

BLA Clinical Review Memorandum

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Applicant	bluebird bio, Inc. concur with recommendation for Accelerated Approval
Established Name	Elivaldogene autotemcel
(Proposed) Trade Name	Skysona
Pharmacologic Class	Gene therapy
Formulation(s), including Adjuvants, etc.	Transduced autologous CD34+ cells, washed, suspended, and cryopreserved, containing 5% dimethyl sulfoxide
Dosage Form(s) and Route(s) of Administration	A suspension for intravenous infusion
Dosing Regimen	Single dose for infusion, with a recommended minimum dose of 5 x 10 ⁶ CD34+ cells/kg
Indication(s) and Intended Population(s)	For the treatment of patients less than 18 years of age with early cerebral adrenoleukodystrophy who do not have an available and willing human leukocyte antigen-matched sibling hematopoietic stem cell donor
Orphan Designated (Yes/No)	Yes

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GLOSSARY

ABCD1	Adenosine triphosphate binding cassette, subfamily D, member 1
AC	Advisory Committee
ALDP	Adrenoleukodystrophy Protein
Allo-HSCT	Allogeneic hematopoietic stem cell transplant
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
AMN	Adrenomyeloneuropathy
AST	Aspartate aminotransferase
BLA	Biologics License Application
BM	Bone marrow
BMCs	Bone marrow cells
c/dg	Copies per diploid genome
CALD	Cerebral adrenoleukodystrophy
CBC	Complete 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
GLP	Good laboratory practice
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
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
NOAEL	No observed adverse effect level
(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
PD	Pharmacodynamics
PE	Platelet engraftment
PK	Pharmacokinetics
PLT	Platelet
PMR	Post-marketing requirement
polyA	Polyadenylation
PrvIQ	Performance/Reasoning/Visual Intellectual Quotient
PS	Propensity Score
qPCR	Quantitative polymerase chain reaction
RCL	Replication-competent lentivirus
REMS	Risk Evaluation and Mitigation Strategy
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
SGE	Special government employee
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
VABS	Vineland Adaptive Behavior Scale
VAF	Variant allele frequency
VCN	Vector copy number
VLCFA	Very long chain fatty acids
WBC	White blood cell
X-ALD	X-linked adrenoleukodystrophy

1. Executive Summary

On October 18, 2021, bluebird bio, Inc. submitted an original Biologics License Application (BLA), STN BL 125755, for licensure of elivaldogene autotemcel (eli-cel) with the proprietary name of SKYSONA. Eli-cel is an autologous hematopoietic stem cell-based gene therapy. The Applicant proposed the indication, “for the 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.”

Childhood CALD is a rare neurodegenerative X-linked metabolic disease in boys 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 adenosine triphosphate binding cassette, subfamily D, member 1 (*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 4-10 years of age. The disease progresses to vision and hearing impairment, gait difficulties, seizures, cognitive impairment, weakness and stiffness of limbs, and incontinence with eventual loss of voluntary movement, loss of communication, deafness, cortical blindness, and death.

Currently, there are no FDA-approved treatments for CALD, but allogeneic hematopoietic stem cell transplant (allo-HSCT) is the standard of care^{12,26} for boys with early, active CALD. As allo-HSCT is associated with significant morbidity and mortality, particularly when a suitable HLA- matched donor cannot be found, there is a substantial unmet medical need for patients with CALD.

Eli-cel consists of an autologous CD34+ cell-enriched population, that contains the patient’s own hematopoietic stem cells (HSCs), transduced ex vivo with the Lenti-D lentiviral vector (LVV) containing the *ABCD1* gene encoding ALDP. Eli-cel is supplied frozen in 20 mL fluoro-ethylene-propylene bags as a suspension for intravenous infusion. Each bag contains between 4×10^6 and 30×10^6 cells/mL (3.6 to 30×10^6 CD34+ cells/mL), frozen in approximately 20 mL of cryopreservation solution. The minimum dose is 5.0×10^6 CD34+ cells/kg patient weight.

In the eli-cel clinical trials, patients underwent HSC mobilization and apheresis followed by full myeloablative and lymphodepleting conditioning. Granulocyte colony stimulating factor (G-CSF) and, in most patients, plerixafor, were used for mobilization, followed by apheresis to harvest the cells. Apheresed cells were shipped to the manufacturing site where CD34+ cells were selected for and transduced with Lenti-D LVV to manufacture eli-cel. After return of the transduced cells to the treatment site, subjects underwent conditioning with busulfan for myeloablation and cyclophosphamide or fludarabine for lymphodepletion. Eli-cel was subsequently infused to reconstitute the hematopoietic system with cells containing the integrated *ABCD1* gene that produces functional ALDP.

Consistent with 21 USC 355(d), substantial evidence of effectiveness of eli-cel for this rare disease with unmet need is based on a single adequate and well controlled investigation with confirmatory evidence. For the purpose of this approval decision, we

considered a subset of pooled clinical data from 2 single-arm, open label clinical studies, ALD-102 (Phase 2/3) and ALD-104 (Phase 3), compared to external control data from a study that included untreated CALD patients, ALD-101, to constitute one adequate and well controlled investigation.

Studies ALD-102 and ALD-104 enrolled subjects ages 4-17 years of age with early, active CALD, defined by a Neurologic Function Score (NFS) ≤ 1 and brain magnetic resonance imaging (MRI) with gadolinium enhancement (GdE+) and a Loes Score 0.5-9. Most subjects (58/61, 95%) were asymptomatic at baseline. The external control data were from an untreated natural history population with early, active disease from Study ALD-101, an historical, retrospective study of untreated CALD subjects and subjects who had been treated with allo-HSCT. The other external control study (ALD-103) was a hybrid retrospective-prospective observational study of subjects who were treated with allo- HSCT. The clinical reviewers recommend accelerated approval of eli-cel for a modified indication of pediatric patients with early, active CALD without an available HLA-matched donor.

The primary efficacy endpoint was the proportion of subjects in Study ALD-102 who were alive and had none of the six defined Major Functional Disabilities (MFDs) at the Month 24 Visit (i.e., Month 24 MFD-free survival). MFDs were defined as loss of communication, cortical blindness, tube feeding, total incontinence, wheelchair dependence, and complete loss of voluntary movement. To be considered a success on the primary efficacy endpoint (i.e., achieve Month 24 MFD-free survival), at the Month 24 visit, subjects must be alive, MFD-free, not have received rescue cells or allo-HSCT, and not withdrawn from the 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 in pre-submission meetings with the primary efficacy endpoint and clinical benchmark for success, FDA emphasized in these pre-BLA meetings that comparability of external control groups to the eli-cel-treated subjects would need to be demonstrated to support the validity of the benchmark. Thirty-two subjects with early, active CALD were enrolled in Study ALD-102 and treated with eli-cel. Six subjects received investigational product for which comparability to the to-be-marketed product was not demonstrable and were excluded from the analysis. Twenty-three out of 26 (88%) subjects achieved Month 24 MFD-free survival (95% CI: 70%, 98%). ALD-102 was thus successful on the primary efficacy endpoint.

Although ALD-102 met the success criterion for the primary efficacy endpoint, results were difficult to interpret due to:

- 1) lack of comparability between the eli-cel-treated subjects and the populations from ALD-101 that were used to determine the clinical benchmark. Untreated subjects were diagnosed at a time when disease understanding was evolving, contrast was not routinely used for MRI assessments, and delayed diagnosis was common.
- 2) insufficient length of study to ensure that the results suggesting superiority of treatment (with allo-HSCT or eli-cel) compared to lack of treatment were not simply an artifact of early case identification.
- 3) an imputation strategy that over-estimated the number of failure events (and thus dropped the lower bound of the 95% CI) in the allo-HSCT-treated early, active disease subpopulation from ALD-101.

Given these limitations, this data could not be relied on to support approval. The main challenges were that few events (MFDs and deaths) occurred in the allo-HSCT and SKYSONA populations, and subjects treated with allo-HSCT and SKYSONA were generally diagnosed and treated at very early stages of disease. In comparison, event rates were high in the untreated natural history population, but the natural history population was older, with more advanced cerebral disease on MRI, and more likely to present with symptomatic disease at time of diagnosis or shortly after diagnosis. As a result, it was difficult to determine if the lower numbers of MFDs and deaths in the treated populations were due to a treatment effect or due to treatment at an early stage of disease with insufficient duration of follow-up to detect progression to MFD or death. It is unclear what the clinical course would have been in subjects with very early stages of disease had they not been treated. In essence, it was not possible to use all the available efficacy data to compare outcomes following treatment with eli-cel (and allo-HSCT) to untreated CALD because of the concern for lead-time bias in comparisons of eli-cel to the natural history of untreated disease.

In an attempt to better understand the natural history of untreated, early active disease, modeling was performed to evaluate the timing of clinical disease progression to MFDs and death from first onset of symptomatic disease in more comparable subsets of the eli-cel, allo-HSCT, and untreated natural history populations at higher risk of rapid clinical progression. This modeling led to the exploratory post-hoc analysis that formed the basis for recommendation of product approval.

The recommendation for accelerated approval is based primarily on an intermediate clinical endpoint reasonably likely to predict long-term benefit. Kaplan-Meier (KM) time to event analysis in a symptomatic subset of eli-cel-treated subjects and similar untreated controls demonstrated a slowed progression of neurologic dysfunction (NFS \geq 1) assessed by major functional disabilities (MFDs) or death at 24 months from time of symptom onset as compared to the natural history population.

The additional confirmatory evidence of efficacy consists of:

- 1) Trends toward delayed symptom onset in a small number (n=5) of eli-cel-treated subjects based on disease modeling.
- 2) Resolution of gadolinium enhancement on brain MRI (i.e., GdE-) at Month 24 following treatment in the majority (33/36, 92%) of eli-cel-treated subjects.

- 3) Pharmacodynamic response data for the number of CD14+ %ALDP+ cells (the functional cells) at Month 6 following treatment which showed differences between subjects who did and did not experience an MFD, death or receive rescue allo-HSCT by Month 24 following treatment.
- 4) Nonclinical data that support a pharmacologic effect on VLCFA metabolism.

We recognize limitations of post-hoc analyses and concerns about reliance on such analyses as the basis of approval, but feel the populations in these analyses are comparable, high-risk populations and that the results plus the above stated confirmatory evidence indicate substantial evidence of efficacy on an intermediate clinical endpoint reasonably likely to predict long-term clinical benefit.

The benefit-risk evaluation of this product was complicated by a worrisome and life-threatening safety finding that is directly attributable to the product: insertional oncogenesis. Three subjects developed hematologic malignancy after treatment with eli-cel. The first two cases were diagnosed approximately 1 and 2 years after treatment with eli-cel, before these patients may have experienced any clinical benefit from the treatment. The third case of hematologic malignancy occurred approximately 7.5 years after treatment with eli-cel, in the first subject to have been treated with eli-cel, who therefore had the longest timeframe for malignancy to potentially develop.

While the incidence of hematologic malignancy in the trials is 4% (3 of 67 subjects), this does not likely reflect the true risk of insertional oncogenesis because of the short period of follow-up for many of the subjects, with 99% having less than 7.5 years of follow-up data. Furthermore, integration into proto-oncogenes is ubiquitous, and although the clinical significance of an integration site in isolation is limited, our observation of the growth of clones with integration sites in proto-oncogenes suggests that some of these clones have a selective advantage and may evolve into cancer. Adding to the concern for insertional oncogenesis are the handful of subjects who have bone marrow dysplasia demonstrated on biopsy in addition to having evidence of clonal expansion.

Because of the novelty of and uncertainties surrounding the assessment of LVV-mediated hematologic malignancy, the clinical team consulted with a special government employee (SGE), Dr. Lucy Godley, a physician with expertise in hematologic malignancy. She provided support in the assessment of causality of the vector in the three cases of malignancy, and advice about how subjects should be evaluated for the development of hematologic malignancy after treatment with eli-cel.

External input was also sought through an Advisory Committee meeting that occurred on 09 Jun 2020. The review team presented concerns about insertional oncogenesis and uncertainties in the efficacy data, and sought input the AC's input regarding the benefit-risk calculation for eli-cel. The AC agreed that insertional oncogenesis is a serious and important risk of eli-cel and that patients treated with eli-cel should be monitored closely for the development of hematologic malignancy. Despite the risk of malignancy, the AC voted unanimously (with one abstention) that the benefit-risk calculation for eli-cel is favorable. The population determined by the AC to have a favorable benefit-risk included boys without an available HLA-matched hematopoietic stem cell donor; the AC did not provide a clear consensus about the benefit-risk in boys with a matched non-sibling donor.

Additionally, because of the serious risk of hematologic malignancy, the clinical review team recommends approval with a Risk Evaluation and Mitigation Strategy (REMS). A REMS would ensure that subjects are followed closely and that cases of hematologic malignancy are identified early, and may improve patient outcomes through (1) diagnosis of malignancy when it is less likely to be refractory to treatment, (2) more time to find a better match for allo-HSCT to treat the malignancy.

Office leadership did not support a REMS and instead there is a Medication Guide as part of labeling and a Post-Marketing Requirement (PMR) to conduct a study to characterize the risk of hematologic malignancy, including its incidence, risk factors, prognosis, and outcomes. The study will enroll 120 subjects and require monitoring for malignancy via blood tests every 3 to 6 months during the first 15 years after treatment with eli-cel.

Although the primary evidence of effectiveness is based on a subset of subjects who had mild symptoms, we believe it is reasonable to extrapolate efficacy to asymptomatic (i.e., NFS=0) early, active CALD (brain MRI with Loes score 0.5-9 and presence of gadolinium enhancement) in boys 4-17 years of age due to the disease pathophysiology being the same. There are clinical data from patients treated with allo-HSCT to suggest that early replacement of functional ALDP offers increased clinical benefit.^{8,13,14,26} Additionally, there is evidence from Studies ALD-102 and ALD-104 of decreased brain inflammation detected by resolution of gadolinium enhancement on brain MRI at Month 24 in 33/36 (92%) of the entire early, active CALD study population treated with eli-cel who had MRI data at Month 24 following treatment, indicating that eli-cel could be favorably altering the disease course for boys with asymptomatic and symptomatic early, active CALD.¹¹

Despite this extrapolation of efficacy to the entire asymptomatic and symptomatic early, active CALD population, there are two populations for whom there is greater uncertainty regarding a favorable benefit-risk determination given the uncertainty of durability of effect and the magnitude of hematologic malignancy risk. Specifically:

- 1) Boys with CALD who present with the isolated pyramidal tract pattern of disease on brain MRI are known to have a slower progression of radiographic and clinical disease, typically with stable Loes score over time and prolonged duration between radiographic diagnosis and the onset of clinical disease (usually in adulthood).¹⁰ Two boys in the untreated natural history population with isolated pyramidal tract disease developed first symptoms at 19 and 20 years old despite diagnosis at 9 and 11 years old, respectively. Worse outcomes have been seen in boys with isolated pyramidal tract disease who have been treated with eli-cel. Three (30%) of 10 subjects with isolated pyramidal tract disease treated with eli-cel have received rescue allo-HSCT prior to reaching adulthood. One subject experienced progression of radiographic disease and was withdrawn from the study to receive rescue allo-HSCT and subsequently died of transplant-related causes. Two others developed myelodysplastic syndrome (MDS) and required allo-HSCT as treatment of the malignancy. The remaining 7 subjects, while stable, have not been followed for a sufficient duration to make any efficacy conclusions. Because of these worse outcomes and long latency period from diagnosis to onset of symptoms even without treatment, the benefit-risk profile for treatment of these boys does not appear to be favorable, particularly given uncertainties about durability of effectiveness.

- 2) A large number of subjects treated with eli-cel in the clinical studies had very early cerebral disease with NFS=0 (asymptomatic) and low Loes score (i.e., Loes score 1-2) at Baseline. Boys with very early radiographic and asymptomatic disease are poorly represented in the natural history of disease due to frequent delayed diagnosis at the time the natural history subjects were diagnosed, and thus the time course of expected clinical progression of disease is relatively unknown. Advancement of screening tools and establishment of clinical guidelines now enable early diagnosis and treatment of affected boys. As allo-HSCT is now standard of care upon diagnosis of early, active cerebral disease, there likely never will be an appropriate natural history comparator for this very early disease population. While it is almost certain disease will progress, we are uncertain of the timeframe of such progression. While we believe evidence of effectiveness can be extrapolated to the entire population of early, active CALD, including these patients with NFS=0 and low Loes score, without knowing the timing of expected onset of symptoms and disease progression, the **durability** of effectiveness and risks of treatment factored into the benefit-risk assessment.

Because 45 of 61 (74%) of subjects treated with eli-cel had a baseline Loes score of 1-2 and/or isolated pyramidal tract disease, the magnitude of uncertainty regarding the long-term efficacy of eli-cel is high in the greater population of boys who are diagnosed and treated for CALD. Relative long-term efficacy and benefit-risk assessment in these populations with isolated pyramidal tract disease or very early and asymptomatic disease could only be determined with a longer duration of follow-up.

The clinical reviewers recommend accelerated approval of eli-cel for the modified indication of pediatric boys with early, active CALD without an available HLA-matched donor. While we believe there is substantial evidence of efficacy on an intermediate clinical endpoint from a single adequate and well controlled investigation using external controls, we do not have enough data to understand the durability of effectiveness and the long-term benefit-risk assessment, particularly in subjects with very mild asymptomatic disease and/or isolated pyramidal tract disease at baseline who may not experience disease progression for several years in the absence of treatment. The serious and significant risk of hematologic malignancy further complicates the benefit-risk assessment in these same subjects. Because we feel short-term efficacy results can be extrapolated to the entire early, active CALD population with no symptoms or mild symptoms ($NFS \leq 1$), these concerns about durability of effectiveness and risks of hematologic malignancy primarily affect our decision to not recommend approval for the entire proposed CALD population of those without HLA-matched sibling donors. Although allo-HSCT is not FDA-approved, we feel that the medical literature supports the benefit of allo-HSCT when utilized early in the course of early, active CALD (as discussed in [Section 2.2](#)), including some evidence that the treatment is effective long-term.^{13,25} Additionally, in the KM analysis of MFD-free survival from time of first NFS ≥ 1 , the symptomatic allo-HSCT population had similar results over time to eli-cel, suggesting a similar short-term effectiveness of the two therapies. There were insufficient data for comparisons of relative long-term efficacy between the two treatments. We believe restricting the indication to only those without HLA-matched donors will provide a much-needed option for the early, active CALD patients who do not have a suitable match, as our review of the comparative allo-HSCT data demonstrated a significant early survival benefit of eli-cel as compared to the allo-HSCT population who received stem cells from an HLA-mismatched donor. The allo-HSCT population who received stem cells from

HLA-matched donors (sibling and unrelated) had similar outcomes on overall survival to the eli-cel-treated population during the course of follow-up in the studies; however, relative long-term efficacy of the two treatments is unclear due to insufficient long-term data. At this time, the benefit-risk assessment is most favorable for pediatric patients with early, active CALD who do not have an available HLA-matched HSCT donor. The uncertainties about the magnitude and severity of MDS and durability of effectiveness that complicate the benefit-risk assessment in the greater early, active CALD population regardless of donor can only be resolved with additional time in follow-up.

The clinical reviewers recommend accelerated approval of this BLA for the more limited population of boys with early active CALD without an available HLA-matched donor. Two Clinical PMRs are to provide confirmatory evidence to demonstrate the long-term efficacy of eli-cel in boys with early, active CALD through assessments of efficacy outcomes including MFDs, death, and NFS changes. One PMR will follow subjects treated with eli-cel in Studies ALD-102 and ALD-104 for at least 10 years after treatment and the other PMR will enroll and treat an additional 24 boys with more advanced early, active CALD and assess event-free survival over 5 years. The clinical review team also recommends a REMS to mitigate the risks of insertional oncogenesis to patients treated with this product.

1.1 Demographic Information: Subgroup Demographics and Analysis Summary

Sixty-seven (67) boys with early, active cerebral adrenoleukodystrophy were treated with eli-cel in two single-arm clinical trials. The median (min, max) age at time of treatment across the two studies was 6 (4,14) years; 100% were male; 54% were White/Caucasian, 4% were Black/African American, 1% were Asian, 10% were of other races including mixed race, and 30% did not report race; 25% were of Hispanic ethnicity. There was insufficient information to draw conclusions about differences in effectiveness or safety outcomes by subgroup via analysis of age, race and/or ethnicity.

1.2 Patient Experience Data

Data Submitted in the Application

Table 1: Patient Experience Data Submitted in the Application

Check if Submitted	Type of Data	Section Where Discussed, if Applicable
<input checked="" type="checkbox"/>	Patient-reported outcome	Section 6.1.11.5
<input checked="" type="checkbox"/>	Observer-reported outcome	Section 6.1.11.5
<input checked="" type="checkbox"/>	Clinician-reported outcome	Section 6.1.8
<input checked="" type="checkbox"/>	Performance outcome	Section 6.1.11.5
<input type="checkbox"/>	Patient-focused drug development meeting summary	
<input type="checkbox"/>	FDA Patient Listening Session	
<input type="checkbox"/>	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel)	
<input checked="" type="checkbox"/>	Observational survey studies	Section 5

Check if Submitted	Type of Data	Section Where Discussed, if Applicable
<input checked="" type="checkbox"/>	Natural history studies	Section 5
<input type="checkbox"/>	Patient preference studies	
<input type="checkbox"/>	Other: (please specify)	
<input type="checkbox"/>	If no patient experience data were submitted by Applicant, indicate here.	
Check if Considered	Type of Data	Section Where Discussed, if Applicable
<input type="checkbox"/>	Perspectives shared at patient stakeholder meeting	
<input type="checkbox"/>	Patient-focused drug development meeting	
<input checked="" type="checkbox"/>	FDA Patient Listening Session	Section 2.1
<input type="checkbox"/>	Other stakeholder meeting summary report	
<input type="checkbox"/>	Observational survey studies	
<input checked="" type="checkbox"/>	Other: Public hearing, AC Meeting	Section 2.1

Abbrev.: FDA, Food and Drug Administration; AC, Advisory Committee

2. Clinical and Regulatory Background

Bluebird bio, Inc. (the Applicant) has proposed the indication 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.”

2.1 Disease or Health-Related Condition(s) Studied

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 adrenoleukodystrophy protein (ALDP) needed to transport very long chain fatty acids (VLCFAs) into the peroxisome for degradation.^{1-3, 15-18} The accumulation of VLCFAs are believed to primarily affect the adrenal cortex through direct toxicity and affect the brain white matter by causing perivascular accumulation of cytotoxic T lymphocytes and expression of pro-inflammatory cytokines and chemokines that result in progressive inflammatory demyelination.^{3, 19-21} The most concerning symptoms of CALD are neurologic disability and premature death. The disease course is heterogeneous and marked by variable rates of progression depending on location, extent and contrast enhancement of lesions on brain magnetic resonance imaging (MRI), age at presentation, and presence or absence of neurologic dysfunction or neurocognitive deficits.⁴⁻¹⁴ Boys typically present with inattention, hyperactivity or academic challenges by 4-10 (median 7) years of age.^{4-7,25} Left untreated, the disease progresses to neurologic dysfunction, disability and ultimately to death, typically by the second decade of life from complications of the disease. Death typically occurs within 2-4 years of symptom onset, though some patients may survive in a severely disabled state for many years.²⁶ 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.

The Neurologic Function Score (NFS), a 25-point composite scale that focuses on 15 domains of neurologic function, is traditionally used to evaluate the clinical status of CALD patients.^{4,8} A score of 0 indicates absence of clinical signs of cerebral disease (i.e., asymptomatic), and higher scores correspond to increasing severity of neurological dysfunction. The full scale, including definitions, can be found in [Appendix 1: Neurologic Function Score \(NFS\)](#). The Major Functional Disabilities (MFDs) as defined in the BLA are a subset of the NFS that most significantly impact daily function. The six MFDs are (1) loss of communication, (2) cortical blindness, (3) requirement for tube feeding, (4) total incontinence, (5) wheelchair dependence, or (6) complete loss of voluntary movement.

Approximately 40% of patients with X-linked adrenoleukodystrophy develop cerebral adrenoleukodystrophy. The diagnosis is made once there is evidence of cerebral demyelination on brain MRI. Lesions are graded according to a Loes score,⁹ which assigns a severity score (0-34) score based on location and extent of demyelination, as well as presence/ absence of focal and/or global atrophy. A score of 0 indicates a normal MRI (i.e., no cerebral disease), and higher scores indicate increased severity of cerebral lesions.

Patterns of cerebral disease with prognostic implications have been identified,¹⁰ and are as follows:

- Pattern 1: Parieto-occipital white matter
- Pattern 2: Frontal white matter
- Pattern 3: Isolated pyramidal tract
- Pattern 4: Cerebellar white matter
- Pattern 5: Concomitant parieto-occipital and frontal white matter
- Other: Any pattern other than those characterized by patterns 1-5

It was found by Dr. Loes that MRI patterns appear to predict age of presentation, where patients with patterns 1 or 5 disease typically present in childhood, with patterns 2 or 4 disease present in adolescence, and with isolated pyramidal tract (pattern 3) disease present in adulthood.¹⁰ Patients with pattern 1 or 2 disease experience rapid disease progression if the pattern is present at an early age, particularly if gadolinium enhancement is present. Disease progression is generally slower in patients with pattern 3 or 4 disease. Pattern 5 disease is uncommon and is associated with much more rapid progression than other patterns. Additional literature reports the observation that disease is more likely to progress and/or be more rapidly progressive to neurologic dysfunction, disability and death if patients present in childhood with a greater degree of cerebral involvement (i.e., greater Loes scores) and gadolinium enhancement on brain MRI.^{4,11,13,14} Gadolinium enhancement on brain MRI has been associated with breakdown of the blood-brain barrier and is thought to represent progressive and active inflammatory demyelination associated with increased risk of rapid disease progression.¹¹

A Childhood Cerebral Adrenoleukodystrophy FDA Patient Listening Session was conducted 13 November 2019³² to better understand burden of disease, symptom progression, willingness to being involved and barriers to being involved in clinical trials

and natural history studies, and what aspects of function or daily life were most important to patients and caregivers to preserve. When asked about activities of daily life that were most important to preserve with any potential CALD treatment, caregiver responses varied, but responses from more than one participant included cognitive function and communication. A majority of caregivers were willing to accept severe or life-threatening risks associated with treatments for CALD, acknowledging the progressive nature of disease that would ultimately lead to death if left untreated. The caregiver who was not willing to accept risks had a child with advanced disease and did not want to proceed with something that might cause additional suffering.

Patient and caregivers of patients with CALD spoke during a public hearing at the Cellular, Tissue, and Gene Therapies Advisory Committee Meeting held 9 June 2022.³³ They primarily expressed concerns about progression to disability and losing the ability to “be a kid,” and the progression to death that occurs without treatment. Several speakers addressed concerns about graft versus host disease (GVHD) with allo-hematopoietic stem cell transplant (allo-HSCT) as the current treatment option for CALD, either discussing the significant impact it had on quality of life, or fears that, should allo-HSCT be the only treatment option, GVHD may impact on quality of life, time away from school and family, and potentially death. Several speakers addressed concerns related to delays in treatment due to the time it takes to find a suitable matched donor for allo-HSCT, particularly for racial and ethnic minorities for whom a suitable donor are less likely to be found. Noting rapid progression of disease, they expressed concerns that this delay in treatment could mean progression of neurologic symptoms and ultimately disability while awaiting a donor. One parent spoke about his son losing his life 6 months following his diagnosis while awaiting a suitable donor.

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

There are no FDA-approved treatments for CALD in the United States (US). Allogeneic hematopoietic stem cell transplant (allo-HSCT)¹² has been the standard of care since approximately 2001 and is the only therapy considered by experts to be disease-modifying, i.e., able to slow or stabilize disease progression.^{8,13, 24, 26} Allo-HSCT was first successfully performed for the treatment of CALD in 1988.²⁴ Prior to 2000, mixed results with allo-HSCT led experts to question how effective the treatment was at treating CALD- in the most comprehensive study of allo-HSCT performed around that time reporting on 126 patients who had received allo-HSCT between 1982 and 1999, 5-year and 8-year survival probabilities were both 56%.¹³ However, subgroup analysis revealed more favorable outcomes in patients with early neurologic involvement at time of treatment as compared to those with more advanced disease- with the former having 5-year survival probability of 92%. Peters and colleagues characterized the “early neurologic involvement” group by a score of 0 or 1 on a 4-point neurologic deficit scale, or a Loes score <9. Of those with a neurologic deficit score of 0 or 1 at time of treatment, 53% maintained stable neurologic function at 5 years following allo-HSCT. In addition to Peters and colleagues’ findings, several other 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.^{8,13,14} It has also been observed that allo-HSCT may increase rapidity of disease progression in patients with advanced cerebral disease (Loes score >9), and is no longer recommended for patients who meet this criterion.^{8,13,14}

The major limitation of these studies was that it was unclear if the early cerebral disease group might merely represent a milder phenotype of disease with a better prognosis regardless of treatment. Mahmood and colleagues²⁵ noted that most natural history controls used in comparisons of allo-HSCT effectiveness evaluated entire cohorts of untreated natural history subjects, regardless of baseline disease severity, and thus sought to find untreated controls through retrospective chart review to compare to the early cerebral disease patients treated with allo-HSCT reported by Peters and colleagues.¹³ In an evaluation of 283 CALD patients followed at Kennedy Krieger Institute between 1978 and 2004 who had not received HSCT, it was found that symptoms began in 280 (99%) patients prior to 2000, when allo-HSCT was not routinely an option for treatment.²⁵ Patients were graded on the same 4-point grading scale used by Peters and colleagues, which evaluated neurologic dysfunction across 6 domains of vision, hearing, speech, gait, fine motor skills, and activities of daily living.¹³ Upon exclusion of 115 patients without baseline MRI and 18 patients who had arrested cerebral disease and no symptomatic disease, 150 patients were classified as having mild neurologic involvement (neurologic deficit score of 0 or 1 and Loes score <9) or severely involved (neurologic deficit score of 2 with 2 or more deficits and Loes score ≥9) at time of diagnosis.²⁵ Five-year mortality for the entire untreated cohort was 66%, and 131 (46%) died at a mean age of 12.3 years during a follow up of 5.9 years (range 1 month-30 years). Survival probability was not associated with degree of neurologic deficit at time of diagnosis and was similar for those with deficit scores of 0, 1, and 2. A slightly worse prognosis was found for baseline Loes score >9 at baseline as compared to score <9 (51% survival probability and 61%, respectively). A greater association was seen for age, where boys diagnosed prior to age 10 had worse 5-year survival (61%) compared to boys diagnosed after 10 years of age (75%). Adrenal insufficiency was present in 91% of patients, with no association found with survival. Mean age at last follow-up in survivors was 12.3 years, similar to mean age of death for those who died, suggesting the two were not representative of distinct disease phenotypes. Progression of neurologic deterioration within 5 years of symptom onset occurred for the majority, as 79 (94%) of 84 patients had a neurologic deficit score of 2 or more at last assessment. Neurologic deficit score maintenance of 0 or 1 at 5 years only occurred in 5 (6%) of patients. Of 127 boys with more than 5 years of follow-up from onset of disease, 18 (14%) maintained stable neurocognitive course through a mean follow up of 12.7 years, all of whom were diagnosed due to unrelated reasons and thus determination of their comparability to other patients was difficult. Twenty-six patients were alive at least 10 years from onset of disease – 20 had multiple neurologic deficits and the majority were disabled and required extensive care, and insufficient data was present for the remaining 6.²⁵

Six of 25 transplanted subjects were excluded from the comparative analysis due to lack of disease manifestations at time of transplant. Outcomes for the 30 untreated patients with mild neurologic involvement²⁵ were compared to the 19 similar patients in the HSCT cohort described by Peters and colleagues.¹³ Untreated and treated patients had similar age of disease onset. One patient (5%) treated with allo-HSCT died of transplant-related complications in the first year following treatment, and no other patients died in the 5-year follow-up period for a 5-year survival rate of 95%. In contrast, untreated subjects died throughout the course of follow-up from progressive disease, with a 5-year survival rate of 54%. Neurologic deficit score did not increase (i.e., was stable) in 53% of surviving transplant patients at 5 years following disease onset, compared to 6% in the untreated group.²⁵

In a study evaluating long-term outcomes following allo-HSCT for the treatment of CALD, 12 of 18 (66%) of patients survived and had at least 5 years of follow-up after treatment with allo-HSCT.¹³ In these patients, transplantation at an early stage of disease resulted in stabilization or even reversal of cerebral demyelination, with complete resolution in 2 (17%) patients. Abnormal motor function present in 5 subjects at time of treatment resolved in 3 (60%), and stabilization or improvement in neurocognitive function was demonstrated in 7 (58%) patients. Two patients (17%) had worsening of visual impairment and one other patient developed seizures and vision loss following treatment- two of these patients had cortical blindness, which is considered a major functional disability. Despite most missing a year of school due to allo-HSCT treatment, all 12 patients were in school: 8 in mainstream classes (including one who graduated and was in college), and four who were receiving general help or tutoring in specific subjects.

Due to significant evidence of effectiveness of allo-HSCT when performed at early stages of disease, allo-HSCT is performed in the early, active radiographic course of disease (Loes score 0.5-9 with gadolinium enhancement on brain MRI), which often corresponds to a time when patients are asymptomatic or mildly symptomatic (NFS 0 or 1). The goal of treatment in this early, active phase of disease is to treat prior to the onset of significant neurologic dysfunction in an effort to prevent progression to disability and death, which is often rapid and more difficult to stabilize once disease is symptomatic.

Most studies that have demonstrated efficacy of allo-HSCT have assessed MRI and neurocognitive changes and/or progression to disability and death following treatment as compared to the natural history of disease. With increasing identification of X-ALD cases due to newborn screening and genetic testing of family members of affected individuals, routine MRI screening now allows for diagnosis of CALD at some of the earliest stages of cerebral disease, often prior to onset of neurologic dysfunction or neurocognitive changes.^{7, 28-30} There is lack of an appropriate natural history (i.e., untreated) population that has been followed from such an early stage of disease to understand the clinical course of asymptomatic very early, active cerebral disease if left untreated. Therefore, despite matching of early cerebral disease patients by Mahmood and colleagues,²⁵ many patients were still symptomatic at time of diagnosis in the untreated population, and impact of gadolinium enhancement was not evaluated in that study to understand how the disease trajectory may be different in populations with radiographic evidence of active disease. Therefore, while numerous studies have demonstrated benefit of allo-HSCT over the natural history of disease in symptomatic CALD patients with more risk factors for rapidly progressive disease, the long-term efficacy of allo-HSCT as compared to the natural history of disease in the earliest asymptomatic disease stages with minimal radiographic active cerebral disease burden is unknown. Additionally, because studies have demonstrated that radiographic and clinical disease progression may occur in the initial 12-24 months following treatment with allo-HSCT before disease stabilization is achieved,^{8,9,12-14} allo-HSCT is now routinely performed without delay upon diagnosis of CALD in an effort to prevent neurologic dysfunction, disability and death. Few (if any) patients are expected to go untreated upon diagnosis of active CALD unless disease is already advanced, and as a result there likely will never be an appropriate untreated comparator with very early asymptomatic disease to understand the time course of disease progression in such a population if left untreated.

The preferred allo-HSCT donor is a human leukocyte antigen (HLA)-matched unaffected sibling, but these HLA-matched sibling donors are only available for $\leq 30\%$ of patients.⁸ Patients of minority racial and ethnic background are less likely to find a suitable donor. Allo-HSCT is associated with known risks, including graft rejection, graft versus host disease, and infection, and these risks are believed to be increased with alternative (HLA- mismatched or HLA- matched unrelated) donors. Morbidity and mortality following allo-HSCT are significant, with 5-year survival cited as varying between 50-95%, depending on donor type, conditioning regimens and stage of disease at time of treatment, with percentages reflecting death from disease progression and transplant-related causes.^{13,14,25} However, as noted by Mahmood and colleagues,²⁵ who found a 95% survival rate when allo-HSCT is administered at an early stage of disease, some of the lower 5-year survival rates may reflect the rapidly progressive disease in subjects with advanced disease who were treated too late in the disease course.

2.3 Safety and Efficacy of Pharmacologically Related Products

Insertional oncogenesis is the primary safety concern with lentiviral vectors (LVVs). Insertional oncogenesis is the consequence of permanent alteration of the host genome by the vector. LVV integration into the DNA of target cells has the potential to affect the expression of nearby genes and may provide those cells with a growth advantage. Cells with a growth advantage may undergo preferential expansion and transform into a hematologic malignancy.

Four genetic mechanisms for insertional oncogenesis that have been described with LVVs 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. 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 a vector type that is related to LVVs (a γ -retroviral vector). Several SCID-X1 patients developed a T-cell leukemia that appears to have been caused by the inserted vector switching on an adjacent oncogene. Similar insertional oncogenesis events have also been observed in patients who were treated for chronic granulomatous disease.³⁹ In addition, MDS and acute myeloid leukemia have occurred after administration of a LVV gene therapy, lovo-cel, that is related to eli-cel, although causality in those cases is not clear.

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

Eli-cel received marketing authorization 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 on 16 July 2021. 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 myelodysplastic syndrome being reported.

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

Investigational New Drug Application 15433 for the use of eli-cel in the treatment of CALD was filed to the United States Food and Drug Administration (US FDA) on 27 March 2013.

Eli-cel was granted an orphan drug designation for the treatment of ALD on 19 April 2012 (#12-3682), received a Rare Pediatric Disease Designation on 09 August 2017 (#RPD 2016-79), and was granted a Breakthrough Therapy Designation on 21 May 2018.

Throughout development, bluebird bio has met with the Agency to agree on the overall design of the development program, including primary clinical efficacy endpoint of Month 24 major functional disability-free survival, as well as the comparison to the benchmark value to demonstrate efficacy (November 2018) and the importance of demonstrating lack of GVHD as compared to allo-HSCT. Although the Agency agreed with the primary clinical efficacy endpoint and comparison to the benchmark value, it was noted that comparability of populations would need to be demonstrated. During BLA review, lack of comparability in study populations complicated analysis of the primary efficacy endpoint, discussed further in [Section 6.1.11.1](#).

Final guidance for BLA content was issued in a pre-BLA meeting on 21 June 2021.

Additional regulatory history:

- 17 November 2015 – Type C Meeting - Clinical - CRMTS #9978
- 22 February 2018 – Type C Meeting – Clinical – CRMTS #11016
- 15 November 2018 – Type B Meeting – BTD and CMC – CRMTS #11453
- 16 September 2020 – Type B Meeting – Clinical and CMC – CRMTS #12618
- 15 January 2021 – Type B CMC WRO- LVV PPQ Package – CRMTS #13047
- 21 June 2021 – Type B Pre-BLA Meeting – CRMTS #13347
- 16 July 2021 – European Commission grants marketing authorization (Refer to 2.4 Previous Human Experience with the Product (Including Foreign Experience) for additional details)
- 08 August 2021 – IND 15433 placed on full clinical hold due to myelodysplastic syndrome diagnosed in one subject
- 15 September 2022 – IND 15433 full clinical hold removed

BLA review dates:

- 19 July 2021 – Rolling Review Granted
- 18 October 2021 – DCC Receipt
- 17 December 2021- Filing Notification
- 14 January 2022 – Major Amendment
- 9 June 2022 - Cellular, Tissue, and Gene Therapies Advisory Committee Meeting

3. SUBMISSION QUALITY AND GOOD CLINICAL PRACTICES

3.1 Submission Quality and Completeness

The submission was adequately organized and integrated to accommodate the conduct of a complete clinical review without unreasonable difficulty.

However, there were shortcomings with regard to availability of data that pertained to adverse events. The submission included laboratory results and vital signs obtained at time points specified in the protocol. However, vital signs and laboratories corresponding with adverse events were not collected, limiting our ability to independently assess adverse event severity and time course.

3.2 Compliance With Good Clinical Practices And Submission Integrity

The two interventional studies, ALD-102 and ALD-104, and the long term follow-up study, LTF-304, were performed in compliance with good clinical practice.

The Bioresearch Monitoring Branch inspected the Applicant and two clinical sites that were the highest enrollers for both ALD-102 and ALD-104. The enrollment at the two sites together accounted for 43% of subjects (26 of 60) who were enrolled prior to the data cut for the BLA submission. The Sponsor Establishment Inspection Report and preliminary the preliminary Establishment Inspection Reports for the clinical sites did not reveal problems that impact the data submitted in the BLA.

3.3 Financial Disclosures

Table 2: Financial Disclosures

Covered clinical study (name and/or number): ALD-101, ALD-102, ALD-104, LTF-304
Was a list of clinical investigators provided? <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No (Request list from applicant)
Total number of investigators identified: <u>126</u>
Number of investigators who are sponsor employees (including both full-time and part-time employees): <u>0</u>
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): <u>1</u>
<p>If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)):</p> <p>Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: _____</p> <p>Significant payments of other sorts: <u>X</u></p> <p>Proprietary interest in the product tested held by investigator: _____</p> <p>Significant equity interest held by investigator in sponsor of covered study: _____</p> <p>Is an attachment provided with details of the disclosable financial interests/arrangements? <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No (Request details from applicant)</p> <p>Is a description of the steps taken to minimize potential bias provided? <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No (Request information from applicant)</p>
<p>Number of investigators with certification of due diligence (Form FDA 3454, box 3): <u>0</u></p> <p>Is an attachment provided with the reason? <input type="checkbox"/> Yes <input type="checkbox"/> No (Request explanation from applicant)</p>

The Applicant has adequately disclosed financial arrangements with clinical investigators. The one investigator with a financial arrangement with the Applicant does not raise questions about the integrity of the data because that investigator provided minimal contribution to the study data.

4. SIGNIFICANT EFFICACY/SAFETY ISSUES RELATED TO OTHER REVIEW DISCIPLINES

4.1 Chemistry, Manufacturing, and Controls

Comparability to the to-be-marketed product was not able to be demonstrated for of product administered to 6 subjects in Study ALD-102. While these subjects were included in the safety analysis, they were excluded from the efficacy analysis.

4.2 Assay Validation

While the recommendation for approval of this BLA is based on clinical outcomes, the results of several assays were considered in the assessment of both safety and efficacy. These included assay of percent of ALDP+ cells in peripheral blood, that has not been demonstrated to correlate to clinical outcomes but could inform efficacy, vector copy number in peripheral blood, that could inform safety and efficacy, and integration site analysis, a critical tool for identifying subjects at risk for serious safety adverse events. The assays above were evaluated by the CMC reviewer, Dr. Anna Kwilas. Dr Kwilas concluded that the assays for percent ALDP+ cells and for vector copy number were suitable. For the integration site analysis assay, S-EPTS/LM-PCR (shearing extension primer tag selection ligation-mediated PCR), Dr. Kwilas concluded that the assay was accurate across the (b) (4) range and was suitable for the analysis of study samples. However, she recommended that the Applicant perform additional studies to potentially lower the limits of quantification of the assay.

4.3 Nonclinical Pharmacology/Toxicology

The following is excerpted and adapted from the Summary Basis of Regulatory Action:

In vitro pharmacology studies were conducted with healthy human donor CD34+ HSCs, CD34+ HSCs obtained from patients with adrenomyeloneuropathy (AMN), and adrenoleukodystrophy protein (ALDP)-deficient fibroblasts obtained from patients with CALD, transduced using the Lenti-D vector used in eli-cel. These studies demonstrated that vector-driven *ABCD1* transgene expression and ALDP production resulted in improvements in very-long-chain fatty acid metabolism in CALD fibroblasts and AMN patient CD34+ HSCs. In vivo assessment of Lenti-D-transduced healthy donor derived CD34+ HSCs transplanted in myeloablated immunodeficient mice demonstrated bone marrow and brain engraftment that was associated with stable *ABCD1* transgene expression and ALDP production for the 3-month study duration.

A 92-day good laboratory practice (GLP) toxicology study in myeloablated immunodeficient mice evaluated a single administration of 1×10^6 Lenti-D transduced healthy human CD34+ HSCs/mouse. There were early mortalities in both the test article (Lenti-D transduced CD34+ HSCs) and control (non-transduced CD34+ HSCs) groups. Although the cause of death was undetermined, the early mortalities occurred at a higher frequency in the control group and human cell engraftment was confirmed in both groups. No other test article related toxicities were observed in this study. Although a true no observed adverse effect level (NOAEL) could not be determined, the dose level administered in this study, approximately 5×10^7 CD34+ cells/kg, represents the maximum feasible dose and a 10-fold multiple of the minimum recommended dose level for patients with CALD $\geq 5 \times 10^6$ CD34+ cells/kg).

Traditional carcinogenicity studies were not conducted for eli-cel and were not warranted. However, integration site analysis was performed on Lenti-D transduced pre-transplant human CD34+ HSCs and post-transplant engrafted bone marrow cells (BMCs) from animals in the GLP toxicology study harvested at 29 and 92 days. Lenti-D transduced HSCs demonstrated the expected integration profile for self-inactivating lentiviral vectors, with preferred integration

in gene-coding regions across the whole genome, no preference for integration in transcriptional start sites, and no bias for the 5' or 3' end of genes. There were no notable differences between the pre- and post-transplant samples.

An in vitro immortalization assay was conducted with mouse lineage-depleted (hematopoietic non-lineage committed; Lin-) BMCs (the murine equivalent of human CD34+ HSCs) transduced with Lenti-D. There was a reduced potential for immortalization induced by insertional mutagenesis of the clinical vector, Lenti-D, compared to positive control vectors.

Reproductive and developmental toxicity studies were not performed for eli-cel which is acceptable based on the product characteristics and safety profile.

Refer to the Pharmacology/Toxicology memo for additional details.

4.4 Clinical Pharmacology

4.4.1 Mechanism of Action

Eli-cel is a biological product containing genetically modified autologous HSCs transduced with Lenti-D LVV encoding ALDP. Following engraftment into the bone marrow, transduced HSCs differentiate into various cell types, including monocytes, that are capable of producing functional ALDP. The functional ALDP can then locally degrade very long chain fatty acids (VLCFAs), which is thought to slow and possibly prevent further inflammation and demyelination.

4.4.2 Human Pharmacodynamics (PD)

The following is excerpted and adapted from the Summary Basis of Regulatory Action:

General Pharmacodynamics

- One month after infusion of eli-cel, lentiviral vector copy was detected in peripheral blood leukocytes (PB VCN) and CD14+ cells (CD14+ VCN), demonstrating the early presence of transduced cells. Levels of PB VCN and CD14+ VCN stabilized by Month 6. Subjects had a Month 6 median (min, max) PB VCN level of 0.38 (0.07, 2.23) c/dg in Study ALD-102 (N=25) and 1.04 (0.03, 3.13) c/dg in Study ALD-104 (N=32). Median CD14+ VCN levels at Month 6 were 0.61 (0.07, 3.96) c/dg (N=29) and 1.41 (0.04, 3.82) c/dg (N=28), for Studies ALD-102 and ALD-104 respectively. VCN levels in peripheral blood and CD14+ cells generally remained stable as of the data cut-off date, although high inter-subject variability of PB VCN and CD14+ VCN kinetic profiles was observed.
- All subjects who received eli-cel with at least 1 month of follow-up produced ALDP in peripheral blood leukocytes and CD14+ cells, demonstrating early expression of the transgene. The %ALDP+ cell counts stabilized at 6 months after eli-cel infusion. Subjects had a Month 6 median (min, max) %ALDP+ CD14+ cell count of 16% (2%, 71%) in Study ALD-102 (N=23) and 26% (2%, 86%) in Study ALD-104 (N=25) respectively. The %ALDP+ CD14+ cells generally remained stable through Month 24 with a median (min, max) of 15% (6%, 45%) in Study ALD-102 (N=23) and 28% (2%, 40%) in Study ALD-104 (N=11). As of the data cut-off date of January 07, 2022, ALDP expression in CD14+ cells was detected in 3 of 7 subjects who had the last follow-up through

Month 60 in Study ALD-102 (N=7), indicating long-term expression of transgenic ALDP in the progeny of hematopoietic stem cells.

- Subjects with higher PB VCNs generally had higher PB %ALDP+ cells at a given timepoint. There was a positive linear relationship between PB VCN and PB %ALDP+ cells at Month 6.
- ALDP is a peroxisomal membrane protein involved in the transport and metabolism of very long-chain fatty acids (VLCFA). VLCFA levels in fasting serum were variable in study subjects treated with eli-cel. There was a decrease in VLCFAs based on decreased median values of C26:0 LysoPC and C26:0/C22:0 ratios from baseline to Month 24 post-administration of eli-cel.

Dosing Characteristics and Responses

- Eli-cel drug product vector copy number (DP VCN) and the percentage of transduced cells in drug product (DP %LVV+ Cells) measure drug product characteristics related to transduction efficiency. There was a positive correlative relationship observed between DP VCN and DP %LVV+ cells: DP %LVV+ Cells shows a linear relationship with DP VCN up to approximately 60% LVV+ Cells, at which point they appear to plateau at higher DP VCNs.
- There was a positive correlation observed DP VCN and pharmacodynamic (PD) parameters in the peripheral blood (PB VCN and PB %ALDP+ cells): subjects with higher DP VCNs generally had higher stable PB VCNs and PB %ALDP+ cells.
- DP %LVV correlated positively with ALDP expression in both peripheral blood leukocytes and CD14+ cells.
- The median (min, max) of eli-cel DP VCN in subjects with 24 months follow up period after infusion of eli-cel was 1.3 (0.5, 3.1) c/dg. The DP VCN values in subjects who experienced an MFD or received allo-HSCT due to disease progression by Month 24 after eli-cel infusion were no more than 1.20 c/dg (median: 0.85 c/dg, range: 0.5 to 1.2 c/dg). were no more than 1.20 c/dg (median: 0.85 c/dg, range: 0.5 to 1.2 c/dg).
- There was no correlation between the total cell dose of eli-cel and engraftment (neutrophil and platelet).

Pharmacodynamic Responses and Clinical Outcomes

- PD responses and Disease Progression Events: Compared to subjects who did not experience disease progression events (MFD or allo-HSCT for disease progression) at Month 24 after eli-cel infusion, the median levels of the following PD parameters were substantially lower in subjects who developed MFD or underwent allo-HSCT due to disease progression: PB VCN at Month 6; 24-month exposure of PB VCN and CD14+ %ALDP+ Cells at Month 6.
- PD responses and Myelodysplastic Syndrome (MDS): among subjects with at least 6 months of follow up, the median levels of PB VCN at Month 6 and maximum PB VCN during the observation period were substantially higher in subjects diagnosed with MDS (N=3), compared to subjects who did not have MDS (N=62). All three subjects diagnosed with MDS had maximum PB VCN levels more than 2.0 c/dg (median (range): 3.13 (2.15, 4.82)). The median (min, max) value of maximum PB VCN was 0.96 (0.11, 3.40) c/dg in subjects who did not have MDS, although there were subjects without MDS who had PB VCN levels greater than 2.0 c/dg.

Refer to Clinical Pharmacology memo for additional details.

4.4.3 Human Pharmacokinetics (PK)

The eli-cel product is an autologous gene therapy derived from hematopoietic stem cells that have been genetically modified. As such, typical evaluations of pharmacokinetics, absorption, distribution, metabolism and elimination are not applicable.

Refer to Clinical Pharmacology memo for additional details.

4.5 Statistical

The primary efficacy analysis of study ALD-102 shows that the success criterion was met for Month 24 MFD-free survival. In the integrated analyses, the comparisons between eli-cel (in ALD-102) and allo-HSCT (in ALD-103) on MFD-free survival and overall survival also support the effectiveness of eli-cel. Due to concerns regarding comparability of populations, Baseline Loes scores and age at time of treatment were used as covariates in the Cox model for hazard ratios comparing eli-cel and allo-HSCT on MFD-free survival and overall survival, with similar results to those not using these covariates. The estimated hazard ratio for overall survival of 0.119 (95% confidence interval: 0.014, 1.020), indicating a lower risk of death following treatment with eli-cel as compared to allo-HSCT, is likely unstable due to the small number of events.

Refer to Statistical memo for additional details.

4.6 Pharmacovigilance

The Division of Pharmacovigilance concluded that the hematologic malignancies observed after treatment with eli-cel warrant a Food and Drug Administration Amendments Act of 2007 Title IX post-marketing requirement (PMR) study. They also concluded that the risks of treatment with Eli-cel can be mitigated through risk communication and risk minimization measures as recommended in the USPI, including a boxed warning for hematologic malignancy, and by routine and enhanced pharmacovigilance activities and adverse event reporting in accordance with 21 CFR 600.80.

Refer to the Pharmacovigilance Plan Review for additional details.

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

5.1 Review Strategy

Pivotal trials ALD-102 and ALD-104 will be discussed individually in Section 6. Discussion of Individual Studies/Clinical Trials. The pivotal trials were each two years in duration. After a subject completed ALD-102 or ALD-104, they were enrolled in the long-term follow-up study, LTF-304. Safety data obtained from LTF-304 will be incorporated into the section of the pivotal trial under which the subject was initially enrolled.

This BLA was evaluated jointly by Dr. Shelby Elenburg, who conducted the efficacy review, and Dr. Leah Crisafi, who conducted the safety review. Both reviewers contributed to the synthesis and documentation of the overall conclusions for the

application. However, due to the complex post hoc analyses that led to determinations about efficacy, compared to a clear, albeit serious and important risk of malignancy, Dr. Elenburg was responsible for the overall conclusions and benefit-risk determination.

In the assessment of safety, the two clinical trials were considered separately and then pooled for certain analyses. The trials were similar in design but differed in the mobilization and conditioning regimens and in their use of post-treatment G-CSF, that would be expected to impact some safety findings, such as engraftment timing and conditioning-associated adverse events. The sample sizes were small, so combining sample sizes increased the likelihood of identifying safety signals and trends for rare outcomes (e.g., myelodysplastic syndrome). Lastly, the drug products used in the trials were considered comparable by CMC, although the multiplicity of infection was (b) (4) leading to a more integration sites per subject in ALD-104, and therefore the integration site analysis data was considered separately for the two trials.

The primary efficacy endpoint of Month 24 MFD-free survival was assessed only in ALD-102, as subjects in ALD-104 had not reached 24 months of follow-up at time of original BLA submission. The analysis of Month 24 MFD-free survival and other endpoints only assessed at Month 24 in ALD-102 is thus discussed in [Section 6](#). The remainder of the efficacy review relied heavily on post-hoc exploratory analyses, and as such the two clinical trials were largely considered together, including data from the long-term follow-up study LTF-304. The post-hoc exploratory analyses are discussed in [Section 7](#). The reasons for integrating are discussed in detail in [Section 7.1.1](#), but are similar to the reasons for combining data from both clinical trials as noted for the safety analysis:

- (1) the trials were similar in design, including eligibility criteria and efficacy endpoint assessments,
- (2) the sample sizes were small, so combining study populations and using interim data cut time points increased the likelihood of having sufficient data to make conclusions about the efficacy of the product, and
- (3) the study populations were similar in demographics and baseline disease characteristics, making the populations comparable for integration purposes.

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

Documents serving as the basis for the clinical review are provided in the following table:

Table 3: Documents That Serve as the Basis for the Clinical Review

Document	STN	Date Received
Introduction; Nonclinical Overview	125755 SN001	22 Jul 2021
Nonclinical Introduction	Module 2.3.1, 125755 SN002	23 Sep 2021
Drug Substance	Module 2.3.S, 125755 SN002	23 Sep 2021
Drug Product	Module 2.3.P, 125755 SN002	23 Sep 2021
Financial Certification and Disclosure (FDA Forms 3454 and 3455)	Module 1.3.4, 125755 SN003	18 Oct 2021

Document	STN	Date Received
Draft Labeling Text	Module 1.14.1.3, 125755 SN003	18 Oct 2021
Foreign Labeling	Module 1.16.5, 125755 SN003	18 Oct 2021
Pharmacovigilance Plan for elivaldogene autotemcel, Version 1.0	Module 1.16.1, 125755 SN003	18 Oct 2021
Clinical Overview	Module 2.5, 125755 SN003	18 Oct 2021
Summary of Clinical Safety	Module 2.7.4, 125755 SN003	18 Oct 2021
Summary of Clinical Safety Late Breaking Safety Listings	Module 2.7.4, 125755 SN003	18 Oct 2021
Synopses of Individual Studies	Module 2.7.6, 125755 SN003	18 Oct 2021
BLA correspondence of "Notification of a Serious Adverse Event (SAE) Report of Myelodysplastic syndrome (MDS)" sent on December 03, 2021	Module 1.2, 125755 SN004	03 Dec 2021
15-Day IND Safety Report for MDS	Module 5.3.5.2, 125755 SN005	16 Dec 2021
Response to Requests from Application Orientation Meeting and Data Monitoring Committee meeting minutes	Module 1.11.3, 125755 SN006	22 Dec 2021
Follow-Up 15-Day IND Safety Reports for MDS	Module 5.3.5.2, 125755 SN007	29 Dec 2021
Information request #1 response – part 1	125755 SN008	03 Jan 2022
Information request #1 response – part 2	125755 SN010	10 Jan 2022
3-Month Safety Update Report	Module 5.3.5.3 125755 SN011	14 Jan 2022
Integration Site \geq 10% Relative Frequency Report	Module 5.3.5.2 125755 SN012	21 Jan 2022
Information request #1 response – part 3	Module 5.3.5.2 125755 SN013	26 Jan 2022
Integration Site \geq 10% Relative Frequency Report	Module 5.3.5.2 125755 SN014	31 Jan 2022
Monthly malignancy report	125755 SN015	01 Feb 2022
Information request #3 response	125755 SN016	04 Feb 2022
Information request #6 response – part 1	125755 SN020	23 Feb 2022
Information request #6 response – part 2	125755 SN021	25 Feb 2022

Document	STN	Date Received
Information request #6 response – part 3	125755 SN023	28 Feb 2022
Monthly malignancy report	125755 SN024	01 Mar 2022
Information request #5 response	125755 SN025	01 Mar 2022
Information request #6 response – part 4	125755 SN026	02 Mar 2022
Information request #6 response – part 5	Module 1.11.3 1252755 SN027	11 Mar 2022
Information request #7 response – part 1	125755 SN028	18 Mar 2022
Information request #7 response – part 2	125755 SN029	21 Mar 2022
Follow-Up 15-Day IND Safety Report	125755 SN030	22 Mar 2022
Information request #9 response	Module 1.11.3 125755 SN031	25 Mar 2022
Follow-Up 15-Day IND Safety Report	Module 5.3.5.2 125755 SN032	28 Mar 2022
Monthly malignancy report	125755 SN34	01 Apr 2022
Updated integration site analysis algorithm	125755 SN035	05 Apr 2022
Information request #10 response – part 1	125755 SN036	07 Apr 2022
Information request #14 response	125755 SN037	08 Apr 2022
Information request #12 response	125755 SN038	08 Apr 2022
Complete response to partial clinical hold for Iovo-cel	125755 SN040	12 Apr 2022
Information request #16 (part 1) response; Integration Site \geq 10% Relative Frequency Reports	125755 SN041	15 Apr 2022
Information request #1 response – part 4	Module 5.3.5.2 125755 SN044	21 Apr 2022
Information request #18 response	125755 SN046	22 Apr 2022
Monthly malignancy report	125755 SN047	29 Apr 2022
Information request #10 response – part 2	125755 SN048	29 Apr 2022
Information request #20 response	125755 SN051	04 May 2022

Document	STN	Date Received
Information request response to 29 Apr 2022 request	125755 SN052	04 May 2022
Information request #21 response	Module 1.11.3 125755 SN053	11 May 2022
Information request #10 response – part 3	Module 1.11.3 1252755 SN054	11 May 2022
Advisory Committee support outputs	125755 SN055	13 May 2022
Integration Site \geq 10% Relative Frequency Report	Module 5.3.5.2 125755 SN056	18 May 2022
Information request #22 response	125755 SN058	23 May 2022
Information request #25 response	125755 SN059	24 May 2022
Information request #10 response – part 4	Module 1.11.3 125755 SN062	31 May 2022
Monthly malignancy report	125755 SN063	01 Jun 2022
Revised integration site analysis reporting criteria	125755 SN064	03 Jun 2022
Bone marrow colony analysis and gene expression report	Module 5.3.5.2 125755 SN066	13 Jun 2022
Integration Site \geq 10% Relative Frequency Report	Module 5.3.5.2 125755 SN068	17 Jun 2022
Information request #28 response – part 1	125755 SN070	22 Jun 2022
Information request #28 response – part 2	Module 1.11.3 125755 SN071	24 Jun 2022
Information request #30 response	125755 SN072	28 Jun 2022
Information request #31 response	125755 SN073	29 Jun 2022
Information request #28 (part 3) & #33 response; monthly malignancy report	125755 SN075	01 Jul 2022
Information request #31 response – part 2	125755 SN076	08 Jul 2022
Information request #35 response – part 1	Module 1.11.3 125755 SN077	12 Jul 2022
Information request #35 (part 2) & #16 response (part 2)	125755 SN079	13 Jul 2022
Information request #36 & #37 response	Module 1.11.3 125755 SN080	15 Jul 2022
Information request #35 response – part 3	Module 1.11.3 125755 SN083	18 Jul 2022

Document	STN	Date Received
Integration Site \geq 10% Relative Frequency Report	125755 SN084	22 Jul 2022
Information request #40 response	125755 SN087	29 Jul 2022
Monthly malignancy report	125755 SN088	01 Aug 2022
Information request #41 response – part 1	125755 SN090	05 Aug 2022
Information request #41 response – part 2	125755 SN091	10 Aug 2022
Integration Site \geq 10% Relative Frequency Report	125755 SN092	12 Aug 2022
Draft medication guide	Module 1.11.4 125755 SN098	23 Aug 2022
MFD, SAE, and AE of neutropenia reporting	125755 SN099	23 Aug 2022
Information request #49 response	125755 S104	30 Aug 2022
Monthly malignancy report	Module 1.11.3 125755 SN108	01 Sep 2022
Information request #56 response	Module 1.11.3 125755 SN111	02 Sep 2022

Abbrev: STN, submission tracking number; SN, sequence number; SAE, serious adverse event; MDS, myelodysplastic syndrome; IND, investigational new drug; MFD, major functional disability

5.3 Table of Studies/Clinical Trials

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, 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.

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.

Additional details regarding the eli-cel studies are found in Section 6. Additional details about the external control studies are as follows:

1. Historical External Control: Study ALD-101: A retrospective non-interventional data collection study conducted between 16 April 2011 and 27 March 2012 that sought to characterize the natural history of disease of untreated CALD (subjects diagnosed between June 27, 1988 and January 14, 2010) to define efficacy endpoints and to characterize the efficacy and safety of subjects with CALD treated with allogeneic hematopoietic stem cell transplant (allo-HSCT, between March 12, 1997 and September 21, 2010) for the purpose of defining safety and efficacy endpoints for trial design.

Subjects had to be males between the ages of 3 and 15 years of age with confirmed diagnosis of CALD by elevated VLCFA levels or genetic mutation and baseline cerebral lesions on brain magnetic resonance imaging (MRI) with a Loes score of >0 and <15 at baseline, and have data available for at least 2 years or until death following:

- a) Allo-HSCT with either bone marrow or umbilical cord blood (allo-HSCT Cohort; n=65); or
- b) Diagnosis (Untreated Cohort, n=72)

2. Contemporaneous ALD-103: An international multicenter mixed retrospective/prospective natural history study of boys with CALD who had undergone allo-HSCT, conducted between 10 April 2015 and 6 December 2019. 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.

Males aged 17 years and younger at time of parent/guardian consent were eligible if they had a confirmed diagnosis of CALD by abnormal VLCFA levels and cerebral lesions on MRI. They were enrolled in one of three cohorts:

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

The 5 clinical studies are summarized in Table 4. The table reflects data through August 2021. Additional efficacy data were obtained for subjects in Studies ALD-102 and ALD-104 through early January 2022, which are not reflected in the table but are discussed in [Section 7](#). Additionally, for select subjects, such as those with concerning integration site analysis results, safety data from after the August 2021 data cut were submitted and reviewed on an ad hoc basis, and are discussed primarily in [Section 8](#).

Table 4: 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	<ul style="list-style-type: none"> 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 hematopoietic stem cell transplant 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 for subjects treated in ALD-102 and ALD-104 from an additional data cut in 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.

Reviewer Comment: *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. 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. Also, because it was retrospective, there may have been selection bias and missing data. The early termination of Study ALD-103 limited the utility of the dataset for long-term comparisons of safety and efficacy. Partial retrospective data collection in Study ALD-103 may have contributed to selection bias and missing data.*

5.4 Consultations

5.4.1 Advisory Committee Meeting

The application was discussed at a Cellular, Tissue, and Gene Therapies Advisory Committee Meeting on 9 June 2022. The primary purpose of the Advisory Committee (AC) meeting was to discuss the risk of insertional oncogenesis and cases of hematologic malignancy that have been attributed to the novel product. In addition to presentations of data from CALD clinical trials, the presentations included data relevant to the risk of insertional oncogenesis in two related lentiviral vector (LVV) products. The clinical review team also presented concerns regarding the efficacy data. Input was sought from the Committee regarding the overall benefit-risk profile for eli-cel.

The Committee agreed that insertional oncogenesis is a serious and important risk of eli-cel. They recommended very close follow-up of treated subjects to identify those appearing to be at high risk of malignancy, allowing for an early search for a bone marrow donor should allogeneic hematopoietic stem cell transplant (allo-HSCT) be indicated to treat a malignancy. The AC also recommended closely following the malignancy cases in order to characterize their aggressiveness and responsiveness to treatment to further inform the benefit-risk profile. Lastly, they voted unanimously that the data from the two related LVV products does not inform the safety of eli-cel.

Regarding the benefit-risk profile, the AC voted unanimously (with one abstention) that the benefit-risk profile for eli-cel is favorable. The population determined by the Committee to have a favorable benefit-risk included boys without an available human leukocyte antigen (HLA)-matched HSCT donor. However, the Committee did not provide a clear consensus about the benefit-risk in boys with a matched non-sibling donor. Several members of the AC commented that additional long-term (i.e., more than 2 years) comparisons of outcomes following treatment with eli-cel and HSCT were warranted, including evaluation of quality of life measures in patients who experienced safety events of concern (hematologic malignancy for eli-cel and graft versus host disease or GVHD for allo-HSCT).

5.4.2 External Consults/Collaborations

Insertional Oncogenesis Consultation

The clinical team consulted with a special government employee (SGE), Dr. Lucy Godley, a physician with expertise in hematologic malignancy. The SGE provided feedback on a wide range of issues pertaining to the risk of hematologic malignancy. She provided input regarding causality in the three cases of myelodysplastic syndrome that have been diagnosed, concluding that the vector had mediated these cases of malignancy and that the cases involving *MECOM* are a particularly concerning signal, given the frequency of *MECOM* integration sites in subjects treated with eli-cel.

The SGE provided recommendations regarding screening subjects for germline predisposition to hematologic malignancy, and regarding the monitoring of subjects who have already been treated with eli-cel. These recommendations are being incorporated into the required post-marketing safety study. Her recommendations include a comprehensive evaluation of the baseline hematologic status, including bone marrow biopsy and aspirate and testing cultured skin fibroblasts for germline predisposition to hematologic malignancy. For monitoring subject for the development of malignancy, she recommended bone marrow biopsies during the first year after eli-cel administration, and scheduled CBC and integration site analysis (ISA) assessments at more frequent intervals for all subjects. She also recommended lowering the clone size threshold that triggers clinical work-up, and that the clinical work-up be standardized.

Lastly, the SGE weighed in on the definitions of neutrophil and platelet engraftment. The Applicant's definitions for engraftment did not address support with G-CSF or eltrombopag, and they considered subjects to have met engraftment criteria if they achieved the target neutrophil or platelet counts, irrespective of G-CSF or eltrombopag administration. We question whether this was appropriate and sought the SGE's input. She explained that the same neutrophil and platelet engraftment definitions are not always used, but that they should be prespecified, and that a patient who is requiring G-CSF or eltrombopag support should not be considered engrafted.

Clinical Outcomes Assessment Consultation

The clinical review team consulted a Clinical Outcomes Assessment (COA) reviewer (Dr. Naomi Knoble) from the Center for Drug Evaluation and Research (CDER) due to the reliance on the Neurologic Function Score (NFS) and Major Functional Disabilities (MFDs) for the efficacy review. The NFS (discussed in [Section 2.1](#) and [Appendix 1](#)) is the primary scoring system used clinically for evaluation of CALD patients^{4,8} and has been used in other clinical trials. While the domains appear to reflect clinically meaningful neurologic functioning changes in CALD patients, the significance of any particular score is unclear due to:

- 1) uneven weighting of varied areas of functioning, e.g., mobility/motor functioning evaluated under 5 domains, communication evaluated under 3 domains, seizures evaluated under 1 domain,
- 2) apparently subjective weighting of individual domains, with a weighted scale across 15 domains, each scaled between 1-3 for a positive response for a total possible score of 25, and
- 3) unclear significance to patients and caregivers of relative impact of individual domains on daily functioning and perceived severity of disease (e.g., if NFS of 1 for running difficulties is the same impact on daily functioning as NFS of 1 for seizures).

The MFDs, a subset of the NFS, do appear to capture valid and clinically meaningful events which significantly impact CALD patient daily functioning. However, 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 Studies ALD-102 and ALD-104, and in the 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.

Additional insight was requested from Dr. Knoble regarding neurocognitive testing results; however, the analysis was limited by significant amounts of missing data for natural history and allo-HSCT external controls due to retrospective data collection in Studies ALD-101 and ALD-103 and early termination of study ALD-103, and insufficient number of assessments following treatment in treatment populations to adequately trend neurocognitive course over time.

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6. DISCUSSION OF INDIVIDUAL STUDIES/CLINICAL TRIALS

6.1 Trial #1

A Phase 2/3 Study of the Efficacy and Safety of Hematopoietic Stem Cells Transduced with Lenti-D Lentiviral Vector for the Treatment of Cerebral Adrenoleukodystrophy (ALD-102)

6.1.1 Objectives

The primary objective of ALD-102 was to evaluate the safety and efficacy of eli-cel in subjects with CALD.

6.1.2 Design Overview

ALD-102 was a non-randomized, open-label, multi-site, international, single-dose, single-arm study in male subjects ≤ 17 years of age with early, active CALD without an available and willing human leukocyte antigen (HLA)-matched hematopoietic stem cell (HSC) donor. Approximately 30 subjects were planned to be treated. The study had four phases after informed consent:

- Screening
- CD34+ Cell Collection, Transduction, Disposition of eli-cel, and Re-confirmation of Eligibility
- Conditioning and Washout, followed by eli-cel Infusion (transplant) on Day 0
- Maintenance (Follow-up) (Day 1 through Month 24)

Following completion of the maintenance phase at Month 24, subjects were to enroll in long-term follow-up study, LTF-304, for an additional 13 years (total of 15 years) of follow-up.

Reviewer Comment: *Weaknesses of the study design include its open-label design and the absence of a randomized controlled design with incomparable external control arms, evidence used to support the primary efficacy endpoint from external controls, and duration of follow-up for efficacy outcomes.*

6.1.3 Population

The key eligibility criteria were as follows:

Inclusion Criteria

- Males 17 years of age or younger
- Active CALD defined by elevated VLCFA levels and brain magnetic resonance imaging (MRI) demonstrating Loes scores between 0.5 and 9 and gadolinium enhancement (GdE+)
- Neurologic Function Score (NFS) of ≤ 1

Exclusion Criteria

- Recipient of an allogeneic transplant or previous gene therapy
- Available and willing 10/10 HLA-matched sibling donor (excluding female heterozygotes)
- Hematological, hepatic, renal, or cardiac compromise

6.1.4 Study Treatments or Agents Mandated by the Protocol

Study treatments were mandated by the protocol for the different phases including CD34+ cell collection (also referred to as mobilization), myeloablative conditioning, and during eli-cel transfusion (transplant).

Mobilization

- G-CSF (starting dose 10 $\mu\text{g/kg}$) administered for 4 to 6 days
 - Dose decreased for WBC $> 70 \times 10^9$ cells/L
- Apheresis on day 5, with additional G-CSF administered and plerixafor administered (0.24 mg/kg daily for up to 4 days) for peripheral blood CD34+ count of < 50 cells/ μL

Conditioning

- Busulfan as a 2-hour intravenous infusion 4 times per day for 4 consecutive days
 - Cumulative busulfan exposure targeted to 17,000 to 21,000 $\mu\text{mol}\cdot\text{min}/\text{L}$ if using cumulative exposure to calculate busulfan dose
 - Target area under the curve [AUC] range of 1190 to 1310 $\mu\text{mol min}/\text{L}$ if using first dose to calculate busulfan exposure
- Cyclophosphamide as a 2-hour intravenous infusion with hydration as per institutional protocol
- Supportive care including administration of prophylactic anti-convulsive, antifungal, and antibiotic treatments per institutional standards

Eli-cel Infusion

- Intravenous administration through a central venous catheter in a volume between 20 and 80 mL, according to institutional practice, approximately 48 hours after completion of conditioning with busulfan and cyclophosphamide
- Dose: $\geq 5 \times 10^6$ CD34+ cells/kg

The following table outlines the actual dose information as provided by the Applicant in the ALD-102 Clinical Study Report.

Table 5: Exposure to G-CSF and Plerixafor During Mobilization/Apheresis and Cell Collection (ITT Population)

Parameter	Statistic	Overall Cohort (N = 32)
Subjects requiring 1 mobilization cycle	n (%)	32 (100.0)
G-CSF average dose per day $\mu\text{g}/\text{kg}/\text{day}$) ^a	n	32
--	Median	10.00
--	Min, Max	8.9, 12.5
Number of days G-CSF administered	n	32
--	Median	6.0
--	Min, Max	4, 8
Plerixafor average dose per day (mg/kg/day) ^a	n	11
--	Median	0.240
--	Min, Max	0.24, 0.24
Number of days plerixafor administered	n	11
--	Median	1.0
--	Min, Max	1, 3
Number of apheresis procedures performed per mobilization cycle	n	32
--	Median	2.0
--	Min, Max	1, 4
Total number of CD34+ cells sent for transduction ($\times 10^6$ cells/kg)	n	32
--	Median	13.350
--	Min, Max	5.70, 32.56

^a Sum of doses divided by number of days taking the medication for mobilization

Source: Original BLA 125755; Clinical Study Report ALD-102, p. 198

Reviewer Comment: *The medications and doses for mobilization are consistent with standard practice. The bone marrow conditioning regimen is notable for including agents for full myeloablation and lymphodepletion. The doses of these agents are also consistent with standard practice.*

6.1.5 Directions for Use

The protocol specified the following directions for relating to product administration:

- The product is to be thawed in a 37°C water bath and must be infused immediately, but no later than 4 hours after it has been thawed
- Do not use an infusion filter
- If more than one lot of Lenti-D Drug Product was manufactured to achieve the minimum cell dose, infusions of each lot occur consecutively
- Consecutive infusions will also occur if a single lot is split into 2 drug product bags due to volume constraints

6.1.6 Sites and Centers

The study was conducted at 8 clinical sites in Argentina, Australia, France, Germany, the United Kingdom, and the United States. Subjects returned to their primary study site for assessments at Month 12 and Month 24. However, for some visits between transplant and the Month 12 and Month 24 visits, a site closer to the subject's home (referred to as a secondary study site) was opened. The list of primary study sites and Principal Investigators as compiled by the Applicant in the ALD-102 Clinical Study Report follows.

Table 6: List and Description of Investigators

Site Number	Study Center	Principal Investigator
102	Mattel Children's Hospital UCLA/Ronald Reagan UCLA Medical Center Los Angeles CA, USA	Donald Kohn, MD Ami Shah, MD Satiro De Oliveira, MD
105	Boston Children's Hospital/ Massachusetts General Hospital Boston MA, USA	David A. Williams, MD Florian Eichler, MD
106	Great Ormond Street Hospital for Children NHS Foundation Trust London, UK	Adrian Thrasher, MBBS, PhD
107	Hôpital Bicêtre Le Kremlin-Bicêtre, France	Patrick Aubourg, MD Caroline Sevin, MD, PhD
109	University of Minnesota, Masonic Children's Hospital Minneapolis MN, USA	Paul Orchard, MD
118	Women and Children's Hospital North Adelaide SA, Australia	Nicholas Smith, MBBS, PhD
121	Fundación Investigar Buenos Aires, Argentina	Hernan Amartino, MD
150	Universitätsklinikum Leipzig AöR Leipzig, Germany	Dietger Niederwieser, MD Jörn-Sven Kühl, MD
156	Medeos SRL Buenos Aires, Argentina	Hernan Amartino, MD

Source: Adapted from Original BLA 125755/0.2; ALD-102 Appendix 16.1.4 List and Description of Investigators, p.1

6.1.7 Surveillance/Monitoring

Subjects were actively monitored via the schedule of events that is included in this section, and data was collected on case reports forms. A Data Monitoring Committee was used to provide an independent assessment of safety during the study.

Within this section are subsections with schedules of events, information about efficacy assessors, and algorithms followed for assessment of clonal predominance.

6.1.7.1 Schedule of Events

Surveillance and monitoring are divided into before eli-cel infusion, from eli-cel infusion through 24 months, and more than 24 months after eli-cel infusion. The following two tables outline the pre-infusion and post-infusion assessments.

Table 7: Schedule of Events: Screening through Drug Product Infusion

--	Screening	Mobilization	CD34+ Harvest	Pre-Conditioning Assessments	Conditioning and Monitoring
Study Day	-60 to -45	-45 to -37	-40 to -37	-11	-10 to -1
Visit Window (Days)	-10 to +5	-10	-	-3	-
Search for allogeneic donor & HLA typing ¹	+	--	--	--	--
ABCD1 genotype ²	(+)	--	--	--	--
Adrenal function ³	+	--	--	--	--
Local lab: Blood for immunological studies	+	--	--	--	--
CSF specialty labs: Lumbar puncture ⁴	+	--	--	--	--
Serology panel	+ (I)	+ (II)	--	--	--
Physical examination, Vital signs ⁵	+	+ ⁶	+ ⁷	+	+ ⁸
Hematology ⁹	+	+ ¹⁰	+ ¹⁰	+	+ ¹¹
Clinical chemistry	+	--	--	+	+ ¹¹
Blood specialty labs: • Dried blood spot collection	+	--	--	--	--
• RCL	(+) ¹²	--	--	(+) ¹²	--
• ALDP (Peripheral Blood)	(+) ¹²	--	--	(+) ¹²	--
• VCN (Peripheral Blood)	(+) ¹²	--	--	(+) ¹²	--
• VLCFA (fasting)	+	--	--	--	--
• Exploratory biomarkers ¹³	(+) ¹²	--	--	(+) ¹²	--
• Optional: blood for storage	+	--	--	--	--
Neurological exam	+	--	--	+	--
NFS assessment ¹⁴	+	--	--	(+) ¹⁴	--
MFD assessment ^{14, 15}	+	--	--	(+) ¹⁴	--
Neuropsychological tests	+ ¹⁶	--	--	+	--
Global assessment	--	--	--	+	--
PedsQL	--	--	--	+	--
Echocardiogram ¹⁷	+	--	--	--	--
Electrocardiogram	+	--	--	--	--
Brain MRI (with and without contrast) ¹⁴	+	--	--	(+) ¹⁴	--
Evoked potentials ¹⁸	+	--	--	--	--
CD34+ count ¹⁹	--	+	+	--	--
Busulfan level monitoring	--	--	--	--	+
Concomitant medication ²⁰	+	+	+	+	+
Adverse event monitoring ²⁰	+	+	+	+	+

¹ A preliminary search for a suitable donor will be initiated at Screening for all subjects in the event that a subject is not eligible for drug product at Day -11, experiences engraftment failure, or cannot receive Lenti-D Drug Product (e.g., drug product does not meet specifications). HLA typing does not need to be performed if historical results are available.

² Genotyping of *ABCD1* gene will occur in subjects for whom no historical data is available; documented *ABCD1* mutation required prior to initiating myeloablative conditioning

³ Adrenal function tests (cortisol and adrenocorticotrophic hormone [ACTH]) are to be performed in the morning (approximately 8:00 am) during Screening before the subject has taken hydrocortisone unless subject is on steroid replacement therapy. If ACTH is significantly elevated, tests should be repeated 3 hours after taking hydrocortisone.

Mineralocorticoid functions (aldosterone and plasma renin activity) are to be performed at the same time points with the subject sitting in an upright position.

⁴ Cerebrospinal fluid samples to measure the expression of MMP and chitotriosidase (central laboratory) and total protein (local laboratory).

⁵ Physical examinations will include measurement of weight at all visits and height at Screening only. Full physical exam to be performed at Screening only. During hospitalization, focused physical examinations will be performed twice per week until discharge; Vital signs will include blood pressure, pulse, respiratory rate, and temperature.

⁶ Focused physical examinations and vital signs will be performed prior to the first dose of G-CSF.

⁷ On each day of apheresis, the subject should have a focused physical exam, including abdominal palpation to rule out splenomegaly, and vital signs performed prior to beginning apheresis and again after completion of apheresis.

⁸ Focused physical examinations and vital signs will be performed each day during conditioning.

⁹ Hematology parameters to be determined include white blood cell (WBC) count with differential, hemoglobin, hematocrit, red blood cell (RBC), and platelet count

¹⁰ Hematology will be performed each day of mobilization and apheresis.

¹¹ Chemistry and hematology parameters will be measured daily during conditioning; blood will be collected prior to infusion of busulfan IV and cyclophosphamide IV.

¹² Blood for measurements of RCL, ALDP, VCN, and exploratory biomarkers will be drawn once, any time from Screening prior to start of conditioning.

¹³ Blood for analyses of chitotriosidase (central laboratory) and for storage for potential analysis of antibodies against the transgene.

¹⁴ NFS assessments, MFD assessments, and brain MRIs may be repeated at any time during the study if there is evidence of clinical decline. These assessments must be repeated if more than 60 days has passed since the NFS and MRI at Screening and the start of Pre-Conditioning assessments. However, if subject requires sedation for MRI, performing this repeat assessment is based on Investigator judgment.

¹⁵ May be performed concurrently with NFS assessment.

¹⁶ Only the Socioeconomic Status test derived from Hollingshead and Redlich will be done at Screening.

¹⁷ Read by the site cardiologist.

¹⁸ Evoked potentials to be performed may include BAER, visual evoked potential (VEP), nerve conduction studies (NCS), and somatosensory evoked potential (SSEP) from all 4 limbs, depending on subject age and ability to participate in the assessments. NCS may be performed in the upper and lower limbs (sural, peroneal, tibial, and median nerves). The VEP will be performed at Screening, Month 12 and Month 24. The BAER, SSEP, and NCS will be performed at Screening and Month 24. It is at PI's discretion whether to perform SSEP and NCS.

¹⁹ Peripheral blood CD34+ count should be performed either the day prior to or on the first planned day of apheresis.

²⁰ Continuous from the time of informed consent.

Source: Adapted from - BLA 125755/0.2; ALD-102 Protocol and Amendments, p.55-57

Table 8: Schedule of Events: Drug Product Infusion Through 24 Months – Part 1

--	Lenti-D Infusion	Week 2	Month 1	Month 2	Month 3	Month 6	Month 9	Month 12	Month 15	Month 18	Month 21	Month 24
Study Day:	0	15 ±7	30 ±7	60 ±4	90 ±4	180 ±4	270 ±4	360 ±30	450 ±30	540 ±30	630 ±30	720 ±30
Physical examination, vital signs ¹	+	+	+	+	+	+	+	+	+	+	+	+
Hematology ²	--	+ ³	+ ³	+	+	+	+	+	+	+	+	+
Clinical chemistry	--	+ ³	+ ³	+	+	+	+	+	+	+	+	+
Local lab: Blood for immunological studies	--	--	--	--	+	+	--	+	--	--	--	+
CSF specialty labs: Lumbar puncture ⁴	--	--	--	--	--	--	--	+	--	--	--	+
Blood specialty labs: • Dried blood spot collection	--	--	--	--	--	--	--	+ ⁵	--	--	--	+ ⁵
• RCL	--	--	--	--	+	+	--	+	--	--	--	+
• Integration site analysis	--	--	--	--	--	+	--	+	--	+	--	+
• ALDP (Peripheral Blood)	--	--	+	+	+	+	+	+	+	+	+	+
• VCN (Peripheral Blood)	--	--	+	+	+	+	+	+	+	+	+	+
• VLCFA (fasting)	--	--	--	--	--	+	--	+	--	+	--	+
• Exploratory biomarkers ⁶	--	--	+	+	--	+	+	+	+	+	+	+

¹ Physical examinations will include measurement of weight at all visits. During hospitalization, focused physical examinations will be performed twice per week until discharge. Vital signs will include blood pressure, pulse, respiratory rate, and temperature. Vital signs are to be monitored concurrently during Lenti-D Drug Product infusion according to institutional practice at the clinical site, but no less frequently than at the start, once during, and upon completion of the infusion. Following infusion, vital signs will be performed daily during hospitalization and at least twice per week after discharge until neutrophil engraftment occurs.

² Hematology parameters to be determined include white blood cell (WBC) count with differential, hemoglobin, hematocrit, red blood cell (RBC), and platelet count. If the results from blood tests are not as expected, additional testing may need to be performed and may include a physical exam, blood tests, imaging tests, or a bone marrow biopsy to allow for further investigation of stem cells.

³ Chemistry and hematology parameters will be measured at least twice per week until neutrophil engraftment occurs.

⁴ Cerebrospinal fluid samples to measure the expression of MMP and chitotriosidase (central laboratory) and total protein (local laboratory).

⁵ Dried blood spot collection is not required for subjects who fail to engraft with drug product.

⁶ Blood for analyses of chitotriosidase (central laboratory) and for storage for potential analysis of antibodies against the transgene.

Source: Adapted from BLA 125755/0.2; ALD-102 Protocol and Amendments, p.58-59

Table 9: Schedule of Events: Drug Product Infusion Through 24 Months – Part 2

--	Lenti-D Infusion	Week 2	Month 1	Month 2	Month 3	Month 6	Month 9	Month 12	Month 15	Month 18	Month 21	Month 24
Study Day:	0	15 ±7	30 ±7	60 ±4	90 ±4	180 ±4	270 ±4	360 ±30	450 ±30	540 ±30	630 ±30	720 ±30
Bone marrow specialty labs⁷	--	--	--	--	--	(+)	(+)	(+)	(+)	(+)	(+)	(+)
• VCN (CD34+)	--	--	--	--	--	(+)	(+)	(+)	(+)	(+)	(+)	(+)
• ALDP (CD34+)	--	--	--	--	--	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Neurological exam	--	+	+	--	+	+	+	+	--	+	--	+
NFS and MFD assessments ⁸	--	+	+	--	+	+	+	+	--	+	--	+
Neuropsychological tests	--	--	--	--	--	--	--	+	--	--	--	+
Global assessment	--	--	--	--	--	--	--	+	--	--	--	+
PedsQL	--	--	--	--	+	+	--	+	--	--	--	+
Electrocardiogram	--	--	--	--	--	--	--	--	--	--	--	+
Brain MRI (w/ and w/o contrast) ⁸	--	--	+	--	--	+	--	+	--	+	--	+
Evoked potentials ⁹	--	--	--	--	--	--	--	+	--	--	--	+
Concomitant medication ¹⁰	+	+	+	+	+	+	+	+	+	+	+	+
Adverse event monitoring ¹⁰	+	+	+	+	+	+	+	+	+	+	+	+

⁷ (+) To be performed only if peripheral blood shows a single clone with integrated lentiviral vector sequences persistently representing > 0% of total PBLs and concurrent presence of leukocytosis (WBC count >30,000 cells/μL/mm³) or at the Investigator's discretion.

⁸ NFS assessments, MFD assessments, and brain MRIs may be repeated at any time during the study for evidence of clinical decline.

⁹ May include BAER, visual evoked potential, nerve conduction studies, and somatosensory evoked potential. NCS may be performed in the upper and lower limbs (sural, peroneal, tibial, and median nerves). The VEP will be performed at Screening, Month 2 and Month 24. The BAER, SSEP, and NCS will be performed at Screening and Month 24. It is at PI's discretion whether to perform SSEP and NCS.

¹⁰ Continuous from the time of informed consent.

Source: Adapted from BLA 125755/0.2; ALD-102 Protocol and Amendments, p.58-59

6.1.7.2 Efficacy Assessors

Most efficacy assessments were performed by a pediatric neurologist or an appropriately trained and qualified physician. The physician performed the neurologic examination to include ophthalmologic and audiologic examinations and the Neurologic Function Score (NFS) assessment with Major Functional Disability (MFD) determination.

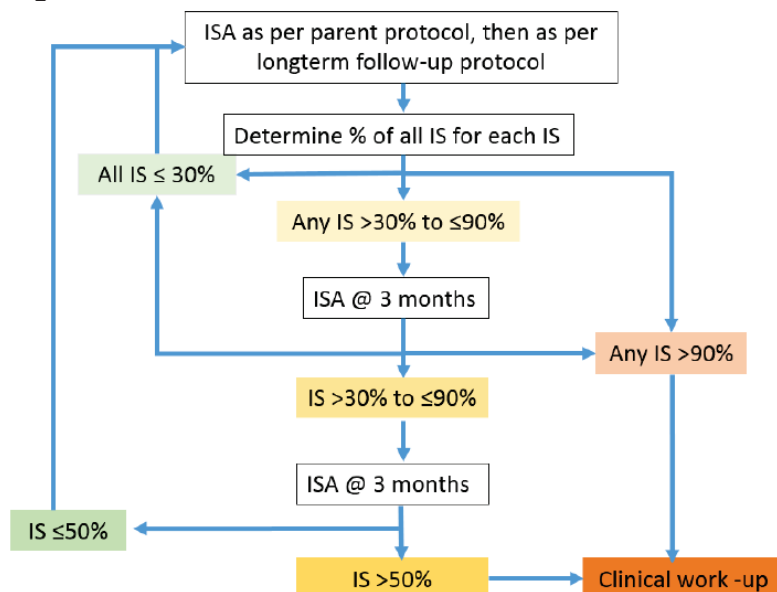
Neuropsychological tests were performed by the same assessor at each study visit if possible.

MRIs were evaluated by a blinded central reviewer.

6.1.7.3 Integration Site Analysis

Integration site analysis (ISA) was scheduled for most subjects at Months 6, 12, 18, and 24 during the two-year follow-up period of ALD-102, and served to evaluate for the development of predominant clones. The schematic for assessment of clonal predominance utilized for most of the duration of the study is provided below. The schematic shows (1) that any integration site (IS) relative frequency > 90% would prompt clinical work-up, (2) that any IS relative frequency >30% that did not prompt clinical work-up would be repeated in 3 months, and (3) that a repeated ISA yielding an IS relative frequency >50% would prompt clinical work-up.

Figure 1: Schematic for Assessment of Clonal Predominance Through September 2020



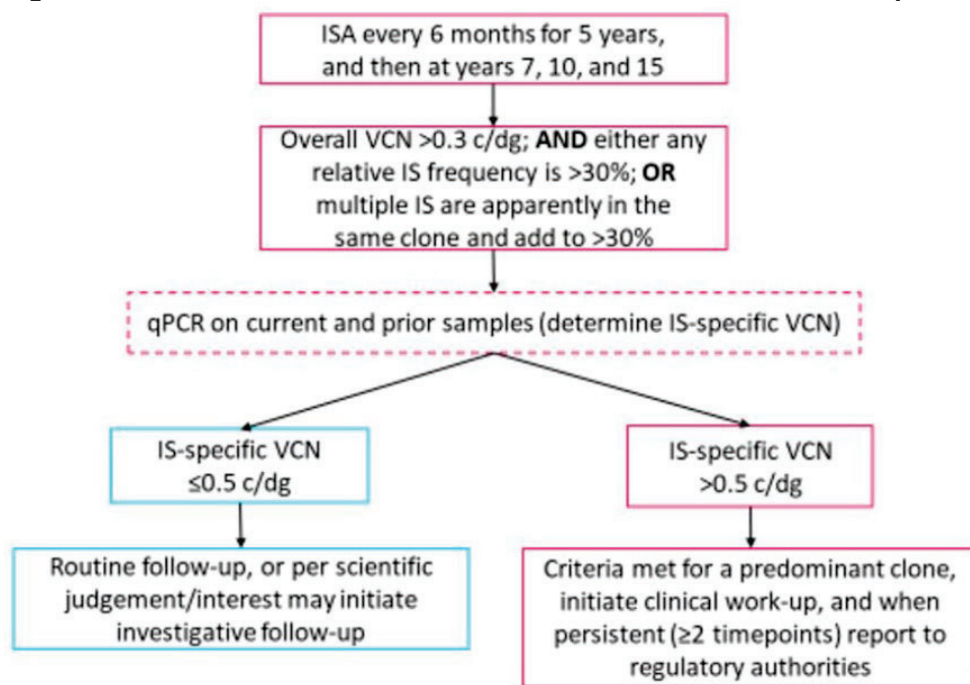
Abbrev: ISA, integration site analysis; IS, integration site

Source: Original BLA 125755; ALD-102 Protocol and Amendments, p.167

The schematic for assessment of clonal predominance was changed in the 23 Sep 2020 version of the protocol, to account for the possibility of multiple integration sites within a single clone. The revised schematic is provided below, which incorporated qPCR performance when overall VCN was > 0.3 c/dg, and either any relative IS frequency >30% or multiple IS appearing to be in the same clone and adding up to >30%. If the IS-specific VCN measured by qPCR was > 0.5 c/dg, then criteria were met for a predominant clone and a clinical work-up for malignancy was initiated. The protocol also

stated that if clonal predominance is observed, bone marrow samples would be used for determination of VCN in bone marrow.

Figure 2: Schematic for Assessment of Clonal Predominance After September 2020



Abbrev.: c/dg, copies per diploid genome; IS, integration site; ISA, integration site analysis; qPCR, quantitative polymerase chain reaction; VCN, vector copy number

Source: Original BLA 125755/0.2; ALD-102 Protocol and Amendments, p.71

Other criteria that would trigger a work-up for malignancy were either of the following:

- Unexplained WBC count > $30 \times 10^9/L$ on two consecutive measurements
- After achievement of a WBC count within the normal range post-drug product infusion and engraftment of gene-modified cells, the development of WBC < 1×10^9 on two consecutive measurements

A work-up for malignancy could include of the following: physical exam, CBC lymphocyte subsets, studies to rule out infectious cause, studies to rule out autoimmune disease, imaging studies, and bone marrow analysis.

6.1.8 Endpoints and Criteria for Study Success

The primary efficacy endpoint was the proportion of subjects who were alive and had none of the six defined Major Functional Disabilities (MFDs) at the Month 24 Visit (i.e., Month 24 MFD-free survival). MFDs were defined as loss of communication, cortical blindness, tube feeding, total incontinence, wheelchair dependence, and complete loss of voluntary movement. The MFDs are further defined in Table 10.

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.

Reviewer Comment: *Although FDA agreed in pre-submission meetings with the primary efficacy endpoint and clinical benchmark for success, FDA emphasized in these pre-BLA meetings that comparability of external control groups to the eli-cel-treated subjects would need to be demonstrated to support the validity of the benchmark. Concerns regarding the comparability of the external control groups are detailed in the analysis of the primary efficacy endpoint in [Section 6.1.11.1](#).*

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 (\geq 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 \geq Grade II acute GVHD or chronic GVHD in Study ALD-102 compared to the Study ALD-103 transplant population.

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.⁴ It is the most commonly used clinical evaluation tool for CALD patient evaluation.^{4,14} A score of 0 indicates absence of clinical signs of cerebral disease, and higher scores correspond to increasing severity of functional deficits in hearing, communication, vision, feeding, mobility and motor function, bowel and bladder continence, and seizures. The entire NFS scale, including definitions, can be found in [Appendix 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 10.

Table 10: 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.

Abbrev: CALD, cerebral adrenoleukodystrophy

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

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.

Reviewer Comment: *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.

6.1.9 Statistical Considerations & Statistical Analysis Plan

Statistical methods were primarily descriptive, including point estimates and confidence intervals where appropriate. Tables and figures were used for demographic, Baseline, efficacy, safety and exploratory parameters when appropriate. Categorical variables were tabulated as number and percentage of subjects for each category or parameter. Continuous evaluations were tabulated as number of observations, mean (including 1-or 2-sided confidence intervals, or CIs), median (including standard deviation), minimum and maximum values. Time to event data was summarized with Kaplan-Meier analyses using 25th, 50th (median), and 75th percentiles with associated two-sided 95% CIs, as well as percent of censored observations and percent events. By-subject listings were provided for all parameters.

The populations evaluated for eli-cel outcomes included:

- the intent-to-treat (ITT) population, who initiated any study procedure beginning with G-CSF mobilization;
- the transplant population (TP), who received eli-cel; and
- the successful neutrophil engraftment population (NEP), who achieved 3 consecutive absolute neutrophil count (ANC) values of $\geq 0.5 \times 10^9$ cells/L (after initial post-infusion nadir) on different days by 42 days post- eli-cel infusion.

All three populations were identical, and are thus reported as TP, or "Overall Cohort TP." The first 17 subjects treated with drug product are presented for some results as the "Initial Cohort TP."

The success criterion for the study, as discussed in [Section 6.1.8](#), was based on comparison of the primary efficacy endpoint results for the Initial Cohort TP (n=17) to the clinical benchmark, such that the lower bound of the 2-sided 95% exact CI of Month 24 MFD-free survival for the cohort must be >50%. This would be met with a point estimate of 76.5% (13 of 17 Initial Cohort subjects).

Month 24 MFD-free survival was summarized and presented as Kaplan-Meier analyses. Overall survival was presented as Kaplan-Meier analyses. Resolution of gadolinium positivity, NFS, and Loes score were summarized and plotted over time by subject. Proportion analyses at 24 months for efficacy endpoints other than MFD-free survival were done for evaluable subjects, or those with data collected during the Month 24 Visit Window.

The number and percent of subjects, along with the exact 95% CI, were presented for the primary safety endpoint of proportion of subjects experiencing either acute \geq Grade II GVHD or chronic GVHD by Month 24. This primary endpoint was intended to support interstudy analysis with CALD subjects treated with HSCT in studies ALD-101 and ALD-103. AEs were listed, and numbers of subjects were summarized for the following time periods:

1. from informed consent/assent up to start of mobilization
2. from start of mobilization to start of conditioning

3. from start of conditioning through neutrophil engraftment (NE)
4. from NE through Month 12
5. from NE through Month 24
6. from drug product infusion through Month 12
7. from Month 12 through Month 24
8. from drug product infusion through Month 24
9. from informed consent through Month 24

Summary statistics were provided for survival status, hospitalizations, intensive care unit (ICU) stays, detection of vector-derived replication competent lentivirus (RCL), and Lenti-D LVV-mediated oncogenesis events.

Pharmacodynamic parameters were summarized using descriptive statistics and plotted over time, with exploratory analyses to determine relationships between parameters at defined time points.

6.1.10 Study Population and Disposition

6.1.10.1 Populations Enrolled/Analyzed

Populations enrolled and analyzed are defined in the statistical plan in [Section 6.1.9](#). The early, active CALD population which eli-cel is intended to treat are adequately represented by the transplant population (TP).

6.1.10.1.1 Demographics

All 32 subjects enrolled and treated in the study were male and between ages 3 and 13 years at time of enrollment, and between 4 and 14 years (median 6) at drug product infusion. Subjects were predominantly white (47%), with 3% of patients being Asian, 3% being Black or African American, and 16% of patients were of other races included mixed race; 38% of patients were of Hispanic ethnicity.

6.1.10.1.2 Medical/Behavioral Characterization of the Enrolled Population

Of the 32 subjects enrolled and treated in the study, 26 were evaluated in analyses of efficacy. The remaining 6 subjects were excluded because comparability to the to-be-marketed product could not be demonstrated for the product administered to these subjects. Disease-specific characteristics of subjects at baseline relevant to the efficacy analysis are discussed in [Section 7.1.2](#).

6.1.10.1.3 Subject Disposition

Table 11: Subject Disposition for Study ALD-102

Parameter	Statistic	ALD-102 TP (N = 32)
Initiated mobilization (ITT)	n (%)	32 (100)
Initiated conditioning	n (%)	32 (100)
Infused with eli-cel (TP)	n (%)	32 (100)
Successful neutrophil engraftment (NEP)	n (%)	32 (100)
Completed Study	n (%)	29 (91)
Discontinued Study	n (%)	3 (9)
Reasons for study discontinuation	--	--
Death	n (%)	1 (3)
Subject to receive allo-HSCT	n (%)	2 (6)
Enrolled in Study LTF-304 ^a	n (%)	29 (91)
Duration of follow-up in study (months)	Median	24
--	Min.	13
--	Max	32
Subject-years of follow-up (years) ^b	Total	64
Last Visit Completed	--	--
Month 9	n (%)	0
Month 12	n (%)	1 (3)
Month 15	n (%)	1 (3)
Month 18	n (%)	0
Month 21	n (%)	1 (3)
Month 24	n (%)	29 (91)

Abbrev.: allo-HSCT, allogeneic hematopoietic stem cell transplantation; ITT, intent-to-treat population; NEP, neutrophil engraftment population; TP, transplant population

^a LTF-304 is the long-term follow-up study to support parent eli-cel studies. Two subjects who completed ALD-102 had not signed consent for LTF-304 by the time of the data cut (26 March 2021).

^b Subject-years were calculated by summing the total of the number of years each subject has been followed after drug product infusion in this study; it does not include subsequent follow-up in Study LTF-304.

Source: Adapted from: Original sBLA 125755/0.3 ALD-102 Study Synopsis p. 5

6.1.11 Efficacy Analyses

6.1.11.1 Analyses of Primary Endpoint(s)

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 6.1.8](#). Key demographic 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 12.

Table 12: 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=26)*
Age (Years) ^a	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 (2,15)	4.5 (0.5, 9)	2 (1,9)
Baseline NFS	Median (Min,Max)	3.5 ^b (0, 25)	0 (0,1)	0 (0,1)

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

*Note that baseline demographics and disease features are the exact same for the TP-102 efficacy population of n=26 (excluding the 6 subjects who received product for which comparability to the to-be-marketed product was not demonstrable) and the TP-102 safety population of n=32 (which was used for some efficacy analyses where noted in this review).

^aAge reflects age at diagnosis for UTG-101 and age at time of treatment for TPES-101 NMSD, TP-102.

^bNFS at baseline only available for 14 of the 21 UTG-101 population.

Source: Reviewer's analysis of ADSL datasets

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 at least of similar efficacy to allo-HSCT (as the lower bound of the 95% CI for TPES- 101 NMSD was 50%).

Eli-cel was successful on the primary efficacy endpoint with a point estimate of 88% (exact 95% CI of 70% to 98%) MFD-free survival at Month-24. Of the 26 subjects evaluated on the primary efficacy endpoint in Study ALD-102, 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).

Reviewer Comment: *Although eli-cel was successful on its primary endpoint, upon review of BLA data, there were several concerns with the clinical benchmark for the primary efficacy endpoint due to the following concerns with Study ALD-101:*

1. *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 (Table 12). In fact, only one subject in the UTG-101 population would have met the criteria for early, active disease*

(as defined by the ALD-102 eligibility criteria) at diagnosis. 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). As seen in Table 12, 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. We do 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. Reviewer-initiated 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 and gadolinium enhancement on MRI) who are asymptomatic or with mild functional limitations (NFS score of ≤ 1). Few MFDs or deaths occurred by 24 months across*

appropriately matched comparator groups (including the untreated group) in all studies.

In a reviewer-initiated exploratory analysis, the UTG-101 subject data were re-coded 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 months). Two subjects maintained MFD-free survival at time of last contact (70 and 187 months from date of diagnosis, respectively). The subject followed for 187 months remained asymptomatic. It is worth noting these subjects had older age at diagnosis compared to the eli-cel population.

Reviewer Comment: *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.*

In summary, although eli-cel was successful on the primary efficacy endpoint, the clinical benchmark value of 50% is not meaningful, in particular because Population #1 had much more severe disease at baseline as compared to the population treated with eli-cel in Study ALD-102, and we do not have an appropriate comparator population to understand what proportion of patients with early, active CALD as defined by the ALD-102 eligibility criteria would progress to MFD or death within 24 months of diagnosis in the absence of treatment. Unfortunately, this makes interpretation of the prespecified primary efficacy endpoint difficult, and success on the primary efficacy is not meaningful in the demonstration of eli-cel efficacy as compared to the natural history of disease.

6.1.11.2 Analyses of Secondary Endpoints

Secondary efficacy endpoints were pre-specified but not hierarchically ordered, and thus were treated as exploratory. Discussion of relevant secondary and exploratory efficacy endpoints are thus discussed jointly in this section. Demographics and baseline disease characteristics for populations used for the efficacy outcome comparisons between allo-HSCT and eli-cel are shown in Table 13 for the entire TP-102 population (N=32), TPES-101, and TPES-103 (entire population and NMSD subpopulation). As the results of these analyses did not weigh significantly into the determination of product effectiveness, results were not updated to remove the 6 subjects who received investigational product for which comparability to the to-be-marketed product was not demonstrable. Results are expected to be similar to those shown/discussed. Demographics and disease characteristics for the entire TP-102 population (N=32) are the same as for the cohort of N=26 (excluding the 6 subjects for which product comparability could not be demonstrated).

Table 13: Demographics and Baseline Disease Characteristics for allo-HSCT Populations and Eli-Cel (TP-102)

Parameter	Statistic	TPES-101 (n=26)	TPES-103 (n=27)	TPES-103 NMSD (n=17)	TP-102 (n=32)
Age (Years) ¹	Median (Min, Max)	8 (4,14)	8 (5,11)	8 (5,11)	6 (4,14)
Age at Diagnosis (Years)	Median (Min, Max)	7 (3,13)	7 (0,11)	7 (0,11)	6 (1,13)
Baseline Loes	Median (Min, Max)	4.5 (0.5, 9)	3 (1, 9)	2 (1, 9)	2 (1, 9)
Baseline NFS	Median (Min, Max)	0 (0,1)	0 (0,1)	0 (0,1)	0 (0,1)

Abbrev.: TP, Transplant Population; TPES, Strictly ALD-102-eligible Transplant Population; NMSD, No Matched Sibling Donor; NFS, Neurologic Function Score.

¹ Age at time of treatment.

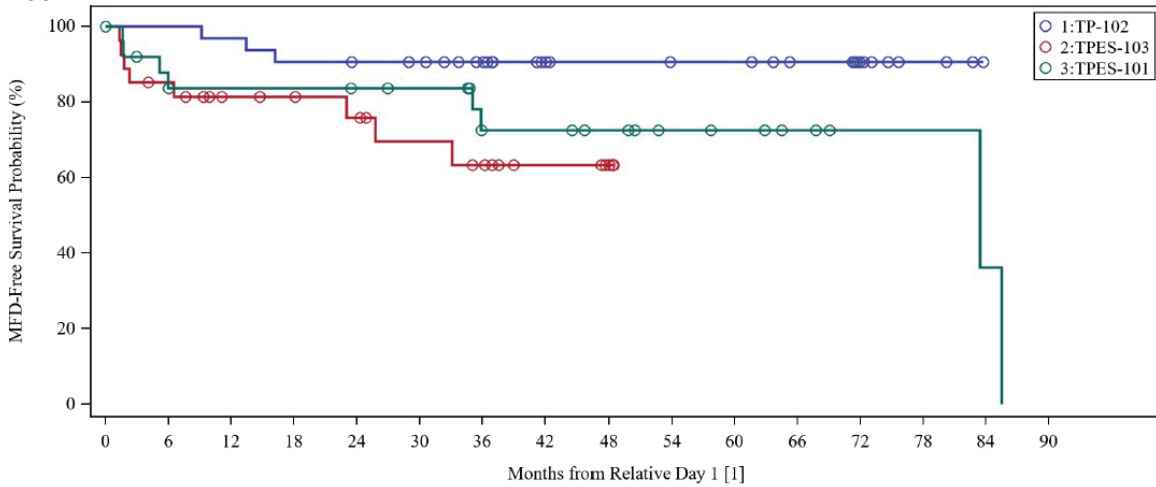
Source: Reviewer's analysis of ADSL datasets.

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. In the Applicant's analysis, repeat HSCT was imputed as a 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 3.

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 4. In both figures, eli-cel appears superior to the similar allo-HSCT populations.

Figure 3: Major Functional Disability (MFD)-Free Survival for TP-102, TPES-101 and TPES-103

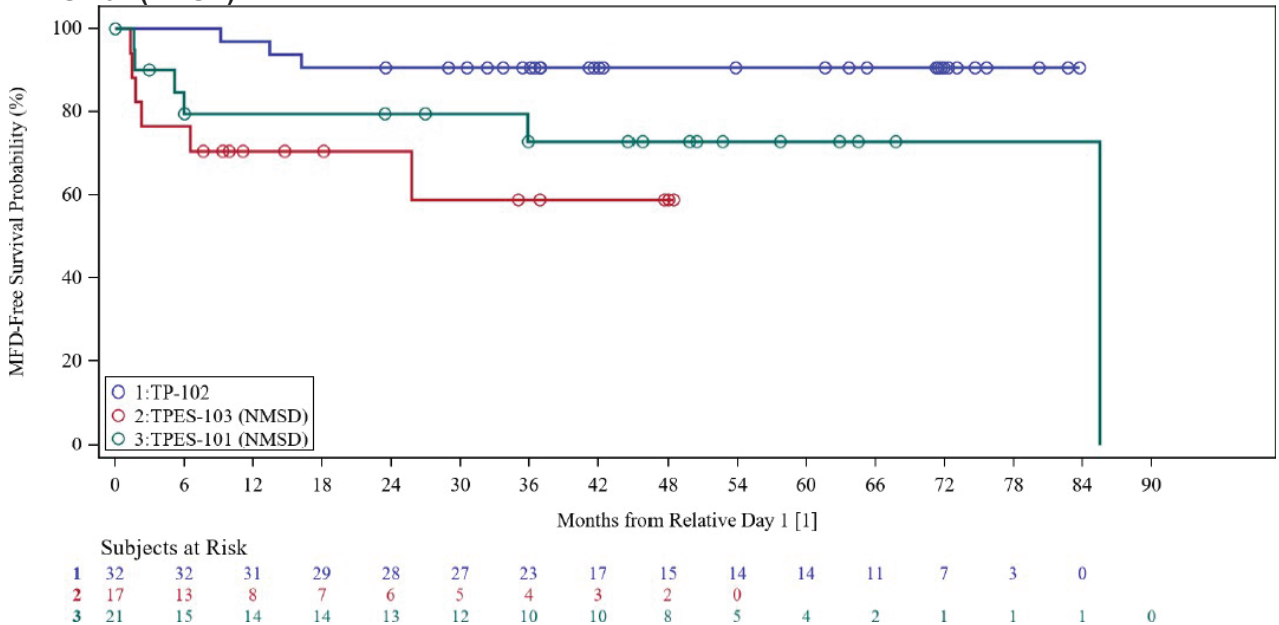


Abbrev.: TP, Transplant Population; TPES, Strictly ALD-102-eligible Transplant Population.
Note: Estimates of MFD-free survival and restricted mean survival time are obtained using the Kaplan- Meier method, where events include deaths, MFDs, and rescue cell administration or second allo-HSCT. For all 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

Source: bluebird bio, Inc., Original BLA submission, Figure 2.1.2.1

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



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

Source: bluebird bio, Inc., Original BLA submission, Figure 2.1.2.1.2

Reviewer Comment: *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 13. The Applicant provided propensity score (PS) adjustments to account for such differences, but we do not believe PS adjustments are sufficient to account for the known and unknown baseline differences between groups. As such, the adjustments were minimal, and therefore results are not shown.*

As previously stated, we do not agree that repeat HSCT for failure of initial HSC graft is an outcome equivalent to MFD or death, and therefore do 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.

Additional Secondary Endpoints: Change in NFS and Gadolinium Enhancement (GdE) at Month 24

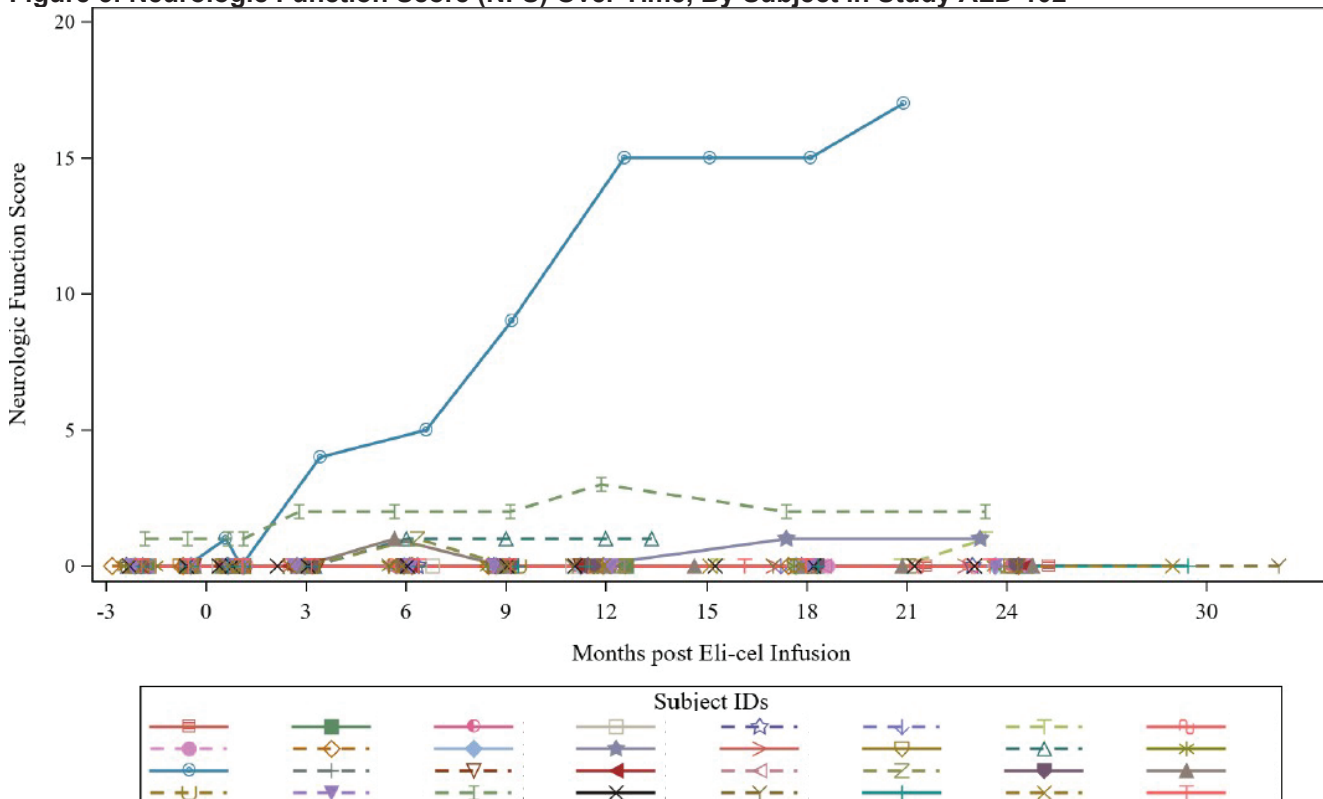
In the primary analysis, 30 of 32 subjects in Study ALD-102 were evaluable for Neurologic Function Score (NFS) and gadolinium enhancement endpoints at the Month 24 visit. Two (2) subjects withdrew from the study to receive allo-HSCT prior to the Month 24 visit. The subject who developed MFDs and subsequently 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 5. 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. TPES allo-HSCT-treated subjects had similar changes in NFS at Month 24 to eli-cel- treated subjects.

Reviewer Comment: *While 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, we are not confident that 24 months is sufficient time to assess stability of NFS.*

Figure 5: Neurologic Function Score (NFS) Over Time, By Subject in Study ALD-102



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

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 (87%) had a GdE- MRI at the Month 24 visit, compared to 100% of TPES-103 subjects evaluable at Month 24.

Reviewer Comment: Clinical implications of resolution of gadolinium positivity on MRI following treatment are unknown at this time and could only be evaluated with additional time in follow-up. Resolution of gadolinium enhancement on MRI would not be expected to occur spontaneously in an untreated CALD population, and thus is supportive of product efficacy. However, due to unknown clinical implications of resolution of contrast enhancement in the short duration of follow-up, it is not an appropriate biomarker surrogate endpoint for the efficacy analysis.

6.1.11.3 Subpopulation Analyses

Eli-cel use was evaluated only in a male pediatric CALD population. Use was not evaluated in infants, the elderly, immunocompromised patients, or pregnant or lactating women. Subjects were primarily white, and other racial or ethnic groups were insufficiently represented to detect substantial differences in the small number of events.

6.1.11.4 Dropouts and/or Discontinuations

There were no drop-outs during the course of the trial, though one subject withdrew from the long-term follow-up study, LTF-304, after the 36 month post-treatment visit.

6.1.11.5 Exploratory and Post Hoc Analyses

Exploratory and post hoc analyses were primarily conducted on data pooled from subjects treated with eli-cel in Studies ALD-102 and ALD-104 and are discussed in [Section 7](#). Results of neuropsychological testing and quality of life assessments were primarily reported for subjects in ALD-102, however, as there was a paucity of data for ALD-104.

Neuropsychological and Quality of Life Analyses

Individual by-subject plots of changes in Performance/Reasoning/Visual Intellectual Quotient Subset (PrvIQ) using the age-appropriate Wechsler panel were trended over time by subject, with a majority of subjects experiencing mild decreases early following treatment and stabilization at later time points. The majority of subjects had normal or near-normal PrvIQ (i.e., 100 ± 15) with relatively stable course throughout the study. Full-Scale Intellectual Quotient trends over time were similar to the PrvIQ. Results were compared to similar subjects who were treated with allo-HSCT in ALD-103, but data from ALD-103 was limited due to partial retrospective study design and early termination of the study.

The Vineland Adaptive Behavior Scale (VABS) composite was assessed throughout ALD-102 but was not assessed in LTF-304 or ALD-103. It assesses long-term quality of life measures as related to daily functioning based on adaptive behaviors. Results remained stable for the majority of subjects and mirrored results with the PrvIQ.

Quality of life was primarily assessed with the PedsQL, and individual by-subject plots of changes were trended over time. The vast majority of subjects had significant variability in the total scale and subscales over time. Only four allo-HSCT subjects in ALD-103 had results for comparison. Caregivers and subjects (when able) completed the global assessment and results were recorded in clinical study reports for each subject. Results were similarly variable over time, and sometimes discordant between subject and caregiver reports.

Reviewer Comment: *Although the majority of subjects maintained relatively stable normal or near-normal PrvIQ and VABS throughout the course of the study, a paucity of data points to trend due to short duration of follow-up and lack of similar assessments in external controls limit the interpretability of results. Quality of life assessments were uninterpretable due to significant variability in results, high potential for bias, and lack of appropriate control group for comparison.*

6.1.12 Safety Analyses

6.1.12.1 Methods

The safety population consists of all 32 subjects who received eli-cel after enrollment in ALD-102. Monitoring for adverse events was ongoing from the time of enrollment. Adverse events could be (1) spontaneously reported, (2) identified in response to an open question from study personnel, or (3) revealed by observation, physical

examination, or other diagnostic procedures. Other safety data were collected through scheduled assessments as outlined in the Schedule of Events (Table 7, Table 8, and Table 9).

6.1.12.2 Overview of Adverse Events.

Adverse events that occurred in one or more subjects in ALD-102 are presented by organ system in the following table. The table also includes a column for serious adverse events (SAEs) and columns for the timeframe when the adverse event occurred. Events that occurred in subjects originally enrolled in ALD-102 that occurred \geq 24 months after treatment with eli-cel in the long-term follow-up study, LTF-304. Note that with the exception of febrile neutropenia, hematologic adverse events are not included in this section, as they are captured as laboratory abnormalities in [Section 8.4.5 Clinical Test Results](#).

Table 14: Adverse Events by Organ System and Time of Onset in Subjects Treated Under ALD-102 (n = 32)

Adverse Event	Subject n (%)	SAE n (%)	Grade 3+	PT	D1 to < NE	NE to < M12	M12 to < M24	M24+
Blood	29 (91%)	8 (25%)	--	--	--	--	--	--
Febrile neutropenia	29 (91%)	8 (25%)	29	--	28	1	--	--
Cardiac	6 (19%)	--	--	--	--	--	--	--
Bradycardia	4 (12%)	--	--	3	1	--	--	--
Tachycardia	3 (9%)	--	--	--	3	--	--	--
Endocrine	5 (16%)	2 (6%)	--	--	--	--	--	--
Adrenal Insufficiency	4 (12%)	2 (6%)	2	2	--	2	--	1
Eye Disorders	5 (16%)	--	--	--	--	--	--	--
Eye swelling	2 (6%)	--	--	--	2	--	--	--
Vision blurred	2 (6%)	--	--	1	--	--	1	--
Gastrointestinal	32 (100%)	3 (9%)	--	--	--	--	--	--
Nausea	30 (94%)	--	4	4	20	12	--	--
Stomatitis	29 (91%)	1 (3%)	10	--	29	--	--	--
Vomiting	28 (88%)	2 (6%)	3	8	17	7	--	1
Abdominal Pain	19 (59%)	1 (3%)	1	8	9	2	--	1
Diarrhea	14 (48%)	--	1	2	10	3	--	--
Constipation	9 (28%)	--	--	3	4	2	--	--
Proctitis	3 (9%)	--	--	--	3	--	--	--
Toothache	2 (6%)	--	--	1	1	--	--	--
Hematochezia	2 (6%)	--	--	--	2	--	--	--
General Disorders	20 (62%)	8 (25%)	--	--	--	--	--	--
Pyrexia	10 (31%)	7 (22%)	2	--	1	8	1	1
Catheter site pain	8 (25%)	--	--	8	--	--	--	--
Fatigue or lethargy	5 (16%)	1 (3%)	--	--	1	3	--	1
Catheter site hemorrhage	2 (6%)	--	--	2	--	--	--	--
Infections	19 (28%)	7 (22%)	--	--	--	--	--	--
Vascular device infection	8 (25%)	3 (9%)	2	4	2	3	--	--
Respiratory tract infection	8 (25%)	1 (3%)	--	5	1	2	1	--
Oral candidiasis	3 (9%)	--	--	--	2	1	--	--
Enterobiasis	2 (6%)	--	--	1	1	--	--	--
Otitis media	2 (6%)	1 (3%)	1	--	--	2	--	--
Sinusitis	2 (6%)	1 (3%)	1	--	--	2	--	--

Adverse Event	Subject n (%)	SAE n (%)	Grade 3+	PT	D1 to < NE	NE to < M12	M12 to < M24	M24+
Bacterial infection/bacteremia	2 (6%)	--	1	--	1	1	--	--
Injury, Poisoning and Procedures	18 (56%)	3 (9%)	--	--	--	--	--	--
Procedural pain or back pain	8 (25%)	1 (3%)	--	7	1	--	--	--
Allergic transfusion reaction	4 (12%)	--	--	--	3	1	--	--
Head injury	3 (9%)	1 (3%)	--	1	1	1	--	--
Fall	2 (6%)	--	--	1	--	1	--	--
Investigations	8 (25%)	--	--	--	--	--	--	--
C-reactive protein increased	2 (6%)	--	--	--	2	1	--	--
INR increased	2 (6%)	--	--	2	--	--	--	--
Metabolism	23 (72%)	1 (3%)	--	--	--	--	--	--
Decreased appetite	22 (69%)	1 (3%)	12	--	17	5	--	--
Fluid retention	4 (12%)	--	--	4	--	--	--	--
Iron deficiency	2 (6%)	--	--	--	--	--	1	1
Protein total decreased	2 (6%)	--	--	1	1	--	--	--
Musculoskeletal and Connective	8 (25%)	--	--	--	--	--	--	--
Bone pain	2 (6%)	--	--	--	1	1	--	--
Arthralgia	2 (6%)	--	--	--	1	1	--	--
Nervous System	19 (59%)	10 (31%)	--	--	--	--	--	--
Headache	13 (41%)	--	2	6	3	1	--	--
Seizure	5 (16%)	9	5	--	--	--	1	5
Visual field defect	3 (9%)	--	--	--	--	1	1	1
Cognitive disorder	2 (6%)	--	--	--	--	--	--	1
Dizziness	2 (6%)	--	--	2	--	--	--	--
Dyskinesia	2 (6%)	1	--	--	1	--	1	--
Dystonia	2 (6%)	--	--	1	1	--	--	--
Sensory loss	2 (6%)	--	--	--	2	--	--	--
Psychiatric	13 (41%)	1 (3%)	--	--	--	--	--	--
Irritability	5 (16%)	--	--	1	3	--	1	--
Fatigue or lethargy	5 (16%)	1 (3%)	--	--	1	3	--	1
Enuresis	3 (9%)	--	--	1	1	1	--	--
Depression	2 (6%)	1 (3%)	--	--	--	1	1	1
Encopresis	2 (6%)	--	--	1	1	--	--	--
Insomnia	2 (6%)	--	--	--	1	--	1	--

Adverse Event	Subject n (%)	SAE n (%)	Grade 3+	PT	D1 to < NE	NE to < M12	M12 to < M24	M24+
Renal and Urinary	6 (19%)	--	--	--	--	--	--	--
Incontinence	3 (9%)	--	--	2	1	1	--	--
Respiratory	13 (41%)	--	--	--	--	--	--	--
Cough	5 (16%)	--	--	4	1	--	--	--
Epistaxis	4 (12%)	--	3	--	--	4	--	--
Fluid retention	4 (12%)	--	--	4	--	--	--	--
Skin and Subcutaneous	29 (91%)	--	--	--	--	--	--	--
Alopecia	23 (72%)	--	--	--	21	2	--	--
Rash	11 (34%)	--	--	8	3	1	--	--
Pruritis	7 (22%)	--	--	4	3	--	--	--
Skin hyperpigmentation	4 (12%)	--	--	--	--	4	--	--
Vascular	5 (16%)	--	--	--	--	--	--	--
Hypertension	3 (9%)	--	--	2	1	1	--	--

Abbrev: PT, prior to eli-cel administration; D1 to < NE, occurring after eli-cel administration and before neutrophil engraftment; NE to < M12, occurring after neutrophil engraftment and before one year post-eli-cel administration; M12 to < M24, occurring from one year to less than two years after eli-cel administration; M24+, occurring at least two years after eli-cel administration

Febrile neutropenia includes 4 subjects with AEs coded as neutropenia or pyrexia

Bradycardia includes bradycardia and sinus bradycardia

Tachycardia includes sinus tachycardia and tachycardia

Adrenal insufficiency includes adrenal insufficiency and adrenocortical insufficiency acute

Eye swelling includes eye swelling and periorbital edema

Vision blurred includes vision blurred and visual acuity reduced

Stomatitis includes oral pain and stomatitis

Abdominal pain includes abdominal discomfort and abdominal pain

Hematochezia includes hematochezia and occult blood positive

Vascular device infection includes catheter site discharge and vascular device infection

Respiratory tract infection includes adenovirus test positive, influenza, nasopharyngitis, rhinorrhea, rhinovirus infection, and viral infection

Oral candidiasis includes oral candidiasis and oropharyngeal candidiasis

Irritability includes anxiety, attention deficit disorder, aversion, and irritability

Incontinence includes incontinence and urinary incontinence

Rash includes dermatitis contact, rash, rash erythematous, and rash maculo-papular

Pruritus includes catheter site pruritus, pruritus, and pruritus allergic

Source: Reviewer's analysis, derived from ADAE dataset

The majority of adverse events occurred early in the study, between the start of conditioning and the time of engraftment. After neutrophil engraftment but within the first year, adverse events were most commonly gastrointestinal or infections. After the first year, nervous systems disorders were the most frequent.

The most common adverse events that occurred prior to eli-cel administration were vomiting, catheter site pain, procedural pain, headache, and rash. The most common adverse events occurring between eli-cel administration and neutrophil engraftment were febrile neutropenia, nausea, stomatitis, vomiting, abdominal pain, diarrhea, decreased appetite, and alopecia. The most common adverse events occurring after neutrophil engraftment were nausea, vomiting, pyrexia, and decreased appetite.

The majority of adverse events were Grade 1 or 2. Grade 3 adverse events that occurred in at least one subject were the following: febrile neutropenia, adrenal insufficiency, nausea, stomatitis, vomiting, pyrexia, vascular device infection, decreased appetite, seizure, and epistaxis.

Some of the adverse events were attributable to progression of CALD. They have been included in Table 14 above and are also being accounted for in the efficacy review, and include many of the neurologic AEs, such as seizures, visual field defect, and cognitive disorder.

Not included in Table 14 above is causality determination. The Applicant attributed the majority of adverse events to conditioning (586 out of the total 912 AEs), and classified the following three adverse events as possibly or definitely related to eli-cel. They are the following:

- Viral cystitis SAE, possibly related to eli-cel
- Vomiting AE, related to eli-cel
- Vomiting AE, possibly related to eli-cel

Important adverse events where eli-cel may have a causal role include those adverse events that do not align with the expected timeline for bone marrow reconstitution: late infections are discussed in [Section 6.1.12.4 Nonfatal Serious Adverse Events](#), delayed engraftment in [Section 6.1.12.5 Adverse Events of Special Interest \(AESI\)](#), and cytopenias in [Section 8.4.5 Clinical Test Results](#). Also related to eli-cel but occurring after the data cut for the BLA is myelodysplastic syndrome, which occurred in one subject in ALD-102 and is described in [Section 8.4.2 Nonfatal Serious Adverse Events](#).

Reviewer Comment: *The adverse event profile of eli-cel overall reflects the adverse events due to conditioning. Because the conditioning is necessary for product administration, conditioning-related adverse events should be taken into consideration in the assessment of the overall safety of the product.*

6.1.12.3 Deaths

Two subjects who were enrolled in ALD-102 died. One death was due to an adenovirus infection in a patient who had rapid progression of CALD after treatment with eli-cel. The second death was a complication of HSCT in subject who underwent allogeneic HSCT after eli-cel failed to stabilize the subject's CALD:

- Subject (b) (6) had clinical progression of CALD at his Month 12 visit. At 21 months, he was admitted to the intensive care unit with fever and respiratory “discomfort.” He developed multisystem organ failure and died of cardiorespiratory arrest. His illness was attributed to an adenovirus infection. In addition to CALD, he had adrenal insufficiency and reactive airway disease.
- Subject (b) (6) was withdrawn from ALD-102 because of CALD progression, and 13 months after treatment with eli-cel he underwent allo-HSCT. The first allo-HSCT failed to engraft, and 22 days after the second allo-HSCT, the subject died from complications of HSCT and chemotherapy leading to multisystem organ failure.

Reviewer Comment: *Survival was a component of efficacy endpoints and therefore these deaths are considered within the efficacy analysis in [Section 6.1.11](#). However, it is notable that the cause of death in the subject with rapid progression of CALD, complications of a viral infection, is not typical in CALD and could be related to impaired immune function.*

6.1.12.4 Nonfatal Serious Adverse Events

Fifty-five nonfatal serious adverse events (SAEs) occurred in ALD-102 subjects. The SAEs that occurred during the first month after eli-cel administration were generally consistent with myeloablative conditioning. Further out from treatment, the most notable SAEs were opportunistic infections. SAEs also included normal childhood illnesses and complications of CALD. In this section, the adverse events are divided into those occurring prior to eli-cel administration, between eli-cel administration and neutrophil engraftment, and after neutrophil engraftment.

Prior to eli-cel administration, four SAEs were reported. There were two instances of adrenal insufficiency, one of procedural pain, and one of vascular device infection. Adrenal insufficiency is a common manifestation of ALD, and thus adrenal crisis is not a surprising event in this population. Likewise, a central line infection would be a relatively frequent complication in subjects undergoing apheresis. The procedural pain SAE was unusual in that pain from a minor procedure (lumbar puncture) resulted in a one-week hospitalization.

Between eli-cel administration and neutrophil engraftment, 11 SAEs were reported. Eight were cases of febrile neutropenia, and the other three were stomatitis, dyskinesia, and anorexia. While febrile neutropenia and stomatitis are clinically important, they are common after myeloablation. The dyskinesia SAE was attributed to a scopolamine patch, although it may have reflected progression of CALD in this subject. The anorexia case was severe, with the subject requiring total parenteral nutrition and during a 15-day hospitalization. The Investigator attributed this SAE to conditioning, which is reasonable although eli-cel cannot be ruled out as a contributor.

Between neutrophil engraftment and 12 months after eli-cel administration, 18 SAEs were reported. All were classified as serious because they required hospitalization. Most were either pyrexia or some type of infection. All seven cases of pyrexia were managed with intravenous antibiotics, and five of the seven cases (including the one described in the preceding paragraph) occurred within the first month after neutrophil engraftment, with the others occurring on Day 152 and Day 341. Three of the infections

seemed to be relatively common and uncomplicated infections that are normal in children: otitis media on Day 93, gastroenteritis on Day 169, and sinusitis on Day 299. The other infections are not normal childhood infections and therefore presented in greater detail:

- Cystitis Viral, Day 42: Subject (b) (6), who also experienced pyrexia attributed to a viral process on Day 27, presented with penile pain and dysuria. Urinalysis revealed was positive for protein, blood and WBCs, and mural thickening of the bladder compatible with cystitis was seen on ultrasound. The subject was treated with oxycodone, pyridoxine, and antibiotics. Two days after admission, the BK virus test result was positive at 9×10^8 DNA copies/mL, and the subject was diagnosed with BK virus hemorrhagic cystitis. Symptomatic management continued and hyperhydration was provided, and the subject recovered and was discharged on Day 48.
- Device related infection, Day 37: Subject (b) (6) was admitted with a fever on Day 37. Blood cultures from both lumens of his vascular catheter were positive for *Enterococcus faecalis*. He was treated with intravenous antibiotics and catheter removal. The subject was discharged from the hospital with oral antibiotics on Day 41, and considered recovered on Day 46 when oral antibiotics were discontinued.
- Device related infection, Day 167: Subject (b) (6), who also experienced pyrexia attributed to a viral process from Day 152 to 155, presented with fever, abdominal pain, and vomiting. Blood cultures from both lumens of his vascular catheter were positive for *Mycobacterium chelonae*. He was treated with intravenous antibiotics and the catheter was removed. The subject was discharged from the hospital on Day 197.

Reviewer Comment: *These three infections are opportunistic infections demonstrate opportunistic infection was a significant risk of eli-cel in ALD-102. BK cystitis is a relatively common post-engraftment complication of allogeneic stem cell transplant, although relatively uncommon after autologous transplants, making its occurrence after eli-cel notable. However, it is associated with higher intensity myeloablation regimens such as used prior to treatment with eli-cel. Ongoing treatment of adrenal insufficiency with hydrocortisone may also have increased his risk of post-engraftment hemorrhagic viral cystitis, although this subject is not unique in this regard. Enterococcus faecalis and Mycobacterium chelonae are bacterial opportunistic infections. They should be less common after autologous HSCT for gene therapy than after allogeneic HSCT because autologous HSCs more rapidly reconstitute the immune system. In the case of Enterococcus faecalis, the subject was receiving exogenous glucocorticoids that may have increased his risk. In contrast, the subject who developed Mycobacterium chelonae was not being treated with steroids.*

Non-infection SAEs that occurred between neutrophil engraftment and 12 months after eli-cel administration were a spinal fracture from a motorbike accident, an overnight hospitalization for vomiting approximately nine months after eli-cel administration, and, in a subject with progression of CALD, events of head injury after a fall and later total

incontinence. None of these events are likely related to eli-cel nor significant considerations in eli-cel's safety profile.

Between 12 and 24 months after eli-cel administration, three subjects had SAEs. One was (b) (6) who died after rapid progression of CALD; prior to his death he had SAEs of cortical blindness, loss of communication, wheelchair dependence, respiratory distress, hepatic failure, renal failure, rhabdomyolysis, viral infection, and cardiorespiratory arrest. Other SAEs between 12 and 24 months were influenza and seizure, each occurring in one subject. The subject with influenza was noted to have a low lymphocyte count of $0.56 \times 10^9/L$ at the time of the SAE, and was hospitalized overnight while being treated with intravenous hydrocortisone and hydration. The subject with a seizure SAE had no seizure history and experienced his first seizure almost 2 years after eli-cel. This seems to have been an early indicator of CALD progression rather than a direct effect of eli-cel.

Beyond 24 months, six subjects each had one or more SAEs. One subject had a fatigue SAE that was attributed to psychological anxiety, but resulted in an overnight hospitalization during which the subject was diagnosed with iron deficiency anemia. A second subject had SAEs of depression and suicidal ideation and spent one year in an alcohol and drug rehabilitation program. He also had an SAE of abdominal pain that was accompanied by fever and vomiting, for which he was treated with intravenous fluids and stress dose steroids during a three day hospitalization; he was ultimately diagnosed with food poisoning complicated by adrenal insufficiency. This subject later experienced an SAE of pyrexia; he was hospitalized with fevers, rigors, rhinorrhea, cough, tachycardia and hypotension, treated with stress dose steroids, and discharged the following day. The remaining four subjects all had seizure SAEs that were attributed to CALD.

Reviewer Comment: *Early SAEs aligned with the expected side effects of myeloablative conditioning. Among the later SAEs, most notable are the opportunistic infections, that were not expected in these subjects who were transplanted with peripherally derived autologous HSCs and should have immune function restored relatively quickly. Also notable were four subjects with seizures that were attributed to CALD.*

6.1.12.5 Adverse Events of Special Interest (AESI)

Adverse events of special interest in this study were myelodysplastic syndrome (MDS) and failed neutrophil or platelet engraftment. Neutrophil and platelet engraftment are discussed in this section and MDS is discussed together for the entire development program in Section 8.4.2 Nonfatal Serious Adverse Events

Neutrophil engraftment was defined a priori as 3 consecutive absolute neutrophil count (ANC) laboratory values of $\geq 0.5 \times 10^9$ cells/L (after initial post-infusion nadir) obtained on different days by 42 days post-infusion of eli-cel. All subjects met these criteria, and the Applicant reported a median time to neutrophil recovery of 13 days. However, 24 of the 32 subjects received G-CSF, and many of those had significant declines in neutrophil levels (i.e., to $< 0.5 \times 10^9/L$) after G-CSF was discontinued.

Calculating time of neutrophil recovery as three consecutive absolute neutrophil counts $\geq 0.5 \times 10^9/L$ on three different days within 42 days of eli-cel administration while not

receiving G-CSF, the median day of engraftment was 27 and the range between 15 and 41 days, and therefore, no subject had delayed engraftment by the Applicant's definition.

Eight subjects in ALD-102 did not receive G-CSF: (b) (6). For these subjects, the median neutrophil engraftment day was Day 23, and the range was Day 15 to Day 39.

Reviewer Comment: *Because G-CSF administration hastens recovery of neutrophil counts, I also calculated day of neutrophil recovery based on achievement of $ANC \geq 0.5 \times 10^9$ while **not** receiving G-CSF. The median day of engraftment was 27 and the range between 15 and 41 days. Therefore, even using a more conservative definition for neutrophil engraftment, all subjects had engrafted within the prespecified time frame of 42 days.*

Platelet engraftment was defined as 3 consecutive platelet counts of $\geq 20 \times 10^9/L$. The Applicant did not prespecify a cut-off date for delayed or failed engraftment, and the median platelet engraftment day was Day 32. However, using a 42 day duration for defining platelet engraftment failure, four subjects failed to engraft, giving an incidence of platelet engraftment failure of 12.5%. These four subjects eventually achieved platelet counts of $\geq 20 \times 10^9/L$ with the following timeframe: Day 44 for Subject (b) (6), 47 for Subject (b) (6), 55 for Subject (b) (6), and 60 for Subject (b) (6).

6.1.12.6 Clinical Test Results

Neutrophil and platelet engraftment are discussed in Section 6.1.12.5 Adverse Events of Special Interest (AESI) above. This Clinical Test Results section includes Integration Site Analysis results and testing for replication competent lentivirus. Laboratory results and vital signs are covered Section 8.4.5 Clinical Test Results.

Integration Site Analysis

The Applicant utilized scheduled integration site analysis to evaluate for clonal predominance and to identify subjects in need of clinical work-up for malignancy. The assessment method changed during the course of the study. NR(LAM-PCR) was utilized through 31 May 2019, and thus for the majority of assessments. After 31 May 2019, the more quantitative method of S-EPTS/LM-PCR was utilized. In ALD-102, integration site analysis was performed 244 times, with the first 164 tests conducted with (NR)LAM-PCR and the remaining 80 with S-EPTS/LM-PCR.

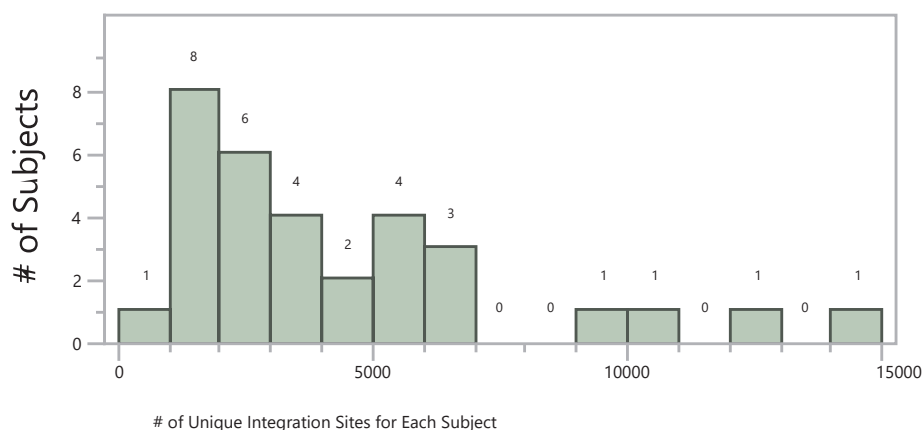
The change in analysis method, while improving the accuracy of the results, increase the difficulty of identifying trends over time. Furthermore, the data demonstrate the different likelihood of detecting certain genes when using the different methods. For instance, (b) (6) had a MECOM integration repeatedly confirmed by qPCR, but it was never identified as a Top 10 integration site by (NR)LAM-PCR. When the testing changed to S-EPTS/LM-PCR, the relative frequency was much higher, with this MECOM integration site detected with a relative frequency of 26% and thereafter persisted in the top 3 integration sites in S-EPTS/LM-PCR.

The algorithm that determines the timing of ISA and the criteria for initiating a clinical work-up changed several times prior to the completion of ALD-102. The algorithm in place at the time of submission of the BLA is provided as Figure 2 above.

The algorithm outlines ISA performance every 6 months through Year 5, and then at years 7, 10, and 15. Results of an overall vector copy number of > 0.3 c/dg and any relative integration site frequency $> 30\%$ or multiple integration sites apparently in the same clone adding to $> 30\%$ prompted qPCR testing to determine the frequency and vector copy number of that integration site more accurately. If the integration site-specific vector copy number measured by qPCR was > 0.5 c/dg, the clone was classified as a “predominant clone,” an integration site analysis was repeated with a new sample, and a clinical work-up was initiated. A predominant clone was classified as persistent if it met criteria for predominance at ≥ 2 timepoints. No subject met criteria for clonal predominance during the two-year follow-up period of ALD-102. However, at year 5, one ALD-102 subject ((b) (6)) enrolled in long-term follow-up developed a predominant clone.

The number of unique integration sites for each subject varied widely. The mean number of integration sites was 4321, the median 3676, and the range 4343 to 14500. The distribution of the number of unique integration sites for each subject is presented in the following figure, which shows the majority of subjects having between 1000 and 4000 unique integration sites, and only four having more than 7000 unique integration sites.

Figure 6: Distribution of Unique Integration Site Number in ALD-102 Subjects



Source: Reviewer’s Analysis of ADISAVCN dataset

Due to the high number of total integration sites, the integration site data submitted in the BLA were limited to the ten most prevalent integrations for each subject at each timepoint (referred to as the Top 10). Across all ALD-102 subjects, the Top 10 integration sites occurred in 821 different genes. The most frequent genes for integration, the total number of times the gene was identified as being in the Top 10, and the number of subjects in ALD-102 with integration into that gene are presented in the following table. The high percentage of subjects with integration sites in *MECOM* and *MPL*, two proto-oncogenes that are involved in hematologic malignancy, is concerning for potentially increasing risk of malignant transformation.

Table 15: Genes with the Highest Number of Integration Sites in ALD-102

Gene	# of Top 10 Instances	# of Subjects with Top 10 Instances (%)
<i>SMG6</i>	117	21 (66%)
<i>MPL</i>	45	9 (28%)
<i>MECOM</i>	41	12 (38%)
<i>CCND2-AS1</i>	32	12 (38%)
<i>KMT2A</i>	14	7 (22%)
<i>MIR100HG</i>	10	4 (12%)
<i>TIE1</i>	7	3 (9%)
<i>HMGA2</i>	7	2 (6%)
<i>PARP11</i>	6	5 (16%)
<i>IGF2BP2</i>	6	3 (9%)
<i>ADAM10</i>	6	2 (6%)

Source: Reviewer's analysis of ADISAVCN dataset

ISA demonstrated many integration sites with high relative frequencies. One hundred four integration sites had a relative frequency of $\geq 10\%$. Eleven integration sites had a relatively frequency of $\geq 30\%$, and two integration sites had a relative frequency of $\geq 50\%$. The 19 subjects where these increased relative frequencies were observed are demonstrated in the table below, along with the number of times an integration site result was above a certain threshold by subject.

Table 16: Instances of Integration Site Relative Frequency $\geq 10\%$ by Subject in ALD-102

Subject	$\geq 10\%$	$\geq 20\%$	$\geq 30\%$	$\geq 40\%$	$\geq 50\%$
(b) (6)	28	21	8	3	1
	10	4	--	--	--
	7	2	--	--	--
	7	3	--	--	--
	7	3	--	--	--
	6	--	--	--	--
	6	2	1	1	1
	5	1	--	--	--
	5	--	--	--	--
	5	1	1	--	--
	3	--	--	--	--
	3	--	--	--	--
	3	--	--	--	--
	2	--	--	--	--
	2	1	1	1	--
	2	--	--	--	--
	2	--	--	--	--
	2	1	--	--	--
	1	--	--	--	--
	106	39	11	5	2

Source: Reviewer's analysis

Changes over time in relative frequency in individual subjects were also evaluated. The following table includes results of $\geq 10\%$ relative frequency, the assessment type used (NR(LAM)-PCR or S-EPTS/LM-PCR), and confirmatory qPCR results when qPCR was performed. This table demonstrates that the ISA and qPCR frequency are not always well correlated, and that correlation is generally better for S-EPTS/LM-PCR than for (NR)LAM-PCR. The table also shows relative frequency increases for Subject (b) (6) and Subject (b) (6) that are concerning for progression to clonal predominance. Subject (b) (6) appears to have a clone with integration sites in *MECOM* and *KDM4B* that are increasing in relative frequency. Subject (b) (6) has a clone with integration sites in *PUM3*, *PLAG1*, and *SECISBP2*, that together have a relative frequency of $\sim 50\%$. Of note, the table does not include Subjects (b) (6), who either died or received a HSCT for CALD progression.

Table 17: ISA Data by Subject for Relative Frequency $\geq 10\%$ in ALD-102

Subject	Gene	ISA Method	Time of Analysis	ISA Relative Frequency (%)	qPCR Relative Frequency* (%)
(b) (6)	<i>MF1</i>	NR	1 year	16.2	--
--	<i>MECOM</i>	S-EPTS	5 years	10.5	--
(b) (6)	<i>SMG6</i>	NR	5 months	29.7	2.64
--	<i>SMG6</i>	NR	1 year	54.6	27.4
--	<i>SMG6</i>	NR	1.5 years	15.4	28.9
--	<i>ZNF26</i>	NR	1.5 years	12.6	--
--	<i>SMG6</i>	NR	2 years	13.7	24.2
--	<i>ZNF26</i>	NR	3.5 years	11.0	--
(b) (6)	<i>SMG6</i>	NR	2 years	14.5	10.8
--	<i>SMG6</i>	NR	2.5 years	11.0	15.5
--	<i>SMG6</i>	NR	3.5 years	18.8	14.5
--	<i>SMG6</i>	NR	4 years	13.9	18.3
--	<i>SMG6</i>	NR	60	15.7	15.0
--	<i>SMG6</i>	S-EPTS	69	10.2	11.8 WB, 19.8 CD15
(b) (6)	<i>MDS1</i>	NR	8.7	19.0	--
--	<i>CAPN7</i>	NR	8.7	11.3	--
--	<i>SMG6</i>	NR	2 years	15.6	4.5
--	<i>SMG6</i>	NR	2.5 years	24.3	4.9
--	<i>INO80</i>	NR	2.5 years	17.7	--
--	<i>SMG6</i>	NR	34.7	20.6	4.0
--	<i>INO80</i>	NR	34.7	14.2	--
(b) (6)	<i>MAN1A1</i>	NR	1.5 years	16.8	--
--	<i>ITFG3</i>	NR	2 years	21.8	--
--	<i>MAN1A1</i>	NR	2 years	14.7	--
--	<i>FAM234A</i>	NR	2.5 years	13.2	--
--	<i>MAN1A1</i>	NR	2.5 years	12.4	--
(b) (6)	<i>RIMKLB</i>	NR	1 year	40.5	--
--	<i>RIMKLB</i>	NR	14 months	17.0	--
(b) (6)	<i>IFT140</i>	NR	6 months	12.0	--
--	<i>PKN2</i>	NR	1 year	12.2	--
--	<i>CCND2-AS1</i>	NR	2 years	11.1	--
--	<i>CCND2-AS1</i>	NR	2.5 years	16.0	--
--	<i>CCND2-AS1</i>	NR	3 years	10.4	--
(b) (6)	<i>C6ORF10</i>	NR	9 months	16.1	--
--	<i>CD4</i>	NR	1 year	11.2	--
--	<i>SMG6</i>	NR	2 years	11.0	--
(b) (6)	<i>SMG6</i>	NR	6 months	11.1	3.1

Subject	Gene	ISA Method	Time of Analysis	ISA Relative Frequency (%)	qPCR Relative Frequency* (%)
--	SMG6	NR	1 year	15.6	11.5
--	DEDD2	NR	1 year	13.6	--
--	ATF6	NR	1 year	10.5	--
--	ACER3	NR	2 years	13.7	2.3
--	SMG6	NR	2 years	11.0	6.2
--	ACER3	NR	2.5 years	30.3	7.2
--	ACER3	NR	3 years	46.0	19.3
--	RFX3	NR	3 years	17.3	14.2
--	ACER3	NR	3.5 years	50.1	23.5
--	RFX3	NR	3.5 years	27.0	24.1
--	ACER3	NR	4 years	42.7	22.4
--	RFX3	NR	4 years	30.2	21.1
--	MECOM	S-EPTS	4.5 years	26.8	23.1
--	ACER3	S-EPTS	4.5 years	24.3	24.3
--	RFX3	S-EPTS	4.5 years	22.6	22.9
--	ACER3	S-EPTS	5 years (59 months)	27.7	26.8 WB, 36.8 CD15
--	MECOM	S-EPTS	5 years (59 months)	25.4	23.8 WB, 32.1 CD15
--	RFX3	S-EPTS	5 years (59 months)	25.4	24.6 WB, 32.2 CD15
--	ACER3	S-EPTS	5 years (61 months)	33.3	31.4 WB, 32.6 CD15
--	MECOM	S-EPTS	5 years (61 months)	27.2	30.2 WB, 30.9 CD15
--	RFX3	S-EPTS	5 years (61 months)	25.9	24.1 WB, 27.0 CD15
--	ACER3	S-EPTS	5.5 years	37.1	30.5 WB, 32.8 CD15
--	RFX3	S-EPTS	5.5 years	29.0	27.8 WB, 35.4 CD15
--	MECOM	S-EPTS	5.5 years	20.7	25.7 WB, 29.7 CD15
--	ACER3	S-EPTS	6 years	34.2	25.8 WB, 29.6 CD15
--	MECOM	S-EPTS	6 years	28.2	24.4 WB, 27.2 CD15
--	RFX3	S-EPTS	6 years	26.4	23.6 WB, 27.6 CD15
--	ACER3	--	6.5 years	--	34.8 WB, 38.5 CD15
--	MECOM	--	6.5 years	--	34.1 WB, 29.7 CD15
--	RFX3	--	6.5 years	--	31.6 WB, 28.8 CD15
(b) (6)	CASC3	NR	6 months	10.5	--
--	SULT1E1	NR	1 year	18.3	--
(b) (6)	KDM4B	NR	4 years	20.7	7.1
--	KDM4B	S-EPTS	4.5 years	15.9	10.9
--	MECOM	S-EPTS	4.5 years	13.9	9.1
--	KDM4B	S-EPTS	5 years	20.4	14.6
--	MECOM	S-EPTS	5 years	19.5	12.6
--	MECOM	S-EPTS	6 years	21.8	15.2
--	KDM4B	S-EPTS	6 years	16.3	15.0
(b) (6)	CYTH1	NR	6 months	37.5	--
--	KDM2A	NR	6 months	16.9	--
--	EWSR1	NR	6 months	11.6	--
--	OR7C2	NR	1 year	10.5	--
--	SMG6	NR	2 years	10.6	2.9
(b) (6)	KNTC1	NR	6 months	17.0	--
--	PUM3	NR	1 year	26.1	18.3
--	PLAG1	NR	1 year	17.6	18.7
--	SECISBP2	NR	1 year	15.5	18.1
--	SECISBP2	S-EPTS	1.5 years	23.7	22.8
--	PLAG1	S-EPTS	1.5 years	23.4	21.0
--	PUM3	S-EPTS	1.5 years	22.0	20.8
--	PLAG1	S-EPTS	2.5 years	19.6	17.6

Subject	Gene	ISA Method	Time of Analysis	ISA Relative Frequency (%)	qPCR Relative Frequency* (%)
--	<i>SECISBP2</i>	S-EPTS	2.5 years	19.1	18.5
--	<i>PUM3</i>	S-EPTS	2.5 years	18.6	16.1
(b) (6)	<i>KNTC1</i>	NR	1 year	13.5	--
(b) (6)	<i>HMG2A</i>	NR	3.5 years	14.5	--
--	<i>HMG2A</i>	NR	4 years	13.6	--
(b) (6)	<i>SARDH</i>	NR	3 months	18.6	--
--	<i>SLC5A20</i>	NR	3 months	17.2	--
--	<i>AGPS</i>	NR	6 months	10.3	--
(b) (6)	<i>MECOM</i>	NR	6 months	15.4	2.1
--	<i>MECOM</i>	NR	1 year	18.7	4.2
--	<i>MECOM</i>	NR	1.5 years	10.3	1.1
--	<i>SMG6</i>	S-EPTS	2 years	11.5	6.3
--	<i>SMG6</i>	S-EPTS	2.5 years	21.9	13.0
--	<i>SMG6</i>	S-EPTS	3 years	24.2	13.6
--	<i>SMG6</i>	S-EPTS	3.5 years	20.6	17.3

Abbrev: ISA, integration site analysis; qPCR, quantitative polymerase chain reaction; NR, linear amplification polymerase chain reaction plus non-restricted linear amplification polymerase chain reaction (NR)LAM-PCR; S-EPTS, Shearing extension primer tag selection ligation-mediated polymerase chain reaction (S-EPTS/LM-PCR)*measured in whole blood unless two results are provided in the cell, in which case the first value is in whole blood and the second value is in CD15+ cells

Source: Reviewer's analysis

One subject met criteria for clonal predominance while enrolled in ALD-102. At year 5, a predominant clone was identified with integrations in *MECOM*, *ACER3*, and *RFX3* in subject (b) (6). He had a relatively uneventful clinical course, to include timely neutrophil and platelet engraftment, and slow but steady recovery of CBC values to the normal range, with the notable exception of neutrophil counts that declined to below normal on most assessments from year 4.5 through 6. The subject also had several bone marrow biopsy and aspirates as a result of his ISA findings. Bone marrow studies have revealed moderately hypocellular marrow with maturing trilineage hematopoiesis, and no increase in blasts or overt features of myelodysplasia.

Recombinant lentivirus assessment

Blood samples were evaluated for recombinant lentivirus at 3, 6, and 12 months after elice infusion, and there were no instances of positive results for vector-derived recombinant lentivirus.

6.1.12.7 Dropouts and/or Discontinuations

Three of 32 treated subjects did not complete the ALD-102. They were Subject (b) (6) who died, and Subjects (b) (6) who were discontinued to receive allo-HSCT due to disease progression. These three subjects not having completed the study does not significantly impact the evaluation of safety, because of the relatively small number of discontinuations. Furthermore, two of the subjects died (including (b) (6) who died of HSCT complications) and those deaths are considerations in the overall assessment of safety.

A fourth subject dropped out from the long-term follow-up study. However, no information about the reason for discontinuation nor his status was available.

6.1.13 Study Summary and Conclusions

This study enrolled 32 subjects and 29 completed the 2-year follow-up. The safety data demonstrate that eli-cel has a side effect profile that largely reflects the safety of the myeloablative and lymphodepleting medications that are administered prior to eli-cel. However, several subjects had unexpected opportunistic infections between one and six months after eli-cel administration. Additionally, one of the 29 subjects followed for at least 2 years went on to develop MDS, and many others have large clones identified by integration site analysis. With additional follow-up time, the diagnosis of additional cases of hematologic malignancy seems likely.

6.2 Trial #2

A Phase 3 Study of Lenti-D Drug Product After Myeloablative Conditioning Using Busulfan and Fludarabine in Subjects ≤ 17 Years of Age with Cerebral Adrenoleukodystrophy (ALD-104) (24 Jan 2019 to ongoing at time of review)

6.2.1 Objectives

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

6.2.2 Design Overview

The study 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.

6.2.3 Population

The key enrollment criteria were the same as in Study ALD-102, provided in Section 6.1.3 Population except subjects are not excluded for having an available and willing matched sibling donor.

6.2.4 Study Treatments or Agents Mandated by the Protocol

Study treatments were mandated by the protocol for the different phases including CD34+ cell collection (also referred to as mobilization), myeloablative conditioning, and during eli-cel transfusion (transplant).

Mobilization

- G-CSF (starting dose 10 $\mu\text{g/kg}$) was administered for 4 to 7 days
 - Dose decreased for WBC $> 70 \times 10^9$ cells/L
- Plerixafor 0.24 mg/kg daily for up to 3 days

Conditioning

- Busulfan IV on Days -6, -5, -4, and -3, dose as follows:
 - If based on cumulative exposure:

- Dosed every 6, 12 or 24 hours, with choice of dosing frequency at the investigator's discretion.
- Cumulative exposure targeted to 20,706 to 23,180 $\mu\text{mol}\cdot\text{min}/\text{L}$
- Target area under the curve [AUC] range of 1335 to 1491 $\mu\text{mol}\cdot\text{min}/\text{L}$ if using first dose to calculate busulfan exposure
- Fludarabine
 - Through Nov. 27, 2019, fludarabine IV 30 mg/m^2 on Days -8, -7, and one hour before busulfan on Days -6, -5, -4, and -3
 - After Nov. 27, 2019, fludarabine IV 40 mg/m^2 one hour before busulfan on Days -6, -5, -4, and -3

Eli-cel Infusion

- Intravenous administration through a central venous catheter in a volume between 20 and 80 mL, according to institutional practice, at least 48 hours after completion of conditioning with busulfan and fludarabine
- Dose: $\geq 5 \times 10^6$ CD34+ cells/kg

G-CSF

- Starting 5 days after eli-cel administration with dose at the Investigators' discretion
- Stop after 3 consecutive days with an ANC $> 0.5 \times 10^9/\text{L}$, or any time thereafter at the Investigator's discretion

The following table outlines the conditioning dose information as provided by the Applicant in the ALD-104 Clinical Study Report.

Table 18: Exposure to Busulfan and Fludarabine During Conditioning in Study ALD-104

Parameter	Statistic	Result (N = 23)
Average Daily Dose Busulfan ($\text{mg}/\text{kg}/\text{day}$) ^a	n	23
--	Median	4.20
--	Min, Max	3.0, 5.3
Estimated Average Daily Busulfan AUC ($\mu\text{M}\cdot\text{min}/\text{day}$)	n	21
--	Median	5339.0
--	Min, Max	3478, 5695
Total Ordered Dose Fludarabine, (mg/m^2)	--	--
154	n (%)	1 (4.3)
160	n (%)	9 (39.1)
170.8	n (%)	1 (4.3)
180	n (%)	11 (47.8)
196.4	n (%)	1 (4.3)

Abbrev: AUC, area under the curve, Max, maximum; Min, minimum; TP, Transplant Population.

^a Calculated as the sum of busulfan dose infused divided by weight prior to conditioning and number of days of conditioning.

Source: Reviewer's analysis of ADPP dataset

6.2.5 Directions for Use

Directions for use were the same as in Study ALD-102, provided in Section 6.1.5 Directions for Use.

6.2.6 Sites and Centers

Study ALD-104 was conducted at 8 clinical sites in France, Germany, Italy, Netherlands, the United Kingdom, and the United States. The list of primary study sites and Principal Investigators as compiled by the Applicant in the Study ALD-104 Clinical Study Report follows.

Table 19: Primary Study Sites and Principal Investigators Study ALD-104

Site Number	Study Center	Principal Investigator
105	Boston Children's Hospital/ Massachusetts General Hospital Boston, MA, USA	Christine Duncan, MD, Msc Florian Eichler, MD
106	Great Ormond Street Hospital for Children NHS Foundation Trust London, UK	Adrian Thrasher, MBBS, PhD Robert Chiesa, MD
107	Hôpital Robert Debré Paris, France	Jean-Hugues Dalle, MD, Msc, PhD
109	University of Minnesota, Masonic Children's Hospital Minneapolis, MN, USA	Paul Orchard, MD
119	Lucile Packard Children's Hospital Palo Alto, CA, USA	Ami Shah, MD
135	Ospedale Pediatrico Bambino Gesù Rome, Italy	Franco Locatelli, MD
150	Universitätsklinikum Leipzig AöR Leipzig, Germany	Jörn-Sven Kühl, MD
152	Prinses Maxima Centrum Utrecht, Netherlands	Caroline Lindemans, MD, PhD

Source: Adapted from Original BLA 125755; ALD-104 Appendix 16.1.4 Description of Investigators and Sites, p.1

6.2.7 Surveillance/Monitoring

As with Study ALD-102, subjects were actively monitored via the schedule of events that is included in this section, and data were collected on case reports forms. A Data Monitoring Committee was used to provide an independent assessment of safety during the study. Efficacy assessments were performed by a pediatric neurologist or someone determined to be an appropriately trained and qualified physician. Also unchanged between Study ALD-102 and ALD-104 is the schematic for assessment of clonal predominance.

The schedule of events is divided into three tables, representing time before eli-cel infusion, post-eli-cel infusion to two years, and during long-term follow-up:

Table 20: Schedule of Events - Screening through Drug Product Infusion in Study ALD-104

--	Screening	Mobilization ¹	Apheresis	Pre-Conditioning	Conditioning and Monitoring
Study Day:	-60 to -45	-44 to -37	-40 to -37	-7	-6 to -1
Informed Consent ²	+	--	--	--	--
Search for allogeneic donor & HLA typing ³	+	--	--	--	--
Demographics & Medical History	+	--	--	--	--
ABCD1 genotype ⁴	(+)	--	--	--	--
Adrenal function ⁵	+	--	--	--	--
Local lab: Blood for immunological studies	+	--	--	--	--
Sperm / testicular tissue banking, if requested ⁶	+	--	--	--	--
Serology panel	+	+	--	--	--
Physical examination, Vital signs, Weight ⁷	+	+ ⁸	+ ⁹	+	+ ¹⁰
Hematology ¹¹	+	+ ¹²	+ ¹²	+	+ ¹³
Clinical chemistry	+	--	--	+	+ ¹³
Glomerular Filtration Rate ¹⁴	+	--	--	+	--
Blood specialty labs:	--	--	--	--	--
RCL	(+) ¹⁵	--	--	(+) ¹⁵	--
ALDP (Peripheral Blood)	(+) ¹⁵	--	--	(+) ¹⁵	--
VCN (Peripheral Blood)	(+) ¹⁵	--	--	(+) ¹⁵	--
VLCFA (fasting)	+	--	--	--	--
Neurological exam	+	--	--	+	--
NFS assessment ¹⁶	+	--	--	(+) ¹⁶	--
MFD assessment ^{16,17}	+	--	--	(+) ¹⁶	--
Neuropsychological tests	--	--	--	+	--
Global assessment	--	--	--	+	--
PedsQL	--	--	--	+	--
Echocardiogram	+	--	--	--	--
Electrocardiogram	+	--	--	--	--
Brain MRI ¹⁶	+ ¹⁸	--	--	(+) ¹⁶	--
Evoked potentials ¹⁹	+	--	--	--	--
Confirmation of eligibility	+	--	--	+	--
G-CSF and plerixafor	--	+	--	--	--
CD34+ count ²⁰	--	+	+	--	--
Busulfan and Fludarabine administration	--	--	--	--	+
Busulfan level monitoring	--	--	--	--	+
Concomitant medication	+	+	+	+	+
Adverse event monitoring	+	+	+	+	+

Note: (+) denotes an optional assessment

¹ If more than one mobilization cycle is required, they must be separated by an interval of at least 2 weeks.

² If a subject is < 18 years of age at ICF signing and turns 18 while on study, the subject must be re-consented at the next scheduled study visit, prior to the collection of additional study data.

³ A preliminary search for a suitable donor will be initiated at Screening for all subjects in the event that a subject is not eligible for drug product during Pre-Conditioning Assessments, experiences engraftment failure, or cannot receive eli-cel. HLA typing does not need to be performed if historical results are available.

⁴ Genotyping of *ABCD1* gene will occur in subjects for whom no historical data are available; documented *ABCD1* mutation required prior to initiating myeloablative conditioning.

⁵ Adrenal function tests (cortisol and adrenocorticotrophic hormone [ACTH]) are to be performed in the morning (approximately 8:00 am) during Screening before the subject has taken hydrocortisone unless subject is on steroid replacement therapy. If ACTH is significantly elevated, tests should be repeated 3 hours after taking hydrocortisone. Mineralocorticoid functions (aldosterone and plasma renin activity) are to be performed at the same time points with the subject sitting in an upright position.

⁶ May occur any time before conditioning; hormonal treatment, if applicable as part of banking, should stop at least 7 days prior to conditioning.

⁷ Full physical examination, including height and weight measurements, will be performed at Screening only. Vital signs will include blood pressure, pulse, respiratory rate, and temperature. During hospitalization, focused physical examinations and vitals to be performed as standard of care. AEs identified during this time will be entered into the clinical database.

⁸ Focused physical examinations and vital signs will be performed prior to the first dose of G-CSF.

⁹ On each day of apheresis, the subject should have a focused physical exam, including abdominal palpation to rule out splenomegaly, and vital signs performed prior to beginning apheresis and again after completion of apheresis.

¹⁰ Focused physical examinations and vital signs will be performed each day during conditioning.

¹¹ Hematology parameters include white blood cell (WBC) count with differential, hemoglobin, hematocrit, red blood cell (RBC), and platelet count.

¹² Hematology will be performed each day of mobilization and apheresis.

¹³ Chemistry and hematology parameters will be measured daily during conditioning; blood will be collected prior to infusion of busulfan IV and fludarabine IV.

¹⁴ Calculated from sex, age, height, weight, and creatinine. Unit = mL/min/1.73m²

¹⁵ Blood for measurements of RCL, ALDP, and VCN will be drawn once, any time from Screening prior to start of conditioning.

¹⁶ NFS assessments, MFD assessments, and brain MRIs may be repeated at any time during the study if there is evidence of clinical decline. These assessments must be repeated if more than 60 days has passed between the assessment at Screening and the start of Pre-Conditioning. However, if subject requires sedation for MRI, performing this repeat assessment is based on Investigator judgment.

¹⁷ May be performed concurrently with NFS assessment.

¹⁸ MRI performed within 5 days of signing of ICF can be used as the Screening MRI.

¹⁹ The Brain Stem Auditory Evoked Response (BAER) and the Visual Evoked Potential (VEP) P100 latency will be performed at Screening.

²⁰ Peripheral blood CD34+ count should be performed either the day prior to or on the first planned day of apheresis.

Source: Adapted from BLA 125755; ALD-104 Protocol and Amendments, p.55-56

Table 21: Schedule of Events - Drug Product Infusion through End of Study Year 2

--	Eli-cel Infusion	Follow-Up Week 2	Follow-Up Month 1	Follow-Up Month 2	Follow-Up Month 3	Follow-Up Month 6	Follow-Up Month 12	Follow-Up Month 18	Follow-Up Month 24	Early Termination
Study Day:	1	15 ±7	30 ±7	60 ±14	90 ±14	180 ±14	360 ±30	540 ±30	720 ±30	-
Eli-cel infusion ¹	+	--	--	--	--	--	--	--	--	--
Physical examination, Vital signs, Weight ²	+	+	+	+	+	+	+ ³	+	+ ³	+ ³
Hematology ⁴	--	+ ⁵	+ ⁵	+	+	+	+	+	+	+
Clinical chemistry	--	+ ⁵	+ ⁵	+	+	+	+	+	+	+
Local lab: Blood for immunological studies	--	--	--	--	--	--	+	--	+	+
Blood specialty labs:	--	--	--	--	--	--	--	--	--	--
RCL ⁶	--	--	--	--	+	+	+	--	+ ⁷	+ ⁷
Integration Site Analysis ⁸	--	--	--	--	--	+	+	+	+	+
ALDP (Peripheral Blood Populations)	--	--	+	+	+	+	+	+	+	+
VCN (Peripheral Blood Populations) ⁸	--	--	+	+	+	+	+	+	+	+
VLCFA (fasting)	--	--	--	--	--	--	+	--	+	+
Neurological exam	--	--	+	--	+	+	+	+	+	+
NFS assessment ⁹	--	--	+	--	+	+	+	+	+	+
MFD assessment ^{9, 10}	--	--	+	--	+	+	+	+	+	+
Neuropsychological tests	--	--	--	--	--	--	+	--	+	+
Global assessment	--	--	--	--	--	--	+	--	+	+
PedsQL	--	--	--	--	+	+	+	--	+	+
Electrocardiogram	--	--	--	--	--	--	--	--	+	+
Brain MRI (with and without contrast) ⁹	--	--	--	--	--	+	+	+	+	+
Evoked potentials ¹¹	--	--	--	--	--	--	+		+	+
Concomitant medication	+	+	+	+	+	+	+	+	+	+
Adverse event monitoring	+	+	+	+	+	+	+	+	+	+

- ¹ Start G-CSF 5 days after infusion (Day 6; Day 1 is defined as day of eli-cel infusion), with dose at Investigator's discretion per usual institutional practice. GCSF may be held to start later at the Investigator's discretion if the WBC has not fallen to nadir.
- ² During hospitalization, focused physical examinations and vitals to be performed as standard of care. AEs identified during this time will be entered into the clinical database. Vital signs are to be monitored concurrently during eli-cel infusion according to institutional practice at the clinical site, but no less frequently than at the start, once during, and upon completion of the infusion.
- ³ Full physical examination, including height and weight measurements, will be performed. Focused physical examinations may be performed at other visits.
- ⁴ Hematology parameters include white blood cell (WBC) count with differential, hemoglobin, hematocrit, red blood cell (RBC), and platelet count. If the results from blood tests are not as expected, additional testing may need to be performed and may include a physical exam, blood tests, imaging tests, or a bone marrow biopsy.
- ⁵ Chemistry and hematology parameters will be measured at least twice per week until neutrophil engraftment occurs.
- ⁶ Two samples are required, one for RCL screening test, another for potential co-culture of PBLs if RCL screening test is positive.
- ⁷ If a subject's previous RCL tests were all negative, this sample will be archived.
- ⁸ Additional blood may be collected for analysis in cell subtypes.
- ⁹ NFS assessments, MFD assessments, and brain MRIs may be repeated at any time during the study if there is evidence of clinical decline.
- ¹⁰ May be performed concurrently with NFS assessment.
- ¹¹ The BAER will be performed at Month 24. The VEP P100 latency will be performed at Month 12 and Month 24.
- Source: Adapted from BLA 125755; ALD-104 Protocol and Amendments, p.57-58

Table 22: Schedule of Events - Long-Term Following-Up

Post-drug product infusion Timepoint ^a :	Y2.5	Y3	Y3.5	Y4	Y4.5	Y5	Y6	Y7	Y8	Y9	Y10	Y11	Y12	Y13	Y14	Y15
Visit Window (Days):	±30	±30	±30	±30	±30	±30	±90	±90	±90	±90	±90	±90	±90	±90	±90	±90
Local lab: Blood for immunological studies	--	+	--	+	--	+	--	--	--	--	(+)	--	--	--	--	(+)
Physical Exam, Vital signs ^b	+	+	+	+	+	+	(+)	(+)	(+)	(+)	(+)	--	--	--	--	(+)
Neurological Exam	+	+	+	+	+	+	(+)	(+)	(+)	(+)	(+)	--	--	--	--	(+)
NFS assessment	+	+	+	+	+	+	+	+	+	+	+	--	--	--	--	+
MFD assessment ^c	+	+	+	+	+	+	+	+	+	+	+	--	--	--	--	+
Global Assessment	+	+	+	+	+	+	(+)	(+)	(+)	(+)	(+)	--	--	--	--	(+)
Brain MRI	--	+	--	+	--	+	--	+	--	--	+	--	--	--	--	+
Quality of Life Assessment	--	+	--	+	--	+	(+)	(+)	(+)	(+)	(+)	--	--	--	--	(+)
IQ Assessment	--	+	--	+	--	+	--	--	--	--	(+)	--	--	--	--	(+)

Post-drug product infusion Timepoint ^a :	Y2.5	Y3	Y3.5	Y4	Y4.5	Y5	Y6	Y7	Y8	Y9	Y10	Y11	Y12	Y13	Y14	Y15
Visit Window (Days):	±30	±30	±30	±30	±30	±30	±90	±90	±90	±90	±90	±90	±90	±90	±90	±90
Laboratory tests ^d :	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Complete blood count ^e	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Vector Copy Number (VCN) ^{f,g}	+	+ ^f	+	+ ^f	+	+ ^f	+	+	+	+	+	--	--	--	--	+
Integration Site Analysis (ISA) ^{g,h}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Replication Competent Lentivirus (RCL)	--	+	--	+	--	+	--	--	--	--	--	--	--	--	--	--
ALDP Expression	--	+	--	+	--	+	--	--	--	--	--	--	--	--	--	--
Very Long Chain Fatty Acids (fasting)	--	+	--	+	--	+	--	--	--	--	--	--	--	--	--	--
Adverse event monitoring ⁱ	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Concomitant medication ^j	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Note: (+) denotes assessments that are not required; data would be collected from chart review for assessments that were performed per standard of care

^a Visits are based on time post-eli-cel infusion in parent study.

^b Vital signs will include weight, height, blood pressure, pulse (heart rate), respiratory rate, and temperature.

^c May be performed concurrently with the NFS assessment.

^d If the results from blood tests are not as expected, additional testing may need to be performed and may include a physical exam, blood tests, imaging tests, or a bone marrow biopsy.

^e CBC includes hematocrit, hemoglobin, RBC count, WBC count with differential, and platelet count.

^f VCN will be performed in whole blood at each timepoint, and also in CD14+ cells at visits indicated by this footnote (i.e. visits that coincide with those in which a sample is taken for ALDP expression only).

^g Additional blood may be collected for analysis in cell subtypes.

^h After identification of a persistent predominant clone, ISA is to be performed at least every 6 months

ⁱ Includes all drug product-related AEs, all SAEs regardless of attribution to the drug product, CALD-related ≥ Grade 2 AEs, and immune-related AEs and new or worsening hematologic or neurologic disorders or malignancies.

^j ALD-related concomitant medications and therapies, such as adrenal insufficiency medications, speech therapy, physical therapy, cytotoxic/chemotherapeutic agents, radiotherapy, other potentially mutagenic agents, or investigational medications received by the subject while participating in LTF-304. In addition, concomitant medications used to treat SAEs or DP-related AEs.

Source: Adapted from BLA 125755; LTF-304 Protocol v. 7.0, p. 30-31

6.2.8 Endpoints and Criteria for Study Success

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.

Reviewer Comment: *At the time of BLA submission, no subjects had reached 24 months of follow-up to assess the primary efficacy endpoint in this population.*

6.2.9 Statistical Considerations & Statistical Analysis Plan

The statistical analysis plan was similar to that of Study ALD-102.

6.2.10 Study Population and Disposition

Study subjects in ALD-104 were similar to subjects in ALD-102 and the two populations were pooled for integrated analyses of efficacy. Therefore, subject demographics, baseline disease characteristics, and disposition are discussed in [Section 7, Integrated Overview of Efficacy](#).

6.2.11 Efficacy Analyses

Study subjects in ALD-104 were similar to subjects in ALD-102 and the two populations were pooled for integrated analyses of efficacy. Therefore, analyses of efficacy are discussed in [Section 7, Integrated Overview of Efficacy](#).

6.2.12 Safety Analyses

6.2.12.1 Methods

The safety population consists of all 35 subjects who received study product in ALD-104, although the study was ongoing at the time of datacut for the BLA submission and no subject had completed two-year follow-up. In the safety review, data from the 90-day safety update were used; at that time, all subjects had been treated and 5 had completed the 24-month follow-up visits.

Monitoring for adverse events (AEs) was ongoing from the time of enrollment and AEs could be (1) spontaneously reported, (2) identified in response to an open question from study personnel, or (3) revealed by observation, physical examination, or other

diagnostic procedures. Other safety data were collected through scheduled assessments as outlined in [Section 6.2.7 Surveillance/Monitoring](#).

6.2.12.2 Overview of Adverse Events

The following table includes non-laboratory adverse events (AEs) that occurred in a minimum of two subjects treated under ALD-104. The AEs are presented by overall incidence, number of serious adverse events (SAEs), number of severe AEs (i.e., Grade 3 or 4), and time of onset. Most AEs occurred prior to neutrophil engraftment and are consistent with mobilization and conditioning. After neutrophil engraftment but within the first year, adverse events occurred most commonly in the gastrointestinal and skin and subcutaneous organ systems. After the first year, the number of subjects with safety data was limited, however the infections and nervous system disorders were the most frequent classes of adverse events.

Table 23: Non-laboratory Adverse Events by Organ System and Time of Onset in ALD-104

Adverse Event	Subjects n (%)	SAEs n (%)	Grade 3+ (n=35)	PT (n=35)	D1 to <NE (n=35)	NE to <M12 (n=35)	M12+ (n=14)
Blood	--	--	--	--	--	--	--
Febrile neutropenia	24 (69%)	4 (11%)	23	1	24	--	--
Pancytopenia	5 (14%)	2 (6%)	2	--	3	2	1
Cardiac	--	--	--	--	--	--	--
Bradycardia	1 (3%)	1 (3%)	1	--	--	--	1
Tachycardia	7 (20%)	--	--	1	6	1	--
Ear and Labyrinth Disorders	--	--	--	--	--	--	--
Ear pain	3 (9%)	--	--	2	--	--	1
Endocrine	--	--	--	--	--	--	--
Adrenocortical insufficiency acute*	2 (6%)	--	--	1	--	1	--
Eye Disorders	--	--	--	--	--	--	--
Dry eye	5 (14%)	--	--	1	1	3	--
Vision blurred*	8 (23%)	--	--	2	3	3	--
Gastrointestinal	--	--	--	--	--	--	--
Nausea	28 (80%)	1 (3%)	13	27	9	4	--
Mucositis*#	34 (97%)	1 (3%)	23	1	33	--	1
Vomiting	26 (74%)	1 (3%)	8	22	10	6	1
Abdominal Pain*	14 (40%)	--	1	6	12	3	--
Diarrhea	6 (17%)	--	--	2	3	1	--
Constipation	20 (57%)	1 (3%)	--	13	7	6	--
Dental caries	3 (9%)	--	--	1	--	1	1
General Disorders	--	--	--	--	--	--	--
Pyrexia	13 (37%)	5 (14%)	1	4	6	8	--
Catheter site pain	18 (51%)	--	1	18	--	--	--
Fatigue	3 (9%)	--	--	1	1	1	--
Motor dysfunction*#	4 (11%)	1 (3%)	1	3	--	1	--
Immune System Disorders	--	--	--	--	--	--	--
Drug hypersensitivity	2 (6%)	--	--	--	2	--	--

Adverse Event	Subjects n (%)	SAEs n (%)	Grade 3+ (n=35)	PT (n=35)	D1 to <NE (n=35)	NE to <M12 (n=35)	M12+ (n=14)
Infections	--	--	--	--	--	--	--
Device related infection	2 (6%)	--	1	--	1	1	--
Viral respiratory tract infection*	5 (14%)	1 (3%)	--	3	3	--	2
COVID-19	2 (6%)	--	--	1	--	--	1
Candidiasis*	2 (6%)	--	--	1	--	1	--
Bacteremia*	3 (9%)	3 (9%)	3	--	--	3	--
Pneumonia	2 (6%)	--	1	1	1	--	--
Gastroenteritis viral	2 (6%)	--	--	--	--	1	1
Injury, Poisoning and Procedures	--	--	--	--	--	--	--
Procedural pain*	7 (20%)	--	--	6	--	--	1
Transfusion reaction*	4 (11%)	1 (3%)	2	--	1	3	--
Fall	2 (6%)	--	--	1	1	--	--
Arthropod bite	2 (6%)	--	--	--	--	1	1
Skin abrasion	2 (6%)	--	--	--	--	1	1
Investigations	--	--	--	--	--	--	--
Weight decreased	2 (6%)	--	--	--	2	--	--
Metabolism	--	--	--	--	--	--	--
Decreased appetite	21 (60%)	--	15	12	9	1	--
Musculoskeletal and Connective	--	--	--	--	--	--	--
Musculoskeletal pain	5 (14%)	--	--	1	1	1	1
Bone pain	3 (9%)	--	--	3	--	--	--
Nervous System	--	--	--	--	--	--	--
Headache	13 (37%)	--	--	5	5	3	1
Seizure	3 (9%)	1 (3%)	--	1	--	--	2
Paresthesia	2 (6%)	--	--	2	--	--	--
Dizziness	2 (6%)	--	--	--	--	1	1
Psychiatric	--	--	--	--	--	--	--
Anxiety*#	8 (23%)	--	--	1	5	4	--
Autism spectrum disorder	2 (6%)	1 (3%)	--	1	--	1	--
Aversion	2 (6%)	1 (3%)	1	1	--	1	--

Adverse Event	Subjects n (%)	SAEs n (%)	Grade 3+ (n=35)	PT (n=35)	D1 to <NE (n=35)	NE to <M12 (n=35)	M12+ (n=14)
Insomnia	3 (9%)	--	--	3	--	--	--
Renal and Urinary	--	--	--	--	--	--	--
Urinary incontinence	4 (11%)	--	--	3	--	2	--
Cystitis noninfective	2 (6%)	--	--	1	--	1	--
Dysuria	2 (6%)	--	--	--	--	2	--
Reproductive System	--	--	--	--	--	--	--
Penile pain	2 (6%)	--	--	--	1	1	--
Respiratory	--	--	--	--	--	--	--
Cough	2 (6%)	--	--	1	--	2	--
Oropharyngeal pain*#	7 (20%)	--	3	1	5	1	--
Epistaxis	9 (26%)	--	2	1	8	1	--
Hypoxia*#	3 (9%)	--	1	1	3	--	--
Rhinorrhea	3 (9%)	--	--	2	--	1	--
Tachypnea	2 (6%)	--	--	--	2	--	--
Skin and Subcutaneous	--	--	--	--	--	--	--
Alopecia	25 (71%)	--	1	--	12	13	--
Rash*#	11 (31%)	--	--	5	3	3	--
Pruritis*#	8 (23%)	--	--	1	7	1	--
Skin hyperpigmentation	8 (23%)	--	--	--	2	6	--
Dry skin	2 (6%)	--	--	--	--	2	--
Skin exfoliation	2 (6%)	--	--	--	--	2	--
Vascular	--	--	--	--	--	--	--
Hypertension	5 (14%)	--	2	3	2	1	1
Hypotension	4 (11%)	--	--	1	2	1	--

Abbrev: PT, prior to eli-cel administration; D1 to < NE, occurring after eli-cel administration and before neutrophil engraftment; NE to < M12, occurring after neutrophil engraftment and before one year post-eli-cel administration; M12+ occurring at least one year after eli-cel administration

*Tachycardia includes sinus tachycardia and tachycardia

Adrenocortical insufficiency acute includes adrenal insufficiency

Vision blurred includes vision blurred and visual acuity reduced

Mucositis includes anal inflammation, colitis, gastrointestinal inflammation, mucosal inflammation, and stomatitis

Abdominal pain includes abdominal pain and abdominal pain upper

Motor dysfunction includes gait disturbance, tetany, and tic
Viral respiratory tract infection includes viral upper respiratory tract infection and rhinovirus infection
Candidiasis includes anal candidiasis and oral candidiasis
Bacteremia includes pseudomonal bacteremia, *Stenotrophomonas* infection, and streptococcal bacteremia
Procedural pain includes post procedural discomfort and procedural pain
Transfusion reaction includes allergic transfusion reaction and anaphylactic transfusion reaction
Anxiety includes agitation, akathisia, and anxiety
Incontinence includes enuresis and urinary incontinence
Oropharyngeal pain includes mouth ulceration, oral pain, and oropharyngeal pain
Hypoxia includes hypoxia and oxygen saturation decreased
Rash includes catheter site dermatitis, dermatitis contact, rash, rash maculo-papular, and urticaria
Pruritus includes catheter site pruritus, pruritus, and pruritus allergic
Hypotension includes hypotension and orthostatic hypotension
Includes dictionary-derived terms from multiple organ systems
Source: Reviewer's analysis

Reviewer Comment: *The adverse event profile of eli-cel overall reflect the adverse events due to conditioning. Because the conditioning is necessary for product administration, conditioning-related adverse events should be taken into consideration in the assessment of the overall safety of the product.*

6.2.12.3 Deaths

No subject died as of the time of the writing of this review.

6.2.12.4 Nonfatal Serious Adverse Events

Thirty-two nonfatal serious adverse events (SAEs) occurred in subjects treated under ALD-104. The most common SAEs were febrile neutropenia in the second week after eli-cel administration and pyrexia in the second month after eli-cel administration. Also prominent among SAEs were infections with onset between two and eleven months after eli-cel administration. In this section, all 32 SAEs are briefly presented.

Prior to eli-cel administration, there was one SAE of autism leading to hospitalization. This SAE was Grade 2 and occurred between Days -18 and -15 (i.e., 15 to 18 days before eli-cel administration).

Ten SAEs occurred after eli-cel administration but prior to neutrophil recovery. Four were febrile neutropenia. Two were delayed hematopoietic reconstitution. Also occurring prior to neutrophil engraftment was one SAE each of oral mucositis, oral medication aversion, anaphylactic transfusion reaction, and pyrexia. Of the ten SAEs occurring prior to neutrophil engraftment, all were Grade 3 except for the one of the delayed hematopoietic reconstitution SAEs and the pyrexia SAE (Grade 1).

Seventeen SAEs occurred between neutrophil engraftment and 12 months. Six subjects had an SAE of fever. One of those subjects also had concomitant SAEs of vomiting and nausea. Three subjects had bacteremia SAEs: one with pseudomonas and stenotrophomonas, one with pseudomonas, and one with streptococcus. One subject had concomitant SAEs of transverse myelitis and transaminitis. The three remaining SAEs were constipation, tic, and upper respiratory tract infection.

Two SAEs occurred between 12 and 24 months. The first was an SAE of delayed hematopoietic reconstitution occurring for the second time in one subject, (b) (6), that progressed to myelodysplastic syndrome (MDS). The second SAE was MDS in a different subject, (b) (6).

Two SAEs occurred after 2 years. The first was an SAE of bradycardia that occurred in early in the Year 3 while the subject was receiving sedation. The second was an SAE of seizure 2.2 years after eli-cel administration, that was attributed to CALD.

6.2.12.5 Adverse Events of Special Interest (AESI)

Adverse events of special interest in this study were myelodysplastic syndrome and failed neutrophil or platelet recovery (also referred to as engraftment). Neutrophil and platelet recovery are discussed in this section and MDS is presented for the entire development program in Section 8.4.2 Nonfatal Serious Adverse Events.

The Applicant used the same definitions for neutrophil engraftment and platelet engraftment in this study and for ALD-102. (Refer Section 6.1.12.5 Adverse Events of Special Interest (AESI) for definitions.) All subjects met the criteria for engraftment and the median time to neutrophil engraftment was 14 days. However, in ALD-104, G-CSF was mandated. Calculating time of neutrophil recovery as three consecutive absolute

neutrophil counts $\geq 0.5 \times 10^9/L$ on three different days within 42 days of eli-cel administration while not receiving G-CSF, the median day of engraftment was 28 and the range between 13 and 189 days, with seven subjects meeting engraftment criteria later than 42 days after treatment with eli-cel. Data regarding those seven subjects are presented in the following table.

Table 24: Subjects That Did Not Meet Neutrophil Engraftment Criteria Without Concomitant G-CSF in ALD-104

Subject #	Engraftment day with G-CSF	Post-engraftment ANC nadir Day(s)	Post-engraftment ANC nadir ($\times 10^9/L$)	Last Day of G-CSF	Engraftment
(b) (6)	12	24	0.3	104	188
	12	87	0.1	174	167
	13	46	0.4	98	189
	14	19	0.8	30	62
	17	45	0.4	39	48
	16	26	0.36	23	60
	24	38 & 40	0.29	45	50

Abbrev: G-CSF, granulocyte colony stimulating factor; ANC, absolute neutrophil count
Source: Reviewer's analysis

This table demonstrates that after the Applicant's criteria for engraftment were met while supported by G-CSF, some subjects had declines in their ANC to below the threshold for engraftment. Additionally, six of the seven subjects who had not met engraftment criteria in the absence of concomitant G-CSF administration by Day 42 had declines in their ANC values to $< 0.4 \times 10^9/L$, raising the possibility of secondary engraftment failure. For platelet engraftment, the median day was 29 and the range was 14 to 108 days, and 9 of 35 subjects (26%) did not achieve unsupported platelet engraftment by Day 42. Two subjects were supported with filgrastim when engraftment criteria were met. An additional seven subjects did not meet engraftment criteria by Day 42, achieving platelet counts of $\geq 20 \times 10^9$ on the following days: Day 48 for Subjects (b) (6), Day 49 for Subject (b) (6), Day 50 for Subject (b) (6), Day 53 for Subject (b) (6), Day 58 for Subject (b) (6), and Day 106 for Subject (b) (6).

6.2.12.6 Clinical Test Results

This Clinical Test Results section includes Integration Site Analysis results and replication competent lentivirus test results. Laboratory results and vital signs for the entire safety population, i.e., subjects enrolled under ALD-102 and ALD-104, are covered in Section 8.4.5 Clinical Test Results.

Integration Site Analysis

As in ALD-102, the Applicant utilized integration site analysis to evaluate for clonal predominance and to identify subjects in need of a clinical work-up for malignancy. In ALD-104, all integration site analysis (ISA) was performed using S-EPTS/LM-PCR.

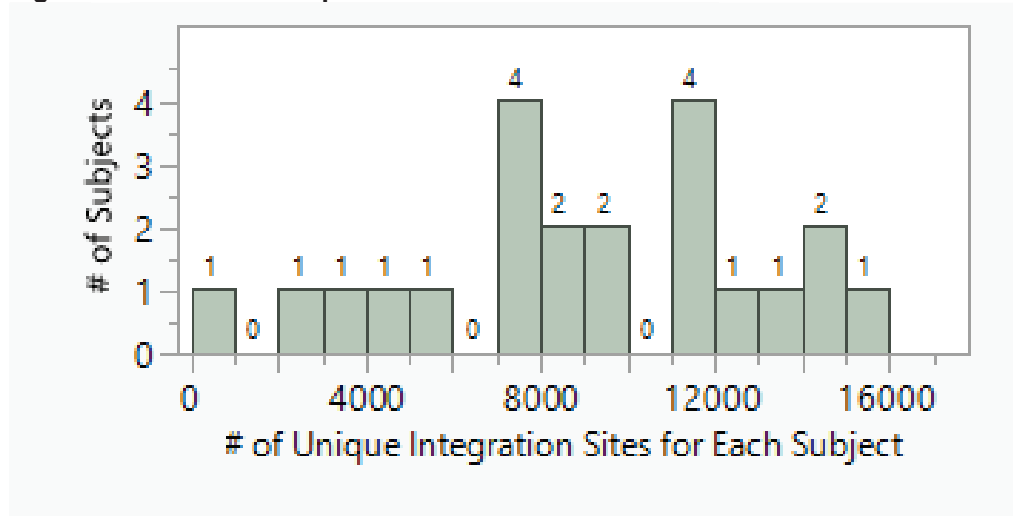
The algorithm including the timing of ISA and criteria for initiating a clinical work-up are the same as in ALD-102 (Refer to Section 6.1.7.3 Integration Site Analysis) until July 2021, when the frequency was increased to yearly during years 5 through 15, and the

requirement added for immediate repeat ISA upon meeting criteria for a predominant clone.

Twenty-two subjects in ALD-104 had data from at least one integration site analysis.

The number of unique integration sites was generally higher in ALD-104 than in ALD-102. Twenty-two subjects had a mean and median number of unique integration sites of 9000 and 8633, respectively. The range was 770 to 15,683, and the interquartile range was 6766 to 11845. The distribution of unique integration sites per subject is also presented in the following figure.

Figure 7: Number of Unique Integration Sites by Subject in ALD-104



Source: Reviewer's analysis

In ALD-104, ISA yielded 378 Top 10 integration sites in 268 different genes across the 22 subjects. The most frequent genes for integration, the total number of times the gene was identified as being in the Top 10, and the number of subjects in ALD-104 with integration into that gene are presented in the following table.

Table 25: Genes with the Highest Number of Integration Sites in ALD-104

Gene	# of Top 10 Instances	# of Subjects with Top 10 Instances (%)
<i>SMG6</i>	64	13 (60%)
<i>MECOM</i>	33	13 (60%)
<i>MPL</i>	12	6 (27%)
<i>CCND2-AS1</i>	7	3 (14%)
<i>KMT2A</i>	7	2 (9%)
<i>PBX3</i>	6	3 (14%)
<i>ACTR3</i>	6	2 (9%)
<i>PRDM16</i>	6	2 (9%)
<i>RAP2C-AS1</i>	6	2 (9%)

Source: Reviewer's analysis

The frequent occurrence of *MECOM* as a top integration site of particular concern. Of the 13 subjects with *MECOM* integration sites in their Top 10, two developed MDS and two have bone marrow findings concerning for evolving MDS. In addition, the number of

subjects with integration sites in other genes known to be involved in hematologic malignancy, including *MPL*, *KMT2A*, and *PRDM16*, is of concern.

Recombinant lentivirus assessment

Blood samples were evaluated for recombinant lentivirus (RCL) and 3, 6, and 12 months after eli-cel infusion. Two subjects (Subject (b) (6)) each had one instance where RCL was detectable, and the RCL value in both cases <10 copies/0.2 µg DNA. For Subject (b) (6), this occurred at Month 6, however, RCL was not detectable at other time points (screening, Month 3, and Month 12). For Subject (b) (6), this occurred at screening, and RCL was not detectable at subsequent time points (Months, 3, 6, and 12).

6.2.12.7 Dropouts and/or Discontinuations
No subject has discontinued from the study. However, as the study is ongoing, only six subjects had completed the 24-month follow-up. The 35 treated subjects include one who underwent allogeneic HSCT due to efficacy failure, and most product-specific risks should have been eliminated with that treatment.

6.2.13 Study Summary and Conclusions

This study enrolled 35 subjects and 6 have completed 2-year follow-up. As with ALD-102, the safety data demonstrate that eli-cel has a side effect profile that largely reflects the safety of the myeloablative and lymphodepleting medications that are administered prior to eli-cel. However, two subjects have developed MDS, and others have large clones identified by integration site analysis. With additional follow-up time, the diagnosis of additional cases of hematologic malignancy seems likely.

7. INTEGRATED OVERVIEW OF EFFICACY

7.1 Indication #1

Insert text here

7.1.1 Methods of Integration

The studies evaluated in the efficacy review are discussed in [Section 5.3](#). The clinical studies evaluating efficacy of eli-cel were Studies ALD-102 and ALD-104, discussed in [Sections 6.1](#) and [6.2](#), respectively. After completing 24 months of follow-up in each study, subjects were to enroll in the long-term follow-up study, LTF-304, for a total of 15 years of follow-up after treatment with eli-cel.

The primary efficacy endpoint of Month 24 MFD-free survival is discussed separately in [Sections 6.1.8](#) and [6.1.11.1](#). No subjects in Study ALD-104 had completed 24 months of follow-up following treatment with eli-cel at the time of original BLA submission and thus were not included in the analysis of Month 24 MFD-free survival. Additionally, due to concerns about comparability of populations used to determine the benchmark, as discussed in [Sections 6.1.8](#) and [6.1.11.1](#), success on the primary efficacy endpoint was not meaningful to the efficacy analysis. Secondary efficacy endpoint analysis of MFD-free survival over time by Kaplan-Meier estimates was also difficult to interpret, due to concerns about comparability of populations and imputation methods. The efficacy review therefore relied heavily on exploratory post-hoc analyses.

For comparative analyses of efficacy, subjects treated with eli-cel were compared to two external control populations: to untreated subjects in Study ALD-101 and to allo-HSCT-treated subjects in Studies ALD-101 and ALD-103. To be considered a comparable subject for efficacy comparisons, or, “strictly eligible for enrollment in ALD-102” (i.e., similar to subjects enrolled and treated with eli-cel in ALD-102 by the same disease-related eligibility criteria that define early, active CALD), a subject in ALD-101 or ALD-103 had to meet the following criteria:

1. NFS ≤ 1
2. Loes score between 0.5-9
3. Gadolinium enhancement (GdE+) on MRI

For the untreated population, this population was referred to as UTES, and for the allo-HSCT population, as TPES. Because gadolinium was not routinely assessed on brain MRIs for CALD when many subjects in ALD-101 were diagnosed, only one subject met criteria for the UTES-101 population. For allo-HSCT subjects, there were 26 subjects that met TPES criteria in Study ALD-101 (TPES-101 population), and 27 in Study ALD-103 (TPES-103).

In these exploratory analyses, subject populations were pooled to increase the robustness of the analysis of the data for eli-cel and allo-HSCT populations, particularly given a rare disease with limited duration of follow-up following treatment, and small sample sizes that were further reduced with exploratory subgroup analyses. Subjects from studies ALD-102 and ALD-104 were pooled to constitute the eli-cel Efficacy Population (n=61, which excludes the 6 subjects in ALD-102 who received product for which comparability to the to-be-marketed product was not demonstrable), and the ALD-101 and ALD-103 allo-HSCT TPES populations were pooled (n=53). Pooling the allo-HSCT TPES populations helped overcome some concerns that arose due to early termination of ALD-103 and allowed for evaluation of some longer-term outcomes following allo-HSCT. Interim data cuts were used to allow for additional long-term data for subjects treated in ALD-102 and now being followed in LTF-304, as well as to allow for the inclusion of subjects in ALD-104 who had reached 24 months of follow-up after eli-cel administration.

Untreated Subpopulations

Although efficacy of allo-HSCT in the treatment of early, active CALD has been well-documented in the literature, we still struggled to understand the relative benefit of allo-HSCT compared to lack of treatment in the clinical course of asymptomatic, very early cerebral disease (i.e, Loes scores 1-3). Prior to implementation of newborn screening, widespread genetic screening of family members of affected individuals, and allo-HSCT becoming routine, many CALD patients were diagnosed when presenting with symptoms rather than through MRI screening, and as such were often at more advanced stages of disease at diagnosis than those being diagnosed and treated now. Therefore, we don't know the expected clinical course of asymptomatic, very early active cerebral disease if left untreated, because such an untreated comparator is likely never to exist. While comparison of outcomes in untreated and eli-cel- and allo-HSCT-treated subjects with baseline NFS=1 and/or higher Loes scores is reasonable, given what we know from the medical literature, few subjects met these criteria and had been followed for a sufficient duration to make such comparisons. Many of the subjects treated with eli-cel had very early disease with NFS=0 and Loes score=1 at baseline. Thus, we simultaneously

sought to understand the benefit of eli-cel as compared to the natural history of disease, but also the relative efficacy comparing eli-cel to allo-HSCT, and allo-HSCT to the natural history. The evaluations and analyses involved in this process are discussed in [Section 7.1.10](#). The untreated populations used in exploratory post-hoc analyses are described below.

1. rUTES-101 (N=7): As discussed briefly in [Section 6.1.11.1](#), a reviewer-initiated re-coding of subjects in the untreated population from Study ALD-101 resulted in a population referred to as rUTES-101, which consisted of 7 untreated subjects who met the ALD-102 eligibility criteria of NFS ≤ 1 and Loes score 0.5-9 at time of diagnosis, and who had a documented gadolinium-enhancing (GdE+) MRI during the course of follow-up. These subjects had re-coding of Baseline values to be time of diagnosis rather than time of first GdE+ MRI. Because gadolinium was not routinely used in MRI assessments at the time many CALD patients in ALD-101 were diagnosed, gadolinium status was unknown at diagnosis for many. By imputing first GdE+ MRI to be the MRI at diagnosis, it allowed for a more conservative comparison between eli-cel and a more comparable untreated population than the entire GdE+ untreated population (UTG-101, or Population #1 of the benchmark), some of whom had very advanced disease and thus were not appropriate comparators.

This population primarily helped to better understand the natural history of disease in an early, active cerebral disease population in a general sense, which helped to understand that 24 Months from Baseline (either diagnosis or time of treatment depending on study group) may be an insufficient time to assess MFD-free survival due to limited MFD or death events across groups (including the untreated) during that time. However, due to concerns that rUTES-101 still had more severe disease at Baseline than the eli-cel population, this population was not used for any of the exploratory post-hoc analyses discussed in this section comparing outcomes for eli-cel to those of an untreated natural history population.

2. UTE-101 (N=14): UTE-101 was a pre-defined untreated subpopulation in ALD-101 that met the NFS ≤ 1) and Loes score 0.5-9) criteria for which they would have been eligible to enroll in ALD-102, but did not necessarily have gadolinium enhancement on MRI, as for many patients gadolinium status was unknown. Instead, to be included in UTE-101, a subject had to have documented gadolinium enhancement with the NFS and Loes criteria at time of GdE+ MRI (n=1) or had to have the NFS and Loes criteria at diagnosis if GdE- or unknown GdE status. Because this population was suspected to have a significant number of subjects with GdE- MRIs, there was concern that this population was not comparable because they might not have active disease and thus may have arrested or less advanced disease with less risk of progression compared to the eli-cel population. This population was used in one post-hoc analysis of MFD-free survival (not shown) in an attempt to compare outcomes in a more conservative analysis. However, there were still concerns about comparability because of varied baseline disease characteristics and features, poorly defined time zero and continued concerns for lead-time bias. As such, a new natural history population was sought.

3. Natural History Population (N=10): The untreated population ultimately drawn from for post-hoc analyses comparing eli-cel to the natural history of disease was derived from a reviewer-initiated assessment of clinical courses in similar treated and untreated subjects to understand which untreated subjects were most similar to eli-cel subjects with early, active disease, and contains a mix of subjects in rUTES-101 and UTE-101. The process to defining and analyzing the Natural History Population is described in [Section 7.1.10](#).

Allo-HSCT by Donor Type Subpopulations

Matched sibling donors (MSD) are the preferred HSCT donors, and therefore the Applicant focused their comparative analyses on the TPES allo-HSCT MSD and NMSD (no matched sibling donor) subgroups. NMSD includes matched unrelated donors and mismatched donors (either related or unrelated). In this analysis, “matched” refers to any full HLA-matching of all evaluated alleles (e.g., 6 out of 6, 10 out of 10). “Mismatched” includes mismatch on 1 or more alleles (e.g., 4 out of 6, 9 out of 10). Because HSCT outcomes differ between matched and mismatched donors, we included these populations in the post-hoc exploratory sub-group analysis. Table 26 describes the donor characteristics for the allo-HSCT comparator populations.

Table 26: Donor HLA-Matching and Relatedness 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)
Mismatched Donor	10 (38)	7 (26)	17(32)
Unknown Matching of Donor	2 (8)	0	2 (4)
Matched Sibling Donor (MSD)	5 (19)	10 (37)	15 (28)
No Matched Sibling Donor (NMSD)	21 (81)	17 (63)	38 (72)
Matched Unrelated Donor	9 (35)	10 (37)	19 (36)
Mismatched Related Donor ¹	1 (4)	0 (0)	1 (2)
Mismatched Unrelated Donor	9 (35)	7 (26)	16 (30)

Abbrev.: TPES, Strictly ALD-102-eligible Transplant Population; MSD, Matched Sibling Donor; NMSD, No Matched Sibling Donor

¹All unmatched related donors, including unmatched sibling donors

Source: Reviewer’s analysis of ADHSCT dataset

7.1.2 Demographics and Baseline Characteristics

Subject demographics and baseline disease characteristics are provided in Table 27 for the subpopulations used in the post-hoc analysis that contributed most significantly to the substantial evidence of efficacy on an intermediate clinical endpoint, a Kaplan-Meier analysis of MFD-Free Survival in subpopulations who had NFS=1 at baseline or developed NFS changes during the course of follow-up. This analysis is discussed in [Section 7.1.10](#). The remaining 3 Natural History Population subjects who were not in the analysis are described in narrative or tabular form for any other relevant analyses as applicable in [Section 7.1.10](#). Baseline demographics and disease characteristics for the subpopulations used for comparative efficacy analyses of eli-cel and allo-HSCT are in Table 28.

Table 27: Demographics and Disease Characteristics for eli-cel, allo-HSCT, and Natural History Symptomatic Subpopulations

Parameter	Statistic	Eli-Cel (N=11)	Allo-HSCT (N=16)	Natural History (N=7)
Age at CALD Diagnosis	Median (min, max)	6 (1, 10)	7 (2, 13)	9 (5, 15)
Age at Treatment	Median (min, max)	6 (4, 10)	8 (5, 13)	NA
Age at Time of First NFS ≥ 1	Median (min, max)	7 (4, 10)	8 (5, 14)	10 (5, 17)
Baseline Loes Score	Median (min, max)	2.5 (1, 9)	5.8 (1, 9)	5 (2, 9)
MRI Pattern: Parieto-Occipital	N (%)	10 (91)	12 (75)	4 (57)
MRI Pattern: Frontal	N (%)	0	4 (25)	2 (29)
MRI Pattern: Pyramidal Tract	N (%)	1 (9)	0	1 (14)
Baseline NFS=0	N (%)	9 (82)	9 (56)	6 (86)
Baseline NFS=1	N (%)	2 (18)	7 (44)	1 (14)
NFS Total Score = 1 at Time of First NFS ≥ 1	N (%)	10 (91)	13 (81)	6 (86)
NFS Total Score > 1 at Time of First NFS ≥ 1 ^a	N (%)	1 (9) ^a	3 (19) ^a	1 (14) ^a

Abbrev: allo-HSCT: allogeneic hematopoietic stem cell transplant; CALD: cerebral adrenoleukodystrophy; NFS: Neurologic Function Score; MRI: magnetic resonance imaging

^a At time of first NFS ≥1, one eli-cel subject had a total score of 2, three allo-HSCT subjects had scores of 2,4 and 5, and one natural history subject had a total score of 3.

Source: Reviewer analysis of bluebird bio, Inc. BLA 125755 ADL, ADMRI, and ADEFF3 datasets

Table 28: Demographics and Baseline Disease Characteristics for Efficacy Populations

Parameter	Eli-cel population (N=61)	Allo-HSCT population, HLA- mismatched donor (N=17)	Allo-HSCT population, HLA-matched unrelated donor (N=19)	Allo-HSCT population, HLA-matched sibling donor (N=15)
Age at treatment in years Median (Min, Max)	7 (4, 14)	7 (5, 13)	8 (4, 14)	8 (6, 13)
Baseline NFS Score: 0 N (%)	58 (95)	16 (94)	16 (84)	13 (87)
Baseline NFS Score: 1 N (%)	3 (5)	1 (6)	3 (16)	2 (13)
Baseline Loes score Median (Min, Max)	2 (1, 9)	3.5 (0.5, 8)	3.5 (1, 9)	4 (1, 9)

Abbreviations.: allo-HSCT, allogeneic hematopoietic stem cell transplantation; GdE, gadolinium enhancement; HLA, human leukocyte antigen; NFS, neurologic function score.

Source: Adapted from bluebird bio, Inc. BLA 125755 Draft Package Insert from Applicant on 02 September 2022

7.1.3 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 29. 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 months for TPES-103 subjects (approximately half the 52-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 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%) and repeat HSCT (19%).

Table 29: 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	52	12	53	24
Study Status:	--	--	--	--
Ongoing, n (%) ³	28 (88)	35 (100)	0	0
Completed Study, n (%)	0	0	22 (85) ⁵	4 (15)

Parameter	TP-102	TP-104	TPES-101	TPES-103
Discontinued Study, n (%)	4 (13) ⁶	0 ⁶	4 (15) ⁵	23 (85)
Reason for Study Discontinuation:	--	--	--	--
Rescue/ Repeat HSCT, n (%)	2 (6)	0	2 (8)	5 (19)
Death, n (%) ⁴	1 (3)	0	2 (8)	3 (11)
Lost to/Refuses Follow-Up, n (%)	1 (3)	0	0	1 (4)
Termination of Study by Sponsor, n (%)	0	0	0	13 (48)
Protocol Deviation, n (%)	0	0	0	1 (4)

Abbrev.: TP, Transplant Population; TPES, Strictly ALD-102-eligible Transplant Population; HSCI, hematopoietic 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.

¹The 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). For TP-102, the full cohort of subjects treated with eli-cel are included in this table, including the 6 subjects otherwise excluded from the efficacy analyses as they received drug product for which comparability to the to-be-marketed product was not demonstrable.

²For TP-102 and TP-104, median duration of follow-up is updated for most recent data cut through January 2022.

³LTF-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.

⁴For 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)

⁵For 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.

⁶Subjects who have received allo-HSCT for treatment of MDS are not discontinued.

Source: Adapted from bluebird bio, Inc. original BLA submission, interstudy TLFs Table 1.1.2; updated with data through January 2022 data cut.

7.1.4 Analysis of Primary Endpoint(s)

Analysis of the primary endpoint, which was evaluated only in the ALD-102 population, is addressed in [Section 6.1.11.1 Analyses of Primary Endpoint\(s\)](#)

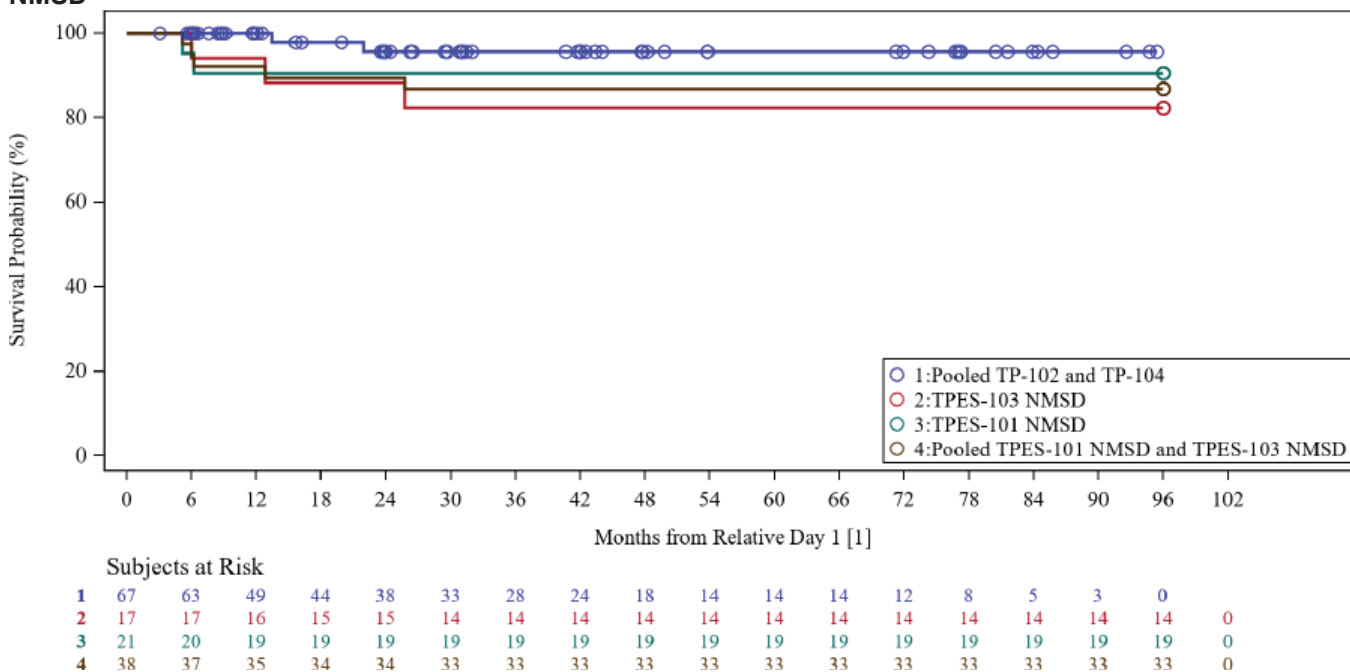
7.1.5 Analysis of Secondary Endpoint(s)

Secondary endpoints as defined in [Sections 6.1.8](#) and [6.2.8](#) for Studies ALD-102 and ALD-104, respectively, were pre-specified but not hierarchically specified and thus were treated as exploratory. The two pre-specified secondary endpoints with supportive efficacy evidence were MFD-free survival over time and overall survival (OS). MFD-free survival over time was primarily evaluated as post-hoc analyses for subgroups of the natural history, allo-HSCT, and eli-cel efficacy populations, and as such is discussed in [Section 7.1.10](#). As the results of post-hoc subgroup exploratory analyses weighed more significantly into the determination of product effectiveness than overall survival analysis discussed in this section, results in this section were not updated to remove the 6 subjects who received investigational product for which comparability to the to-be-marketed product was not demonstrable.

Overall Survival

The Applicant did not include the death of one subject in the analysis of overall survival. The rationale was that the subject was not enrolled in the study at time of death because he had withdrawn to receive rescue allo-HSCT due to progressive disease on brain MRI. However, his death occurred following treatment with eli-cel, and we did not agree with his exclusion. Kaplan-Meier (KM) estimates of OS comparing pooled eli-cel subjects from ALD-102 and ALD-104 and comparing to TPES-101 and TPES-103 NMSD populations (pooled and separated by study population) are shown in Figure 8. The second subject death in ALD-102 is hard-coded in this analysis, and due to paucity of long-term data for allo-HSCT populations, survival to 96 months was imputed for allo-HSCT subjects alive at last contact. For subjects treated with eli-cel (TP-102 and TP-104), censoring was done at date of last contact. Here it appears that OS is similar between eli-cel and allo-HSCT in CALD patients who have early, active cerebral disease and have no available matched sibling donor.

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



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 (b) (6), 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.

Source: bluebird bio, Inc.,BLA ad hoc Figure 80.2.2.1.1.2

Reviewer Comment: Although Kaplan-Meier estimates of time to event for MFD-free survival and overall survival were important to the efficacy review, we did not agree with the imputation methods used for these analyses in the original BLA submission, as noted in the discussion of MFD-free survival for ALD-102 in [Section 6.1.11.2](#). MFD-free survival is addressed separately in [Section 7.1.10](#) due to heavy reliance on exploratory post-hoc analyses for this endpoint, and the original BLA submission comparing eli-cel and allo-HSCT on MFD-free survival over time is thus not shown. This is largely due to clinical review team disagreement with imputation methods used by the Applicant in the analysis of the endpoint, namely that repeat allo-HSCT in the allo-HSCT population was imputed as a failure event. The clinical review team did not agree with this imputation as all repeat HSCT was done for graft failure rather than for progressive disease. Additionally, two subjects developed an MFD after the original BLA submission, and 3 subjects developed myelodysplastic syndrome (MDS) and received rescue allo-HSCT as treatment. Post-hoc analyses of MFD-free survival were conducted pooling study population subgroups as described in [Section 7.1.1](#), with more conservative imputations that did not include repeat allo-HSCT as an event (but did impute rescue allo-HSCT for eli-cel-treated subjects as events), and including the interim events of MFD cases and allo-HSCT for treatment of MDS. These post-hoc analyses are reviewed in [Section 7.1.10](#).

Although overall survival (OS) appears similar for subjects treated with eli-cel and subjects treated with allo-HSCT from an NMSD, comparability between groups and paucity of long-term data made interpretation of data difficult. Additional post-hoc analysis of overall survival was done hard-coding the second death in the eli-cel population and using pooled populations and subpopulations. Subpopulations used in the post-hoc analyses are discussed in [Section 7.1.1](#). The post-hoc analyses of overall survival are reviewed in [Section 7.1.10](#).

7.1.6 Other Endpoints

Other pre-specified secondary and exploratory endpoints did not weigh significantly into the efficacy analysis, so are only briefly addressed here. As the results of these analyses did not weigh significantly into the determination of product effectiveness, results were not updated to remove the 6 subjects who received investigational product for which comparability to the to-be-marketed product was not demonstrable. Results are expected to be similar to those discussed.

Change in NFS from Baseline to Month 24

This was addressed for Study ALD-102 subjects in [Section 6.1.11.2](#). Changes in the pooled population were similar. The majority of subjects maintained a stable NFS from Baseline to Month 24. Results were similar for the populations and as compared to results from subjects treated with allo-HSCT.

Reviewer Comment: As noted in [Section 6.1.11.2](#), it is not clear that 24 months is sufficient time to assess change in NFS from Baseline in an early, active disease population, and it is not clear that the definition of stability is appropriate. Further post-hoc analysis of NFS changes is discussed in [Section 7.1.10](#).

Change in Loes Score from Baseline to Month 24

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.

Reviewer Comment: *The clinical significance of this greater increase in Loes score at Month 24 following treatment is unknown. While disease progression may be expected in the 2 years following allo-HSCT, followed by stabilization of disease,^{9,13,14} 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, it is unclear if 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.*

7.1.7 Subpopulations

Subpopulations used in the ad hoc exploratory analyses of efficacy have already been described in [Section 7.1.1](#). No subgroup analysis was performed based on sex (as 100% of subjects were male), race, ethnicity, country of origin, or treatment center. No concerns were identified with respect to differences between treatment centers in these multinational clinical trials. As the majority of subjects were White/Caucasian, and sample sizes were small, no differences based on race or ethnicity were expected due to limited data for comparison in other races/ethnicities.

7.1.8 Persistence of Efficacy

Although there was no evidence to suggest loss of therapeutic effects over time in subjects treated with eli-cel, there was insufficient long-term data at the time of the review to draw conclusions about persistence or durability of efficacy. One subject demonstrated loss of product efficacy (or possibly failure to achieve efficacy), but loss of therapeutic effect occurred early in this subject. The subject was the only subject in the eli-cel clinical studies who had a full deletion of the *ABCD1* gene, and it was hypothesized that the lack of or loss of efficacy in this subject may have been related to an immune response to the investigational product.

7.1.9 Product-Product Interactions

Although concomitant medications were documented for all subjects, no product-product interactions were expected or observed during the course of the clinical studies.

7.1.10 Additional Efficacy Issues/Analyses

The efficacy review relied heavily on post-hoc exploratory endpoints as discussed in [Section 7.1.1](#). Because the comparator populations in the external control studies were generally considered not comparable to the eli-cel-treated populations in Studies ALD-102 and ALD-104 (discussed in [Section 6.1.11](#)), subgroup analysis on more comparable populations was sought in an effort to make meaningful comparisons from the available data. As such, the comparator subgroups were subsets of the natural history and allo-HSCT populations with early, active disease as defined by the eligibility criteria for ALD-102 (NFS 0 or 1, Loes 0.5-9, and Gadolinium enhancement [GdE+]¹ on brain MRI) at baseline. Subpopulations are defined and relevant baseline demographic and disease characteristics for the subpopulations are provided for each analysis, as relevant.

Post-Hoc Evaluations to Better Define a Comparable Natural History Control

In an effort to first better understand and compare the natural history of disease in the untreated and treated early, active CALD populations, reviewer-initiated assessment of clinical courses of subjects who ultimately developed MFDs was pursued. Subjects who developed MFDs in the eli-cel Efficacy Population, allo-HSCT Efficacy Population, and in the untreated (rUTES-101 and UTE-101 pooled) population were evaluated and clinical courses were mapped over time. Throughout the remainder of this section, these populations will be referred to simply as eli-cel, allo-HSCT, and untreated/natural history populations, respectively, with subpopulations defined relative to each analysis. Subjects were grouped based on baseline MRI patterns of disease since these are understood to be prognostic (both as related to age and to rate of disease progression)¹⁰ and courses over time were compared. These subpopulations of subjects who developed MFDs were chosen because they clearly had progression of disease, and thus could be compared in an attempt to understand rates and risk factors for disease progression.

To understand the most appropriate time zero to use in post-hoc analyses, subject clinical courses were compared at 3 different time zeros:

1. Baseline as defined in the BLA: diagnosis for the untreated subjects, and time of treatment for allo-HSCT and HSCT subjects,
2. Time of diagnosis for all untreated and treated subjects, and
3. Birth, so that courses were mapped according to age at events.

For most MRI pattern groupings, birth appeared to be the appropriate time zero, particularly for determining typical time of symptom onset (first NFS ≥ 1) for the varied patterns of disease. As it has been documented that radiographic and clinical disease progression can occur in the first 1-2 years following allo-HSCT prior to disease stabilization,^{9,14} it was reasonable to conclude that treated subjects who had symptoms at baseline or shortly after treatment with allo-HSCT or eli-cel might have symptom timing consistent with the natural history of untreated disease. With this in mind, any subjects who had NFS ≥ 1 at any time during the course of follow-up were added to the subject modeling, even if they had not had an MFD. By evaluating untreated subjects with NFS changes, and subjects treated with allo-HSCT and eli-cel who either had NFS 1 at baseline or developed NFS changes shortly after treatment, the analysis of timing of

¹ Or for natural history subjects where gadolinium enhancement status was unknown, a clinical course consistent with active disease (i.e., suspected GdE+)

symptom onset helped to more clearly see patterns that have been documented in the literature – those with patterns inclusive of parieto-occipital involvement were the youngest at time of symptom onset, with trends noted for symptom onset generally between 6-9 years of age. Subjects with frontal involvement (but no parieto-occipital) trended a little older with symptom onset generally between 9-12 years of age. This is consistent with reports of adolescent presentation for frontal pattern disease, but as a majority of patients were diagnosed and treated prior to 10 years of age, might be expected, based on the literature, to have more rapid disease progression due to presentation in childhood.^{10,27} Two subjects in the untreated population had isolated pyramidal tract disease, and although they were diagnosed at 11 and 9 years old, did not develop first NFS changes until 20 and 19 years old, respectively. This is consistent with the literature in that symptom onset is largely in adulthood for this pattern.¹⁰ Trends for ages are noted here because descriptive statistics are not appropriate when factoring in possible treatment effect in delaying onset of symptoms for the allo-HSCT and eli-cel subjects. It was not possible to determine an appropriate time following treatment that would delineate symptoms related to early progression prior to a functioning graft consistent with the natural history of disease and symptoms after uptake of a functioning graft that might represent a treatment effect of slowed or delayed neurologic dysfunction.

With this understanding, all subjects in the pooled rUTES-101 and UTE-101 untreated population were assessed individually to determine which subjects most clearly had a disease course consistent with active disease. As a reminder, all subjects in this pooled population had a Loes score between 0.5-9 and NFS ≤ 1 at time of diagnosis. All rUTES-101 subjects were included in the ultimate natural history population by virtue of having documented GdE+ MRI consistent with active disease with the exception of 1 subject who had spontaneous resolution of GdE+ MRI and had clinical and radiographic disease course more consistent with arrested disease. Four subjects in UTE-101 had a clinical and/or radiographic course consistent with active disease despite never having had a GdE+ MRI- as gadolinium was not routinely used at that time, it was reasonable to deduct an MRI likely would have been GdE+ had contrast been used in the MRI assessment.

This newly formed Natural History Population (N=10) was then used for subject-specific matching with eli-cel subjects and in some cases, allo-HSCT subjects, to compare clinical and radiographic findings between untreated and treated subjects matched for age, MRI pattern of disease, Loes score, and NFS at baseline (where in this case baseline was time of diagnosis for untreated subjects and time of treatment for eli-cel and allo-HSCT). If close matching was not possible, matching was done to bias against eli-cel (e.g., the eli-cel subject had baseline NFS=1 and the natural history subject NFS=0). Though few subjects were able to be closely matched due to small sample sizes and heterogeneity of disease, this matching allowed for better understanding of timing of symptom onset and progression to MFD or death in the early, active CALD population, which helped guide the most meaningful exploratory post-hoc analyses with the most confidence in comparability of populations.

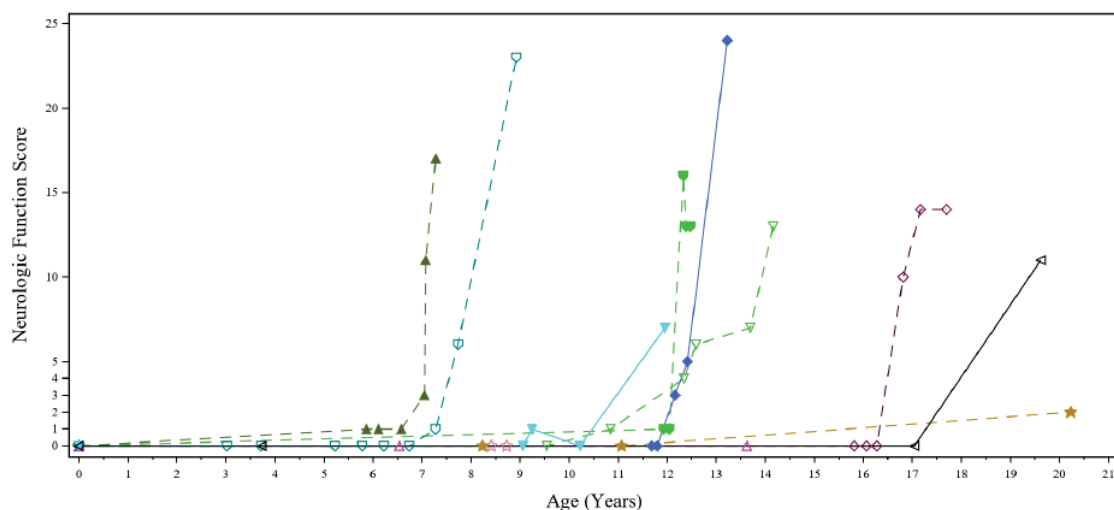
Exploratory Analysis of NFS Changes and MFD-Free Survival Comparing Symptomatic Eli-Cel and Natural History Populations

The main challenges in previous analyses of MFD-free survival were large numbers of asymptomatic, very early disease subjects in the eli-cel and allo-HSCT populations, and few MFDs and deaths occurred in these populations. In comparison, event rates were

high in the untreated natural history population, but the natural history population was also older with more advanced cerebral disease on MRI, and more likely to present with symptomatic disease at time of diagnosis. As a result, it was difficult to determine if the lower numbers of MFDs and deaths in the treated populations were due to a treatment effect or due to treatment at an early stage of disease with insufficient duration of follow-up to detect progression to MFD or death. It is unclear what the clinical course would have been in subjects with very early stages of disease had they not been treated. In essence, it was not possible to use all the available efficacy data to compare outcomes following treatment with eli-cel (and allo-HSCT) to untreated CALD because of the concern for lead-time bias in the natural history untreated population.

Following the disease modeling described above, the Applicant was asked to provide graphical representations of NFS changes over time with individual lines for each subject in the early, active CALD natural history population, allo-HSCT population, and eli-cel population. These graphics demonstrated a rapid trajectory of NFS increase for untreated subjects after first NFS ≥ 1 , typically peaking to maximum documented NFS within 24 months (Figure 9). In comparison, lines either stabilized or had a lesser degree of incline for the eli-cel (ALD-102) population (Figure 10) or allo-HSCT population (Figure 11). Although timing of progression from symptomatic disease to disability or death in these early, active disease populations has not specifically been demonstrated in the literature, the literature supports what was seen in the natural history populations that even asymptomatic children with Loes scores >1 have high rates of progression to neurologic dysfunction, disability and death, all within 5 years of initial presentation.⁴ It has also been described that, once symptomatic, death typically occurs in 2-4 years, though some patients may survive long-term in a severely disabled state.^{26, 31} Although the efficacy analysis was complicated by large numbers of eli-cel- treated subjects who were asymptomatic and with mild cerebral disease (i.e., Loes score 1-3) at time of treatment, this modeling demonstrated a trend of slowing or stabilization of the progression of neurologic dysfunction once disease had become symptomatic in the treated populations as compared to the natural history of disease.

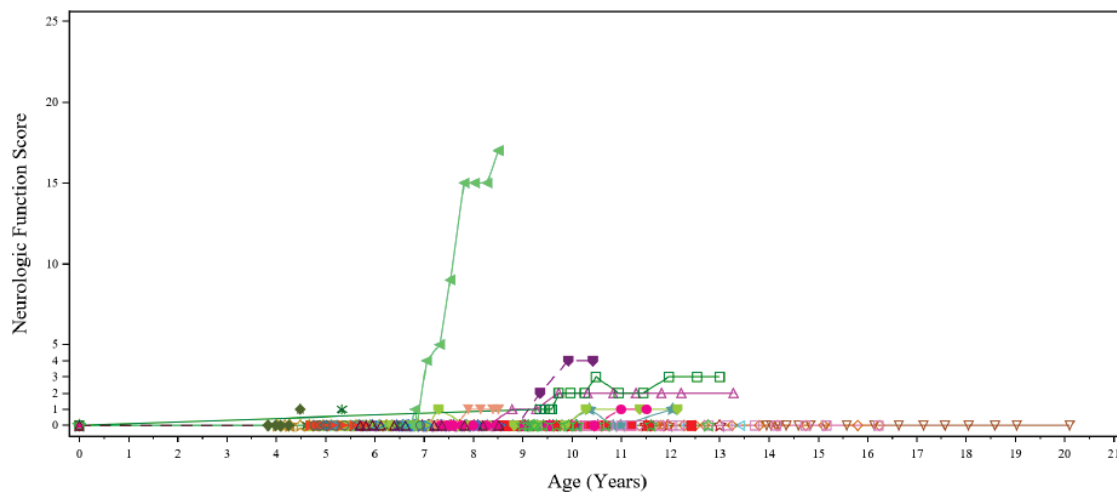
Figure 9: Neurologic Function Score (NFS) Over Time by Subject- Natural History Subjects



Abbrev: NFS: neurologic function score

Notes: Each line represents a different subject. In this analysis, one allo-HSCT subject who experienced disease progression during the pre-transplant conditioning period was evaluated as a natural history subject, but in all other analyses is evaluated as an allo-HSCT subject.
Source: bluebird bio, Inc. BLA 125755 ad hoc analysis, Figure 80.2.3.1.1

Figure 10: Neurologic Function Score (NFS) Over Time by Subject- Study ALD-102 eli-cel Subjects

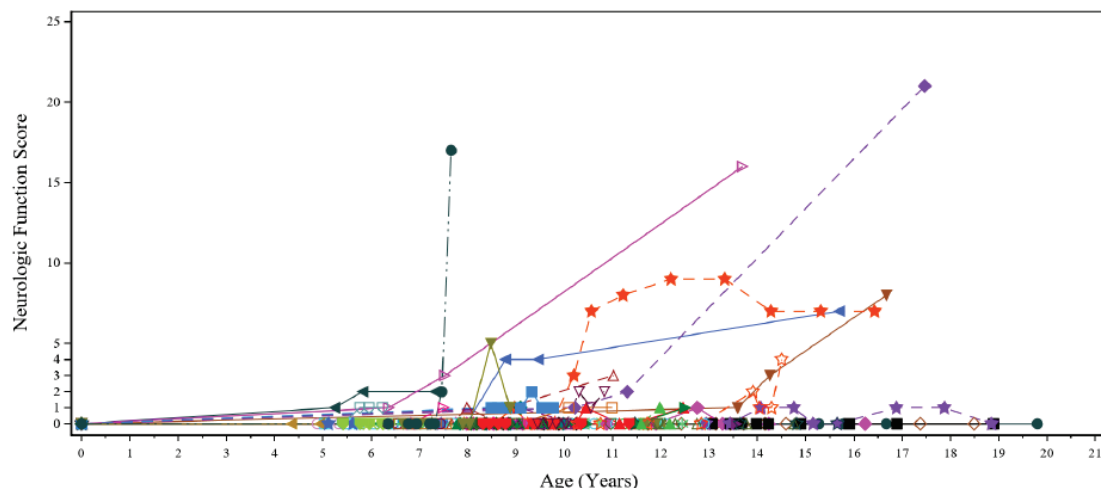


Abbrev: NFS: neurologic function score

Note: Each line represents a different subject; in this analysis, the bright green line with sharp incline of line is the subject who developed rapid disease progression shortly after treatment.

Source: bluebird bio, Inc. BLA 125755 ad hoc analysis, Figure 80.2.3.1.2

Figure 11: Neurologic Function Score (NFS) Over Time by Subject- Successful Engraftment allo-HSCT Subjects



Abbrev: NFS: neurologic function score

Notes: Each line represents a different subject. In this analysis, one allo-HSCT subject who experienced disease progression during the pre-transplant conditioning process was evaluated as a natural history subject, as thus is not included in this graphic. The dark line that essentially goes straight up between 7 and 8 years of age is an allo-HSCT subject who experienced rapid disease progression shortly after treatment.

Source: bluebird bio, Inc. BLA 125755 ad hoc analysis, Figure 80.2.3.1.3

The decision was made to cull from the datasets an enriched population in an attempt to identify a group of patients/ subjects who were expected to already be on a more rapid trajectory of disease progression by virtue of having developed symptomatic disease, based on the modeling just discussed. The strategy was intended to identify a more homogeneous, prognostically enriched subpopulation in the eli-cel-treated subjects and in the untreated and allo-HSCT external control populations, judging that they would be more comparable, and to compare them on MFD-free survival (i.e., time to develop an MFD or die) in an exploratory analysis.

To be included in this analysis, the subjects had to meet the following criteria:

1. meet the eligibility criteria for ALD-102 at time of diagnosis (untreated subjects) or treatment (subjects treated with eli-cel or allo-HSCT): NFS of 0 or 1, Loes score 0.5-9, gadolinium enhancement on brain MRI (or unknown status and clinical course suggestive of active disease).
2. have had symptomatic disease at some time during the study: either NFS=1 at baseline or development of symptoms (NFS \geq 1) during the course of follow-up.
3. have been followed for at least 24 months after onset of symptomatic disease (NFS \geq 1) or had an MFD or death.

The exploratory MFD-free survival analysis compared subsets of the early active disease natural history population (N=7), allo-HSCT population (N=16), and eli-cel population (N=11). In this analysis, time zero was date of first NFS \geq 1.

The demographics and disease characteristics of these subpopulations are discussed in [Section 7.1.2](#). Although not identical, these subpopulations have been “homogenized” by selecting subjects/ patients with similar prognostic features. Values for the natural history population are somewhat skewed due to the inclusion of a subject with isolated pyramidal tract disease on brain MRI, as this pattern typically is slowly progressive and becomes symptomatic in adulthood.¹⁰ The eli-cel subpopulation subject who had isolated pyramidal tract disease in this analysis had an atypical course, with rapidly progressive disease on brain MRI and development of symptoms in childhood, and ultimately died of transplant-related causes following rescue allo-HSCT due to progression of disease. If the subject with isolated pyramidal tract disease in the natural history population had been excluded from the analysis, the natural history population would have been more comparable to the treated populations.

Additionally, conservative imputations were used for 2 of the natural history subjects who had first NFS \geq 1 and MFD at the same time after a period of being lost to follow-up (including the pyramidal tract subject noted above). First NFS \geq 1 for these subjects were imputed as: (1) the date after last documented NFS of 0 for one whose exact assessment date was known, and (2) as the mid-way point of the year for the subject with pyramidal tract disease discussed above whose date of last NFS of 0 was only documented as the year (i.e., NFS 0 was documented in the year 2004, so NFS 1 was imputed as occurring on June 30, 2004). With this conservative imputation strategy, 3 (43%) of the 7 natural history subjects maintained MFD-free survival at 24 months following first NFS \geq 1, compared to 29% (2 of 7) without the conservative imputation

strategy. Regardless, more than 50% of the natural history subpopulation experienced an MFD within 24 months of first NFS ≥ 1 , and median time from first NFS ≥ 1 to MFD in this population was 20 months even with conservative imputations, and thus the choice to perform Kaplan-Meier estimates of MFD-free survival comparing subjects followed at least 24 months from time of first NFS ≥ 1 was reasonable. Event details are provided in Table 30. It should be noted that results for allo-HSCT skew as worse than eli-cel, partially due to 2 deaths (of which 1 was from transplant-related causes and the other is unclear if it was related to disease progression, treatment, or neither), and partially due to longer duration of follow-up and 2 subjects developing MFDs >80 months after first NFS ≥ 1 in the allo-HSCT group. If evaluating just the number of MFDs (i.e., removing subjects who only had death without MFD from the analysis), and evaluating at an earlier time point to account for eli-cel subjects not being followed to >80 months after first NFS ≥ 1 (i.e., imputing the two allo-HSCT subjects with MFDs >80 months after first NFS ≥ 1 as successes), incidence of MFDs would have been similar in the two populations: 3/10 eli-cel subjects (30%) and 5/14 allo-HSCT subjects (36%).

Table 30: Event Details and Duration of Follow-Up for Symptomatic Subpopulations

Parameter	Statistic	Eli-Cel (N=11)	Allo-HSCT (N=16)	Natural History (N=7)
Total Events	N (%)	4 (36)	9 (56)	7 (100)
First Event = MFD	N (% of total subjects)	3 (27)	7 (44)	7 (100)
First Event = Death	N (% of total subjects)	1 (9)	2 (13)	0
Time from First NFS ≥ 1 to MFD (months)	Median (min, max)	24 (9, 24)	27 (0, 88)	20 (6, 40)
Duration of Follow-Up Since First NFS ≥ 1 (months)*	Median (min, max)	26 (7, 58)	37 (0, 88)	20 (11, 40)

Abbrev: allo-HSCT: allogeneic hematopoietic stem cell transplant; CALD: cerebral adrenoleukodystrophy; NFS: Neurologic Function Score; MFD: Major Functional Disability

*To last recorded NFS assessment

Source: reviewer analysis of bluebird bio, Inc. BLA 125755 ADSL, ADMRI, and ADEFF3 datasets

Reviewer Comment: *Although all concerns for lead time bias cannot be eliminated with an enriched analysis, the confidence in comparability of populations is increased by the similarities in NFS at baseline and NFS at time of first NFS ≥ 1 for the eli-cel and natural history populations, indicating similar proportions of subjects with asymptomatic disease at baseline and similar number of NFS changes at initial onset of symptoms (first NFS ≥ 1) between the two populations. With these similarities, we can be more confident that any differences between eli-cel and the natural history populations in the analysis of MFD-free survival are truly treatment effect.*

Additional details regarding specific outcomes for subjects matched on baseline disease characteristics that strengthen the argument of treatment effect are provided following the KM analysis below. The matching of these subjects provides additional support for comparability of populations in this analysis.

Analysis of MFD-free survival:

The Applicant, at our request, conducted a Kaplan-Meier (KM) time-to-event analysis that compared estimated time to progression to MFD or death from first NFS ≥ 1 among the untreated and treated subpopulations (Figure 12). The KM curves showed a striking difference between treatment groups (eli-cel, allo-HSCT) and lack of treatment (untreated natural history group). MFD-free survival KM estimates at the 24-month time point were 43% (95% CI: 10%, 73%), 69% (95% CI: 41%, 86%), and 72% (95% CI: 35%, 90%) for the untreated, allo-HSCT treated and eli-cel-treated symptomatic subpopulations, respectively. It is notable that 28% of eli-cel-treated symptomatic subjects experienced an MFD or death within 24 months of first NFS ≥ 1 , as compared to 57% of the untreated natural history subpopulation. In essence, twice as many symptomatic natural history subjects progressed to MFD or death within 24 months of symptom onset as compared to a similar eli-cel population.

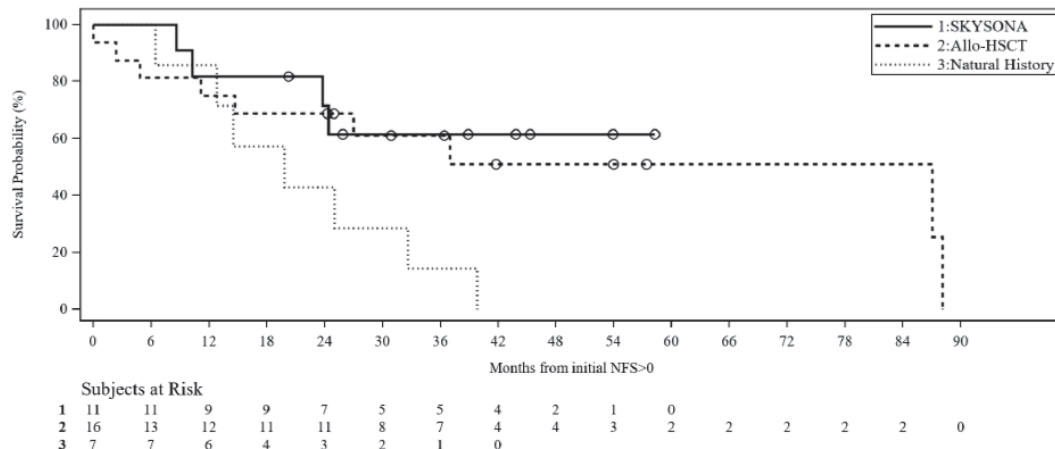
KM estimates of MFD-free survival at 48 months following first NFS change were 61% (95% CI: 27%, 84%) and 51% (95% CI: 22%, 74%) for subjects treated with eli-cel and allo-HSCT, respectively, compared to 0% for untreated subjects. It is notable that **all** untreated natural history subjects who became symptomatic during the course of follow-up in this analysis experienced an MFD or death within 42 months of first NFS ≥ 1 . Trends at 48 months and beyond for the few treated subjects followed to these later time points provide additional evidence of the slowed progression from symptomatic disease to MFD or death following treatment due to the stability of these lines for several years following first NFS ≥ 1 .

Reviewer Comment: *Eli-cel and allo-HSCT demonstrated similar estimated treatment effects on slowing progression to MFD or death over time, though the analysis is limited by short duration of follow-up after first NFS ≥ 1 in the majority of subjects treated with eli-cel and allo-HSCT. Although the estimated MFD-free survival for eli-cel at 48 months following first NFS ≥ 1 is encouraging, it is inconclusive due to the small number of subjects available for evaluation of the endpoints at 48 months in the symptomatic eli-cel population. The results at the 24 month time point provide substantial evidence of effectiveness on an intermediate clinical endpoint that is reasonably likely to predict long-term clinical benefit, and thus this analysis provides the basis for the recommendation of accelerated approval of eli-cel.*

Outcomes on MFD-free survival comparing the eli-cel subpopulation to the allo-HSCT and natural history subpopulations were also evaluated using a Cox regression model. The observed nominally statistically significant hazard ratio of 0.27 (95% CI: 0.08, 0.93) for eli-cel showed that eli-cel may reduce the risk of MFD or death by 73% as compared to similar subjects in the untreated natural history symptomatic subpopulation.

Reviewer Comment: *Though the hazard ratio is encouraging and provides supportive evidence of the efficacy of eli-cel, insufficient long-term follow-up in the treatment populations and a wide confidence interval due to small number of events decrease the confidence in this statistic.*

Figure 12: Kaplan-Meier Curve of MFD-Free Survival from Time of Initial NFS ≥ 1 Between eli-cel, allo-HSCT and Natural History Symptomatic Subpopulations



Abbreviations: MFD, Major Functional Disability; NFS, Neurologic Function Score; allo-HSCT, allogeneic hematopoietic stem cell transplant

Note: SKYSONA is the proprietary name for eli-cel

Source: bluebird bio, Inc. BLA 125755 Ad hoc analysis Figure 80.56.2.1

Specific subject-level matching of subjects in this analysis provides additional evidence of the treatment effect, particularly in those with higher-risk baseline features. Four subjects were similar at baseline with MRI patterns consistent with parieto-occipital and auditory involvement, a Loes of 8.5-9, and diagnosed in childhood (prior to age 10). Both the eli-cel subject ((b) (6)) and the untreated subject ((b) (6)) were 9 years old at baseline. The other two subjects were treated with allo-HSCT ((b) (6)). All 3 treated subjects had a baseline NFS of 1, and the untreated subject had a baseline NFS of 0. The treated subjects were at even higher risk of disease progression compared to the natural history subject (and biasing against eli-cel). In Figure 12 above, the eli-cel subject is the one censored at ~44 months following first NFS ≥ 1 with no MFD or death at last follow-up. The other 3 subjects developed MFDs and thus failed MFD-free survival: the untreated subject at 40 months, one allo-HSCT subject at 36 months and the other at ~85 months. Subject ((b) (6)) treated with eli-cel with high baseline Loes and NFS 1 at baseline has evidence of slowed progression of disease that would not be expected had he not been treated. Additionally, although he experienced increase of NFS to 3 by his Month 12 post-treatment visit, he has maintained a stable NFS for ~30 months, and his Loes score has remained stable since his Month 24 post-treatment visit.

Reviewer Comment: Subject ((b) (6)) disease course provides evidence of stabilization and slowing of disease progression as compared to a matched natural history comparator, and he has also had slower progression than one of the similar allo-HSCT subjects. This supports comparability of populations in this analysis and provides supportive evidence of product efficacy.

Lastly, not only did eli-cel slow progression to MFD or death from first NFS ≥ 1 as compared to the natural history of disease, but all eli-cel subjects who maintained MFD-free survival at Month 24 had a period of NFS stability with no further increase in NFS for at least 24 months.

Reviewer Comment: *Though numbers are small, the stabilization of NFS with no further increase in score over a period of at least 24 months further supports a treatment effect of slowed progression of neurologic dysfunction.*

In summary, although the analysis was post-hoc, not pre-specified and not randomized, careful matching of subjects provided assurance that the populations were comparable, with early symptomatic disease and high risk of rapid disease progression. There is a chance the populations are inherently different at baseline; however, this analysis provided a comparison of the most similar natural history and eli-cel populations evaluated throughout the course of the BLA review and included comparison of subject level-matched outcomes that demonstrated a benefit of eli-cel over the natural history control. The analysis of MFD-free survival at 24 months following first NFS ≥ 1 establishes an effect of eli-cel on an intermediate clinical endpoint that is reasonably likely to predict long-term clinical benefit on MFD-free survival and slowing of progression of neurologic dysfunction as compared to the natural history of disease in symptomatic subpopulations. Success on this intermediate clinical endpoint forms the basis of accelerated approval, and confirmatory PMR studies will be required to assess long-term efficacy in early, active CALD.

Supportive Evidence of Delayed Symptom Onset and MRI Changes

Delayed Symptom Onset

Individual subject matching of eli-cel subjects with high-risk prognostic disease features at baseline to similar subjects in the untreated natural history population provided additional evidence of efficacy through comparisons of clinical course. Evidence of delayed symptom onset was present in two subsets of eli-cel- treated subjects with a baseline NFS of 0 (asymptomatic at time of treatment) who:

- 1) remained asymptomatic during a period of time when symptomatic disease progression would have been expected in the absence of treatment, or
- 2) developed neurologic dysfunction at an age and duration of follow-up after treatment that was later than would have been expected when compared to the natural history of disease.

Subjects who were already included in the MFD-free survival analysis are not duplicated here. High risk factors in this evaluation included a parieto-occipital pattern of disease diagnosed in childhood (10 years of age and younger) and followed at least 2 years after treatment. In addition to evidence as compared to untreated natural history subjects with early, active disease from Study ALD-101, rapid radiographic and clinical disease progression in children 10 years of age or younger presenting with parieto-occipital pattern of disease and gadolinium enhancement on brain MRI is also supported by the literature.^{10,11,14, 27}

Asymptomatic Subjects:

Five subjects treated in childhood (age 10 years or younger) with a parieto-occipital pattern of disease on brain MRI reached adolescence (11 years of age and older) after at least 24 months of follow-up and remained asymptomatic (NFS=0) at last follow-up. Of these 5 subjects, 2 (40%) had a baseline Loes score of 1-2 and the remaining 3 (60%) had Loes scores between 4-9. Of the 3 subjects in the higher Loes score (4-9)

group, 2 (66%) had improved (decreased) Loes score at Month 24 and a stable neurocognitive course. The third higher baseline Loes score subject (Loes 7.5) had an initial worsening (increase) in Loes score at Month 24, after which time it stabilized.

Reviewer Comment: *The asymptomatic course of the subjects with baseline Loes scores ≥ 4 for 24 months or more following treatment with SKYSONA is unexpected based on the natural history of disease. Loes scores are not expected to spontaneously improve in the natural history of disease, so improvement in 2 of the subjects provides additional evidence of efficacy. Stable neurocognitive course in those with higher baseline Loes scores at baseline is also unexpected, based on literature suggesting those with Loes scores >2 at baseline experience some cognitive decline in the 1-2 years following treatment with allo-HSCT, and greater decline might be expected in those with Loes scores >4 at baseline.^{22,23} Assuming a similar treatment effect to allo-HSCT for eli-cel, a neurocognitive decline following treatment might be expected for subjects with higher baseline Loes scores treated with eli-cel. The stable neurocognitive course for 2 of the subjects provides additional supportive evidence of efficacy.*

The two subjects with baseline Loes score of 1-2 had a longer duration of follow-up (approximately 3-6 years following treatment with SKYSONA), though the expected time of progression to symptomatic disease from identification of mild cerebral disease (Loes score 1-3) is unknown, as it is not well-represented in the natural history population or clearly described in the medical literature.

Symptomatic Subjects:

Two subjects treated in childhood with a parieto-occipital pattern of disease on brain MRI developed first symptoms (NFS of 1 or more) more than 24 months following treatment and at an age that would not be expected based on the natural history of disease. One subject, treated at 5 years of age, developed first NFS changes 74 months following treatment at 10 years of age. The other subject was treated at 7 years of age and developed first NFS changes at 11 years of age and 42 months following treatment. These subjects are not represented in the analysis of MFD-free survival because they had not been followed for at least 24 months from time of symptom onset.

Reviewer Comment: *Despite evidence of delayed symptom onset in a small number of eli-cel-treated subjects, there is insufficient evidence to determine if treatment is able to delay symptom onset in the entire population of patients with early, active CALD. Additionally, as numbers are small, it is not clear if these subjects could represent a disease phenotype with slower progression than expected. The analysis is limited by insufficient long-term data beyond 24 months in treated subjects and the large percentage of subjects with very early cerebral lesions (Loes score 1-2) and asymptomatic disease (NFS=0) at baseline for whom the time course of disease progression is relatively unknown due to poor representation in the natural history of disease. Of 61 subjects in the eli-cel Efficacy Population, 44 (72%) had baseline NFS=0 and Loes score 1-2. Only a longer duration of follow-up could provide more information about delayed symptom onset in the population of early, active CALD with mild cerebral lesions and asymptomatic disease and increase confidence that such a prolonged asymptomatic course would not be expected in the natural history of untreated disease. There are also insufficient data to determine if eli-cel might prevent*

onset of neurologic dysfunction in patients treated very early in the disease course.

Resolution of Gadolinium Enhancement at Month 24 Following Treatment

Of 36 subjects with data available for gadolinium enhancement assessment on brain MRI at Month 24 following treatment, 33 (92%) had resolution of enhancement (GdE-). The three subjects who had GdE+ MRIs at Month 24 had resolution of enhancement (GdE-) at the Month 36 visit. In comparison, 27 of 27 (100%) allo-HSCT subjects with Month 24 values for gadolinium assessment had GdE- MRIs. These results with pooled ALD-102 and ALD-104 data are similar to the results seen for ALD-102 alone (discussed in [Section 6.1.11](#)), suggesting a similar effect of eli-cel across both studies.

Reviewer Comment: *Spontaneous resolution of gadolinium enhancement is not expected to occur in the natural history of disease, though lack of routine and regular use of contrast media for MRI assessments in CALD at the time many of the natural history subjects were diagnosed limits the interpretation of these results in comparison to the natural history population. As gadolinium enhancement of demyelinating lesions is indicative of active inflammatory disease at high risk of progression, the resolution of enhancement (GdE-) in the majority of subjects is supportive evidence of a treatment effect of SKYSONA, with reduced cerebral inflammation that predicts slowing of progressive demyelination.*

Extrapolation of Evidence of Effectiveness to the Entire Early, Active CALD Population

The primary evidence of efficacy lies in the outcomes of subjects with parieto-occipital disease, as the pattern was the most common across studies, presents the earliest (in childhood) and is one of the most rapidly progressive if left untreated. Although numbers of subjects are small, there is evidence for efficacy in frontal patterns of disease, as well. The treatment effect of slowed progression of symptomatic disease to MFD or death as compared to the natural history of disease appears similar between allo-HSCT and eli-cel, regardless of baseline pattern of disease or age at onset of symptoms. Additionally, the CALD literature suggests that, although typical presentation of frontal disease is in adolescence, earlier childhood frontal disease is rapidly progressive,¹⁰ and there is evidence of this in the study populations. Of 10 subjects across study populations (2 untreated, 3 treated with eli-cel, 5 treated with allo-HSCT) with early, active frontal disease at baseline, all were younger than 13 years at time of diagnosis and/or treatment, and all were expected to experience rapid disease progression. All but 2 subjects (1 subject treated with eli-cel and 1 treated with allo-HSCT) became symptomatic within 24 months of diagnosis (for the untreated) or treatment (for the populations treated with eli-cel or allo-HSCT), regardless of baseline Loes score. Therefore, with 80% of subjects with early, active frontal disease presenting in childhood developing symptomatic disease within 24 months of presentation, the asymptomatic course in the one (33%) eli-cel-treated subject who has remained asymptomatic at 2

years following treatment provides additional supportive evidence of delayed onset of neurologic dysfunction.

Experts in the CALD community stress that early intervention prior to clinical progression is crucial, despite uncertainty regarding expected time to progression if asymptomatic disease is left untreated due to heterogeneity of disease. Allo-HSCT literature suggests disease progression may occur in the 12-24 months following treatment, followed by clinical and radiographic disease stabilization.¹⁴ The trends following treatment with eli-cel appear to be similar. This variability of clinical course despite early treatment supports early treatment of asymptomatic patients upon first detection of early, active cerebral disease to slow or possibly delay the progression to symptomatic disease, disability, and death.

Reviewer Comment: *Although many of the subjects treated with eli-cel in the clinical trials were treated earlier in the disease course and with lower-risk baseline disease features than the subjects characterized in these analyses, we believe efficacy of eli-cel can be extrapolated to the entire population with early, active disease, regardless of baseline MRI pattern, Loes score, or NFS. The underlying pathophysiology of disease is believed to be the same and results from pharmacodynamic factor analysis and resolution of gadolinium enhancement on brain MRI for most subjects supports this extrapolation. Similar efficacy results for allo-HSCT and eli-cel further support the extrapolation, as the mechanism of action of both treatments is believed to be similar. The comparisons between allo-HSCT and eli-cel are primarily interpretable in the short-term (24 months) and long-term relative efficacy cannot be assessed due to insufficient long-term data for the two treatment populations. We therefore feel the substantial evidence of efficacy in symptomatic subjects at Month 24 following symptom onset and supportive evidence in asymptomatic high-risk subjects can be extrapolated to the entire asymptomatic or mildly symptomatic population with early, active CALD, regardless of MRI pattern of disease (with the possible exception of those with isolated pyramidal tract disease as discussed below).*

Efficacy Concerns in Subjects with Isolated Pyramidal Tract Disease

The small subpopulation of CALD subjects with isolated pyramidal tract disease on brain MRI had worse outcomes following treatment with eli-cel as compared to the natural history of disease. In the untreated natural history population in ALD-101, two (2) subjects met criteria for early, active CALD with isolated pyramidal tract disease at time of diagnosis at 9 and 11 years of age. Despite lack of treatment, both remained asymptomatic for approximately a decade, first developing NFS changes at 19 and 20 years of age, respectively. Adult presentation of symptomatic CALD in patients with isolated pyramidal tract disease is supported by the literature.¹⁰ Ten (16%) subjects treated with eli-cel had isolated pyramidal tract disease at time of treatment, and 3 (30%) have subsequently received rescue allo-HSCT prior to age 20 years – 2 received allo-HSCT as treatment for MDS, and 1 received allo-HSCT as rescue therapy at the investigator's discretion due to progressive cerebral disease on brain MRI, and subsequently died of transplant-related causes. The remaining 7 subjects with isolated pyramidal tract disease, although stable since treatment with eli-cel, have not been followed for a sufficient duration to determine their long-term neurofunctional outcomes.

Reviewer Comment: *Because of these findings, patients with isolated pyramidal tract disease, whose disease generally doesn't become symptomatic until adulthood, may need to be considered differently than patients with other MRI patterns of disease as far as timing of intervention. Careful consideration as to whether and when to treat boys with isolated pyramidal tract disease is warranted, weighing the benefit-risk profile for individual patients based on baseline disease factors, age, and available HSC donor options.*

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Reviewer Comment: *Because of these findings, patients with isolated pyramidal tract disease, whose disease generally doesn't become symptomatic until adulthood, may need to be considered differently than patients with other MRI patterns of disease as far as timing of intervention. Careful consideration as to whether and when to treat boys with isolated pyramidal tract disease is warranted, weighing the benefit-risk profile for individual patients based on baseline disease factors, age, and available HSC donor options.*

Comparative Analyses of Eli-Cel and Allo-HSCT

Exploratory Analyses of MFD-Free Survival and Overall Survival Comparing Eli-Cel and Allo-HSCT:

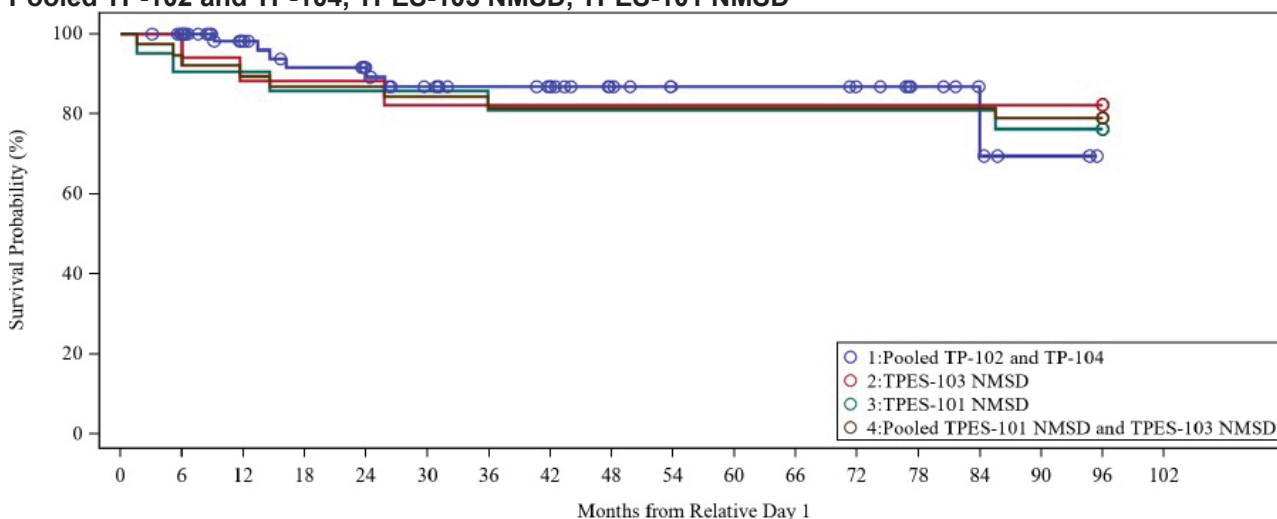
1. Pooled populations and revised imputation schemes:

Additional analyses were performed pooling eli-cel subjects treated 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 after treatment. 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. We felt it was important to impute MDS as failure due to associated morbidity and mortality and in an effort to use conservative imputation methods to be more sure of differences between treatments (if any are seen). We therefore asked the Applicant to perform an analysis of MFD-free survival with pooled TP-102 and TP-104 eli-cel treated subjects and pooled TPES-101 and TPES-103 allo-HSCT treated subjects without a matched sibling donor (NMSD subpopulation), with the following imputation scheme:

- a) 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.
- b) 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.

With these imputations, results for MFD-free survival over time appeared nearly identical for eli-cel and allo-HSCT from a NMSD (Figure 13).

Figure 13: Major Functional Disability (MFD)-Free Survival Over Time Sensitivity Analysis, Pooled TP-102 and TP-104, TPES-103 NMSD, TPES-101 NMSD



Subjects at Risk																	
1	67	63	48	42	38	32	28	24	18	14	14	14	12	8	4	2	0
2	17	17	15	15	15	14	14	14	14	14	14	14	14	14	14	14	0
3	21	19	19	18	18	18	17	17	17	17	17	17	17	17	17	16	0
4	38	36	34	33	33	32	31	31	31	31	31	31	31	31	31	30	0

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.

Source: bluebird bio, Inc., BLA 125755 ad hoc Figure 80.2.6

Reviewer Comment: Due to similar issues already addressed regarding incomparability of populations, the known and unknown differences between populations could not be accounted for simply by pooling data, adjusting the imputation scheme or utilizing propensity score adjustments. Additionally, 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, and many subjects from ALD-103 and ALD-104 did not even have 24 months of data. Similar concerns already discussed regarding selection bias due to retrospective nature of some of the allo-HSCT data and possible bias in the assessment of MFDs without blinding of assessors further complicated the analysis and decreased confidence in the results.

2. Subgroup Analysis by HLA-Matching of Hematopoietic Stem Cell Donor

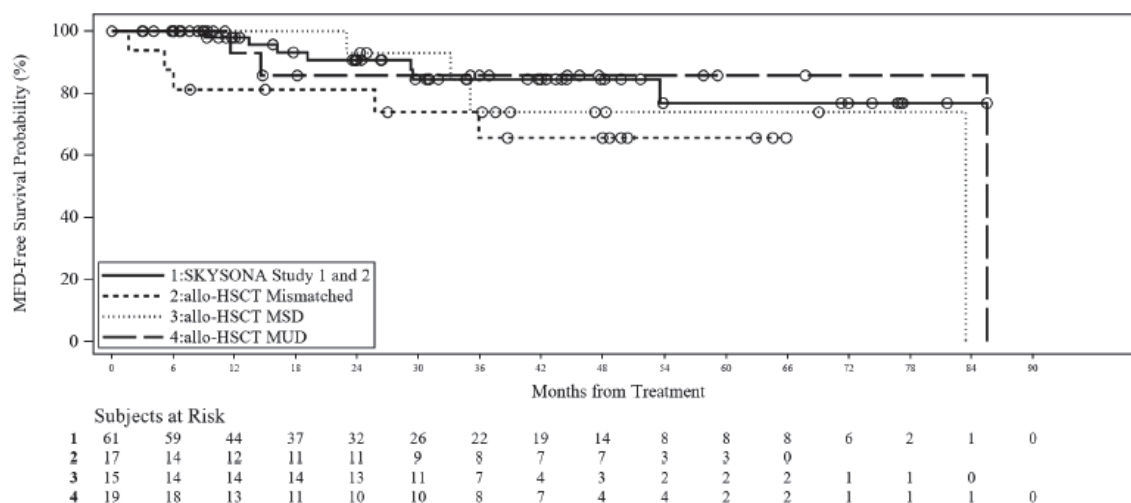
Additional analysis of the allo-HSCT study population subgroups identified a population for which risks of allo-HSCT appear to be greater and for whom events (including death) appear to occur sooner. While it has been traditionally

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 and patients with HLA-mismatched donors. Analyses of events in Studies ALD-101 and ALD-103 demonstrate trends toward worse outcomes for those with mismatched donors compared to those with matched donors, regardless of relatedness of donor to the subject.

KM curves comparing MFD-free survival over time for eli-cel and allo-HSCT from subjects with matched sibling donors, matched unrelated donors, and mismatched donors are shown in Figure 14. Demographics for these donor type allo-HSCT subpopulations and the pooled eli-cel population are in Table 28. Table 31 compares HLA-matched versus HLA-mismatched donor outcomes, rather than specifically breaking down matched donor to matched sibling versus matched unrelated donor. The groups are well-matched aside from the treatment they received. Imputation schemes used in this analysis were as follows:

- a) 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.
- b) Failures of MFD-free survival for eli-cel cohorts include MFD, rescue allo-HSCT for disease progression or MDS, and death. Subjects who did not experience an event were censored at date of last contact.

Figure 14: Kaplan-Meier Curve of MFD-Free Survival Between eli-cel and Allo-HSCT Treated Donor Subpopulations



Abbreviations: MFD, Major Functional Disability; allo-HSCT, allogeneic hematopoietic stem cell transplant; MSD, matched sibling donor; MUD, matched unrelated donor

Note: SKYSONA is the proprietary name for eli-cel, and Study 1 and 2 refers to ALD-102 and ALD-104, respectively

Source: bluebird bio, Inc. BLA 125755 Ad hoc analysis Figure 80.50.4.1.1

Table 31: 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-Mismatched Donor (n=17)	HLA-Matched Donor (n=34)
Subjects with at least one MFD	n (%)	2 (12)	5 (15)
Time to first MFD (months)	Median Min, Max	19 2, 36	35 12, 86
Deaths	n (%)	4 (24)	3 (9)
Time to death (months)	Median Min, Max	6 5, 26	23 13, 33
Duration of follow-up from HSCT (months)	Median Min, Max	48 5, 109	38 4, 108

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.

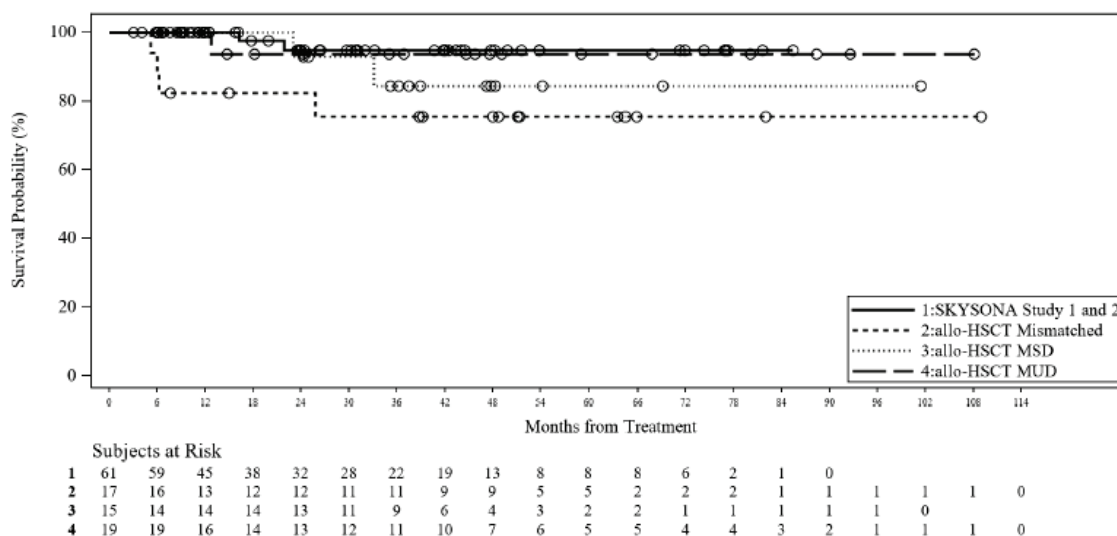
Source: Reviewer's analysis of ADSL, ADBASE, and ADHSCT datasets

Time to MFD or death was prolonged by approximately double (or more) in subjects with HLA-matched donors. As seen in Figure 14 and Table 31, there is steep drop off for the population who received allo-HSCT from a mismatched donor during the first 6 months after which the curves are similar. This is primarily due to deaths. More deaths occurred in the allo-HSCT population with mismatched donors (23.5%, compared to 8.8% for matched donors).

The large number of deaths in the MFD-free survival analysis are mirrored in the analysis of overall survival (OS) in the same populations (Figure 15). Overall survival after treatment with eli-cel was 95% (95% CI: 81%, 99%) through up to 7 years of available follow-up, and the hazard ratio of 0.15 (95% CI: 0.03, 0.83) indicates eli-cel may reduce the risk of death by 85% compared to treatment with allo-HSCT from an HLA-mismatched donor.

Reviewer Comment: *The Statistical reviewer noted the hazard ratio is likely unstable due to wide confidence interval and small number of events, which decreases our confidence in this statistic. The most notable finding in this analysis was the early mortality through 6 months following treatment in the allo-HSCT population who received transplant from an HLA-mismatched donor.*

Figure 15: Kaplan-Meier Curve of Overall Survival Between Eli-Cel and Allo-HSCT Donor Subpopulations



Abbreviations: allo-HSCT, allogeneic hematopoietic stem cell transplant; MSD, matched sibling donor; MUD, matched unrelated donor

Note: SKYSONA is the proprietary name for eli-cel, and Study 1 and 2 refers to ALD-102 and ALD-104, respectively

Source: bluebird bio, Inc. BLA 125755 Ad hoc analysis Figure 80.2.4.7.2.s

These differences in MFD-free survival and OS for subjects receiving allo-HSCT from mismatched donors were largely due to allo-HSCT-related toxicities contributing to early transplant-related mortality, as opposed to CALD disease progression. While small numbers of subjects and events are limitations of the analysis, the results are supported by biologic plausibility, in that increased rates of graft failure and graft versus host disease (GVHD) would be expected in those with mismatched donors compared to those with matched donors. No subjects treated with eli-cel experienced GVHD.

To support the biological plausibility of a difference in prognosis between subjects with mismatched donors, further analysis on GVHD and repeat allo-HSCT was done. Subjects who were treated with allo-HSCT from mismatched donors experienced more transplant-related events compared to those with matched donors, as seen in Table 32. Time to repeat HSCT was shorter for those with mismatched donors than those with matched donors (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 mismatched donors compared to those with matched donors. Incidence of acute GVHD >Grade 2 and/or chronic GVHD by Month 24 was also increased in those with mismatched donors.

Table 32: Graft Failure, Repeat HSCT, and Acute or Chronic GVHD by Donor HLA Matching

Parameter	HLA-Mismatched 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)

Abbrev: HLA, Human Leukocyte Antigen; HSC, hematopoietic stem cell; GVHD, graft versus host disease

Source: Reviewer's analysis of ADSL and ADHSCT datasets

Reviewer Comment: *Eli-cel appears to offer a survival advantage over allo-HSCT from a mismatched donor by virtue of avoiding certain transplant-related toxicities. Small numbers of subjects decrease confidence in these results. However, there is biological plausibility (one would predict poorer prognosis in mismatched donors compared to matched donors because of HLA-mismatch and increased risk for graft rejection and graft versus host disease, GVHD.)*

Additional Analyses and Evaluations: Loes Score Changes and Neurocognitive Trends

As noted in [Section 7.1.5](#), Loes score changes from Baseline to Month 24 following treatment were greater in the eli-cel population than the allo-HSCT population. The clinical significance of this is not known, and Loes changes did not appear to correlate with changes in NFS, though noted limitations included insufficient duration of follow-up to understand if early Loes score changes might predict later clinical changes. Previous allo-HSCT studies ^{8,14,24,25} have not found correlations between post-treatment changes in Loes score, neurocognition, and neurologic function, and instead found pre-treatment Loes scores to be more predictive of later, post-treatment neurocognitive and neurologic function status. However, it was not clear if the same was true of eli-cel.

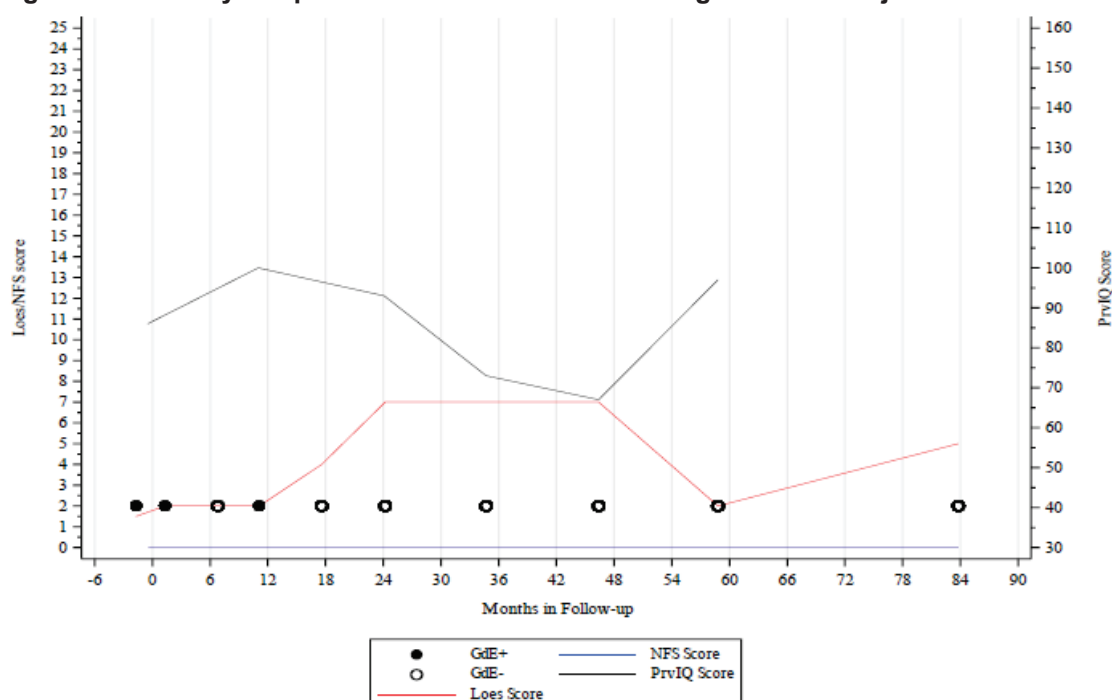
In an effort to better understand the significance of these increased Loes scores at 24 months and the greater overall clinical picture of subjects evaluated in the efficacy analysis, we asked the Applicant to provide individual graphics for each subject mapping NFS, Loes score, and performance IQ (PrvIQ) over time, with specific notations for events (MFD, MDS, rescue allo-HSCT) and notations of specific domains that were present at each NFS assessment. We hoped this would help us better understand on a subject level if certain MRI changes predicted later clinical changes in subjects who had follow-up beyond 24 months.

No formal analysis was done to analyze correlation between MRI changes and clinical endpoints, though trends were noted. In eli-cel-treated subjects who did have longer duration of follow-up (i.e., beyond 24 months following treatment), Loes scores that initially increased by 4 or more at 24 months could either stabilize, decrease, or continue to increase at later time points. In general, Loes scores that did not change or that increased by less than 4 from Baseline to Month 24 remained stable or decreased at later time points. There was insufficient information to determine if greater increases in Loes score in the first 24 months predicted later clinical deterioration, though the

available data did not suggest this was the case. One striking finding was that Loes scores coursed inversely with PrvIQ in many subjects, suggesting MRI changes might correlate with neurocognitive testing. An example is shown in Figure 16, where the subject had increase in Loes score to Month 24 after which time it stabilized to Month 48 and then improved, trending back toward baseline at Month 60. The PrvIQ declined (decreased) along with Loes score declines (increases) and the PrvIQ improved (increased) as Loes score improved (decreased). NFS remained 0 throughout.

Reviewer Comment: *Insufficient neurocognitive data were available to compare outcomes between eli-cel and either natural history or allo-HSCT, or to conduct formal correlation analyses between Loes score and PrvIQ changes, but further data from the Clinical Efficacy PMRs will hopefully provide more insight with time.*

Figure 16: Efficacy Endpoint Trends Over Time in a Single Eli-Cel Subject



Source: Adapted from bluebird bio, Inc. BLA 125755 ad hoc Figure 80.9.1.1 (b) (6) in response to IR#28

7.1.11 Efficacy Conclusions

In summary, this clinical reviewer concludes that there is evidence of effectiveness on an intermediate clinical endpoint for eli-cel in pediatric patients with early, active CALD from a single adequate and well-controlled investigation comprised of pooled data from two clinical studies, compared to external controls, that demonstrate slowed progression to MFDs or death from time of first NFS ≥ 1 to Month 24 in symptomatic subjects who have been treated with eli-cel as compared to the natural history of disease, and confirmatory evidence. Supportive evidence of efficacy is based upon subjects with parieto-occipital or frontal MRI patterns who would have been expected to experience rapid disease progression in childhood in the absence of treatment but had evidence of delayed onset of neurologic dysfunction either with asymptomatic course or onset of symptoms in

adolescence and/or at least 24 months of follow-up after treatment. There is additional supportive evidence of effectiveness from improvement in Loes score in 2 subjects and resolution of gadolinium enhancement in the majority of subjects at Month 24 following treatment with eli-cel.

There is additional confirmatory evidence of effectiveness from in vitro pharmacology studies (discussed in [Section 4.3](#)) wherein fibroblasts from patients with CALD that were transduced with the Lenti-D vector demonstrated vector-driven *ABCD1* transgene expression, ALDP production, and improved VLCFA metabolism. In vivo studies of Lenti-D-transduced HSCs from healthy donors transplanted into myeloablated immunodeficient mice demonstrated stable *ABCD1* transgene expression and ALDP production in bone marrow and brain during the study's duration. Clinical pharmacodynamic response data (discussed in [Section 4.4.2](#)) are also supportive. The median (min, max) value of %ALDP+ CD14+ cells at Month 6 in subjects who maintained MFD-free survival at 24 months following infusion of eli-cel was 23.1 (2.0, 71.4) which was more than twice as high as the median value of %ALDP+ CD14+ cells at Month 6 in subjects who failed MFD-free survival by Month 24, which was 10.9 (10.8, 18.2).

This clinical reviewer also is of the opinion that evidence of effectiveness can be extrapolated to the entire asymptomatic or early symptomatic (i.e., NFS ≤ 1) population with early, active CALD regardless of baseline Loes score or pattern of disease, with the possible exception of patients with pyramidal tract disease who do not typically have symptom onset until adulthood and who had worse outcomes following treatment with eli-cel than the natural history of disease. Additionally, there is an early survival benefit of eli-cel compared to allo-HSCT from HLA-mismatched donors due to avoidance of allo-HSCT-related toxicities in that population.

Despite the limitations of heavy reliance on post-hoc exploratory analyses, the totality of efficacy evidence supports accelerated approval of eli-cel for the indication of slowing the progression of neurologic dysfunction in boys with early, active CALD, with careful consideration given to timing of treatment based on available HSC donor options, the relative risks of treatment, and the CALD disease phenotype of the patient (including but not limited to MRI pattern of disease, Loes score, and presence or absence of early neurologic dysfunction at time of intended treatment). Significant uncertainty remains about durability of effectiveness, which is of particular concern given the large number of subjects treated with eli-cel in the clinical studies who had baseline NFS=0 and Loes scores of 1-2, because the time course of disease progression to symptomatic disease, disability and death is poorly understood in patients diagnosed at such an early stage of disease. Given the uncertainties about the benefit-risk assessment in subjects with baseline NFS=0 and Loes scores of 1-2, as well as subjects with isolated pyramidal tract disease, additional time in follow-up is warranted to understand the long-term benefit and durability of effectiveness of eli-cel. Two PMR studies will be required to confirm efficacy of SKYSONA, and will evaluate long-term outcomes on MFDs, death, need for rescue allo-HSCT, NFS, MRI findings, neurocognitive outcomes, quality of life measures, %ALDP+ CD14+ cells, and peripheral blood VCN. Because of significant uncertainties regarding durability of effect and magnitude of risk of myelodysplastic syndrome, the most favorable benefit-risk is in patients with early, active CALD who do now have an available HLA-matched donor. Eli-cel offers a clear early survival benefit over allo-HSCT from an HLA-mismatched donor, and thus the recommendation of this

clinical reviewer is to approve for the population of boys with early, active CALD who do not have an available HLA-matched donor.

8. INTEGRATED OVERVIEW OF SAFETY

8.1 Safety Assessment Methods

The safety assessments for evaluating the major identified risk with eli-cel, hematologic malignancy, were adequate given our evolving understanding of the factors that contribute to the development of malignancy and limitations in the available methods for assessing those factors. The safety assessments and algorithm for further assessment changed over the course of the trials as their limitations were identified and as technology improved. However, as outlined in this section, tests to better characterize cases of malignancy and clonal expansion (e.g., gene expression studies and RNA sequencing) could have been employed in order to better understand clone characteristics in more subjects who have integration site analysis results that suggest clonal expansion.

Complete blood count and integration site analysis (ISA) were the primary means of monitoring for the development of malignancy in the trials. Further clinical work-up was performed when certain ISA criteria were met. While not specified in the protocol, bone marrow biopsy and aspirate, the definitive test for detecting hematologic malignancy, was generally performed as a component of the clinical work-up. Bone marrow studies were not standardized, but often included some combination of flow cytometry, cytogenetics, next generation sequencing, and viral studies. As it became clear that patients were developing malignancy mediated by the lentiviral vector (LVV), the frequency of follow-up for all subjects increased. However, there is no consensus regarding optimal schedule and components of follow-up, whether routine or enhanced due to some finding(s) of concern.

While we are heavily reliant upon ISA for understanding LVV behavior, it has a number of limitations that have become apparent through review of this BLA. First, relative frequency obtained from ISA does not identify multiple integration sites in a single clone, leading to underestimation of the relative size of a clone when it is not recognized that the clone has multiple integration sites. Second, the lower limit of reliable results for the S-EPTS/LM-PCR method of ISA is 5% relative frequency. This may not be sufficiently low to provide reliable data for evaluating potentially problematic clones. Third, the Applicant has reported a range of six weeks to three months for obtaining ISA results, which is longer than desirable for making clinical decisions in the setting of possible malignancy.

The primary strategy for the identification of multiple integration sites present within a clone was noting those integration sites whose relative frequencies tended to rise and fall together over time. The primary method for identifying multiple integration sites in clone is colony-forming unit assays, as utilized for subject (b) (6) to determine which of five genes were integration sites within the same clone. An alternative method that was not employed and could be considered for confirming the presence of integration sites within a single clone is single cell sequencing.

Gene expression studies were performed in select subjects with a *MECOM* integration site in a large clone. *EVI1* is an oncoprotein encoded by *MECOM*, and gene expression studies that demonstrate increased expression of *EVI1* suggest a potentially deleterious *MECOM* integration site. Because integration into *MECOM* was ubiquitous, gene expression studies could have been performed more widely to identify *EVI1* overexpression as a component of assessing a subject's risk for progressing to malignancy. Among those subjects in whom gene expression studies were performed, all had overexpression of *EVI1*.

RNA sequencing is used for characterizing long-range chromatin interactions and resultant changes in gene expression. It was performed in the subject who developed MDS but did not have *MECOM* integration in the clone. RNA sequencing in that subject provided information about increased expression of numerous genes and several fusion transcripts, all of which may have contributed to the development of malignancy. RNA sequencing could have been helpful for characterizing the factors that contributed to clonal expansion and the development of malignancy aside from *EVI1* overexpression in the other two subjects with MDS.

Whole genome sequencing is one additional method that could have been utilized. In subjects with malignancy, it can define chromosomal and molecular changes, including the identification of multiple integration sites within a malignant clone, and could be employed as an alternative to conventional karyotyping and next generation sequencing.

8.2 Safety Database

8.2.1 Studies/Clinical Trials Used to Evaluate Safety

The safety database includes 67 subjects treated with eli-cel in Studies ALD-102 (N=32) and ALD-104 (N=35). All subjects (excluding drop-outs) were still undergoing follow-up and had not completed the long-term follow-up safety study.

8.2.2 Overall Exposure, Demographics of Pooled Safety Populations

Demographics of the pooled safety population follow:

Table 33: Demographics of ALD-102 and ALD-104 Safety Population

Characteristic	ALD-102/104 (N=67)
Sex, n (%)	
Male	67 (100%)
Race, n (%)	
White	36 (54%)
Black or African American	3 (4%)
Asian	1 (1%)
Other (includes mixed race)	7 (10%)
Not reported	20 (30%)
Ethnicity, n (%)	
Hispanic	17 (25%)
Non-Hispanic	41 (61%)
Not reported	9 (13%)
Age at Treatment	

Median	6 years
Minimum, Maximum	4, 14

Source: Adapted from BLA 125755/011; 3 Month Safety Update Report, p. 13

This demonstrates that the majority of patients treated with eli-cel are non-Hispanic and white. Safety data in non-white populations are limited, a significant shortcoming because non-white patients generally have greater difficulty in finding HLA-matched HSC donors, and are therefore most likely to benefit from the availability of eli-cel.

8.2.3 Categorization of Adverse Events

During review of this BLA, verbatim terms were compared to dictionary-derived terms. In most cases, the coding was appropriately and consistently performed. The following table provides a few examples where coding was revised by FDA either because there were more suitable dictionary-derived terms available or so that similar adverse events would be lumped together and recognized as occurring in multiple subjects.

Table 34: Examples of Adverse Event Recoding

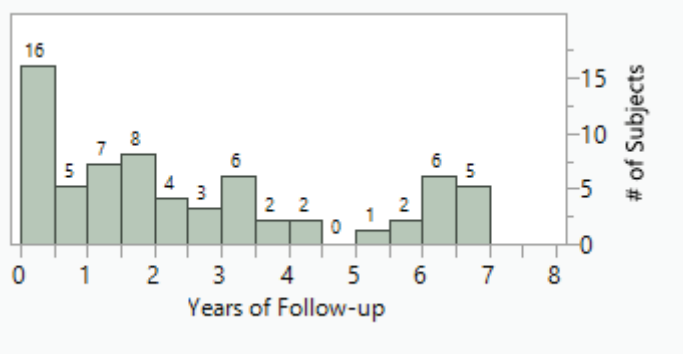
FDA Recode	Dictionary-Derived Terms	Verbatim terms
Abdominal pain	Abdominal discomfort	Abdominal discomfort
Abdominal pain	Abdominal pain upper	Pain: LUQ; stomach pain; stomach ache
Adrenocortical insufficiency acute	Adrenal insufficiency	Adrenal insufficiency exacerbation; adrenal insufficiency crisis
Bradycardia	Sinus bradycardia	Sinus bradycardia; intermittent sinus bradycardia
Candidiasis	Anal candidiasis	Rectal culture, candida; candidiasis of anus
Candidiasis	Oral candidiasis	Oral thrush
Candidiasis	Oropharyngeal candidiasis	Throat culture, candida

Prior to conducting final adverse event analyses, a total of 265 of 1762 adverse events were coded to an alternate dictionary-derived term either for better characterization of the adverse event or to minimize splitting. All available information, including subject narratives and comments provided in the datasets, considered when making coding changes.

8.3 Caveats Introduced by Pooling of Data Across Studies/Clinical Trials

At the time of BLA submission, ALD-104 was ongoing and no subject had completed LTF-304, the long-term follow-up study. The distribution of follow-up duration is depicted in the following figure.

Figure 17: Years of Follow-Up for ALD-102 and ALD-104 safety population



Source: Reviewer's analysis

This figure demonstrates that the safety data for many of the subjects is of limited duration, i.e., 50% of subjects have less than 2 years of follow-up. For delayed adverse events, the calculation of incidence based on total enrolled subjects will yield a value lower than the true incidence. Therefore, adverse event incidence calculations were sometimes performed based on the number of subjects that were followed to that point in time, and therefore with a denominator less than the full population of 67 treated subjects.

8.4 Safety Results

8.4.1 Deaths

Three subjects died after treatment with eli-cel. All were treated under ALD-102, and are discussed in Section 6.1.12.3 Deaths.

8.4.2 Nonfatal Serious Adverse Events

This section presents the important serious adverse event (SAE) of myelodysplastic syndrome (MDS) and cases of where hematologic malignancy appears to be developing due to clonal expansion.

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 patients with CALD. However, the Applicant found a single reported case of chronic myelogenous leukemia reported in a patient with adult adrenomyeloneuropathy,⁵³ a phenotype of adrenoleukodystrophy that is distinct from childhood CALD.

Three children in the eli-cel development program have been diagnosed with myelodysplastic syndrome (MDS). Two of these cases occurred within two years of eli-cel administration and are the result of expansion of a clone that has lentiviral vector (LVV) integration into a proto-oncogene. Both subjects had a predominant clone² with integration into the *MDS1* and *EVI1* complex locus, also referred to as *MECOM*. Both subjects also had overexpression of *EVI1*, the oncoprotein produced by *MECOM*. The third subject had LVV integration into multiple genes that appear to have contributed to

² A clone was considered predominant when IS-specific VCN measured by qPCR was >0.5 c/dg.

his developing MDS. Selected information about these three subjects is presented in the following table, after which follows a description of the individual cases.

Table 35: Overview of Subjects Who Developed Myelodysplastic Syndrome

	(b) (6)		
Age at eli-cel administration	11 years	13 years	5 years
Age at malignancy	12 years	14 years	12 years
Time of malignancy relative to eli-cel administration	14 months	22 months	7.5 years
Eli-cel cells administered	5.7 x 10 ⁶ /kg	12.1 x 10 ⁶ /kg	6 x 10 ⁶ /kg
Eli-cel % LVV+	Not reported	70%	62%
Eli-cel vector copies per transduced cell	Not reported	2.6 c/dg	2.6 c/dg
Eli-cel VCN	Not reported	1.8 c/dg	1.8 c/dg
Neutrophil engraftment day*	27	188	37
Platelet engraftment day*	106	Not Engrafted	37
Key integration sites	<i>MECOM, SLC6A16</i>	<i>MECOM, ACTR RAP2C, STGAL6</i>	<i>PRDM16, GAB3, SNX12</i>
Gene expression studies	Increased <i>EVI1</i>	Increased <i>EVI1</i>	Increased <i>PRDM16, GAB3, and CAM2KA</i>
Bone marrow at malignancy diagnosis	MDS with single lineage dysplasia	MDS with single lineage dysplasia	MDS with excess blasts 2 (MDS-EB-2)
Cellularity	10-20%	80%	60-70%
Other features	Dysmegakaryopoiesis	Dysmegakaryopoiesis	15% blasts
Flow cytometry	Negative	Negative	15% myeloblasts
FISH	Normal	Normal	Normal
Karyotype	del(14)(q11.2q13) versus inv(14)(p11.2q11.2)	Normal	Normal
Rapid Heme Panel next generation sequencing	<i>CDKN2A</i> SNV** – 41% VAF	Normal	<i>KRAS</i> SNV – 14% VAF <i>NRAS</i> SNV – 3% VAF <i>JAK</i> SNV** – 48%
PB WBC (x 10⁹) / Hgb (g/dL) / Plt (x 10⁹)	2.6 / 13 / 123	2.2 / 10.7 / 19	14.9 / 10 / 17

Abbrev: LVV, lentiviral vector; VCN, vector copy number; c/dg, copies per diploid genome; MDS, myelodysplastic syndrome; FISH, fluorescence in situ hybridization; del, deletion; inv, inversion; SNV, single nucleotide variant; VAF, variant allele frequency; PB peripheral blood; WBC, white blood cell; Hgb, hemoglobin; g/dL, grams per deciliter; Plt, platelet

Source: Reviewer's analysis

Subject (b) (6)

Subject (b) (6) was treated with eli-cel on (b) (6), at the age of 11 and was diagnosed with MDS with unilineage dysplasia 14 months later. His MDS is attributed 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 CD15+ cells.

Subject (b) (6) 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 (b) (6) 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 *SLC6A16* at all timepoints are provided in the table below:

Table 36: Relative Frequencies of *MECOM* and *SLC6A16* by qPCR in Subject (b) (6)

Time Post-Eli-Cel	<i>MECOM</i> Primers	<i>SLC6A16</i> 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

Note: Clonal contribution is estimated by IS-specific qPCR.

Source: BLA 125755 Listing 80.1.46 Integration Site Analysis Subject (b) (6)

In addition to the demonstration of integration into *MECOM*, increased *EVI1* expression of the *MECOM* locus was present in CD15+ cells.

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. Rapid Heme Panel next generation sequencing (NGS) 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, and his course post-HSCT was complicated by SAEs of diabetes at three months post-HSCT

and fever and hypotension at six months post-HSCT. His bone marrow biopsy at six months confirmed his MDS was in remission, with a hypocellular marrow with maturing trilineage hematopoiesis with no overt dysplasia or increase in blasts, normal flow cytometry, and negative FISH and NGS. Blood and bone marrow chimerism showed 100% donor cells. CBC demonstrated mild anemia and thrombocytopenia but normal WBC (WBC 4.9×10^9 , Hgb 10.8 g/dL, PLT 101×10^9).

Subject (b) (6)
Subject (b) (6) 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 attributed 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 *EV11* expression in the *MECOM* locus in CD15+ cells.

Details regarding Subject (b) (6) 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 1×10^9 /L, finally meeting engraftment criteria on Day 188. The subject also had poor engraftment of platelets; his post-treatment platelet count peaked at 53×10^9 /L while he was receiving eltrombopag approximately 3.5 months after eli-cel administration. He achieved an unsupported platelet count of 45×10^9 /L around Month 14, that technically did not meet engraftment criteria because it was not sustained on three consecutive measurements, having declined to 19×10^9 /L by Month 22 when he met criteria for MDS. In addition to the low platelet counts, Subject (b) (6) had leukocyte, neutrophil, and hemoglobin levels that were abnormally low at most or all assessments.

Integration site analysis (ISA) for Subject (b) (6) 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 a persistent predominant clone were met beginning at 12 months. Increased *EV11* expression in the *MECOM* locus was present in CD15+ cells, 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 NGS) were normal.

After a bone marrow biopsy at 2.5 years demonstrated persistent MDS the subject underwent treatment with allogenic hematopoietic stem cell transplant from a paternal haploidentical donor. His post-transplant course was complicated by mucositis, febrile neutropenia, nausea, anorexia requiring total parenteral nutrition, *Clostridium difficile* infection, and cytopenias requiring intermittent packed red blood cells and platelet

transfusions as well as growth factor support. The last report from two months post-HSCT was that the subject was in remission. His bone marrow biopsy demonstrated hypocellular marrow (20-30%) with trilineage hematopoiesis and normal flow cytometry. CBC demonstrated mild anemia and thrombocytopenia but normal WBC and ANC (WBC 4.5×10^9 , ANC 3.3×10^9 , Hgb 10.9 g/dL, PLT 142×10^9).

Subject (b) (6)

Subject (b) (6) was treated with eli-cel on (b) (6), at the age of 5, and he was diagnosed with MDS approximately 7.5 years later. This case of MDS appears to be caused by eli-cel given the integration into several proto-oncogenes and increased expression of those genes in the predominant clone. The Applicant has concluded the vector likely caused the malignancy.

Subject (b) (6) had a comparatively uneventful early clinical course in that neutrophil and platelet engraftment occurred on Day 37, and blood counts fully recovered to the normal range. However, it is notable that he is one of only four subjects who had a platelet count of $< 100 \times 10^9/L$ more than 100 days after eli-cel administration ($91 \times 10^9/L$ on Day 135). He was also slower than average in recovering WBC and hemoglobin values, as one of eight subjects with WBC $< 2 \times 10^9/L$ 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 $25 \times 10^9/L$, WBC normal).

Integration site analysis (ISA) for Subject (b) (6) 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 of 19% in *MDS1* at Month 8, 26% in *SMG6* at Month 30, and 18% in *INO80* at Month 30), none of them appeared to persist or increase in the latter assessments.

In 2019, the ISA method for the study was changed and when the subject presented with thrombocytopenia at Year 7.5, his ISA was performed using S-EPTS/LM-PCR for the first time. S-EPTS/LM-PCR identified integration sites in *PRDM16*, *MIR106A*, *CAMK2A*, *GAB3*, *TYK2*, and *SNX12* with relative frequencies between 13 and 18 percent. These six integration sites were likely present in the same clone given each had an integration site-specific vector copy number, as determined by qPCR, of > 0.5 copies per diploid genome.

Table 37: Integration Site-Specific Vector Copy Numbers in CD34+ Cells for Subject (b) (6)

Cell Source	<i>SNX12</i> (c/dg)	<i>PRDM16</i> (c/dg)	<i>CAMK2A</i> (c/dg)	<i>MIR106A</i> (c/dg)	<i>TYK2</i> (c/dg)	<i>GAB3</i> (c/dg)
Whole blood, CD34+	0.94	0.84	0.88	0.79	0.68	0.56
Bone marrow, CD34+	1.03	0.93	0.91	0.79	0.67	0.53

Abbrev: VCN, vector copy number; c/dg, copies per diploid genome

Source: Derived from Clinical Information Amendment received May 4, 2022

Bulk RNA sequencing was also performed on (b) (6) CD34+ cells from bone marrow, with two important findings. First was greater than three-fold increased expression of three of the six genes where LVV integration occurred. Second was the

detection of with HIV-derived sequence fused with *TYK2* and *PRDM16*. The increased expression of genes containing the LVV integration and the detection of fusion transcripts in the malignant clone support causality of the vector in this case of malignancy.

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. 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 significance showed *JAK c269T>c (p.I889T)* at 48% variant allele frequency. He was diagnosed with MDS with excess blasts, worrisome for evolving acute myeloid leukemia. Blast cells from peripheral blood and bone marrow aspirate were positive for the lentiviral vector.

The subject was initially treated with chemotherapy and total body irradiation. Subsequent bone marrow biopsy demonstrated hypocellular marrow with trilineage hematopoiesis including paucity of maturing myeloid population and 1% CD34+ blasts. Approximately two months after being diagnosed with MDS, the subject underwent treatment with allo-HSCT with a cord blood donor. His course was complicated by an SAE of septic shock with onset five days after HSCT. Blood cultures grew *Streptococcus mitis/oralis* and *Clostridium perfringens*, and he was treated with antibiotics and G-CSF. He reportedly achieved neutrophil engraftment on Day 26, and was discharged to an inpatient rehabilitation facility 5 weeks post-transplant. Four months post-transplant, he with had an SAE of fever with central line. He was hospitalized for 5 days and subsequently transferred back to the inpatient rehabilitation facility.

The patient had bone marrow studies reported at 5 weeks and 100 days post-transplant. The bone marrow studies at 5 weeks 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. At Day 100 bone marrow studies demonstrated maturing trilineage hematopoiesis and blood and bone marrow chimerism showed 100% donor cells.

In addition to the three subjects who have been diagnosed with malignancy, this review section includes information about nine additional subjects with findings suggesting they may progress to malignancy. Key information about four of those nine subjects where concern for malignancy is greatest are also presented in Table 38, and all nine subjects are described in the subsequent text.

Table 38: Overview of Subjects Of Greatest Concern for Future Development of Malignancy

	(b) (6)			
Age at eli-cel administration	4 years	9 years	7 years	13 years
Date of eli-cel Administration	Apr 2018	Aug 2019	Feb 2015	Sep 2020
Cells administered	5 x 10 ⁶ /kg	14.5 x 10 ⁶ /kg	10.5 x 10 ⁶ /kg	14.4 x 10 ⁶ /kg
% LVV+	62%	67%	59%	47%
Vector copies per transduced cell	3.4 c/dg	2.7 c/dg	2.7 c/dg	3.2 c/dg
Eli-cel VCN	2.1 c/dg	1.8 c/dg	1.6 c/dg	1.5 c/dg
Neutrophil engraftment day*	32	167	27	13
Platelet engraftment day*	60	356	41	29
Key integration sites	<i>MECOM, EVI5, SECISBP2, PLAG1, PUM3</i>	<i>LINC00982, SMG6, MECOM, MPL</i>	<i>MECOM, ACER3, RFX3</i>	<i>MECOM, MPL</i>
Gene expression studies	Increased <i>EVI1</i>	Not reported	Increased <i>EVI1</i>	Not reported
Bone marrow	Maturing trilineage hematopoiesis	Trilineage hematopoiesis, atypical megakaryopoiesis, 2% blasts	Maturing trilineage hematopoiesis	n/a
Cellularity	40-50%	30-40%	30-40%	n/a
Other features	Unremarkable megakaryocytes	Small megakaryocytes with monolobated nuclei and very rare forms with widely spaced nuclei	Megakaryocytes with overall normal morphology and include some small forms	n/a
Flow cytometry	Negative	No definitive abnormal myeloid blast population	Negative	n/a
FISH	Normal	Not reported	Normal	n/a
Karyotype	Normal	Normal	Normal	n/a
Next Generation Sequencing	Rapid Heme Panel Normal	MDS Panel – <i>MPL</i> SNV – 47% VAF <i>CALR</i> SNV – 47% VAF	Rapid Heme Panel Normal	n/a
PB WBC / Hgb / Plt	5.1 / 11.2 / 184	5.1 / 14.6 / 100	6.4 / 14.9 / 307	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 10⁹), hemoglobin (g/dL), platelets (x10⁹)

Based on FDA definitions for engraftment that did not permit concomitant G-CSF or eltrombopag at time of engraftment

*Variant of unknown significance

Source: Reviewer's analysis

Subject (b) (6)

Subject (b) (6) 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 (b) (6) 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 $114 \times 10^9/L$ at 15 months). His last CBC, ~4 years post-eli-cel, was normal except for mild anemia (WBC $5.1 \times 10^9/L$, ANC $3.5 \times 10^9/L$, Hgb 11.2 g/dL, PLT $184 \times 10^9/L$).

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%. The protocol specified criteria for clonal predominance were not met at Month 48, when the VCN for *MECOM* and *EVI5* in CD15⁺ cells exceeded the required threshold of 0.5 c/dg.

Table 39: Integration Site Frequency and Vector Copy Number for Two Likely Clones in Subject (b) (6)

Time Point	Cell Type	Method	<i>MECOM</i> Freq (%)	<i>MECOM</i> VCN (c/dg)	<i>EVI5</i> Freq (%)	<i>EVI5</i> VCN (c/dg)	<i>SECISBP2</i> Freq (%)	<i>SECISBP2</i> VCN (c/dg)	<i>PLAG1</i> Freq (%)	<i>PLAG1</i> VCN (c/dg)	<i>PUM3</i> Freq (%)	<i>PUM3</i> VCN (c/dg)	PB VCN All IS (c/dg)
Month 6	WB	qPCR	--	--	--	--	1.9	.03	1.8	0.03	1.5	0.02	1.8
Month 12	WB	qPCR	--	--	--	--	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	qPCR	--	--	--	--	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	qPCR	--	--	--	--	18.5	0.30	17.6	0.30	16.1	0.25	--
--	CD3	qPCR	--	--	--	--	16.2	0.34	15.5	0.17	14.5	0.17	--
--	CD15	qPCR	--	--	--	--	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	qPCR	18.7 [†]	0.33	19.6 [†]	0.34	12.3	0.20	11.8	0.20	11.7	0.18	--
--	CD3	qPCR	0.2	0.002	0.2	0.003	11.3	0.14	10.8	0.13	10.5	0.14	--
--	CD15	qPCR	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	qPCR	NR	0.41	NR	0.48	NR	0.14	NR	0.14	NR	0.13	--
--	CD3	qPCR	NR	0.05	NR	0.41	NR	0.23	NR	0.23	NR	0.21	--
--	CD15	qPCR	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; qPCR, quantitative polymerase chain reaction; Freq, relative frequency; VCN, vector copy number; c/dg, copies per diploid genome; IS, integration sites; NR, not reported

Integration sites in *SECISBP2*, *PLAG1*, and *PUM3* are in the same clone and add up to > 30%.

[†] Integration sites in *MECOM* and *EVI5* are in the same clone and add up to > 30%.

Source: Reviewer's analysis derived from Listing 80.1.29 Integration Site Analysis Subject (b) (6) & 72-Hour Reporting Form – ISA Tier 1 & Tier 2, CBC

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 (b) (6) had a bone marrow biopsy and aspirate around Month 48 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.

A second bone marrow biopsy was performed on 3 months later. Anatomic pathology demonstrated dysmegakaryopoiesis and equivocal dysgranulopoiesis, “highly concerning for an evolving myelodysplastic syndrome without an increase in blasts, however the suboptimal samples precludes optimal evaluation.” Flow cytometry was normal and FISH and NGS (Rapid Heme Panel) were negative. Concurrent CBC had borderline low ANC and was otherwise normal (WBC $4.6 \times 10^9/L$, ANC 1.18, Hgb 13.5 g/dL, PLT $240 \times 10^9/L$, lymphocytes 2.83, MCV 81.4 fL).

Subject (b) (6)

Subject (b) (6) was treated with eli-cel (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, his 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 (b) (6) 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 (b) (6) 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.⁶⁹

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.⁴⁶

The severity and timeframe for Subject (b) (6) cytopenias do not support parvovirus as the cause of his cytopenias. While the predominant hematologic manifestation of parvovirus is anemia, Subject (b) (6) 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 $100 \times 10^9/L$ when last measured 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 (b) (6) 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. A second opinion on this bone marrow biopsy confirmed cellularity (70%) however determined 40% of megakaryocytes were abnormal.

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 blast population; however, some CD34⁺ cells with increased CD7. Karyotype was normal. A second opinion on this bone marrow biopsy found lower cellularity (20%) and determined 50% of megakaryocytes were abnormal.

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 ($300 \times 10^9/L$) 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.

The second opinion pathology assessment is that this subject has idiopathic dysplasia of undetermined significance, based on mildly low or normal blood counts and the absence of somatic pathogenic mutations. The pathologist opines that the significance of the parvovirus test is uncertain given the preservation of the erythroid lineage, although parvovirus has been associated with thrombocytopenia in the post-transplant setting, and the abnormal megakaryocyte morphology could be related to chronic parvovirus or other infection.

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 and 3.9 at Month 30; at Month 30, the IS-specific VCN by qPCR was 0.1349 in whole blood, suggesting *MECOM* is one of several integration sites in a clone that contributes 13.5% of peripheral blood cells.

ISA identified two integration sites that appear to be a single clone that is larger than the *MECOM*-containing clone and expanding. The integration sites are in *LINC00982* and *SMG6*, with relative frequencies at Month 30 of 14.1% and 11.5%, respectively. The IS-specific VCN by qPCR for *LINC00982* at Month 30 was 0.31 c/dg in whole blood and 0.5933 in CD15+ cells. For *SMG6*, the IS-specific VCN at Month 30 was 0.3781 in whole blood and 0.5345 in CD15+ cells. Therefore, this subject has a clone that is contributing > 30% of peripheral blood cells and >50 % of CD15+ cells in peripheral blood.

This subject also has integrations into *MPL* that are at a comparatively low, but increasing frequency:

Table 40: *MPL* Integration Site Data for Subject (b) (6)

Time	Method	Relative Frequency
Month 6	S-EPTS/LM-PCR	0.113574
Month 12	S-EPTS/LM-PCR	0.445554
Month 18	S-EPTS/LM-PCR	1.616869
Month 24	S-EPTS/LM-PCR	2.151184

Abbrev: S-EPTS/LM-PCR, Shearing extension primer tag selection ligation-mediated polymerase chain reaction

Source: Reviewer's analysis from dataset (b) (6) _allISA_Nov2021

In summary, Subject (b) (6) has ongoing thrombocytopenia that is unlikely due to parvovirus, because it is not suggested by the type and 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 (b) (6)
Subject (b) (6) 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 (b) (6) 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 (b) (6) 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, 7 years after treatment with eli-cel, was normal (WBC $6.4 \times 10^9/L$, ANC $2.2 \times 10^9/L$, Hgb 14.9 g/dL, PLT $307 \times 10^9/L$).

Subject (b) (6) 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) had a combined integration site relative frequency of 97% 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 41: Integration Site-Specific Frequency and Vector Copy Number for *MECOM*, *ACER3*, and *RFX3* in Subject (b) (6)

Time Point	<i>MECOM</i> Primers Frequency / VCN (% / c/dg)	<i>ACER3</i> Primers Frequency / VCN (% / c/dg)	<i>RFX3</i> 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
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
Met criteria for predominant clone
Source: Reviewer's analysis

In addition to almost 100% clonal predominance and a rising vector copy number, Subject (b) (6) has increased expression of *EV11*, which is concerning for malignancy.

Subject (b) (6) 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. A second opinion on the anatomic pathology reveals moderate dysmegakaryopoiesis (20% of all megakaryocytes) at the last two assessments in July 2020 and July 2021.

Subject (b) (6)
Subject (b) (6) 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 also has an ongoing adverse event of thrombocytopenia and below normal neutrophil count.

Subject (b) (6) achieved neutrophil engraftment on Day 13 and platelet engraftment on Day 29. Platelet counts have not returned to normal levels and have recently declined. ANC at the most recent assessment is also below normal. The following table demonstrates the subject's laboratory results at his three most recent visits.

Table 42: Complete Blood Count Results for Subject (b) (6)

Date of CBC (Visit)	WBC (10 ⁹ /L)	Hemoglobin (g/dL)	Platelets (10 ⁹ /L)	ANC (10 ⁹ /L)	Lymphocytes (10 ⁹ /L)
(b) (6) (M12)	3.9	14.1	112	1.81	1.4
(b) (6) (M18)	4.9	14.5	118	2.74	1.48
(b) (6) (M24)	4.0	14.9	86	1.28	1.35

Abbrev: WBC, white blood count; ANC, absolute neutrophil count; M, month
Source: Reviewer's analysis

Integration site analysis results for the top two sites for Subject (b) (6) 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.

Table 43: Relative Frequencies of *MECOM* and *MPL* by S-EPTS/LM-PCR for Subject (b) (6)

Time Post-Eli-Cel	<i>MECOM</i> Relative Frequency (%)	<i>MPL</i> 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	0.15

Abbrev: S-EPTS/LM-PCR, shearing extension primer tag selection ligation-mediated polymerase chain reaction; c/dg, copies per diploid genome

Source: Reviewer's analysis derived from Listing 80.1.49 Integration Site Analysis Subject (b) (6) and FDA 72-Hour Reporting Form – ISA Tier 1 & Tier 2, CBC

Integration site-specific vector copy number by qPCR for *MPL*, provided for Month 18, was 0.0124 in whole blood, equating to a 1% clonal contribution.

(b) (6)

Subject (b) (6) was treated with eli-cel on (b) (6) at the age of 8. He had post-transplant adverse events of Grade 4 thrombocytopenia and neutropenia of at least 3 months' duration. All CBC values have improved over time, with WBC and lymphocytes at the most recent assessment (Month 12) below normal and platelet and neutrophil counts within the normal range. CBC trends are provided in the following table.

Table 44: Complete Blood Count Results for Subject (b) (6)

Date	WBC (10 ⁹ /L)	Hemoglobin (g/dL)	Platelets (10 ⁹ /L)	ANC (10 ⁹ /L)	Lymphocytes (10 ⁹ /L)
(b) (6) (M2)	1.6	9.9	30	0.8	0.5
(b) (6) (M3)	2.3	10.8	45	1.2	0.7
(b) (6) (M6)	2.2	12.1	95	1.0	0.9
(b) (6) (M12)	2.8	14.1	151	1.5	1.0

Abbrev: WBC, white blood cells; ANC, absolute neutrophil count; M, month

Source: Reviewer's analysis

Because of his cytopenias, the subject had bone marrow biopsies performed at 6 weeks and 3, ~6, and 12 months post-transplant. They demonstrated varying cellularity including hypo and hypercellularity, and at the most recent (Month 12) assessment, 50% cellularity and 20% abnormal megakaryocytes. At Month 12, a single, small, interstitial benign appearing lymphoid aggregate seen in the marrow sections, noted to be a nonspecific finding that can be associated with inflammation. Flow cytometry revealed hematogones present at all timepoints. FISH and karyotype were normal.

ISA data for this subject are limited to the 3 and 6 months after eli-cel administration, with select information provided about the Month 12 assessment. Integration site relative frequencies at Month 3 are all $\leq 0.2\%$, and there is a *MECOM* integration site with relative frequency of 0.1%. At Month 6, that *MECOM* integration site has increased in relative frequency to 2.3%. A second *MECOM* integration site at Month 6 has a relative frequency of 1.5% and the overall VCN is 1.03 c/dg.

This subject also had ISA performed on bone marrow at Month 12. An integration site in *PRPSAP1* was identified in bone marrow with a relative frequency of 11.4%. This site was not detected in peripheral blood with enough frequency to be reported in the Top 10 most common integration sites at the Month 3, 6, or 12 assessments.

(b) (6)

Subject (b) (6) was treated with eli-cel on (b) (6) at the age of 7. Between 1 week and almost 2 years post-transplant, he did not have any adverse events. However, beginning at approximately 2 years, he had seizure adverse events and later cognitive decline that were attributed to CALD.

Subject (b) (6) labs have been within the normal range since approximately 6 months post-transplant. He appears to have a clone with integration sites in *MECOM* and *KDM4B* that are increasing in relative frequency. ISA data are provided in the following table.

Table 45: ISA Results Including S-EPTS/LM-PCR and qPCR for *MECOM* and *KDM4B* for Subject (b) (6)

Time Post-Eli-Cel	Method			
Sep 2019 (Y4.5)	S-EPTS/LM-PCR	13.9	15.9	0.29
Sep 2019 (Y4.5)	qPCR	9.1	10.9	0.18
Jan 2020 (Y5)	S-EPTS/LM-PCR	19.5	20.4	0.27
Jan 2020 (Y5)	qPCR	12.6	14.6	0.20
Feb 2021 (Y6)	S-EPTS/LM-PCR	21.8	16.3	0.27
Feb 2021 (Y6)	qPCR	15.2	15.0	0.26
Apr 2022 (Y7)	S-EPTS/LM-PCR	21.7	17.6	--

Abbrev: VCN, vector copy number; c/dg, copies per diploid genome; Y, year; S-EPTS/LM-PCR, shearing extension primer tag selection ligation-mediated polymerase chain reaction; qPCR, quantitative polymerase chain reaction
Source: Reviewer's analysis

Corresponding to the qPCR results are clonal contributions that are increasing over time but still relatively small. The June 2019 results correspond to an estimated clonal contribution of <2% in Sept. 2019 that increased to 4% in Feb. 2021 and in April 2022 had declined to 2.8%.

(b) (6)
Subject (b) (6) was treated with eli-cel on (b) (6) at the age of 14. He had post-treatment adverse events of neutropenia, anemia, and thrombocytopenia, that had resolved within 7 weeks of treatment with eli-cel. CBC values between Year 2 and Year 7 visits have been normal. However, he appears to have an expanding clone with integration sites in *MECOM* and another proto-oncogene *MIR100HG*. ISA results are in the following table.

Table 46: ISA Results for *MECOM* and *MIR100HG* for Subject (b) (6)

Time Post-Eli-Cel	<i>MECOM</i> Relative Frequency (%)	<i>MIR100HG</i> Relative Frequency (%)	Overall VCN (c/dg)
Month 54	9.0	7.7	0.4
Month 66	9.9	8.5	0.4
Month 78	15.2	Not reported	Not reported

Abbrev: VCN, vector copy number; c/dg, copies per diploid genome
Source: Reviewer's analysis

(b) (6)
Subject (b) (6) is another subject with concerning integration site analysis results. He was treated with eli-cel on (b) (6) at the age of 12. He had serious adverse events of febrile neutropenia and HHV6 infection after treatment with eli-cel. His absolute neutrophil count was low through Month 6 and his lymphocyte count was low through Month 12, but all reported CBC values were within normal limits at the most recent assessment (Month 18). He appears to have a clone with integrations sites in *MECOM* and two other genes. ISA values follow.

Table 47: ISA Results for *MECOM*, *MIR99AHG*, and *EPB41L3* for Subject (b) (6)

Time Post-Eli-Cel	<i>MECOM</i> Relative Frequency (%)	<i>MIR99AHG</i> Relative Frequency (%)	<i>EPB41L3</i> Relative Frequency (%)	Overall VCN (c/dg)
Month 12	4.90	4.50	7.07	1.90
Month 18	15.26	14.36	20.23	Not reported

Abbrev: VCN, vector copy number; c/dg, copies per diploid genome

Source: Reviewer's analysis

(b) (6)
Subject (b) (6) was treated with eli-cel on (b) (6) at the age of 5. All CBC results were within the normal range by 6 months post-treatment. He appears to have a clone with integration sites in *SMG6*, *ACSF3*, and *PDE3A*. ISA values follow.

Table 48: ISA and qPCR Results for *SMG6*, *ACSF3*, and *PDE3A* for Subject (b) (6)

Time Post-Eli-Cel	<i>SMG6</i> RF (%)	<i>SMG6</i> IS-qPCR (c/dg)	<i>ACSF3</i> RF (%)	<i>ACSF3</i> IS-qPCR (c/dg)	<i>PDE3A</i> RF (%)	Overall VCN (c/dg)
Month 6	4.82	0.036	3.32	0.038	2.96	1.15
Month 12	15.25	0.189	14.42	0.198	10.96	1.91
Month 18	20.33	0.162	12.44	0.162	10.90	pending

Abbrev: RF, relative frequency, IS-qPCR, integration site-specific quantitative polymerase chain reaction; c/dg, copies per diploid genome, VCN, vector copy number

Source: Reviewer's analysis

Based on qPCR data, the clonal contribution of this clone increased from ~4% at Month 6 to ~19% at Month 12 and then decreased to ~16% at Month 18. The recent decrease in size is encouraging that this may not progress to malignancy, however, it is still a large clone warranting close follow-up.

Reviewer Comment: *It is likely that there will be more cases of hematologic malignancy that are diagnosed with additional follow-up time. Many subjects have findings that are highly concerning and may be evolving into malignancy. Furthermore, hematologic malignancy usually develops over a period of years, and subjects who were more recently treated with eli-cel may not have had enough time for a clone to expand and evolve to the point where it becomes clinically important.*

8.4.3 Study Dropouts/Discontinuations

Study discontinuations have not significantly impacted the review of safety. Only one of three subjects who were discontinued could have potentially informed safety of the product. The Applicant has no rationale for discontinuation or follow-up information on this subject's status since his final visit that occurred approximately 4.5 years after treatment. Two other subjects were discontinued from the study, however the reason for discontinuation was treatment failure and both underwent rescue allogeneic HSCT.

8.4.4 Common Adverse Events

Non-laboratory adverse events that occurred in $\geq 10\%$ of subjects with onset any time between the start of conditioning and 24 months after eli-cel administration are included in the following table, that also includes a column with the percentage of subjects with the adverse event classified as Grade 3 or 4 in severity.

Table 49: Non-laboratory Adverse Events that Occurred in $\geq 10\%$ of Subjects in ALD-102 and ALD-104 Safety Population

Adverse Reaction	Any Grade N (%)	Grade 3 or Higher N (%)
Blood and lymphatic	--	--
Febrile neutropenia ^a	49 (73%)	49 (73%)
Cardiac	--	--
Tachycardia ^b	10 (15%)	0
Eye	--	--
Vision blurred	7 (10%)	0
Gastrointestinal	--	--
Mucositis ^{c#}	62 (92%)	34 (51%)
Nausea	56 (84%)	17 (25%)
Vomiting	51 (76%)	12 (18%)
Abdominal pain ^d	30 (45%)	2 (3%)
Constipation	28 (42%)	0
Diarrhea	19 (28%)	1 (1%)
General disorders and administration site conditions	--	--
Pyrexia	24 (36%)	3 (4%)
Injury, poisoning and procedural complications	--	--
Transfusion reaction ^e	8 (12%)	2 (3%)
Metabolism and nutrition	--	--
Decreased appetite	43 (64%)	27 (40%)
Nervous system	--	--
Headache	19 (28%)	0
Anxiety ^{f#}	10 (15%)	0
Respiratory, thoracic, mediastinal	--	--
Epistaxis	13 (19%)	5 (7%)
Oropharyngeal pain ^{h#}	12 (18%)	3 (4%)
Cough	7 (10%)	0

Adverse Reaction	Any Grade N (%)	Grade 3 or Higher N (%)
Skin and subcutaneous	--	--
Alopecia	48 (72%)	1 (1%)
Rash ⁱ	14 (21%)	0
Pruritus ^j #	13 (19%)	0
Skin hyperpigmentation	12 (18%)	0
Vascular	--	--
Hypertension	8 (12%)	1 (1%)

Includes adverse events associated with conditioning.

^a Febrile neutropenia includes febrile bone marrow aplasia and febrile neutropenia.

^b Tachycardia includes sinus tachycardia and tachycardia.

^c Mucositis includes anal inflammation, colitis, gastrointestinal inflammation, mucosal inflammation, proctitis, and stomatitis.

^d Abdominal pain includes abdominal discomfort, abdominal pain, and abdominal pain upper.

^e Transfusion reaction includes allergic transfusion reaction and anaphylactic transfusion reaction.

^f Anxiety includes akathisia, agitation, anxiety, and irritability.

^g Seizure includes epilepsy and seizure.

^h Oropharyngeal pain includes mouth ulceration, oral pain, and oropharyngeal pain.

ⁱ Pruritus includes anal pruritus, pruritus, and pruritus allergic.

^j Rash includes rash, rash erythematous, rash maculo-papular, and urticaria.

Encompasses more than one system organ class.

Source: Reviewer's analysis

Some of the adverse events with onset prior to eli-cel administration are included in the table above. Across the period of time from enrollment through apheresis and conditioning until eli-cel administration, adverse events that occurred in $\geq 20\%$ of subjects were nausea (79%), vomiting (72%), decreased appetite (42%), catheter site pain (39%), constipation (30%), headache (24%), abdominal pain (21%), and rash (21%).

Adverse events with onset after treatment with eli-cel can be summarized by timeframe as follows:

- In the first 60 days after treatment in $\geq 10\%$ of patients: mucositis (88%), febrile neutropenia (73%), abdominal pain (33%), vomiting (31%), decreased appetite (31%), pyrexia (27%), nausea (27%), constipation (21%), diarrhea (21%), headache (16%), tachycardia (13%), transfusion reaction (12%)
- Between 60 days and 1 year after treatment in $\geq 5\%$ of patients: pyrexia fever (9%) and vomiting (6%)
- At least 1 year after treatment in $\geq 5\%$ of patients: seizure 15%) and myelodysplastic syndrome (6%)

Reviewer Comment: *There was as high number of adverse events, which is expected for a study of this duration and given a disease and therapy associated with high morbidity. The common adverse events were consistent with the adverse events expected with full myeloablation for HSCT.*

8.4.5 Clinical Test Results

Complete Blood Count

Prolonged cytopenias, including pancytopenia, were observed across eli-cel clinical studies. Myeloablative conditioning is known to cause cytopenias, however the severity of prolonged cytopenias suggests eli-cel may be a contributing factor.

The incidence of Grade 3 or higher cytopenias at different timepoints is presented in the following table:

Table 50: Incidence of Grade 3-4 Cytopenias at Day 60 and Day 100 After Eli-cel Administration in ALD-102 and ALD-104 Safety Population

--	Any Grade 3-4 Cytopenia	Grade 3-4 Thrombocytopenia	Grade 3-4 Neutropenia	Grade 3-4 Lymphopenia	Grade 3-4 Anemia
Day 60 (n = 64)	30 (47%)	9 (14%)	14 (22%)	17 (27%)	1 (2%)
Day 100 (n = 54)	8 (15%)	4 (7%)	5 (9%)	3 (6%)	0

Source: Reviewer's analysis

Reviewer Comment: *The CBC abnormalities were common and severe, and are important because of the bleeding and infection risks associated with cytopenias. They are also important because they can provide evidence that a patient has a malignancy. The persistence of severe CBC abnormalities at Day 60 and Day 100 in some subjects is an important risk of eli-cel.*

Chemistries

Electrolyte abnormalities after eli-cel administration were also common, although in most cases they were mild.

Hypocalcemia occurred in all subjects and persisted beyond one year in 41 subjects. Twenty-one subjects had Grade 2 or higher hypocalcemia and two subjects had Grade 3 or higher hypocalcemia. The Grade 2 or higher instances of hypocalcemia all occurred within the first 3 weeks of eli-cel administration, with the exception of four instances of Grade 2 hypocalcemia in four unique subjects that occurred at approximately 2 months, 3 months, 1 year, and 1.5 years after eli-cel administration.

Fifty-six subjects had 407 recorded instances of hypokalemia after eli-cel administration. Grade 3 and 4 abnormalities accounted for 47 of the results and occurred in 24 subjects. The Grade 3 and 4 abnormalities all occurred during the first month after eli-cel administration. The other instances of hypokalemia were Grade 1 abnormalities. Approximately 300 of the Grade 1 abnormalities occurred during the first month, another 45 occurred during the second and third months, and the remaining 12 occurred between 3 months and 2 years after eli-cel administration.

Eight subjects had nine instances of hyperkalemia after eli-cel administration. Ten were Grade 1 abnormalities and two were Grade 2 abnormalities. The Grade 1 instances occurred during Months 1, 3, 6, 9, 15, 18, and 24, and the Grade 2 instances occurred during Months 1 and 24.

Seven subjects had 17 instances of hypomagnesemia after eli-cel administration. All were Grade 1 abnormalities and occurred within the first 3 weeks.

Sixteen subjects had 30 instances of hypermagnesemia after eli-cel administration. Four were classified as Grade 3 or 4 and the remainder were Grade 1. The four Grade 3 or 4 instances occurred during the first month after treatment with eli-cel. Twenty-one Grade 1 instances occurred during the first month, two during the second month, three during the remainder of the first year, and two during the second year after eli-cel administration.

Thirty subjects had 87 instances of hyponatremia after eli-cel administration. All were Grade 1 abnormalities. Seventy-four occurred within the first month, 8 in the second month, 3 in the third month, and one each at one and two years after eli-cel administration.

Forty-seven subjects had 176 instances of hypophosphatemia after eli-cel administration. Grade 2 and 3 abnormalities accounted for 14 of the results and occurred in 10 subjects. The Grade 2 and 3 abnormalities all occurred during the first month after eli-cel administration. The other instances were all Grade 1 abnormalities. One hundred forty-one of the Grade 1 abnormalities occurred during the first month, another 12 occurred during the second and third months, and the remaining nine occurred between 3 months and 2.5 years after eli-cel administration.

Fourteen subjects had 24 instances of elevated creatinine. Nineteen were Grade 1, four were Grade 2, and one was Grade 3. Of the Grade 1 creatinine elevations, four occurred in the first month, one in the second month, one in the third month, and one in the fourth month after treatment with eli-cel. Five occurred thereafter in the first year and four occurred without explanation in a single subject (Subject (b) (6)) during the second year after eli-cel. The Grade 2 elevations occurred in two subjects at approximately 6 months after eli-cel and in two other subjects at approximately 1 years after eli-cel. The sole Grade 3 elevation occurred approximately one month after eli-cel.

Fourteen subjects had 23 instances of hypoglycemia after eli-cel administration. All were Grade 1 abnormalities. Thirteen occurred within the first 3 weeks and the other ten occurred between approximately one month and two years after eli-cel.

Reviewer Comment: *Chemistry abnormalities were common, with most occurring during the first month after eli-cel administration and a small number being severe. These perturbations are likely attributable to HSCT including conditioning. Close monitoring and electrolyte correction is standard in this setting, and patients are unlikely to suffer sequelae of the observed abnormalities.*

Liver Function Tests

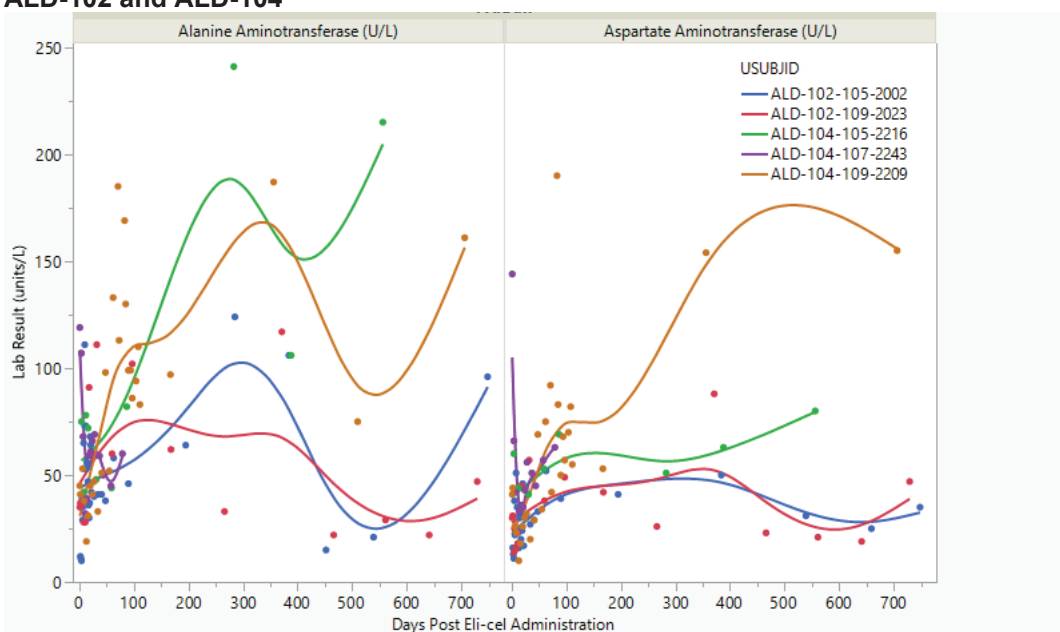
Forty-eight subjects had 204 instances of elevated alanine aminotransferase (ALT). Grade 2 and 3 abnormalities accounted for 10 of the results and occurred in seven subjects. The other instances were all Grade 1 abnormalities. One hundred two of the Grade 1 abnormalities occurred during the first month after eli-cel administration, 32 during the second month, 19 during the third month, and 14 during the fourth month. Visit frequency declined thereafter, with 18 of the remaining 25 Grade 1 abnormalities recorded during the remainder of the first year, six in the second year, and one early in the third year after eli-cel administration. The nine Grade 2 abnormalities occurred in six

subjects over the first year after eli-cel administration; two occurred in the first week, one in the second month, two in the third month, one in the fourth month, one in the fifth month, one in the ninth month, and one in the twelfth month. The sole Grade 3 abnormality occurred in Subject (b) (6) at 1.5 years and was attributed by the investigator to conditioning.

Aspartate aminotransferase (AST) elevations were less common than ALT elevations, but AST and ALT appear to trend together. Thirty-eight subjects had 103 instances of elevated AST. One hundred of those instances were grade 1 abnormalities. Twenty-seven Grade 1 abnormalities occurred during the first month after eli-cel administration, 23 during the second month, 18 during the third month, and nine during the fourth month. Fifteen occurred thereafter during the first year, 10 during the second year, and one early in the third year. Two subjects had two Grade 2 abnormalities, which occurred during the third and fourth months after eli-cel administration. One subject had a single Grade 3 abnormality that occurred during the first week.

ALT and AST are plotted over time for five individual subjects where the values are trending up at last measurement.

Figure 18: AST and ALT Results Plotted Over Time for Five Subjects Showing Increases in ALD-102 and ALD-104



Source: Reviewer's analysis

Two of these subjects had adverse events recorded that overlapped with depicted LFT elevations:

- (b) (6) – Grade 2 hepatic cytolysis from Day -1 to Day 7
- (b) (6) – Grade 1 ALT increased from Day -1 and ongoing, and Grade 1 AST increased with the same start and end date, Day 556

Three subjects had three instances of Grade 1 alkaline phosphatase elevations. They occurred in the sixth, eighth, and twelfth months after eli-cel administration. The subjects were (b) (6).

Six subjects had seven instances of bilirubin elevations. One was a Grade 2 elevation and the other seven were Grade 1 elevations. Four of the Grade 1 elevations occurred within the first two weeks after eli-cel administration, and the other two occurred during the second and third years after eli-cel administration in subjects (b) (6) respectively. The sole Grade 2 elevation occurred in Subject (b) (6) early in the second year after eli-cel administration, and many months before his diagnosis of MDS. Two subjects each had one instance of direct bilirubin elevations. Both occurred during the first two weeks after eli-cel administration and normalized with subsequent assessments.

Reviewer Comment: *Mild LFT abnormalities that returned to normal were relatively common in the first few months after transplant. However, there were a few instances of rising LFTs, particularly AST, that were delayed relative to conditioning and without good explanation. It is possible that as additional data accumulate in these ongoing studies, a risk of lasting liver injury may become clear.*

Vital Signs

With the exception of fever/elevated temperature, few subjects had adverse events related to vital signs abnormalities, they occurred in most subjects.

Heart rate values above the 90th percentile for age were reported in 48 of 55 subjects for whom vital sign data were provided. Similarly, few subjects had adverse events of bradycardia, however, heart rate values below the 90th percentile for age were reported in 21 of 55 of subjects.

Respiratory rate abnormalities were also common. Thirty-nine subjects had respiratory rate values above the 90th percentile for age, and 20 had respiratory rate values recorded at or above the 99th percentile for age. Twenty subjects had respiratory rate values recorded below the 10th percentile for age, and twelve had values at or below the first percentile for age.

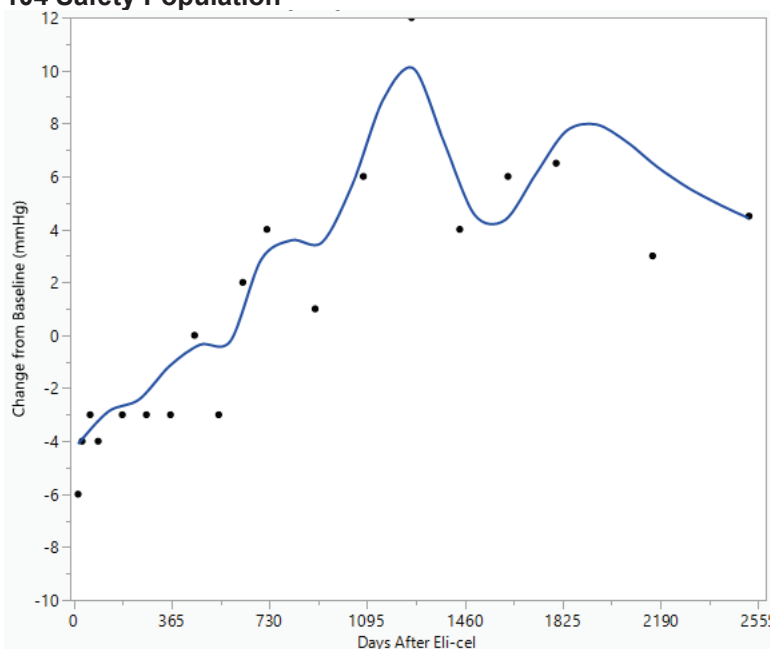
Temperature abnormalities were rarely reported in vital sign data. One subject had a temperature of 39.1°C at one month post-eli-cel. A second subject had a temperature of 38.1°C at 2.5 years post-eli-cel. No other instances of temperature $\geq 38^{\circ}\text{C}$ were reported in vital sign data. Regarding low temperature, two subjects had instances of temperature $< 35^{\circ}\text{C}$. One occurred during drug product infusion and the other at one month post-eli-cel.

Blood pressure abnormalities were more challenging to assess because the normal range for a child depends on his height and age, however height data at the time of vital signs measurement was not consistently provided. Using the formula of $[70 + (\text{age in years} \times 2)]$ to determine the fifth percentile for systolic blood pressure, nine subjects were identified as having a blood pressure recorded that was below the fifth percentile for age. To identify subjects with elevated systolic blood pressures, measurements were compared to values corresponding to the 95th percentile of blood pressure for boys at the 95th percentile of height by age. This is a conservative approach that likely

underestimates the number of subjects with high blood pressures. Using this approach, 22 subjects were identified as having a blood pressure recorded that was above the 95th percentile for age.

Systolic blood pressure was also evaluated by change from baseline over time. The following figure demonstrates that systolic blood pressure was generally lower than baseline for more than one year after eli-cel administration. The blood pressure generally increased over time, as would be expected in childhood, although the rate of increase in the first and second year after eli-cel administration may have been more rapid than would be ideal or expected.

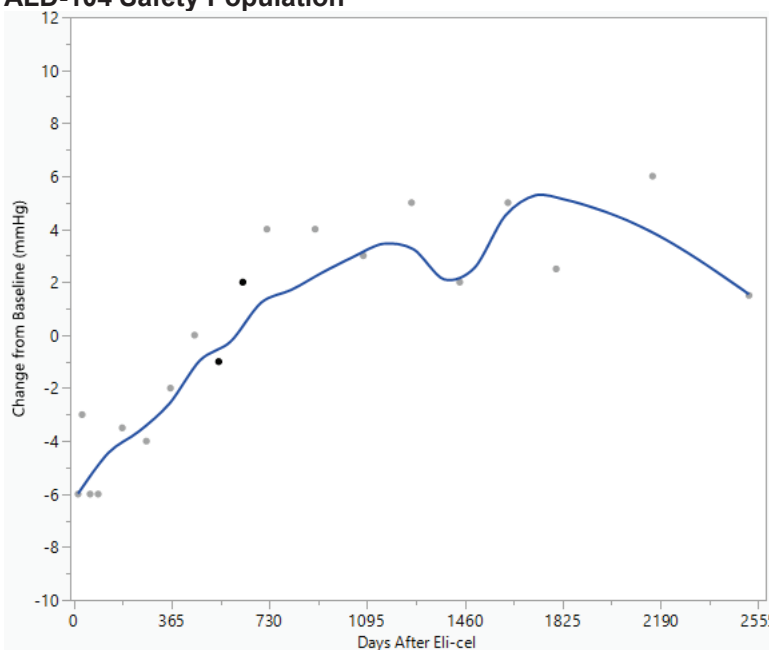
Figure 19: Median Change from Baseline for Systolic Blood Pressure in ALD-102 and ALD-104 Safety Population



Source: Reviewer's analysis

Diastolic blood pressures were also often higher than would be considered normal. Thirteen subjects had a diastolic blood pressure > 80 mmHg, and five subjects had a diastolic blood pressure ≥ 90 mmHg. The change in diastolic blood pressure from baseline over time is depicted in the following figure, which demonstrates that diastolic blood pressure was below normal for most of the first two years after eli-cel administration, and continued to increase overall for the first five years, although to a lesser extent than the systolic blood pressure increase.

Figure 20: Median Change from Baseline for Diastolic Blood Pressure in ALD-102 and ALD-104 Safety Population



Source: Reviewer's analysis

Reviewer Comment: *The vital sign data do not reveal a safety concern. Early abnormalities in vital signs were largely consistent with the physiologic effects of myeloablation and later changes in blood pressure were not dramatic and are attributable in no small part to normal childhood growing.*

8.4.6 Systemic Adverse Events

Refer to adverse events discussion in sections 6.1.12.2 Overview of Adverse Events and 6.1.12.5 Adverse Events of Special Interest (AESI) for ALD-102, 6.2.12.2 Overview of Adverse Events and 6.2.12.5 Adverse Events of Special Interest (AESI) for ALD-104, and 8.4.4 Common Adverse Events for the overall safety population.

8.4.7 Local Reactogenicity

The BLA submission does not include evidence of local reactogenicity of the product.

8.4.8 Adverse Events of Special Interest

Delayed or failed neutrophil or platelet engraftment and myelodysplastic syndrome were adverse events of special interest.

Neutrophil engraftment failure was defined by the Applicant as failure to achieve 3 consecutive absolute neutrophil counts (ANC) $\geq 0.5 \times 10^9$ cells/L obtained on different days by Day 43. While no subject met these criteria, G-CSF was administered in the majority of subjects in Study ALD-102, and was mandated for subjects in ALD-104, promoting earlier, and arguably false, achievement of neutrophil engraftment. In the ISS Population, 7 of 67 subjects (10%) required G-CSF beyond Day 42. All were subjects enrolled in ALD-104, and included the following: (b) (6)

Two of 54 required G-CSF beyond Day 100 (4%).

In addition to the seven subjects with prolonged G-CSF use, and demonstrating the effect of G-CSF on ANC values, was subject (b) (6), who per the Applicant met neutrophil engraftment criteria on Day 16. He received G-CSF from Day 10 to 18 and Day 21 to 23, after which he had several ANC values below 0.5×10^9 cells/L (on Day 26 and Day 53). This subject's ANC values at each post-nadir timepoint are shown in the following table, with values obtained during G-CSF shown in bold and values outside of normal ranges in italics.

Table 51: ANC Results By Day for Subject (b) (6)

--	Day 14	Day 16	Day 17	Day 19	Day 22	Day 26	Day 31	Day 53	Day 60	Day 66	Day 95
ANC ($\times 10^9$ /L)	0.39 ‡	0.71 ‡	0.57 ‡	1.83	5.87	0.36†	0.65†	0.38†	1.3†	0.77†	1.02†

Abbrev: ANC, absolute neutrophil count

*Concomitant G-CSF administration

‡Value below the lower limit of normal

Source: Reviewer's analysis

This subject's ANC appears to have decreased more than 10-fold between Day 22, when G-CSF was being administered, and Day 26, 3 days after G-CSF discontinuation. For the remainder of the study period for which the Applicant has provided data, ANC has not returned to normal.

In addition, it is possible that this subject, if he had more laboratory data available between Day 31 and Day 60, may have met criteria for secondary neutrophil engraftment failure, which is defined as achievement of neutrophil engraftment followed by sustained decline in ANC to $< 0.5 \times 10^9$ cells/L for 3 consecutive measurements on different days after 42 days post-infusion of eli-cel, without alternate etiology.

The Applicant defined platelet engraftment as 3 consecutive platelet values $\geq 20 \times 10^9$ /L on different days and no platelet transfusions administered for 7 days immediately preceding and during the evaluation period. Platelet engraftment was not achieved by Day 43 after eli-cel administration in 13 of 63 subjects (21%). These subjects were the following: (b) (6)

(b) (6) Patients treated with eli-cel achieved platelet engraftment at median (min, max) Day 29 (14, 108) in clinical studies. These platelet engraftment summary statistics include two subjects who were being treated with eltrombopag at the time they met criteria for platelet engraftment. For these subjects, one had eltrombopag through Day 312 (b) (6) and the other through Day 440 (b) (6).

Infections were also adverse events of special interest. 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 six infections that were serious adverse events. They were BK cystitis, pseudomonal and *Stenotrophomonas maltophilia* bacteremia, EBV viremia and 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 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 ((b) (6)) – Days 127 to 133
- Mycobacterium central venous catheter infection ((b) (6)) – Days 167 to 194
- Pseudomonal bacteremia ((b) (6)) – Days 240 to 251
- Epstein-Barr virus infection reactivation ((b) (6)) – Day 547 and ongoing

There were also several central venous catheter infections that were not classified as serious or severe, in addition to the serious and catheter infections that are presented above.

Focusing on type of pathogen among severe (Grade 3 or 4) infections, bacterial infections predominated, occurring in 12% of subjects, vs. 3% for viral infections, and 9% for infections with unspecified pathogen type. Nine infections in 8 subjects were bacterial. These were five bacteremias and four vascular device infections. Two were specified as viral infections. These were BK cystitis and adenovirus in the nasopharynx. The remaining seven infections were largely treated with intravenous antibiotics, and suggesting bacterial infection, although the infection was not otherwise denoted as bacterial. These were two line infections, and one case each of thumb soft tissue infection, pneumonia, infectious enterocolitis, otitis media, and sinusitis.

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.

8.5 Additional Safety Evaluations

8.5.1 Dose Dependency for Adverse Events

The dependency of dose determination for adverse events is challenging in that there are many variables of dose that to account for. The simplest was to consider dose is the number cells administered to a patient. Number of cells administered in HSCT is understood to affect time to engraftment, and could theoretically impact engraftment times and count recovery after eli-cel administration. However, engraftment and recovery of cell counts were less robust than expected for the number of cells

administered. Therefore, it does not appear that cell number impacted that safety outcome. No other adverse outcomes could be linked to number of cells administered.

Vector copy number per cell and percentage of transduced cells in the drug product are variables reflecting the vector component of dose. An increased amount of vector per cell, while helpful for efficacy, could adversely affect safety because of the greater chance of aberrant integration(s) within a cell that could lead to malignancy or other dysfunction within the cell line. Vector copy number data for the development program overall and for the subjects who developed malignancy is presented in the following table, demonstrating that drug product vector copy number and percent of LVV positive cells were at or above the median values but were not the highest observed values.

Table 52: Drug Product Assays - Median for ALD-102 and ALD-104 Safety Population Compared with Subjects Diagnosed with MDS

--	All Subjects Median (range)		(b) (6)	
Drug Product Vector Copy Number (c/dg)	1.3 (0.5 - 3.1)	1.8	1.3	1.6
LVV+ Cells in Drug Product (%)	51 (b) (4)	70	Not done	62
Vector Copy Number per Transduced Cell (c/dg)	2.6 (b) (4)	2.6	Not done	2.6

Abbrev: c/dg, copies per diploid genome; LVV, lentiviral vector

Source: Reviewer's analysis

Reviewer Comment: *These data show that not all subjects had the same drug product assays performed, but that when performed, subjects who developed MDS had median or higher values for DP VCN, % LVV+ cells, VCN per transduced cell. There is not sufficient data to conclude a relationship between these assays and risk of malignancy, nor does there appear to be a strong correlation, because subjects with higher results for each of the assays have not been diagnosed with malignancy.*

8.5.2 Time Dependency for Adverse Events

Most adverse events were temporary cytopenias and related occurrences (e.g., fever) and occurred in the weeks following conditioning. Adverse events that occurred outside that timeframe and were potentially related to eli-cel are cytopenias that did not resolve during the first few weeks after eli-cel administration, myelodysplastic syndrome, and infections. Each of these topics is discussed elsewhere.

8.5.3 Product-Demographic Interactions

The ability of the data to determine product-demographic interactions is limited due to the small size of the trials, absence of a control arm, and predominance of white males in the enrolled population (30 subjects are white non-Hispanic males; 17 are Hispanic males including 5 white Hispanic males; 7 are males reporting neither race nor ethnicity, and 13 are males in other race/ethnicity groups). Focusing on the development of hematologic malignancy, the three subjects come from different groups, including one white non-Hispanic, one white Hispanic, and one black non-Hispanic.

8.5.4 Product-Disease Interactions

One product-disease interaction has been identified with eli-cel - underlying predisposition to the development of malignancy. While germline mutations linked to hematologic malignancy are not more common in children with CALD than in the general population, the addition of the lentiviral vector to cells containing the germline mutation may greatly increase the risk of malignant transformation of the cells. However, it is not necessarily the case that patients and families would opt against treatment with eli-cel if they had knowledge of a germline predisposition to hematologic malignancy.

8.5.5 Product-Product Interactions

Safety concerns resulting from pharmacokinetic-based product interactions are not expected to occur with eli-cel. There is, however, a possibility of product-product interactions related to the conditioning agents. Because the conditioning agents have varied between the studies are generally standardized within institutions, describing those potential interactions is beyond the scope of this review.

8.5.6 Human Carcinogenicity

Carcinogenicity is apparent in the development of three cases of myelodysplastic syndrome, discussed in Section 8.4.2 Nonfatal Serious Adverse Events.

8.5.7 Overdose, Drug Abuse Potential, Withdrawal, and Rebound

Not applicable.

8.5.8 Immunogenicity (Safety)

Although there is one subject who appears to have had an immune-mediated failure of persistence of eli-cel descendent cells (Subject (b) (6)), immunogenicity does not appear to be a safety risk with eli-cel.

8.5.9 Person-to-Person Transmission, Shedding

Person-to-person transmission and viral shedding do not appear to be risks with eli-cel. Eli-cel includes a replication-incompetent vector, and replication-competent lentivirus not been identified in routine assessment of subjects who have been treated with eli-cel.

8.6 Safety Conclusions

Myelodysplastic syndrome (MDS), acute myeloid leukemia (AML) and insertional oncogenesis are the major safety concerns with eli-cel. MDS has been diagnosed in three subjects and more cases of hematologic malignancy are likely to be diagnosed over time.

Treatment with eli-cel involved not only administration of the eli-cel product, but the administration of chemotherapy. 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 following HSCT.

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. 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 late opportunistic infections in eli-cel-treated subjects.

9. ADDITIONAL CLINICAL ISSUES

9.1 Special Populations

9.1.1 Human Reproduction and Pregnancy Data

There are no available data with eli-cel administration in pregnant women. However, it is not expected or intended to be used in the female population.

9.1.2 Use During Lactation

There are no available data with eli-cel administration during lactation, including no information regarding presence of the product in human milk, effect on the breastfed infant, or effects on milk production. However, it is not expected or intended to be used in the female population.

9.1.3 Pediatric Use and PREA Considerations

Safety and efficacy of eli-cel in children younger than 4 years of age have not been established.

9.1.4 Immunocompromised Patients

There are no available data for eli-cel administration in immunocompromised patients.

9.1.5 Geriatric Use

There are no available data for eli-cel administration in a geriatric population nor is it expected or intended to be used in this population.

10. CONCLUSIONS

In summary, the clinical reviewers conclude that there is substantial evidence of effectiveness and reasonable assurance of safety based on a single adequate and well-controlled investigation with confirmatory evidence including pooled data from two clinical studies, compared to external controls. The reviewers recommend accelerated approval of eli-cel for treatment of boys with early, active CALD based on an intermediate clinical endpoint of slowed progression to MFD or death within 24 months of first symptom onset for eli-cel as compared to the natural history of disease.

There is supportive evidence that eli-cel administration appears to have delayed the onset of symptomatic disease in a few subjects at high risk of symptomatic disease progression as compared to the natural history of disease. There is additional supportive evidence from brain MRI findings: improvement in Loes score for 2 subjects and resolution of gadolinium enhancement for most subjects at Month 24 following treatment with eli-cel. The clinical review team believes that efficacy can be extrapolated to the entire population of early, active CALD with asymptomatic or mildly symptomatic disease. Additionally, eli-cel appears to offer an early survival advantage over allo-HSCT

from an HLA-mismatched donor due to avoidance of HSCT-related toxicities in that population. Confirmatory evidence of efficacy is provided by in vitro and in vivo pharmacologic studies demonstrating transgene engraftment and production of functional ALDP with resultant metabolism of very long chain fatty acids, as well as a pharmacodynamic correlation between median values of %ALDP+ CD14+ cells at Month- 6 and the Month-24 event-free survival rate.

Insertional oncogenesis is the major safety concern with eli-cel. Three subjects have been diagnosed with hematologic malignancy that has been attributed to eli-cel, and more cases are likely to be diagnosed with time. A secondary concern is delayed hematopoietic reconstitution, manifest by opportunistic infections, delayed engraftment, and prolonged cytopenias. These are known risks of HSCT, although their timing and extent after treatment with eli-cel suggest that the HSC manipulation that is inherent in the production of eli-cel may have some deleterious effects on HSC function. Aside from the insertional oncogenesis and delayed hematopoietic reconstitution, eli-cel seems to have a safety profile that is similar to the myeloablative and lymphodepletion conditioning agents that are administered prior to eli-cel.

While the risk of hematologic malignancy is of significant concern, the overall benefit-risk profile of eli-cel is favorable due to the substantial benefit of slowed, clinical disease progression in a disease that ultimately leads to disability and premature death if left untreated.

11. RISK-BENEFIT CONSIDERATIONS AND RECOMMENDATIONS

11.1 Risk-Benefit Considerations

Table 53: Risk-Benefit Considerations

Decision Factor	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	<ul style="list-style-type: none"> Childhood cerebral adrenoleukodystrophy (CALD) is a rare neurodegenerative disorder that affects young boys. Disease is heterogeneous and timing of clinical progression is multifactorial and dependent on presence or absence of neurologic or neurocognitive dysfunction, severity of brain magnetic resonance imaging (MRI) lesions and presence or absence of contrast enhancement on MRI. Despite uncertainty of timing, disease does almost certainly progress to neurologic dysfunction, disability and death, typically by the second decade of life. Treatment with the standard of care (allogeneic hematopoietic stem cell transplant) carries significant risks of morbidity and mortality from transplant complications, namely graft failure and graft versus host disease (GVHD). 	<ul style="list-style-type: none"> Childhood cerebral adrenoleukodystrophy is a progressive, life-threatening disease. CALD is a serious condition, based on the chronic morbidity from neurologic dysfunction and disability, and mortality from eventual progression to death. CALD is a serious condition, based on the debilitating impact on physical and psychosocial well-being for patients, and the psychosocial burden on caregivers and other family of the affected children.
Unmet Medical Need	<ul style="list-style-type: none"> There is no FDA-approved treatment for CALD. The standard of care, despite not being FDA-approved, is allogeneic hematopoietic stem cell transplant (allo-HSCT), but suitable donors are not available for at least half of CALD patients, particularly those of mixed race and other racial or ethnic minorities. Risks of graft rejection and GVHD are increased with HLA-mismatched donors, with significant associated morbidity and mortality. Once symptomatic, disease progresses rapidly without treatment, and delays in treatment while awaiting or searching for a suitable allo-HSCT donor are one of the main barriers to treatment according to parents and caregivers. 	<ul style="list-style-type: none"> In pediatric patients with CALD, there is an unmet medical need for effective therapy to slow, delay or prevent the loss of neurologic function and progression to disability and death.
Clinical Benefit	<ul style="list-style-type: none"> Eli-cel demonstrated benefit in slowed progression from neurologic dysfunction (i.e., development of deficits on the Neurologic Function Score, or NFS) to major functional disabilities (MFDs) or death at Month 24 following symptom onset as compared to the natural history of disease. In eli-cel subjects who maintained MFD-free survival, they also maintained a stable NFS for a period of at least 24 months at some time during follow-up after first NFS ≥ 1. There is confirmatory evidence of delayed symptom onset in some subjects who had baseline features that predict rapid progression who had either remained asymptomatic or developed delayed onset of symptoms. There is confirmatory evidence from MRI findings at Month 24 following treatment: improvement in Loes score in 2 subjects and resolution of gadolinium enhancement in the majority of subjects. There is supportive evidence from pharmacodynamic factors, including CD14+ %ALDP+ cells at Month 6 following treatment. There are concerns about worse outcomes in subjects with isolated pyramidal tract disease who, per natural history of disease and the medical literature, are not expected to become symptomatic until adulthood, even in the absence of treatment. 	<ul style="list-style-type: none"> There is substantial evidence for benefit on an intermediate clinical endpoint with slowed disease progression to MFD or death at Month 24 following first NFS ≥ 1 in symptomatic CALD subjects. There is confirmatory evidence of delayed onset of neurologic dysfunction in subjects who were asymptomatic at baseline and have high risk of disease progression based on other baseline features, and from MRI findings that would be unexpected in the natural history of disease without treatment Pharmacodynamic data is supportive. Careful consideration should be given to the relative benefit-risk for patients with isolated pyramidal tract disease, who had worse outcomes following treatment with eli-cel than the natural history of disease.

Risk	<ul style="list-style-type: none"> • The most substantial risk of eli-cel is the development of hematologic malignancy. The incidence among subjects treated with eli-cel is currently 4%. However, the incidence is expected to increase over time because subjects have been followed for what may be, in many cases, an insufficient amount of time for a clone to evolve into a malignancy. Furthermore, numerous subjects have evidence of clonality that is concerning for evolving malignancy. • Another uncertainty is the prognosis for lentivirus-mediated hematologic malignancy, and whether they will have a pattern of slow evolution and responsiveness to treatment or aggression and refractoriness to treatment. • Other important safety signals are delayed engraftment, cytopenias, and opportunistic infection. These are potential sequelae of conditioning, and it is uncertain whether the eli-cel causes these adverse events to be more common, severe, or prolonged than expected as compared to their occurrence solely from conditioning. 	<ul style="list-style-type: none"> • The risk of eli-cel is serious because hematologic malignancy is life-threatening and occurred in multiple patients with an incidence of 4% likely to increase.
Risk Management	<ul style="list-style-type: none"> • The most substantial risk of eli-cel is the development of hematologic malignancy. The incidence among subjects treated with eli-cel is currently 4%. However the incidence is expected to increase over time because subjects have been followed for what may be, in many cases, an insufficient amount for a clone to evolve into a malignancy. Furthermore, numerous subjects have evidence of clonality that is concerning for evolving malignancy. Another uncertainty is the prognosis for lentivirus-mediated hematologic malignancy, and whether they will have a pattern of slow evolution and responsiveness to treatment or aggression and refractoriness to treatment. • Other important safety signals are delayed engraftment, cytopenias, and opportunistic infection. These are potential sequelae of conditioning, and it is uncertain whether the eli-cel causes these adverse events are more common, severe, or prolonged than expected as compared to their occurrence solely due to conditioning. 	<ul style="list-style-type: none"> • Due to the risk of hematologic malignancy, a REMS is recommended for eli-cel if it is approved. • A REMS would ensure patients and their caregivers are aware of the risk of hematologic malignancy with eli-cel and are able to make informed treatment decisions. • A REMS would ensure that patients have adequate monitoring for development of malignancy and are diagnosed early, which should enable to better outcomes for some patients.

11.2 Risk-Benefit Summary and Assessment

Data submitted to the BLA provide substantial evidence of efficacy for eli-cel to slow the progression of neurologic dysfunction (as represented by MFD-free survival at Month 24 following first NFS ≥ 1) as compared to the natural history of disease in boys 4-17 years of age with early, active cerebral adrenoleukodystrophy (CALD).

The primary risk of eli-cel is life-threatening hematologic malignancy. In light of this serious risk, the available data support a favorable benefit-risk assessment only in those boys who do not have an HLA-matched sibling donor, where the risks of the only alternative therapy, allogeneic HSCT, are greatest.

11.3 Discussion of Regulatory Options

While considering the imperfect efficacy data, the clinical reviewers considered the regulatory options of regular approval, accelerated approval, and complete response. Part of that consideration was whether the two-year time period of study would be sufficient to establish durability of effect.

Another key question was the population for which the product should be approved. Issues included the age of boys treated with eli-cel ranging from 4 to 14 years vs the Applicant's proposed population of the entire pediatric age range. Another question was whether the product should be indicated for boys without an HLA-matched sibling donor, or for the narrower population of boys without any HLA-matched donor option. Lastly is the question of whether there are subtypes of CALD in whom eli-cel should not be indicated, particularly given the risks of hematologic malignancy and uncertainty regarding durability of effectiveness. Little is known about the expected clinical course in CALD patients with very early disease (asymptomatic and minimal cerebral lesions on brain MRI) in the absence of treatment, and subjects in the clinical studies with isolated pyramidal tract disease (which is not expected to become symptomatic until adulthood) had worse clinical outcomes than the natural history of disease, largely related to development of hematologic malignancy.

A final major regulatory question was how to address the risks of insertional oncogenesis. The need for a REMS with ETASU elements or a PMR was the primary consideration. A REMS was not supported by the Office given the uncertainties. A large safety PMR was determined to be most appropriate to characterize the risk factors for hematologic malignancy and understand the outcomes of malignancy associated with the product. The CBER Safety Review Board concurred with issuing this PMR. Additionally, different forms of patient focused labeling were considered to ensure boys with CALD and their families were fully informed of the serious risks and knew symptoms to report and monitoring that was recommended. The development of a Medication Guide outside of a REMS was determined to be most appropriate to mitigate risks.

11.4 Recommendations on Regulatory Actions

The clinical review team recommends accelerated approval with the following indication: to slow the progression of neurologic dysfunction in boys 4-17 years of age with early, active cerebral adrenoleukodystrophy (CALD) who do not have an available and willing human leukocyte antigen (HLA)-matched hematopoietic stem cell (HSC) donor. The uncertainties regarding the magnitude and severity of hematologic malignancy following treatment and regarding durability of effectiveness lead us to believe the benefit-risk is favorable for the population without HLA-matched donors, as the risk of early mortality following allo-HSCT is high in this population. As more safety and efficacy data are available over time from the three required Clinical PMRs, the benefit-risk assessment for the entire population of patients with early, active CALD (regardless of donor) may change.

11.5 Labeling Review and Recommendations

From an efficacy perspective, the most important information to include in the labeling are the results at Month 24 for MFD-free survival from time of symptom onset comparing eli-cel to the natural history population in the analysis that formed the basis for approval. It is also important to address the uncertainty regarding durability of effect and relative long-term efficacy of the product, especially as it pertains to the population of early, active CALD patients with isolated pyramidal tract disease who would not be expected to become symptomatic until adulthood in the absence of treatment. Additionally, it is important to convey the survival advantage eli-cel appears to have over allo-HSCT from an HLA-mismatched donor in order to inform the treatment decision-making process for physicians and caregivers.

From a safety perspective, the most critical information to include in the labeling is the risk of hematologic malignancy. The recommended approach to ensuring clinicians and patients are informed of the risk is to include (1) a boxed warning, (2) recommendations for monitoring for malignancy described in Warnings and Precautions, and (3) detailed information about the risk in the Patient Counseling section and the Medication Guide.

Another important consideration in the labeling is the strategy for displaying adverse reactions. Because the studies did not have a comparator arm, and because the conceivable control data were from external control studies that were conducted differently from the eli-cel trials, comparator data should not be presented.

In addition, the adverse events included in labeling should not be limited to those the Applicant has concluded are related to eli-cel. Many of the adverse events, and particularly those occurring in the weeks after myeloablative conditioning and eli-cel administration, may be entirely due to the conditioning. However, because administration of eli-cel requires conditioning, these types of adverse events are unavoidable and should be included in the assessment of the risk of eli-cel administration. Furthermore, the adverse events that are entirely caused by conditioning cannot be differentiated from adverse events where eli-cel contributed to their occurrence, and instances of later than expected engraftment, cytopenias, and infectious complications suggest a contribution of eli-cel to many of the adverse events that might otherwise be attributed to conditioning.

11.6 Recommendations on Postmarketing Actions

Risk Evaluation and Mitigation Strategy Recommendation

Because of the serious safety concern of myelodysplastic syndrome, the clinical reviewers recommend approval with a Risk Evaluation and Mitigation Strategy (REMS). A REMS could ensure that patients are adequately informed the risk of hematologic malignancy via required distribution of a Medication Guide. It could also mandate monitoring for hematologic malignancy after treatment with eli-cel, which is expected to mitigate the risk through earlier diagnosis.

In the clinical trials, subjects were monitored for evidence of clonality as an indicator of the potential for malignancy, through integration site analysis (ISA). Although ISA can be performed on peripheral blood samples, it is a specialized test performed by limited laboratories and requiring uncommon expertise for interpretation. We are therefore concerned that if ISA performance with some oversight from the Applicant is not required, ISA will not be performed as a matter of routine monitoring.

Patients who are not monitored with ISA would rely on abnormalities of complete blood counts (CBC) as signals of potential malignancy. However, reliance on CBC would likely equate to a later diagnosis of malignancy for some patients. A later diagnosis may portend a worse prognosis because of (1) less opportunity to identify a good HLA match for treatment with allogeneic HSCT, and (2) more opportunity for progression to a hematologic malignancy that is more refractory to treatment.

Two of the three cases of MDS occurred in subjects who were first identified as being at risk based on ISA results. Both had evidence of a sizeable clone with integration into the *MECOM* proto-oncogene in their initial ISA, performed six months after treatment with eli-cel. Both of these subjects were closely followed until they were diagnosed with MDS with single lineage dysplasia at 14 and 26 months after treatment with eli-cel. Several months later, each of these subjects was treated with allogeneic HSCT with a haploidentical paternal donor.

If these two subjects were being monitored only with CBC, it is likely that one of the two would have had a timely diagnosis of malignancy and that the diagnosis for the other subject would have been delayed. The subject who was diagnosed at 26 months after treatment with eli-cel had had significant cytopenias from the time of eli-cel administration through the time of his MDS diagnosis. The cytopenias probably would have led to a bone marrow evaluation, even in the absence of ISA data.

The second subject had thrombocytopenia and anemia at three months after treatment with eli-cel, but a normal CBC (besides low lymphocytes) at 6 months. His platelet count and hemoglobin were below normal at 12 months, but probably not low enough to cause a clinical work-up in the absence of ISA data demonstrating evidence of clonality. Therefore, this subject's diagnosis of MDS would probably have been made at a later point in time when the disease had progressed to cause a more significant cytopenia and confer higher morbidity and mortality risk.

The third subject was diagnosed with MDS 7.5 years after treatment with eli-cel, when he presented with symptomatic thrombocytopenia and anemia. His last ISA had been at Year 5 (using a different method for ISA) and did not identify any clones of particular concern. During the 2.5 year interval when he did not have labs or ISA performed, an

aggressive clone emerged, leading to his diagnosis of MDS with excess blasts-2. MDS with excess blast-2 has a poor prognosis, comparable to acute myeloid leukemia. The malignancy was treated the next month with chemotherapy and total body irradiation, followed by HSCT with umbilical cord blood.

With a REMS that ensures subjects are having ISA performed regularly after treatment with eli-cel, the inevitable cases of hematologic malignancy will be detected early in the malignancy or even before a patient has met criteria for malignancy. Because allogeneic HSCT is the only curative treatment for MDS, early detection will promote better outcomes by providing more time to search for a suitable HSC donor. Early detection will also promote better outcomes in the instances where the malignancy would, with time, progress to a more aggressive form, as may have been the case in the subject who had not been evaluated by ISA during a 2.5 year period before he presented with MDS with excess blasts-2.

Post-Marketing Requirements (PMRs)

Accelerated approval regulations require that the Applicant conduct adequate and well-controlled clinical trials to verify and describe clinical benefit attributable to the eli-cel product. The Applicant agreed to conduct the following studies:

1. Follow all subjects who received elivaldogene autotemcel in Studies ALD-102 and ALD-104 to assess event-free survival (i.e., alive without Major Functional Disability (MFD) or need for hematopoietic stem cell transplant (HSCT)) for a minimum of ten years following administration of elivaldogene autotemcel.

Final Protocol Submission: January 31, 2023

Interim Clinical Study Report Submission: July 31, 2027

Final Study Report Submission: July 31, 2032

2. Investigate event-free survival for at least five years post-treatment in 24 boys with more advanced early active, cerebral adrenoleukodystrophy (CALD) [(based on baseline Loes scores and Neurologic Function Score (NFS)) who will be newly treated with elivaldogene autotemcel.

Final Protocol Submission: January 31, 2023

Study fully enrolled by: June 30, 2033

Study Completion date: June 30, 2038

Final Study Report Submission: December 31, 2038

Because of the risk of hematologic malignancy with eli-cel, the Applicant agreed to conduct the following post-marketing safety study as a PMR:

3. A postmarketing, prospective, multi-center, observational study to assess the long-term safety of elivaldogene autotemcel and the risk of secondary malignancies occurring after treatment with elivaldogene autotemcel. The study will include at least 120 adrenoleukodystrophy patients and the enrolled

patients will be followed for 15 years after product administration. The study design will include monitoring at pre-specified intervals for clonal expansion with adequate testing strategies.

Final protocol submission: December 31, 2022

Study completion date: April 30, 2047

Final study report submission: April 30, 2048

APPENDIX 1: NEUROLOGIC FUNCTION SCORE (NFS)

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.^{4,8} 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 54: 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

Symptom / Neurologic Exam Finding	Definition	Score
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
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)