-16	7	W/ USA/ HIS	0	2	3	HSCT 13	16	Transplant-Related
Eli-cel	n/a	n/a	102-18	6	W/ FRA/ NH	0	6.5	5	MFD 9	22	Viral Infection
Allo-HSCT	MD (MSD)	10/10	103-32	8	W/ GER/ NR	0	9	1	--	23	Transplant-Related
Allo-HSCT	UMD (UURD)	9/10	103-22	8	W/ USA/ NH	0	1.5	3	--	26	Cardiac Arrest[2]
Untreated	n/a	n/a	101-72	11	W/ ARG/ NR	0	5	2	MFD 19	28	Progressive Disease
Allo-HSCT	MD (MSD)	6/6	103-44	6	W/ ARG/ HIS	0	7	1	--	33	Septic Shock
Untreated	n/a	n/a	101-67	15	W/ USA/ NR	0	9	5	MFD 20	52	Progressive Disease
Untreated	n/a	n/a	101-87	9	W /USA/ NR	1	8.5	1	MFD 56	73	Death[3]
Untreated	n/a	n/a	101-56	5	W/ USA HIS	1	4.5	1	MFD 14	92	Progressive Disease

Source: Reviewer's analysis of ADSL, ADBASE, ADHSCT and ADMRI datasets
Abbrev: HLA, human leukocyte antigen; COO, country of origin; Eth, ethnicity; NFS, neurologic function score; O, other; MFD, major functional disability; HSCT, hematopoietic stem cell transplant; UMD, unmatched donor; MD, matched donor; UURD, unmatched unrelated donor; MURD, matched unrelated donor; MSD, matched sibling donor; n/a, not applicable; NR, not reported; W; white; USA, United States of America; FRA, France; NLD, Netherlands; SPA, Spain; GER, Germany; ARG, Argentina; unk, unknown; HIS, Hispanic; NH, non-Hispanic; GVHD, graft versus host disease
[1] Age is age at transplant for allo-HSCT and eli-cel populations, and age at time of diagnosis for untreated subjects
[2] Time of MFD, HSCT or death in months relative to time of transplant for allo-HSCT and eli-cel populations, and relative to time of diagnosis for untreated subjects; HSCT refers to repeat HSCT for HSCT populations and rescue allo-HSCT for eli-cel populations

Subject Deaths in Entire allo-HSCT Populations in Studies ALD-101 and ALD-103 (TP-101 and TP-103 Populations)

In the entire allo-HSCT population in Studies ALD-101 and ALD-103 (pooled TP-101 and TP-103 populations, n=124), 31 (25%) of subjects died. Of 124 subjects, 64 received allo-HSCT from an HLA-unmatched donor (UMD) and 58 received allo-HSCT from an HLA-matched donor (MD). Of the 64 subjects with UMD HSCT, 21 (33%) died. Of the 58 subjects with MD HSCT, 10 (17%) died. The number of deaths in UMD HSCT was double those in MD HSCT during the course of follow-up. It is worth noting that this analysis is limited by early termination of Study ALD-103, and thus deaths may be underestimated.

Of 31 deaths:
-21 (68%) were in subjects who received HLA-unmatched donor (UMD) HSCT – 12 (57%) were transplant related, 8 (38%) were from progressive disease, and 1 (5%) had unknown cause of death.
-10 (32%) were in subjects who received HLA-matched donor (MD) HSCT - 7 (70%) were transplant related, 2 (20%) were due to progressive disease, and, and 1 (10%) had unknown cause of death.

These deaths are summarized and detailed further in Table 5.
At each time point, UMD HSCT was associated with twice as many deaths as MD HSCT. Additionally, more than 1/4 subjects who received UMD HSCT died within the first year, compared to 1/10 of subjects who received MD HSCT. Deaths of all causes tended to occur within the first 12 months for UMD HSCT, whereas only transplant-related deaths occurred during the first 12 months for MD HSCT.

Table 18: Summary of Deaths in Pooled TP-101 and TP-103

Parameter	**HLA-Matched allo-HSCT, TP-101 and TP-103 MD, pooled (n=58)**	**HLA-Unmatched allo-HSCT, TP-101 and TP-103 UMD, pooled (n=64)**
Deaths from any cause, n (%)	10 (17)	21 (33)
Deaths from any cause by 6 Months, n (%)	5 (9)	12 (19)
Deaths from any cause by 12 Months, n (%)	6 (10)	18 (28)
Deaths from any cause by 24 Months, n (%)	8 (14)	18 (28)
Transplant-Related Death, n (%)	7 (12)	12 (19)
Time to Transplant-Related COD (months), median (min, max)	3.5 (1.1, 23.0)	5.6 (0.4, 42.3)
Transplant-Related Deaths by 6 Months, n (%)	5 (9)	7 (11)
Transplant-Related Deaths by 12 Months, n (%)	6 (10)	11 (17)
Transplant-Related Deaths by 24 Months, n (%)	7 (12)	11 (17)
Progressive Disease Cause of Death, n (%)	2 (3)	8 (13)
Time to Progressive Disease Cause of Death (months), median (min, max)	31.4 (12.8, 49.9)	6.2 (3.0, 80.9)
Progressive Disease Deaths by 6 Months, n (%)	0	5 (8)
Progressive Disease Deaths by 12 Months, n (%)	0	6 (9)
Progressive Disease Deaths by 24 Months, n (%)	1 (2)	6 (9)
Unknown Cause of Death, n (%)	1 (2)	1 (2)
Time to Unknown Cause of Death (months)	33.1	9.3
Unknown Cause of Death by 6 Months, n (%)	0	0
Unknown Cause of Death by 12 Months, n (%)	0	1 (2)
Unknown Cause of Death by 24 Months, n (%)	0	1 (2)

Source: Reviewer's analysis of ADSL, ADBASE, and ADHSCT datasets
Abbrev: HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplant; TP, transplant population; MD, matched donor; UMD, unmatched donor.

Comparability of TP-101 and TP-103 by HLA Matching of Donor

Analyses were done to ensure comparability between subjects treated with allo-HSCT from HLA-matched donors (MD) and HLA-unmatched donors (UMD) in the entire transplant population (TP-101 and TP-103, pooled) as described above. In those with HLA-matched donors (TP-MD), transplants occurred between 1997 and 2019. In those with HLA-unmatched donors (TP-UMD), transplants occurred between 2000 and 2019. Transplants between 1997 and 2010 occurred in Study ALD-101, and transplants from 2013 onward were in Study ALD-103. No subjects received their primary (first) transplant in 2011 or 2012 due to the timing of studies.

The median age at time of transplant was 8 years for both groups, and age at diagnosis was similar for both groups, as seen in Table 6. Median time from diagnosis to treatment with allo-HSCT was similar for both groups (3.5 months for TP-MD and 4.0 months for TP-UMD). Analyses were attempted for baseline NFS and Loes score but were limited by significant amounts of missing data. We also do not feel baseline NFS or Loes score should have an impact on transplant-related deaths. Even if the population with HLA-unmatched donors were to have higher baseline NFS or Loes scores, the difference in timing of deaths related to progressive disease is striking (6.2 months for those with unmatched donors and 31.4 months for those with matched donors). This is unlikely to be related to more advanced disease at baseline alone.

Table 19: Age at Diagnosis and Transplant for TP-MD and TP-UMD

Parameter	**HLA-Matched Donor (TP-MD, n=64)**	**HLA-Unmatched Donor (TP-UMD, n=58)**
Age at CALD Diagnosis (years), median (min, max)	8 (2, 14)	7 (0, 14)
Age at Transplant (years), median (min, max)	8 (2, 14)	8 (2, 18)
Time from CALD Diagnosis to Transplant (months), median (min, max)	3.5 (0.4, 56.3)	4.0 (0.6, 78.0)

Source: Reviewer analysis of ADSL, ADBASE and ADHSCT datasets
Abbrev: HLA, human leukocyte antigen; TP, transplant population; MD, matched donor; UMD, unmatched donor; CALD, cerebral adrenoleukodystrophy

Appendix 7: Change from Baseline in NFS and Loes Score at Month 24

Table 20: Change from Baseline in NFS and Loes at Month 24 for eli-cel and TPES Populations

Parameter	Change	TP-102 (n=32)	TP-104 (n=35)	Pooled TP-102 and TP-104 (n=67)	TPES-103 (n=27)	TPES-101 (n=26)	Pooled TPES-101 and TPES-103 (n=53)
NFS Evaluable at Month 24, n	--	30	7	37	12	11	23
Stable NFS at Month 24, n (%) [1]	--	29 (96.7)	7 (100.0)	36 (97.3)	12 (100.0)	11 (100.0)	23 (100.0)
Change in NFS from Baseline at Month 24, n (%)	Decreased	0	0	0	0	0	0
--	No Change	26 (86.7)	7 (100)	33 (89.2)	11 (91.7)	9 (81.8)	20 (87.0)
--	Increased by 1	3 (10.0)	0	3 (8.1)	1 (8.3)	1 (9.1)	2 (8.7)
--	Increased by 2	0	0	0	0	0	0
--	Increased by 3	0	0	0	0	1 (9.1)	1 (4.3)
--	Increased by 4 or More	1 (3.3)	0	1 (2.7)	0	0	0
Loes Evaluable at Month 24, n	--	30	5	35	13	17	30
Stable Loes at Month 24, n (%)	--	24 (80.0)	5 (100.0)	29 (82.9)	12 (92.3)	15 (88.2)	27 (90.0)
Change in Loes from	Decreased	0	1 (20.0)	1 (2.9)	4 (30.8)	0	4 (13.3)

Parameter	Change	TP-102 (n=32)	TP-104 (n=35)	Pooled TP-102 and TP-104 (n=67)	TPES-103 (n=27)	TPES-101 (n=26)	Pooled TPES-101 and TPES-103 (n=53)
Baseline at Month 24, n (%)							
--	No Change	5 (16.7)	2 (40.0)	7 (20.0)	1 (7.7)	3 (17.6)	4 (13.3)
--	Increased by 0.5-1.5	10 (33.3)	0	10 (28.6)	6 (46.2)	5 (29.4)	11 (36.7)
--	Increased by 2-3.5	0	0	0	0	5 (29.4)	5 (16.7)
--	Increased by 4-5.5	7 (23.3)	2 (40.0)	9 (25.7)	1 (7.7)	2 (11.8)	3 (10.0)
--	Increased by 6 or more	8 (26.7)	0	8 (22.9)	1 (7.7)	2 (11.8)	3 (10.0)

Source: Adapted from bluebird bio, Inc., BLA ad-hoc Table 80.2.4.1
Abbrev.: TP, Transplant Population; TPES, Strictly ALD-102-eligible Transplant Population.
Note: The analysis is based on subjects who have non-missing Baseline and Month 24 assessments.
[1] Stable NFS at Month 24 is defined as maintaining a NFS ≤4 without an increase of >3 points from Baseline.
[2] Stable Loes score at Month 24 is defined as either maintaining a Loes score ≤9 or not increasing a Loes score by ≥6 points from Baseline.

Appendix 8: Serious Adverse Events in the ISS Population

Ninety serious adverse events (SAEs) occurred in the ISS population. The 40 most notable SAEs, considering severity and attribution, are presented in Table *8*. SAEs in the ISS population that were excluded from Table 8 are listed subsequently.

Table 21: Treatment-Emergent Grade 3 and 4 SAEs Excluding Early Hematologic SAEs and Disease-Progression/Death-Related SAEs in S102-18 – # of SAEs and % of Subjects

System Organ Class	Serious Adverse Event	# of Subjects (n=67)	Onset prior to NE (n=67)	Onset NE to <D60* (n=67)	Onset D60 to <M12* (n=65)	Onset M12 to <M24 (n=46)	Onset M24 or later* (n=32)
Any	**Any Grade 3 or 4 SAE**	22 (31%)	3 (4%)	8 (12%)	13 (14%)	2 (4%)	11 (19%)
Blood and lymph	Pancytopenia (Grade 4)	2 (3%)	--	2 (3%)	--	--	--
Cardiac	Bradycardia (Grade 4)	1 (1%)	--	--	--	--	1 (3%)
Gastrointestinal	**Any gastrointestinal**	4 (6%)	2 (3%)	--	2 (3%)	--	1 (3%)
--	Vomiting (Grade 3)	2 (3%)	--	--	2 (3%)	--	1 (3%)
--	Oral mucositis (Grade 3)	2 (3%)	2 (3%)	--	--	--	--
General Disorders	Pyrexia/Fever (Grade 3/4)	3 (4%)	--	1 (1%)	1 (2%)	--	1 (3%)
	(Grade 3)	2 (3%)	--	1 (1%)	1 (2%)	--	--
	(Grade 4)	1 (1%)	--	--	--	--	1 (3%)
Infections	**Any infection** (Grade 3)	7 (10%)	--	2 (3%)	5 (7%)	--	--
--	Bacteremia†	3 (4%)	--	--	4 (5%)	--	--
--	Central line infection	2 (3%)	--	1 (1%)	1 (2%)	--	--
--	BK cystitis	1 (1%)	--	1 (1%)	--	--	--
--	Otitis Media	1 (1%)	--	--	1 (2%)	--	--
--	Sinusitis	1 (1%)	--	--	1 (2%)	--	--
Injury/complication	**Any injury/complication**	2 (3%)	--	1 (1%)	1 (2%)	--	--
--	Platelet reaction (Grade 3)	1 (1%)	--	1 (1%)	--	--	--
--	Spinal fracture (Grade 4)	1 (1%)	--	--	1 (2%)	--	--
Investigations	Increased LFTs (Grade 3)	1 (1%)	--	--	1 (2%)	--	--
Metabolism	Anorexia (Grade 3)	1 (1%)	1 (1%)	--	--	--	--
Neoplasms	Myelodysplastic syndrome (Grade 4)	3 (4%)	--	--	--	2 (4%)	1 (3%)
Nervous system	**Any nervous system**	5 (8%)	--	--	1 (2%)	1 (2%)	7 (12%)
--	Seizure (Grade 3/4)	4 (6%)	--	--	--	1 (2%)	7 (12%)
	(Grade 3)	4 (6%)	--	--	--	1 (2%)	5 (12%)
	(Grade 4)	2 (3%)	--	--	--	--	2 (6%)
--	Transverse myelitis (Grade 3)	1 (1%)	--	--	1 (2%)	--	--
Psychiatric disorders	**Any psychiatric disorder** (Grade 3)	3 (4%)	--	1 (1%)	--	--	2 (3%)
--	Aversion	1 (1%)	--	1 (1%)	--	--	--
--	Depression	1 (1%)	--	--	--	--	1 (3%)

*Displays number of events, including multiple distinct events in a single subject; in parentheses is % of subjects with the event among subjects who were followed through the start of the "onset" timeframe.
†Includes bacteremia caused by pseudomonas, stenotrophomonas and streptococcus

Abbrev: SAE, serious adverse event; NE, neutrophil engraftment; D, days post-eli-cel administration; M, months post-eli-cel administration; <, before; LFTs, liver functions tests (transaminases)
Source: Reviewer's analysis, derived from ADAE dataset

Excluded from the 40 SAEs in Table 8 are the following:

- Five SAEs that were not treatment-emergent, occurring during the mobilization period prior to eli-cel administration:
    - Adrenal insufficiency in two subjects
    - Central line infection
    - Back pain
    - "Hospitalization due to psychosocial indication by autism"
- Twelve hematologic SAEs (all febrile neutropenia) in 12 subjects that resolved within 30 days of eli-cel administration; all were febrile neutropenia
- Ten SAEs related to disease progression and death in Subject 102-18 who died from complications related to CALD progression
- Grade 1 and 2 SAEs:
    - Nine pyrexia/fever SAEs
    - Two additional seizure SAEs
    - Two involuntary movement SAEs
    - Two upper respiratory tract infection SAEs
    - One each of the following SAEs: constipation, nausea, fatigue, gastroenteritis, influenza, head injury, and suicidal ideation

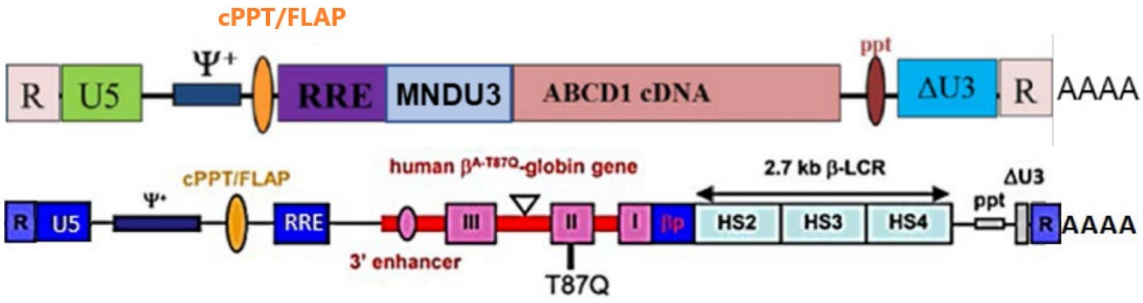
Appendix 9: Product overview of bluebird bio's lentiviral products

The Applicant has three related LVV-based cell products referred to as eli-cel, beti-cel and lovo-cel, used for the treatment of CALD, transfusion-dependent β-thalassemia (TDT) and sickle cell disease (SCD), respectively. Lenti-D LVV is used to generate the eli-cel product, and BB305 LVV is used in the manufacture of beti-cel and lovo-cel.

The Lenti-D LVV is used to manufacture eli-cel via transduction of CD34+ hematopoietic stem cells (HSCs) from patients with CALD, while the BB305 LVV is used to manufacture beti-cel using CD34+ HSCs from patients with TDT and lovo-cel using CD34+ HSCs from patients with SCD.

The Lenti-D LVV production system uses 5 plasmids: a transfer plasmid, a VSV-G-encoding envelope plasmid and 3 separate packaging plasmids encoding the HIV-1 gag-pol, HIV-1 rev and HIV-1 tat proteins, respectively). In addition, Lenti-D contains a gamma retroviral MNDU3 internal enhancer/promoter to control expression of an ABCD1 cDNA transgene. Lenti-D also carries a 3' long terminal repeat (LTR) with deletion of the U3 region (ΔU3) that confers the self-inactivating property (Refer to Figure 2: Diagrams of Lenti-D LVV RNA (top) and BB305 LVV RNA (bottom)Figure 2).

Figure 16: Diagrams of Lenti-D LVV RNA (top) and BB305 LVV RNA (bottom)



From left to right: R = repeat; U5 = unique 5' Long Terminal Repeat (LTR); Ψ+ = Psi packaging signal; cPPT/FLAP = central polypurine tract/DNA flap; RRE = Rev responsive element; [vector-specific components]; ppt = polypurine tract; ΔU3 = unique 3' region of the LTR; AAAA = polyadenylated tail
Lenti-D specific components (top): MNDU3 = promoter derived from the U3 element of the Myelo-proliferative sarcoma virus, negative regional deleted dl1583rec primer binding site LTR; ABCD1 cDNA
BB305-specific components (bottom): 3' enhancer = 3' β-globin enhancer; III, II, I = exons III, II, I; ▽ = 372-bp IVS2 deletion in intron 2; T87Q = $\beta_{A\text{-}T87Q}$ mutation; βp = human B-globin promoter; HS2, HS3, HS4 = DNase I hypersensitive sites HS2, HS3, and HS4; β-LCR = human β-globin locus control region

In contrast, the BB305 LVV production system uses 4 plasmids: a transfer plasmid, a VSV-G-encoding envelope plasmid and 2 separate packaging plasmids encoding the HIV-1 gag-pol and HIV-1 rev proteins, respectively. Additionally, BB305 carries an internal erythroid lineage-specific β-globin locus control region (LCR) and promoter to

control expression of the βA-T87Q-globin transgene, which resembles the intron and exon structure of the wildtype β-globin gene. Like Lenti-D, BB305 also carries a ΔU3 3' LTR that confers the self-inactivating property (Refer to Figure 2).

After integration into the cellular genome, the backbones of the two vector genomes are identical. These include the R and U5 sequences derived from the 5' LTR, the HIV packaging signal (Ψ), a truncated gag region, the central polypurine tract (cPPT), Rev response element (RRE) and the DU3/R sequence (Refer to Figure 2).

However, the internal promoters and transgene sequences are significantly different. The MNDU3 promoter, being of gamma retroviral LTR origin, is a strong, constitutive promoter, with no cell type-specificity for transgene expression. In contrast, the β-globin promoter is a cell-specific promoter with strong activity only in erythroid lineage cells. Therefore, hypothetically, while both promoters may potentially affect the expression of neighboring genes, transgene expression from the LVV BB305 provirus is likely restricted to cells in the erythroid lineage.

The structures of the therapeutic expression cassettes are also different. The Lenti-D transgene cassette contains an *ABCD1* cDNA and uses the polyadenylation (polyA) signal located in the HIV 3' LTR to end the mRNA. In contrast, the BB305 LVV transgene cassette has the β-globin LCR, promoter, and βA-T87Q-globin gene positioned in the reverse orientation, uses a β-globin polyA signal, and uses the genomic globin gene structure, including 2 introns and 3 exons, to produce a spliced transcript. As a result, the BB305 LVV provirus has the potential to structurally alter the expression of the genes in the vicinity of the site where it has integrated, either through altered mRNA splicing involving the splice sites present in the globin gene sequence, or through truncation using the beta globin polyA signal.

Thus, while, the Lenti-D and BB305 proviruses both contain splicing and polyA signals that are integral to the HIV portion of the proviral genome and thus have the potential to alter the structure of integration site (IS) gene transcripts when the proviruses are integrated in the same orientation as the transcription of the IS gene, because the BB305 provirus has polyA and splicing signals in both the sense and anti-sense strands (globin and HIV LTR), it has the potential to truncate transcripts or alter splicing in either orientation. Therefore, hypothetically, the BB305 provirus has more potential to alter the structure of the mRNA of the gene into which it is integrated, as compared to the Lenti-D provirus.

Appendix 10: Hematologic malignancy and integration site data from related products

Cases of malignancy and a summary of integration site analysis (ISA) data from the Applicant's other LVV-based products lovo-cel and beti-cel, that are related to eli-cel, follow.

Sickle Cell Disease Diagnosed Malignancy Cases and Cases of Concern

The drug product (DP) lovo-cel is an autologous CD34+ product developed for the treatment of sickle cell disease. It contains hematopoietic stem cells (HSCs) transduced with a lentiviral vector (LVV) encoding the βA-T87Q globin gene. Forty-nine subjects have been treated with lovo-cel in clinical studies, and two (4.1%) have developed acute myeloid leukemia (AML). Several additional subjects are concerning for development myelodysplastic syndrome (MDS). Select aspects of the subjects who developed AML are presented in the following table.

Table 22: Characteristics of SCD Subjects who Developed AML

Characteristic	Subject 206-A-02	Subject 206-A-01
Exposure to hydroxyurea	Pre-treatment: 2007 to 2014 Post-treatment: 2016 to 2018	Pre-treatment: 2009 to 2017 Post-treatment: 2016 to 2017
Exposure to plerixafor for HSC mobilization	No	No
Exposure to busulfan for conditioning	Yes	Yes
Growth factors post-treatment	G-CSF: 6 days (Days 11-17) Darbepoetin: ~Month 30 to 36	G-CSF: 2 days (Day 16-17)
Time to onset of MDS/AML	3 years to MDS followed 5 months later by AML	5.5 years to AML
Drug Product cell dose (x 10^6 CD34+ cells/kg)	2.8	2.6
Drug Product VCN (c/dg)	1.3	0.55
%LVV cells in Drug Product	29.0	9.3
Month 6 PB VCN (c/dg)	0.128	0.051
Month 6 Hb βA-T87Q (g/dL)	1.02	0.33
Presence of predominant clone	No	Yes (*VAMP4* IS)
Presence of LVV integration sites in blasts	No	Yes (*VAMP4* IS)
Cytogenetic abnormalities in blasts	Monosomy 7 Abnormal 19p *RUNX1* (p.Asp198Gly) *PTPN11* (p.Phe71Leu) *KRAS* (p.Gly12Ala)	Monosomy 7 Partial loss of 11p *RUNX1* (p.Ala149*fs) *PTPN11* (p.Ala72Val) Del/repl at 147,036,771 Del at 231,212,613 4 CNAs on Chr1q

Abbrev: HSC, hematopoietic stem cell; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; kg, kilogram; c/dg, copies per diploid genome; g/dL, grams per deciliter; G-CSF, granulocyte colony stimulating factor; IS, integration site; CNA, copy number alteration
Source: Derived from Original BLA 125755/008, 16.2.8 Clinical IR 01 Question 13 – 3Jan2022, p.12

A description of the AML and possible MDS cases follows:

Subject 206-A-01
A 32-year-old woman with genotype βS/βS developed AML. The blasts contained the lentiviral vector with integration into the *VAMP4* gene and there were multiple gene abnormalities on the same arm of Chr1q as *VAMP4*.Other cytogenetic abnormalities found in the blasts included monosomy 7 and mutations in *RUNX1* and *PTPN11*.

The subject had been treated with hydroxyurea (HU) from 2009 to 2015, and received 2.6 x 10^6 CD34+ cells/kg lovo-cel with vector copy number (VCN) 0.55 c/dg in 2015. Neutrophil engraftment occurred on Day 19 after one day of G-CSF, and platelet engraftment on Day 31. The subject did not respond to gene therapy (GT), requiring transfusions and intermittent HU.

Peripheral blood (PB) blasts in December 2020 were present at 2%; rising to 29% in February 2021 when a diagnosis of AML was made, 5.5 years after GT. Blasts were $CD34^+CD33^+LVV^+$. Bone marrow (BM) blasts were 22%. Chromosomal microarray analysis showed monosomy 7 in 70% of cells and partial loss of 11p involving *WT1* in 50% of cells. Next-generation sequencing (NGS) revealed a *RUNX1* frameshift mutation p.A149*fs with variant allele frequency (VAF) of 26% and a *PTPN11* missense mutation p.A72V with VAF of 30%.

The subject was refractory to induction chemotherapy and underwent salvage chemotherapy, followed by haplo-identical transplant. She died of relapsed disease and transplant complications.

Subject 206-A-01 had ISA every 6 months. Integration into *VAMP4* was present at all assessments and increased over the 5.5-year time period leading up to the diagnosis of AML. The integration site-specific relative frequency increased over time, roughly parallel to the increase in IS-specific VCN. The overall VCN was relatively stable during the first 4.5 years, doubled between Year 4.5 and 5, and had further increased at Year 5.5 when the subject met criteria for a predominant clone and was diagnosed with AML.

Table 23: Subject 206-A-01 ISA and Laboratory Data

Time Point	Date	qPCR Relative Frequency	IS-specific qPCR VCN (c/dg)	S-EPTS/ LM-PCR Relative Frequency	S-EPTS/ LM-PCR VCN (c/dg)	Neutrophils (x 10^9/L)	Platelets (x 10^9/L)
Month 6	Feb 2016	0.2%	0.0001	--	0.0502	6.9	**457**
Month 12	Aug 2016	2.8%	0.0015	--	0.0543	5.85	383
Month 18	Jan 2017	9.5%	0.0043	--	0.0453	5.56	**458**
Month 24	Aug 2017	10.6%	0.006	--	0.0565	**10.79**	**408**

Month 30	Jan 2018	5.1%	0.0027	--	0.0532	**7.58**	324
Month 36	Jul 2018	18.7%	0.0112	--	0.596*	5.31	444
Month 42	Jan 2019	26%	0.0168	--	0.0645	5.44	430
Month 48	Aug 2019	17.7%	0.0105	15.7%	0.0593	**7.42**	313
Month 54	Jan 2020	20.5%	0.0115	20.9%	0.0561	5.1	398
(Month 60)	Aug 2020	51.6%	0.0695	64.9%	0.1246	3.15	361
(Month 65)	Feb 2021	89%	0.685	98.6%	0.7699	--	--

Abbrev: qPCR, quantitative polymerase chain reaction; VCN, vector copy number; c/dg, copies per diploid genome
Note: in **bold** are values outside the normal range; in parentheses are two unscheduled visits and their approximate time points
*This value appears to be misreported.
Source: Reviewer's analysis, derived from ADSL and ADLB datasets

Blasts were positive for LVV integration in *VAMP4*. However, *VAMP4* expression was not increased in the blast cell-enriched cell population relative to the blast cell-depleted cell population. VCN was higher in peripheral $CD34^+$ cells versus unsorted cells (1.12 c/dg vs 0.89 c/dg), and both PB and BM cells showed an increase in the % LVV+ cells in the $CD34^+$ blast-enriched population compared to unsorted cells via single cell PCR. Retrospective microarray and myeloid mutation panel testing on PB leukocyte pellets from screening in 2015, and at months 3, 6, 18 and 24 post-GT, revealed no abnormalities. The Applicant reported that based on transcriptome analysis, the integration into *VAMP4* did not appear to impact the transcription of *VAMP4* or any genes in the vicinity, and that the transgene promoter is not active in tumor-enriched samples.

The Applicant concluded that AML was not related to the *VAMP4* integration for numerous reasons that include the following:

- Classic driver alterations in AML were present
- No substantial change in gene expression around the *VAMP4* integration site
- The LVV is not transcriptionally active in tumor cells
- The transcription profile is consistent with properties of known AML mutations

However, it is not clear that the Applicant's analysis is sufficient to rule out the integration having an effect on genetic elements that could have contributed to the development of AML. For example, genome sequencing identified numerous variants on the same chromosome arm as *VAMP4* that have not been accounted for. They are between 24 and 78 megabase pairs (Mpb) away from the *VAMP4* integration site (IS) and include the following:

- A 2.33 megabase pair (Mbp) deletion and replacement by 10nt at position 147,036,771 - ~24Mbp from the *VAMP4* IS
- A 1.01 kilobase pair deletion at position 231,212,613 - ~59Mbp from the *VAMP4* IS
- Four copy number alterations ~28 to 76 Mbp from the *VAMP4* IS:

- Copy neutral loss of heterozygosity at loc 143208875; 64313 nt
- Loss at loc 221,570,410; 10,502 nt
- Gain at 248,404,967; 122,696 nt
- Loss at 248,575,123; 71,733 nt

Additionally, while the Applicant performed gene expression studies that are useful for understanding the effects of an integration on gene transcription, they did not incorporate internal control spike-in RNAs, which are important for accurately determining differences in gene expression. Furthermore, the gene expression data provided were not sufficient to assess changes in gene expression in the for genes near *VAMP4*, of which there are 33 within 1 Mbp (16 protein-encoding) and 256 within 10 Mpb (127 protein-encoding).

Subject 206-A-02
A 46-year-old man with genotype βS/βS developed AML, although the blasts did not contain the lentiviral vector. Cytogenetic abnormalities in the blasts included monosomy 7, and mutations in *RUNX1* and *PTPN11*.

The subject had been treated with HU from 2007-2014, and received 2.8 x 10^6 $CD34^+$ cells/kg lovo-cel with VCN 1.3 c/dg in 2015. Neutrophil engraftment occurred on Day 17 after G-CSF on days 11 to 17, and platelet engraftment occurred on Day 29.

PB VCN and Hb βA-T87Q were low post-GT (0.08-0.15 c/dg and 0.1-1.2 g/dL). The subject had anemia post-GT treated with transfusions, HU resumption at 1 year, and erythropoietin initiation at ~2.5 years post-GT. At 3 years post-GT, pancytopenia was noted, followed by a diagnosis of MDS (RAEB-2).

The subject was treated with 5 cycles of hypomethylating agent therapy [2 cycles of 5-Azacitidine and 3 cycles of Decitabine], but 5 months after being diagnosed with MDS, he had progressed to AML. AML was treated with induction chemotherapy with 7+3 (idarubicin and cytarabine), but the subject had persistent disease with 15%-19% bone marrow blasts. Subsequent re-induction therapy reduced blasts to 5% prior to allogeneic hematopoietic stem cell transplant (HSCT). The HSCT preparative regimen consisted of melphalan, fludarabine, and total body irradiation followed by haploidentical HSCT. The subject had remission at day 100, with 3% blasts, normal trilineage maturation, and no monosomy 7, however he relapsed 2 months later. At that point, patient was treated with decitabine and venetoclax for 8 cycles, then switched to azacytidine/venetoclax for 3 additional cycles. Following this, he was found to have PB blasts and was placed on hospice care, where he died.

PB at the time of MDS diagnosis showed pancytopenia (WBC 3 x 10^9/L; Hgb 6.7g/dL; plts 111 x 10^9/L; ANC 0.92 x 10^9/L) with circulating myeloblasts at 6.7%. Bone marrow biopsy showed 40% cellularity, megakaryocytic dysplasia with small and hypolobated nuclei, erythroid dysplasia, severe myeloid hypoplasia, and 15% $CD34^+$ myeloblasts.

Flow cytometry showed 10% myeloblasts expressing dimCD45$^+$, CD34$^+$, CD117$^+$, HLA DR$^+$, CD13$^+$, and CD33$^+$. Cytogenetic analysis showed 45,XY,-7,add(19)(p11)[8]/46,XY[12]. NeoTYPE NGS panel testing of BM cells showed *RUNX1* p.Asp198Gly at VAF 8.3%, *PTPN11* p.Phe71Leu at VAF 5.2%, and *KRAS* p.Gly12Ala at VAF 2.7% mutations.

ISA using (NR)LAM-PCR did not reveal any integration sites that had a persistent high relative frequency. Overall VCN was relatively stable ranging between 0.075 and 0.143 c/dg between Month 6 and 36, and then declined to 0.015 c/dg at Month 42.

DP testing by FISH and NGS showed no abnormalities.

VCN analysis demonstrated a lack of enrichment of LVV sequences in the blast cell-enriched population, weakening the case for insertional oncogenesis due to LVV transduction. Furthermore, no LVV was detected in BM cells at the time of relapsed AML post-allogeneic HSCT.

Subject 206-C-27
Subject 206-C-27 is a 20-year-old woman with genotype βS/βS and alpha-thalassemia and a deletion of two *α-globin* genes. She is notable for dyserythropoiesis in the bone marrow, multiple cytogenetic abnormalities (an *ATM* variant and trisomy and tetrasomy 8) that confer risk of hematologic malignancy, and new transfusion dependence.

The subject was treated with HU for ~2 years and received 5.2 x 10^6 CD34$^+$ cells/kg lovo-cel with VCN 5 c/dg in July 2020. She had no growth factor support post-GT. Neutrophil engraftment occurred on Day 35 and platelet engraftment on Day 134. Post-GT, Subject 206-C-27 has had persistent, severe anemia requiring transfusions. A direct antiglobulin test (DAT) was positive for IgG, but there is no report of treatment with immunosuppression. About 6.5 months post-GT in February 2021, PB showed WBC 7 x 10^9/L, Hgb 7.0 g/dL, plts 272 x 10^9/L, with no blasts and low reticulocytes. DAT was negative.

A scheduled bone marrow biopsy at Month 6 revealed hypoplasia of early myeloid precursors with progressive maturation, reduced cellularity with relative erythroid hypoplasia, megakaryocytes without dysplasia, and no abnormal blast population. Karyotype was normal, however FISH demonstrated trisomy 8 in 6% of cells (12/200), and tetraploidy signals in 4.5-9.5% of cells. The subject was given a tentative diagnosis of MDS. However, a repeat bone marrow biopsy performed one month later did not show trisomy 8 by FISH. Karyotype and single nucleotide polymorphism (SNP) microarray were both normal and Trisomy 8 was not seen. NGS (UCSF500 Gene Panel) showed no somatic abnormalities although a heterozygous *ATM* mutation was found in bone marrow and buccal swab. Her diagnosis was changed to “transfusion dependent anemia.”

The subject had two subsequent bone marrow biopsies, at approximately Years 1 and 1.5. Both were interpreted as relative erythroid hyperplasia with dyserythropoiesis, most likely reflecting stress erythropoiesis, however it is not clear whether the pathologist reading the slides was aware of the pathogenic *ATM* mutation. There is also a notation that at Year 1.5, lymphocytes included slightly increased plasma cells (4%).

Cytogenetics at Year 1 (karyotype, FISH and SNP microarray) were normal. At Year 1.5, the following abnormalities were identified on NGS (Rapid Heme Panel): *ATM* (VAF 26.8%), *TERT* (VAF 58.8%), *IKZF1* (VAF 37.8%), and *TET2* (VAF 40.2%). Cytogenetics were otherwise normal.

It appears the *ATM, TERT, IKZF1,* and *TET2* variants were present prior to DP administration, having been retrospectively found with NGS for a specimen from November 2019. In addition, testing on the retained DP and on CD34- cells from bone marrow remaining after CD34+ enrichment, although initially reported to be normal, identified variants in *TET2* and *IKZF1.*

VCN of 4.6 c/dg has remained stable in PB since months 3-6. ISA does not demonstrate any integration sites with high relative frequency, with integration into KDM2A being the most frequent at 0.425% at 18 months.

Subject 206-C-32
Subject 206-C-32 is a 14-year-old boy with genotype βS/βS and alpha-thalassemia, with a deletion of two *α-globin* genes. He is notable for dyserythropoiesis in the bone marrow, cytogenetic abnormalities that are common in myeloid malignancy (trisomy and tetrasomy 8), persistent anemia, and thrombocytopenia.

The subject received 6.3 x 10^6 $CD34^+$ cells/kg lovo-cel with VCN 3 c/dg in July 2020. Neutrophil engraftment was on Day 26 and platelet engraftment was on Day 37. PB at screening and month 9 were reported as normal by SNP microarray. One year post-GT, the subject was noted to have mild pancytopenia and vitamin B12 deficiency (130 pg/mL); PB showed a WBC 4.05 x 10^9/L, hemoglobin 9.2 g/dL; MCV 78; plts 130 x 10^9/L. Occasional sickle cells were seen in the PB. Patient was placed on vitamin B12 supplementation, and several weeks later, the B12 level was 262 pg/mL. Within two months, levels declined again to 154 pg/mL; B12 supplements continued. At Month 18, B12 level remained below normal (169 mg/mL); also found to be below normal were folate (3.9 ng/mL), erythropoietin (24 milliunits/mL), haptoglobin (14 mg/dL) and ANC (1.51 x 10^9/L).

A BM aspirate was collected per protocol at Month 12. Many tri-and bi-nucleate erythroid progenitors were noted, and dysplasia was described as present in ~15%-20% of erythroid cells; blasts < 5%, and ring sideroblasts < 5%. BM biopsy was originally interpreted as “erythroid dysplasia with trisomy 8, highly suggestive of MDS.” A second

pathology read was obtained and interpreted the bone marrow smear as normocellular with mild dyserythropoiesis, likely reflecting stress erythropoiesis secondary to sickle cell anemia, and 5% dysplastic erythroid cells. Karyotype was normal, but a FISH panel had 7.7% trisomy 8 (10/130 nuclei scored) and 6.1% tetrasomy 8 (8/130 nuclei scored).

Follow-up studies were performed two months later in September 2021. BM biopsy (this time including core) showed a normocellular marrow with trilineage hematopoiesis, erythroid hyperplasia and 10-20% of erythroid cells showed dysplasia, with nuclear budding, binucleation, and irregular nuclear contours. Blasts were present at 1%. The morphologic dysplasia in the erythroid lineage, especially the observed binucleation, was concerning for an evolving MDS. No dysplasia was seen in the megakaryocytic or granulocytic lineages. A second pathology read was obtained and interpreted as normocellular with moderate dyserythropoiesis, and 15% dysplastic erythroid cells. Karyotype was normal, and FISH studies were essentially stable, demonstrating persistence of trisomy and tetrasomy for chromosome 8 (5% trisomy (10/200 nuclei scored), and 4% tetrasomy (8/200 nuclei scored)). PB showed WBC 4.78 x 10^9/L, Hgb 9.2 g/dL, MCV 75, plt count 127 x 10^9/L, ANC 1.7 x 10^9/L.

In February 2022 (~Month 18), FISH of the peripheral blood was negative.

NGS (Myeloid Molecular Panel) was performed retrospectively and identified no variants consistent with a myeloid malignancy. A *TET2* c.4946A>G variant of unknown significance was present at VAF 47.5%. Retrospective molecular NGS panel was performed on PB samples at screening and month 9 post-GT revealed the same *TET2* variant at VAF 50%, suggesting a germline nature.

Microarray-based chromosome analysis using the I Scan System with the Global Diversity Array-8 v1.0 Array BeadChip was also performed retrospectively and demonstrated arr(X,Y)x1,(1-22)x2 pre-GT, and unchanged at 9 months post-GT.

VCN of 4.1 c/dg at 18 months was increased from the 6- and 9-month values of 3.3 and 3.1 c/dg, respectively. ISA did not demonstrate any integration sites with high relative frequency, with integration into *KDM2B* being the most frequent at 0.205% at 18 months. ISA at 18 months was also notable for integration into the proto-oncogene *ABL1* with a relative frequency of 0.122%.

VAMP4 Integration in the Lovo-cel and Beti-cel Database

The Applicant concluded that vector integration at the *VAMP4* locus and in genes proximal to it occurs commonly, but that these integrations do not appear drive clonal expansion. They analyzed the frequency of integration into *VAMP4* in other sickle cell disease (SCD) subjects and transfusion-dependent beta-thalassemia (TDT) subjects

treated with lovo-cel and beti-cel, respectively. In SCD, they found that 71% (25/35) of subjects had at least one integration site in *VAMP4* and a total of 60 unique integration sites. Among the 60 integration sites detected across all subjects, 14 (23%) occurred in the intronic region between exons 4-5, like that seen in Subject 206-A-01, and five of these are located within 1 kilobase pair of the integration site in Subject 206-A-01. In TDT, the Applicant found that 56% (31/55) of subjects had at least one IS in *VAMP4*, and that the highest maximum frequency detected was 0.217%.

Because of the large number of total integration sites (between 121 and 54086 unique integration sites per assessment for the 39 SCD subjects and between 981 and 34857 for the 62 TDT subjects) integration site data provided for review were limited to the Top 10 most frequent integration sites for each time point. *VAMP4* did not arise across the database of top 10 integration sites at any time point except in the case of Subject 206-A-01.

Subjects with High-Frequency Integration Sites in the Lovo-cel and Beti-cel Database

In the combined SCD and TDT safety database, 34 subjects had integration sites with > 5% relative frequency. There was one instance of an integration site with > 30% relative frequency, and 41 instances where multiple integration sites that appeared to be in the same clone had a combined relative frequency of > 30%. These 41 instances occurred in three subjects (Subjects 206-A-01, 204-13, and 204-14). Subject 206-A-01 developed MDS and has already been described. Information regarding the other two, who were administered beti-cel and have multiple ISs that appear to be in the same clone, follows:

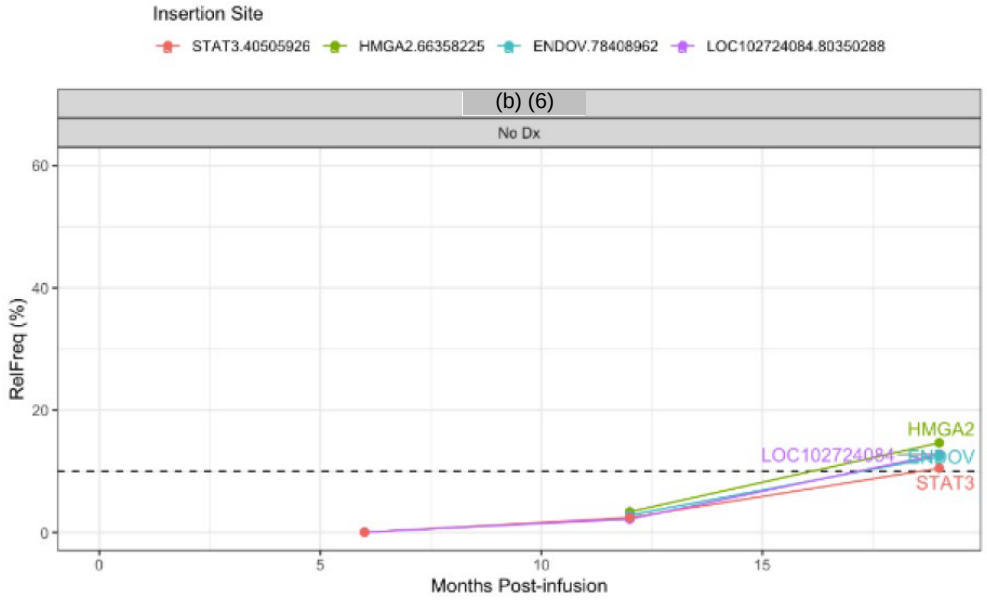
1. Subject 204-14: Integration sites that may be within the same clone include a proto-oncogene, *BCR*, and the following additional genes: *ASH1L-AS1, FNBP1, BTBD7, SACM1L, SFSWAP, PIP5K1A, SELP, TTBK2*, and *ZFAND3*. For *BCR*, the integration site-specific vector copy number in whole blood at the last reported visit on (b) (6), was 0.1245 c/dg.

2. Subject 204-13: Integration sites that may be within the same clone include two proto-oncogenes, *XP07* and *CBFB*, and the following additional genes: *DNAJC13, LINC00430*, and *ZMYM4.* The IS-specific VCN in whole blood at the last reported visit on (b) (6), was 0.0425 c/dg in *XPO7* and 0.0461 c/dg in *CBFB*.

There are several additional subjects who were reported to the FDA as having integration site frequencies of > 10%, although the ISA data for these subjects has not been provided. Based on the available information, the FDA finds Subject 206-C-23 to be most concerning. Subject 206-C-23 was treated with lovo-cel and at Month 18, had

an increase in relative frequency of four genes that may be within a single clone, including two proto-oncogenes, *HMGA2* and *STAT3*, as depicted in the following graphic.

Figure 17: Subject 206-C-23 Integration Sites with ≥ 10% Relative Frequency By Time Post-DP



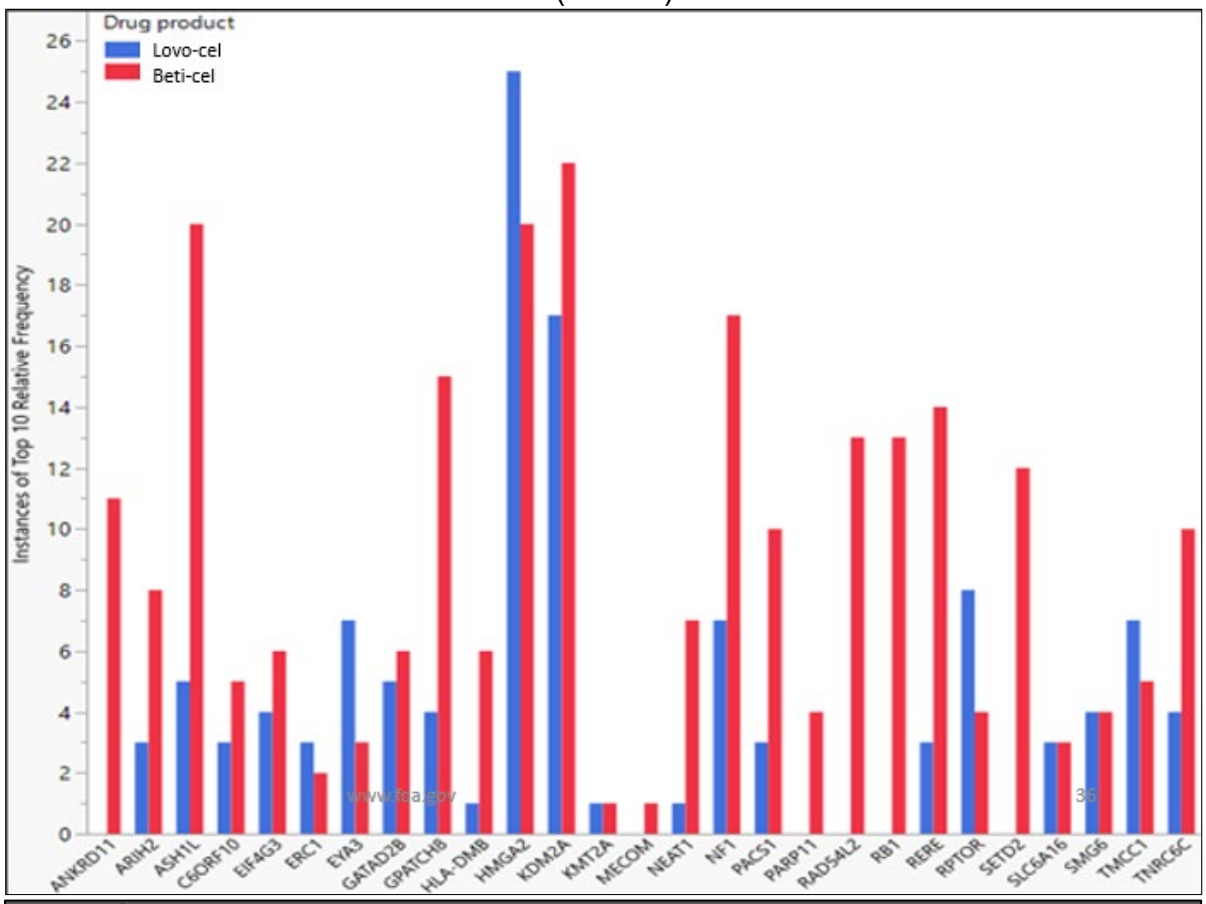
Additional information about the status of this subject is limited to the following:

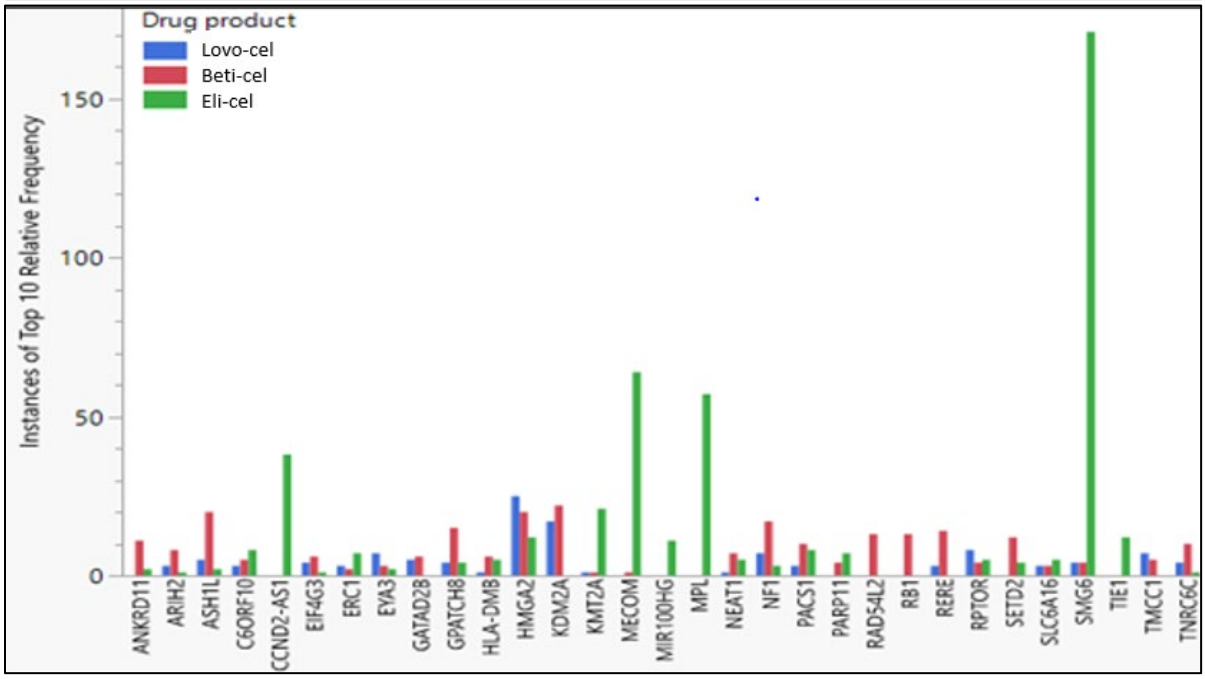
- Peripheral blood VCN increased from 1.3 c/dg at Month 12 to 1.9 c/dg at Month 18
- CBC demonstrated mildly elevated white blood count, and normal differential, hemoglobin, and platelet count
- Repeat ISA is planned to be performed at 21 months

Comparison of Integration Sites for lovo-cel, Beti-cel, and Eli-cel

The following data compare integration site analysis (ISA) results obtained with S-EPTS/LM-PCR at scheduled visits for subjects who received lovo-cel, beti-cel, or eli-cel. Because of the change in methodology for ISA implemented in June 2019, this analysis only includes assessments performed after June 2019. The dataset included 440 such assessments in 155 subjects: 113 assessments in 43 subjects with SCD, 203 assessments in 65 subjects with TDT, and 124 assessments in 47 subjects with CALD. The genes that were among the top 10 integration sites at a given time point for any subject in at least 11 instances are depicted in the following figures. The first compares lovo-cel and beti-cel, and the second also includes eli-cel (labeled as Lenti-D).

Figure 18: Top 10 Integration Sites by Drug Product - Lovo-cel vs. Beti-cel (top) and Lovo-cel vs. Beti-cel vs. Lenti-D/Eli-cel (bottom)





The above analysis has several limitations. First is the different number of datapoints per drug product that factor into the analysis. The greater number of data points for beti-cel than lovo-cel, for example, but equal number of *SLC6A16* integration sites for beti-cel and lovo-cel, reflect a higher prevalence of integrations into *SLC6A16* for lovo-cel than for beti-cel. This analysis is also limited because falling within the top 10 is a relative and not an absolute measure. A third limitation is that the duration of follow-up varies for different subjects, and therefore subjects with a longer duration of follow-up contribute more data to the figures and could skew the patterns toward their most common integration sites.

Despite the above limitations, it can be discerned that patterns of integration for lovo-cel and beti-cel are relatively similar, and that the pattern of integration for eli-cel is relatively different. It can also be discerned that eli-cel has a greater tendency to integrate into the same sites than do lovo-cel and beti-cel. Also important is that all three products have integration sites into proto-oncogenes that are among the highest relative frequency integration sites within certain subjects.

Appendix 11: Subjects with MDS After Treatment with Eli-cel

Subject 104-18
Subject 104-18 was treated with eli-cel on (b) (6), at the age of 11 and was diagnosed with MDS with unilineage dysplasia 14 months later. MDS is attributable to eli-cel because he had a predominant clone with integration into *MECOM*, a known proto-oncogene, and increased *EVI1* expression in the *MECOM* locus in whole blood.

Subject 104-18 achieved neutrophil engraftment on Day 27. Platelet engraftment was delayed, occurring on Day 106. Leukocyte, platelet, and hemoglobin values did not recover until six months after eli-cel and appear to have declined from there until he was diagnosed with MDS with unilineage dysplasia 14 months after treatment with eli-cel.

Integration site analysis (ISA) for Subject 104-18 was performed 6, 12, 14, and 18 months after eli-cel administration. ISA demonstrated integration into *MECOM* and *SLC6A16* with a relative frequency of integration between approximately 19 and 31%. Pre-specified criteria for clonal predominance were met at six months. Relative frequencies of integration sites into *MECOM* and *SLCA16* at all timepoints are provided in the table below:

Table 24: Relative Frequencies of MECOM and SLC6A16 by qPCR in Subject 104-18

Time Post-Eli-Cel	*MECOM Primers*	*SLC6A16* Primers
Month 6	29.1% WB	27.7% WB
Month 12	17.5% WB 18.8% CD15	17.7% WB 18.8% CD15
Unscheduled Relative Month 14	14.5% WB 17.9% CD15	15.9% WB 15.5% CD15
Month 18	17.7% WB 19.5% CD15	16.1% WB 17.6% CD15

Abbrev: CD15, $CD15^+$ subpopulation of peripheral blood as cell source; WB, whole blood as cell source
Source: BLA 125755 Listing 80.1.46 Integration Site Analysis Subject 104-18

In addition to the demonstration of integration into *MECOM*, increased *EVI1* expression of the *MECOM* locus was present in whole blood.

At 12 months, bone marrow biopsy and aspirate were performed, revealing moderate hypocellularity (40-50%) with a subset of dysplastic megakaryocytes. Karyotyping revealed a male chromosome complement with a del(14)(q11.2q13) versus inv(14)(p11.2q11.2) in all cells tested. NGS (Rapid Heme Panel) did not reveal any pathogenic variants. However, a variant of unknown significance in the *CDKN2A* gene (c. 168C>G (p.S56R) was detected at a variant allele frequency of 41%. FISH using

extensive probe set was normal.

At 14 months, bone marrow was markedly hypocellular (10-20%) with dysmegakaryopoiesis, meeting criteria for MDS. At 18 months, a repeat bone marrow biopsy and aspirate was performed with similar results, still consistent with MDS with unilineage dysplasia. The subject subsequently underwent allogeneic HSCT for treatment of MDS. The last report received on February 10, 2022, was that his MDS was in remission.

Subject 104-08
Subject 104-08 was treated with eli-cel on (b) (6), at the age of 13, and met criteria for MDS with single lineage dysplasia (megakaryocytic) approximately two years later. MDS in this case is attributable to eli-cel because the subject had a predominant clone with integration into *MECOM* and the specific *MECOM* integration was found in the megakaryocytes. Also supporting the causality of eli-cel is the identification of increased *EVI1* expression in the *MECOM* locus in whole blood.

Details regarding Subject 104-08's early course and engraftment follow. His conditioning regimen was notable for relatively high busulfan dosing, the area under the curve being higher than all but two subjects across the eli-cel development program. Neutrophil engraftment was significantly delayed and not robust; the subject received his final dose of G-CSF 3.5 months after eli-cel administration and thereafter had numerous ANC values below 1 x 10^9/L, finally meeting engraftment criteria on Day 188. The subject also had poor engraftment of platelets; his post-treatment platelet count peaked at 53 x 10^9/L while he was receiving eltrombopag approximately months after eli-cel administration. He achieved an unsupported platelet count of 45 x 10^9/L on Day 440, that technically did not meet engraftment criteria because it was not sustained on three consecutive measurements, having declined to 19 x 10^9/L by the time he was diagnosed with MDS. In addition to the low platelet counts, Subject 104-08 had leukocyte, neutrophil, and hemoglobin levels that were abnormally low at most or all assessments.

Integration site analysis (ISA) for Subject 104-08 was performed at 6, 12, 18, 24, and 26 months after eli-cel administration. ISA demonstrated integration into *MECOM, ACTR, RAP2C*, and *STGAL6,* each with a relative frequency of integration in $CD15^+$ cells of approximately 15 to 25%. Criteria for clonal predominance were met at six months, and criteria for persistence were met beginning at 12 months. Increased *EVI1* expression in the *MECOM* locus was present in whole blood, and the specific *MECOM* integration (3+168881163) corresponding to the clone was identified in the megakaryocytes.

Multiple bone marrow biopsies and aspirates were performed due to the subject's delayed recovery of blood counts. At Day 60, Year 1, and Year 1.5, bone marrow biopsies were notable only for hypocellularity. At 22 months, the subject's bone marrow was found to be normocellular (80%) with trilineage hematopoietic maturation,

numerous dysplastic megakaryocytes, 1% blasts, consistent with MDS. Flow cytometry was negative and cytogenetics (FISH, karyotyping and rapid heme panel) were normal. After a bone marrow biopsy at 2.5 years demonstrated persistent MDS, the subject underwent treatment with allogenic hematopoietic stem cell transplant. The last report received on February 11, 2022, was that his MDS was in remission.

Subject 102-03
Subject 102-03 was treated with eli-cel on (b) (6), at the age of 5, and he was diagnosed with MDS versus AML approximately 7.5 years later. This case of MDS appears to be caused by eli-cel given the integration into a proto-oncogene of the predominant clone. The Applicant has concluded the malignancy is likely mediated by the Lenti-D LVV.

Subject 102-03 had a comparatively uneventful early clinical course in that neutrophil and platelet engraftment occurred on Day 37, and blood counts returned to the normal range. However, it is notable that he is one of only four subjects who had a platelet count of < 100 x 10^9/L more than 100 days after eli-cel administration (91 x 10^9/L on Day 135). He was also slower than average in recovering WBC and hemoglobin values, as one of eight subjects with WBC < 2 x 10^9/L and the only subject with Hgb < 8.0 g/dL between Day 60 and 100. His CBC values were, nonetheless, completely normal between 1.5 years and approximately 7.5 years post-eli-cel, when he presented with fatigue, pallor, and petechiae, and was found to have thrombocytopenia and anemia (Hgb 10.8 g/dL, PLT 25 x 10^9/L, WBC normal).

Integration site analysis (ISA) for Subject 102-03 was performed eleven times between Month 3 and Month 60 using (NR)LAM-PCR, and while there were several results that might have raised concern (i.e., relative integration frequencies in *MDS1* of 19% at Month 8, *SMG6* of 26% at Month 30, and *INO80* of 18% at Month 30), none of them met criteria for quantitative assessment via qPCR or otherwise appeared to persist or increase in the latter assessments.

In 2019, the ISA method for the study was changed and therefore, when the subject presented with thrombocytopenia at Year 7.5, his ISA was performed using S-EPTS/LM-PCR instead of (NR)LAM-PCR. S-EPTS/LM-PCR identified integration sites in *PRDM16, MIR106A, CAMK2A, GAB3, TYK2,* and *SNX12* with relative frequencies between 13 and 18 percent. As of the time of this writing, partial confirmatory qPCR results are available, demonstrating that most cells in the bone marrow and peripheral blood contain integrations in *PRDM16, GAB3,* and *SNX12.* Vector copy number values are provided in the following table.

Table 25: Vector Copy Numbers for PRDM16, GAB3, and SXN12 for Subject 102-03

Cell Source	Method	***PRDM16*** **VCN (c/dg)**	***GAB3*** **VCN (c/dg)**	***SNX12*** **VCN (c/dg)**
Whole blood	qPCR	0.83	0.76	0.55

Bone marrow	qPCR	0.84	0.87	0.60

Abbrev: VCN, vector copy number; c/dg, copies per diploid genome
Source: Derived from FDA 102-03 ISA & Single Colony BM Form received April 15, 2022

This is strongly supportive of the causal clone containing an integration into the proto-oncogene PRDM16, although RNA sequencing that might confirm eli-cel's causal role in this subject's malignancy is pending.

Bone marrow biopsy and aspirate were performed when the subject presented with severe thrombocytopenia at Year 7.5. Findings were 60-70% cellularity with 15% myeloblasts, and CD34+ cells making up 20-30% of cells in some discrete foci on immunohistochemistry. He was diagnosed with MDS with excess blasts, worrisome for evolving AML. FISH and karyotype were normal. A rapid heme panel showed KRAS and NRAS mutations at 14% and 3% VAF. Analysis of somatic variants of unknown significant showed JAK c269T>c (p.I889T) at 48% variant allele frequency. Blast cells from peripheral blood and bone marrow aspirate collected on (b) (6), were positive for the lentiviral vector.

The subject was initially treated with chemotherapy. Bone marrow biopsy on (b) (6), demonstrated hypocellular marrow with trilineage hematopoiesis including paucity of maturing myeloid population, 1% CD34+ blasts. On (b) (6), the subject underwent HSCT for treatment of MDS/AML that was complicated by septic shock. The last available information about this child, from March 14, 2022, is that he is neutropenic with residual liver function abnormalities attributed to the shock event; and from March 22, 2022, that a bone marrow biopsy demonstrated marked hypocellularity, with markedly reduced myeloblasts compared to pre-transplant, consistent with a bone marrow in early phase of recovery although minimal persistent MDS could not be completely excluded. Flow cytometry demonstrated 0.15% myeloblasts. FISH was negative, karyotype normal, and NGS (Rapid Heme Panel) pending.

Appendix 12: Subjects Concerning for Developing of Malignancy After Treatment with Eli-cel

Subject 102-31

Subject 102-31 was treated with eli-cel on (b) (6), at the age of 4, and has a concerning integration site in the *MECOM* proto-oncogene. This integration site is increasing in relative frequency, currently represents 40% of $CD15^+$ cells in the peripheral blood, and is accompanied by increased *EVI1* expression.

Subject 102-31 achieved neutrophil engraftment on Day 32 and platelet engraftment on Day 60. His blood counts have been normal since Month 6 with the exception of platelet counts that have been mildly reduced (nadir of 114 x 10^9/L at 15 months). His last CBC, on (b) (6), was normal except for mild anemia (WBC 5.1 x 10^9/L, ANC 3.5 x 10^9/L, Hgb 11.2 g/dL, PLT 184 x 10^9/L).

ISA shows LVV integrations into *MECOM* and *EVI5* that have risen in relative frequency at the last three assessments, at Months 24, 42, and 48. The *MECOM* and *EVI5*-containing clone appears to have overtaken an earlier-appearing clone with integration sites in *SECISBP2, PLAG1,* and *PUM3* that peaked in relative frequency at Month 18. The trends in relative frequency of the integration sites corresponding to these two likely clones are demonstrated in the following table, which includes available ISA data for these frequent integration sites by timepoint, to include S-EPTS/LM-PCR results, confirmatory qPCR results, and VCN data. In bold are the instances where the combined relative frequencies of the integration sites in a clone exceed 30%. Despite the high relative frequencies of integration sites apparently in the same clone starting at Month 12, protocol specified criteria for clonal predominance were not met until Month 48, when the VCN for *MECOM* and *EVI5* in CD15+ exceeded the required threshold of 0.5 c/dg.

Table 26: Integration Site Frequency and Vector Copy Number for Two Likely Clones in Subject 102-31

Time Point	Cell Type	Method	*MECOM* Freq (%)	*MECOM* VCN (c/dg)	*EVI5* Freq (%)	*EVI5* VCN (c/dg)	*SECISBP2* Freq (%)	*SECISBP2* VCN (c/dg)	*PLAG1* Freq (%)	*PLAG1* VCN (c/dg)	*PUM3* Freq (%)	*PUM3* VCN (c/dg)	PB VCN All IS (c/dg)
Month 6	WB	Q-PCR	--	--	--	--	1.9	.03	1.8	0.03	1.5	0.02	1.8
Month 12	WB	Q-PCR	--	--	--	--	**18.1**	0.32	**18.7**	0.33	**18.3**	0.28	2.0
Month 18	WB	S-EPTS/ LM-PCR	--	--	--	--	**23.7**	--	**23.4**	--	**22.1**	--	2.1
--	WB	Q-PCR	--	--	--	--	**22.8**	0.48	**21.0**	0.45	**20.8**	0.41	
Month 24	WB	S-EPTS/ LM-PCR	6.2	--	4.8	--	**19.1**	--	**19.6**	--	**18.6**	--	1.7
--	WB	Q-PCR	--	--	--	--	**18.5**	0.30	**17.6**	0.30	**16.1**	0.25	--
--	CD3	Q-PCR	--	--	--	--	**16.2**	0.34	**15.5**	0.17	**14.5**	0.17	--
--	CD15	Q-PCR	--	--	--	--	**17.7**	0.35	**16.9**	0.35	**15.8**	0.31	--
Month 42	WB	S-EPTS/ LM-PCR	**21.9**	--	**17.5**	--	**14.6**	--	**15.3**	--	**14.4**	--	NR
--	WB	Q-PCR	**18.7**	0.33	**19.6**	0.34	**12.3**	0.20	**11.8**	0.20	**11.7**	0.18	--
--	CD3	Q-PCR	0.2	0.002	0.2	0.003	**11.3**	0.14	**10.8**	0.13	**10.5**	0.14	--
--	CD15	Q-PCR	**19.6**	0.41	**21.5**	0.45	**10.9**	0.24	**10.9**	0.26	**8.8**	0.21	--
Month 48	WB	S-EPTS/ LM-PCR	**32.7**	NR	**27.1**	NR	7.6	NR	7.5	NR	7.3	NR	NR
--	WB	Q-PCR	NR	0.41	NR	0.48	NR	0.14	NR	0.14	NR	0.13	--
--	CD3	Q-PCR	NR	0.006	NR	0.41	NR	0.23	NR	0.23	NR	0.21	--
--	CD15	Q-PCR	NR	0.53	NR	0.67	NR	0.13	NR	0.15	NR	0.11	--

Abbrev: WB, whole blood as cell source; CD3, $CD3^+$ population of peripheral blood as cell source; CD15, $CD15^+$ population of peripheral blood as cell source; Freq, relative frequency; VCN, vector copy number; c/dg, copies per diploid genome; IS, integration sites; NR, not reported
Note: Integration site frequencies in **bold** when multiple integration sites are apparently in the same clone and add up to > 30%.
Source: Reviewer's analysis derived from Listing 80.1.29 Integration Site Analysis Subject 102-31 & 72-Hour Reporting Form – ISA Tier 1 & Tier 2, CBC

Subject 102-31 had a bone marrow biopsy and aspirate on (b) (6), that demonstrated 40-50% cellularity with maturing trilineage hematopoiesis, complete maturation of myeloid and erythroid elements, no significant dysplasia, and no increase in blasts. Flow cytometry was negative, and cytogenetics (FISH, chromosomal analysis, and Rapid Heme Panel NGS) were normal.

Gene expression studies performed on PB from Month 24, Month 42, and Month 48 demonstrate overexpression of *MECOM and EVI1* that has increased over time.

Subject 102-11

Subject 102-11 was treated with eli-cel on (b) (6), at the age of 7, and he has a concerning integration site in the *MECOM* proto-oncogene of a predominant clone. Nearly 100% of Subject 102-11's $CD15^+$ cells are derived from a single clone with integration in *MECOM*, and he has and increased *EVI1* expression in $CD15^+$, $CD15^-$, and $CD3^-$ cells from peripheral blood.

Subject 102-11 achieved neutrophil engraftment on Day 27 and platelet engraftment on Day 41. Blood counts rose to the normal range within 3 months after treatment with eli-cel and have largely remained within the normal range. His last CBC, on (b) (6), was normal (WBC 6.4 x 10^9/L, ANC 2.2 x 10^9/L, Hgb 14.9 g/dL, PLT 307 x 10^9/L).

Subject 102-11 has a clone with three integration sites, *MECOM, ACER3,* and *RFX3* that have steadily increased in frequency since first observed at Month 12 and most recently (at Year 6.5) accounts for 97% of integration sites in $CD15^+$ cells. The changes in relative frequency of the three integration sites as well as an increase in vector copy number over time are shown in the following table.

Table 27: Integration Site-Specific Frequency and Vector Copy Number for *MECOM, ACER3,* and *RFX3* in Subject 102-11

Time Point	***MECOM* Primers Frequency / VCN (% / c/dg)**	***ACER3* Primers Frequency / VCN (% / c/dg)**	***RFX3* Primers Frequency / VCN (% / c/dg)**
Month 6 WB	--	--	0.05 / 0.0002
Month 12 WB	0.232 / 0.0013	0.29 / 0.0016	0.256 / 0.0013
Year 2 WB	2.472 / 0.007	2.296 / 0.0075	2.427 / 0.0078
Year 2.5 WB	5.728 / 0.0151	7.171 / 0.0181	5.925 / 0.0147
Year 3 WB	13.637 / 0.0467	19.354 / 0.0561	14.215 / 0.0408
Year 3.5 WB	21.023 / 0.0891	23.483 / 0.1052	24.113 / 0.1005
Year 4 WB	20.772 / 0.0847	22.374 / 0.0958	21.15 / 0.0996
US Year 4.1 BM	24.781 / 0.1926	26.505 / 0.2253	26.974 / 0.211
US Year 4.1 WB	--	25.149 / 0.1582	23.99 / 0.1541

Time Point	*MECOM* Primers Frequency / VCN (% / c/dg)	*ACER3* Primers Frequency / VCN (% / c/dg)	*RFX3* Primers Frequency / VCN (% / c/dg)
US Year 4.1 CD15$^+$	24 / 0.2109	25.599 / 0.2533	28.018 / 0.2384
USV Year 4.2 WB	23.226 / 0.1383	--	22.201 / 0.1355
Year 4.5 WB	23.123 / 0.1132	24.276 / 0.1436	22.886 / 0.1389
Year 5 WB	23.762 / 0.1232	26.792 / 0.145	24.575 / 0.1475
Year 5 CD15$^+$	32.088 / 0.3838	36.8 / 0.4169	32.211 / 0.4149
USV Year 5.25 WB	30.239 / 0.275	31.361 / 0.2686	24.077 / 0.2174
USV Year 5.25 CD15$^+$	30.864 / 0.4791	32.549 / 0.4714	26.975 / 0.4161
USV Year 5.5 WB	25.702 / 0.2806	30.469 / 0.2682	27.838 / 0.2841
USV Year 5.5 CD15$^+$	**29.65 / 0.5886**	**32.776 / 0.4919**	**35.388 / 0.5584**
USV Year 6 WB	24.405 / 0.2988	25.808 / 0.316	23.647 / 0.2895
USV Year 6 CD15$^+$	**27.168 / 0.7408**	**29.576 / 0.8065**	**27.617 / 0.7531**
USV Year 6.5 WB	**34.083 / 0.6913**	**34.849 / 0.7068**	**31.635 / 0.6417**
USV Year 6.5 CD15$^+$	**29.745 / 0.7604**	**38.512 / 0.9846**	**28.781 / 0.7358**

Abbrev: VCN, vector copy number; WB, whole blood as cell source; CD15$^+$, CD15$^+$ population of peripheral blood as cell source; USV, unscheduled visit
Note: Integration site frequency and VCN in **bold** when criteria for predominant clone are met
Source: Reviewer's analysis, derived from BLA 125755 ADISAVCN dataset

In addition to almost 100% clonal predominance and a rising vector copy number, Subject 102-11 has increased expression of *EVI1*, which is concerning for malignancy.

Subject 102-11's numerous bone marrow biopsies have demonstrated moderate hypocellularity (30-40% at last assessment in July 2021). Flow cytometry has been negative, and cytogenetics (FISH, chromosomal analysis, and Rapid Heme Panel NGS) are normal.

Subject 104-09

Subject 104-09 was treated with eli-cel on (b) (6), at the age of 9. He had prolonged, profound, post-transplant pancytopenia which was initially attributed by the investigator to parvovirus infection. However, parvovirus is unlikely to fully explain his hematologic abnormalities because parvovirus typically causes anemia and has characteristic bone marrow findings that were absent in this case. Conversely, Subject 104-09's long-lasting thrombocytopenia, hypocellular bone marrow with atypical platelet progenitor cells, and integration into proto-oncogenes are highly concerning factors that point to evolving malignancy.

Subject 104-09 received numerous platelet and red blood cell transfusions for more than two months after eli-cel treatment, and thereafter low blood counts were treated with bone marrow stimulants, filgrastim and eltrombopag, until approximately four and

ten months post-eli-cel, respectively. Subject 104-09 was found to have parvovirus in the bone marrow two months after eli-cel administration, to which his low blood counts were initially attributed. However, the FDA's thinking is that the relative timing and severity of his cytopenias and his bone marrow findings ultimately do not support parvovirus as the cause of his ongoing thrombocytopenia.

Parvovirus B19 is known to infect the progenitors of red blood cells in the bone marrow and thereby cause cessation of red blood cell production. Bone marrow biopsy characteristics indicating parvovirus infection are an absence of maturing erythroid precursors and the presence of giant pronormoblasts. Parvovirus-induced cessation of red blood cell production is overall short-lived and not problematic in individuals with healthy immune systems and otherwise normal red blood cells. Individuals with immune deficiency and inability to clear the infection may develop anemia.

While anemia is the predominant clinical manifestation of parvovirus, parvovirus can cause a broad spectrum of illness. In immunocompromised individuals, it has also been linked to thrombocytopenia and inflammation of several vital organs. The immunocompromised may not mount an effective immunoglobulin response to be able to clear a parvovirus infection. Therefore, immunocompromised individuals with symptomatic parvovirus infection are usually treated with intravenous immunoglobulin, and in the HSCT subset, intravenous immunoglobulin usually provides long-term resolution of parvovirus signs and symptoms.

The severity and timeframe for Subject 104-09's cytopenias do not support parvovirus as the cause of his cytopenias. While the predominant hematologic manifestation of parvovirus is anemia, Subject 104-09's anemia was comparatively mild and had resolved by six months, whereas his low white blood cell (i.e., neutrophil and lymphocyte) and platelet counts were both more severe and longer lasting. Lymphocytes remained below normal for approximately one year and neutrophils for more than 1.5 years. Platelet counts remained below normal at 100 x 10^9/L when last measured on (b) (6), more than 2 years after eli-cel administration.

Also problematic with attributing this subject's hematologic abnormalities to parvovirus is their failure to resolve after treatment with intravenous immunoglobulin, which was administered approximately 2.5 months post-eli-cel.

Subject 104-09 had numerous bone marrow biopsies to evaluate the etiology of his pancytopenia. None of them demonstrated the pronormoblasts that are pathognomonic of parvovirus. Additionally, each of the bone marrow biopsy reports remarked on the presence of complete erythroid maturation, whereas anemia caused by parvovirus is characterized by an absence of maturing erythroid precursors.

The bone marrow biopsy at two months post-eli-cel demonstrated marked hypocellularity (~5%) with markedly reduced but complete erythroid and granulocytic maturation, and markedly decreased megakaryocytes. Karyotype was normal. Parvovirus was detected by PCR and has remained positive in the bone marrow at all subsequent time points.

Bone marrow biopsy one year post-eli-cel demonstrated cellularity 30-40% with trilineage hematopoiesis, no increase in blasts, and no definitive dysplasia.

Bone marrow biopsy at two years post-eli-cel demonstrated cellularity 60-70% with trilineage hematopoietic maturation, atypical megakaryocytes (with widely spaced nuclei and/or small size, representing < 10% of total megakaryocytes). Cytogenetics (karyotype and Rapid Heme Panel NGS) were normal. Peripheral blood smear was noted to have very rare, atypical cells with morphology suggestive of blasts versus immature granulocytes. Flow cytometry of peripheral blood demonstrated 9% polytypic B cells and no aberrant immunophenotype on T cells.

Bone marrow biopsy at 2.2 years post-eli-cel demonstrated cellularity 30-40% with trilineage hematopoiesis; atypical megakaryopoiesis comparable to the 2-year bone marrow in regard to number of atypical megakaryocytes, morphologic features, and absence of clustering; and 2% blasts. Flow cytometry demonstrated no increase in myeloid blasts and no definitive abnormal myeloid bast population; however, some $CD34^+$ cells with increased CD7. Karyotype was normal.

A myelodysplastic syndrome-focused NGS panel at 2.2 years post-eli-cel revealed a likely pathogenic loss-of-function heterozygous variant in the *MPL* gene (p.R102P) at 0.4669 VAF. This variant had been detected in peripheral blood prior to eli-cel administration, and therefore is not attributable to eli-cel. *MPL* is important for development of platelets, and *MPL* variants may be associated with abnormally low or high platelet counts. However, this subject had a normal platelet count at baseline (300 x 10^9/L) and did not seem to have any effect on his platelet counts prior to eli-cel administration. Also found in the assessment at 2.2 years was an alteration in *CALR* (D165G) at 0.4742 VAF. This is variant of unknown significance.

Integration site analysis (ISA) demonstrated integration into *MECOM,* although the relative frequency declined from 7.1% at Month 12 to 4.3% at Month 26, which was the most recent assessment. ISA identified the clones with highest relative frequency and a slight upward trend at 26 months as *LINC00982* and *SMG6*, at 10.4% and 8.6%, respectively. This subject also has integrations into *MPL* that are at a comparatively low, but increasing frequency:

Table 28: MPL Integration Site Data for Subject 104-09

Time	PCR Method	Relative Frequency
M6	S-EPTS	0.113574
M12	S-EPTS	0.445554
M18	S-EPTS	1.616869
M24	S-EPTS	2.151184

Source: Reviewer's analysis, derived from BLA 125755 dataset 104-09_allISA_Nov2021

In summary, Subject 104-09 has ongoing thrombocytopenia that cannot be attributed to parvovirus, because it is not suggested by the timing of his cytopenias and because the bone marrow biopsy findings are not suggestive of parvovirus infections. Rather, his bone marrow findings are consistent with developing malignancy, particularly in the setting of several clones with integration sites in proto-oncogenes.

Subject 104-22

Subject 104-22 was treated with eli-cel on (b) (6), at the age of 13. He has concerning integration site patterns because of a rising relative frequency of integration into the proto-oncogenes *MECOM* and *MPL*. He has mildly low platelet counts but blood counts are otherwise normal.

Subject 104-22 achieved neutrophil engraftment on Day 13 and platelet engraftment on Day 29. Platelet counts have not returned to normal levels. Other blood counts have been normal except for leukocytes and neutrophils at approximately 3 months post-eli-cel. His last CBC submitted to the BLA, performed on (b) (6), was normal except for mildly low platelets (WBC 4.9 x 10^9/L, ANC 2.7 x 10^9/L, Hgb 14.5 g/dL, PLT 118 x 10^9/L).

Integration site analysis results for the top two sites for Subject 104-22 are summarized in the following table, which demonstrates overall increases in frequency in two proto-oncogenes, *MPL* and *MECOM*, between 6 and 18 months. Because of the difference in relative frequency between the two genes, they do not appear to be in the same clone. However, integration site-specific relative frequency and integration site-specific vector copy number for *MPL* and *MECOM* from Months 12 and 18 are pending.

Table 29: Relative Frequencies of MECOM and MPL by S-EPTS/LM-PCR for Subject 104-22

Time Post-Eli-Cel	*MECOM* Relative Frequency (%)	*MPL* Relative Frequency (%)	Overall VCN (c/dg)
Month 6	1.2	4.1	0.15
Month 12	4.8	19.7	0.13

Month 18	4.1	14.1	NR

Abbrev: S-EPTS/LM-PCR, shearing extension primer tag selection ligation-mediated polymerase chain reaction; c/dg, copies per diploid genome; NR, not reported
Source: Reviewer's analysis derived from Listing 80.1.49 Integration Site Analysis Subject 104-22 and FDA 72-Hour Reporting Form – ISA Tier 1 & Tier 2, CBC