Cellular, Tissue, and Gene Therapies Advisory Committee Meeting

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ADVISORY COMMITTEE

ELIVALDOGENE AUTOTEMCEL BRIEFING DOCUMENT

BLA 125755

CELLULAR, TISSUE, AND GENE THERAPIES ADVISORY COMMITTEE

Meeting Dates: 09 June and 10 June 2022

bluebird bio, Inc.

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Somerville, MA 02145

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LIST OF ABBREVIATIONS AND ACRONYMS

ABCD1	ATP-binding cassette, subfamily D member 1
AE	adverse event
ALD	adrenoleukodystrophy
ALDP	ALD protein
allo-HSCT	allogeneic hematopoietic stem cell transplantation
ANC	absolute neutrophil count
AUC	area under the curve
BLA	Biologics License Application
BLQ	below the limit of quantitation
C	conditioning
CALD	cerebral adrenoleukodystrophy
cDNA	complementary deoxyribonucleic acid
CI	confidence interval
CMV	cytomegalovirus
COI	chain of identity
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DLC	date of last contact
DNA	deoxyribonucleic acid
DP	drug product
DP VCN	drug product vector copy number
eCRF	electronic case report form
eli-cel	elivaldogene autotemcel, formerly known as Lenti-D Drug Product
EOS	end-of-study
FDA	Food and Drug Administration
FSIQ	full-scale IQ
G-CSF	granulocyte colony-stimulating factor
GdE	gadolinium enhancement (on cerebral MRI)
GVHD	graft-versus-host disease
HLA	human leukocyte antigen
HR	hazard ratio
HSC	hematopoietic stem cell (in this briefing document, the CD34+ cell-enriched population which contains hematopoietic stem/progenitor cells, are referred to as HSCs)
HSCT	hematopoietic stem cell transplantation
ICF	informed consent form
IQ	intelligence quotient
IS	insertion site
ISA	integration site analysis
ITT	intent-to-treat
IV	intravenous
LVV	lentiviral vector
LysoPCs	lysophosphatidylcholines
M	mobilization
max	maximum
MDS	myelodysplastic syndrome
MECOM	*MDS1* and *EVI1 c*omplex locus
MFD	major functional disability

min	minimum
MNDU	MNDU3=promoter derived from the U3 element of the MND LTR (Myeloproliferative sarcoma virus, Negative region deleted dl587rev primer binding site)
MRI	magnetic resonance imaging
MSD	matched sibling donor
MUD	matched unrelated donor
NE	neutrophil engraftment
NEP	Neutrophil Engraftment Population
NFS	neurologic function score
NMSD	not a matched sibling donor
OS	overall survival
PB	peripheral blood
PBL	peripheral blood leukocytes
PBMC	peripheral blood mononuclear cell
PB VCN	peripheral blood cell vector copy number
PCS	potential clinical significance
PD	pharmacodynamic(s)
PE	platelet engraftment
PedsQL	Pediatric Quality of Life™
PrvIQ	performance/reasoning/visual IQ
PT	preferred term
QTC	Qualified Treatment Center
RCL	replication-competent lentivirus
Rel Day	Relative Study Day (Rel Day 1 is defined as the day of drug product infusion)
RelFreq	Relative IS-Frequency (as measured by Integration Site Analysis)
SAE	serious adverse event
SD	standard deviation
SE	standard error
SOC	System Organ Class
TEAE	treatment-emergent adverse event
TESAE	treatment-emergent serious adverse event
TP	Transplant Population
TPES	Strictly ALD-102 Eligible Transplant Population
TRM	transplant-related mortality
US	United States
VCN	vector copy number
VLCFA	very long-chain fatty acid

1. EXECUTIVE OVERVIEW

1.1. Introduction

Adrenoleukodystrophy (ALD) is a rare, X-linked, metabolic disease in which dysfunction or lack of the ALD protein (ALDP) is caused by mutations in the ATP-binding cassette, subfamily D member 1 (*ABCD1*) gene (Moser 1997). Defective function of ALDP leads to the accumulation of very long-chain fatty acids (VLCFAs), which occurs in plasma and all tissue types, but most prominently in the adrenal cortex and white matter of the brain and spinal cord. Cerebral ALD (CALD) is the most severe form of ALD, often emerging in early childhood and characterized by rapidly progressive cerebral demyelination leading to irreversible loss of neurologic function and death (Moser et al. 2007). Without intervention, progression of CALD is rapid, causing severe decline in neurologic functions including loss of cognition, vision, hearing, and motor function. Nearly half of patients with CALD die within 5 years of symptom onset (Mahmood et al. 2005).

There are no approved treatments for CALD in the US. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) can stabilize neurologic function, with the best outcomes observed in patients treated at the early stages of cerebral involvement (Raymond et al. 2019), but it is associated with serious immunologic complications, including transplant-related mortality (TRM), graft rejection, and graft-versus-host disease (GVHD).

Immune complications of allo-HSCT are most common in recipients of grafts from donors other than matched siblings. For these patients, autologous transplant with elivaldogene autotemcel (eli-cel) provides a meaningful treatment option.

eli-cel is an innovative one-time, autologous gene addition therapy product for patients with CALD who do not have an available and willing human leukocyte antigen (HLA)-matched sibling donor (MSD). eli-cel is administered with the goal of stabilizing neurologic function without the risks of TRM, graft rejection, GVHD, or the need for post-transplant immunosuppression.

eli-cel consists of a CD34+ cell-enriched population that contains hematopoietic stem cells (HSCs) transduced with a self-inactivating (SIN), replication-incompetent lentiviral vector (LVV) encoding *ABCD1* complementary deoxyribonucleic acid (cDNA) for ALDP, called the Lenti-D LVV. In this briefing document, the CD34+ cell-enriched population which contains hematopoietic stem/progenitor cells, are referred to as HSCs.

The patient's HSCs are first mobilized into the bloodstream for collection by apheresis and then enriched for CD34+ cells, which are then transduced with the Lenti-D LVV to create the eli-cel drug product (DP). Following successful DP manufacture, the patient undergoes full myeloablation and lymphodepletion to permit engraftment of the transduced cells. eli-cel is then administered to the patient via a single intravenous infusion. The minimum recommended dose of eli-cel is 5.0×10^6 CD34+ cells/kg patient weight, with no maximum dose. Following eli-cel infusion, the transduced CD34+ HSCs engraft in the bone marrow and differentiate into various cell types, including monocytes, that are believed to migrate to the brain where they further differentiate into long-lived macrophages and cerebral microglia that produce functional ALDP and replace deficient microglial cells (Varvel et al. 2012; Sevenich 2018; Weinhofer et al. 2018).

The functional ALDP may enable local degradation of VLCFAs. The putative net effect is disease stabilization by the prevention of further inflammation and demyelination.
The expression of functional ALDP after eli-cel treatment and successful engraftment is expected to be lifelong.

The efficacy and safety of eli-cel have been demonstrated in a comprehensive clinical development program, which includes comparator studies of untreated and allo-HSCT treated patients.

The Investigational New Drug (IND) application for eli-cel was submitted to the FDA in March 2013. eli-cel was granted Breakthrough Therapy and Rare Pediatric Disease Designations. Additionally, eli-cel was also granted Orphan Drug Designation for the treatment of adrenoleukodystrophy. The Biologics License Application (BLA) was submitted to the FDA in October 2021 and granted Priority Review in December 2021.

The proposed indication for eli-cel is for the treatment of patients < 18 years of age with early cerebral adrenoleukodystrophy who do not have an available and willing HLA-matched sibling HSC donor.

1.2. Disease Overview

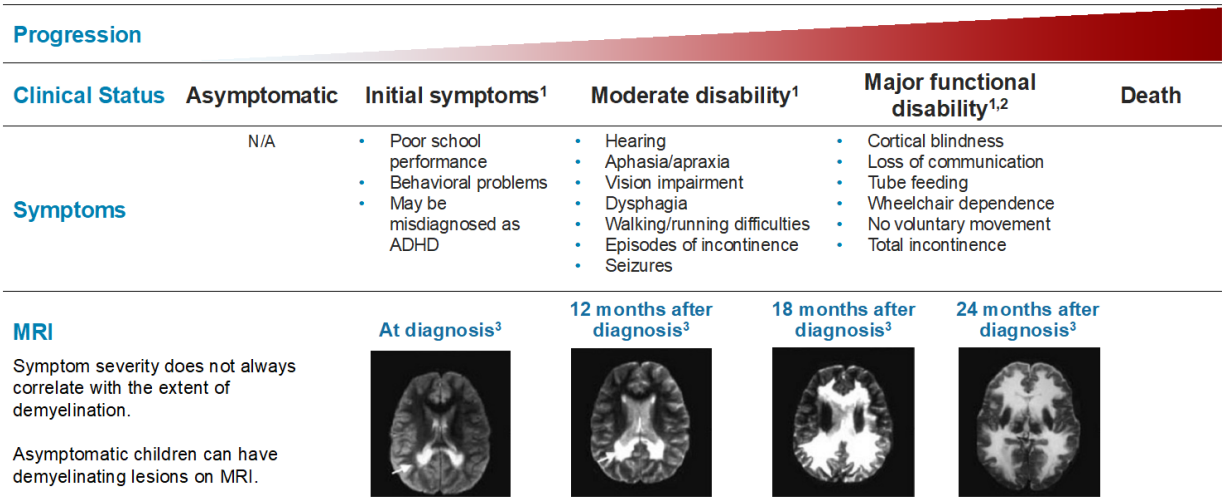
There are 4 forms of ALD; in order of increasing severity these are: asymptomatic, adrenal insufficiency, adrenomyeloneuropathy, and CALD. The worldwide incidence of ALD among males is approximately 1 in 20,000 to 1 in 30,000 (Wiesinger et al. 2015). Although all boys with ALD are born asymptomatic, most (85%) will develop adrenal insufficiency in early childhood (Laureti et al. 1996; Mahmood et al. 2005). Approximately 40% of boys with ALD will develop CALD, typically between 3 and 10 years of age (Moser et al. 2007).

Untreated CALD is a devastating condition for affected boys and their families, with the potential for a rapid decline in neurologic function with profound symptom burden. Initial symptoms of CALD often include learning disabilities and behavioral problems, which may be misdiagnosed as attention-deficit/hyperactivity disorder. The early stages of CALD may be detected by brain magnetic resonance imaging (MRI) in boys with known ALD, even when they do not have noticeable symptoms (Engelen et al. 2012).

As the disease progresses, boys develop vision and hearing problems, seizures, poor coordination, and difficulty swallowing ((Engelen et al. 2012); Figure 1). They eventually develop major functional disabilities (MFDs), which are of particular clinical importance because they compromise a patient's ability to function independently. The 6 MFDs are loss of communication, cortical blindness, tube feeding, total incontinence, wheelchair dependence, and complete loss of voluntary movement (Eichler et al. 2017; Raymond et al. 2019; Miller).

In the absence of treatment, progression to a vegetative state may occur within 2 to 3 years of diagnosis (Moser et al. 2007). Nearly half of patients with CALD die within 5 years of symptom onset (Mahmood et al. 2007; Raymond et al. 2019).

Figure 1. CALD Disease Progression



Abbrev.: ADHD, attention-deficit hyperactivity disorder; CALD, cerebral adrenoleukodystrophy; MRI, magnetic resonance imaging; N/A, not applicable.
1. (Engelen et al. 2012) 2. (Raymond et al. 2019) 3. CALD MRI images from (Cartier et al. 2009).

The diagnosis of CALD is established upon detection of white matter lesions on brain MRI. As it reflects cerebral inflammation, the presence of contrast agent/gadolinium enhancement (GdE+) is a strong predictor of poor prognosis; untreated patients who are GdE+ are at risk of rapid disease progression and death (Melhem et al. 2000; Raymond et al. 2019).

Axonal demyelination caused by accumulated VLCFAs combined with microglial apoptosis and disruptions in the blood-brain barrier likely lead to the development of inflammatory lesions in CALD (Eichler et al. 2008; Engelen et al. 2012; Musolino et al. 2015). However, the precise mechanism by which CALD develops is not known. Assessments commonly used to monitor patients with CALD include the MRI-based Loes score and GdE status, and the clinical Neurologic Function Score (NFS; see Section 5.2.1).

Early CALD

Early CALD is defined as an NFS ≤ 1 and a Loes score ≥ 0.5 and ≤ 9. MFDs, derived from the NFS, provide a convenient and unambiguous clinical assessment framework and are gaining broader use among neurologists managing patients with CALD ((Kuhl et al. 2018); Section 5.2.1).

1.3. Current Treatment Option and Unmet Medical Need

The goal of CALD treatment is to stabilize neurologic function prior to the development of irreversible impairment, and to prevent death. In the US, there is currently no treatment approved for CALD, although allo-HSCT has been shown to have a beneficial effect in terms of disease stabilization and long-term survival. Demyelinating lesions typically continue to progress for 12 to 18 months post–allo-HSCT and clinical deterioration may be observed during this time (Aubourg et al. 1990; Baumann et al. 2003; Saute et al. 2016; Raymond et al. 2019). Outcomes

with allo-HSCT are most favorable when performed at the early stages of cerebral involvement and with a graft from a matched sibling donor (MSD) (Miller et al. 2011; Raymond et al. 2019).

Allo-HSCT carries significant risks, such as TRM, graft failure or rejection, GVHD, and serious opportunistic infections. These risks are reduced if allo-HSCT is performed using cells from an MSD. The ready availability of an MSD generally means the child with CALD is treated without delay, and thus without significant concern for disease progression while awaiting transplant. Unfortunately, only a minority of CALD patients have access to an MSD (Miller et al. 2011; Raymond et al. 2019); 11% of CALD patients treated with allo-HSCT in the US received a graft from an MSD (CIBMTR 2011 to 2017). Among those receiving a graft from a donor other than a matched sibling (NMSD), outcomes differ according to whether the graft is from a matched unrelated donor (MUD) or a mismatched donor. Beyond donor-recipient histocompatibility, other factors such as stem cell source, donor age and gender, donor-recipient cytomegalovirus (CMV) and Epstein-Barr virus (EBV) status, and ABO compatibility may play a role in transplant outcome.

Death due to TRM may be caused by GVHD, infection, or organ toxicity (Gooley et al. 2010). TRM rates of 8 to 12% at 100 days have been reported for patients receiving allo-HSCT for nonmalignant indications (Miller et al. 2011; Mitchell et al. 2013; Raymond et al. 2019). At 2 years following allo-HSCT, Raymond (2019) reported overall survival rates of 92% for patients with an MSD and 72% for those without an MSD. A statistically significant difference in outcomes 6 years after allo-HSCT in patients with leukodystrophies receiving HLA well-matched grafts compared with those receiving mismatched grafts (71% vs 54%; p=0.009) was also reported by van den Broek (2018).

Graft failure rates among CALD patients who received allo-HSCT range from 5 to 22% (Peters et al. 2004; Miller et al. 2011; Raymond et al. 2019; Boelens et al. 2020; Chiesa et al. 2021), and are consistent with rates reported after allo-HSCT in pediatric patients with nonmalignant diseases (Mitchell et al. 2013). The occurrence of graft failure generally necessitates repeat allo-HSCT.

GVHD can be acute (usually within the first 100 days post-transplant) or chronic (usually occurring after 100 days; EBMT 2019). Reported rates of Grades II to IV acute and chronic GVHD following allo-HSCT range from 18 to 39% and 7 to 32%, respectively (Beam et al. 2007; Miller et al. 2011; Mitchell et al. 2013; Tsai et al. 2016; Reinfjell et al. 2017; Eissa et al. 2017; Kuhl et al. 2018; Raymond et al. 2019; Boelens et al. 2020; Chiesa et al. 2021).

To prevent or treat GVHD following allo-HSCT, patients are immunosuppressed for months to years after transplant, depending on the degree of incompatibility between the host and donor cells (Lee and Deeg 2008). This prolonged immunosuppression is associated with a risk for opportunistic infections and additional serious side effects, including hypertension (Reddy et al. 2010; Inagaki et al. 2016; Garcia-Cadenas et al. 2017; Sevilla et al.). Serious infections following allo-HSCT have been reported in 11 to 29% of patients (Miller et al. 2011; Mitchell et al. 2013; Raymond et al. 2019).

There is a clear and immediate need for effective treatment options for CALD patients without an MSD that avoid the immunologic complications of allo-HSCT.

1.4. Clinical Development Program

The eli-cel clinical development program consists of 1 interventional completed pivotal Phase 2/3 study (ALD-102), 1 interventional ongoing Phase 3 study (ALD-104) and 1 ongoing long-term follow up study (LTF-304) for patients who completed ALD-102 or ALD-104. In addition, two completed clinical studies (ALD-101 and ALD-103) provided background and comparative information about the natural history of untreated CALD and outcomes of allo-HSCT.

The eli-cel treatment studies are single-arm, open-label trials. The severity of CALD, the rarity of the disease, the lack of authorized treatment options, the inability of transplant to be blinded, and the risk of disease progression during the time required to conduct a donor match for an allo-HSCT comparator arm precluded the conduct of a randomized controlled trial in the target patient population. Therefore, as agreed with the Food and Drug Administration (FDA) in 2018, an external control approach (ALD-101 and ALD-103) was used to provide context for the eli-cel data.

In the eli-cel clinical studies, drug product infusion was preceded by mobilization/apheresis with granulocyte colony-stimulating factor (G-CSF) with or without plerixafor, myeloablative conditioning using busulfan, and lymphodepletion using either cyclophosphamide (ALD-102) or fludarabine (ALD-104).

In **ALD-101**, data were collected retrospectively on patients who had follow-up after diagnosis (Untreated Cohort) or transplant (Allo-HSCT Cohort) for at least 2 years or until death. Long-term follow-up data were collected when available. These data enabled the identification of the 6 MFDs as reliably identifiable and clinically meaningful indicators of neurologic disease progression, and helped define the efficacy endpoints for ALD-102, ALD-103, and ALD-104. Supported by the literature (Melhem et al. 2000; Moser et al. 2000; Loes et al. 2003; Miller et al. 2016), these data helped inform the choice of the primary endpoint and benchmark to define the primary success criterion for ALD-102.

ALD-103 was a multinational, multisite, prospective and retrospective study designed to evaluate outcomes of allo-HSCT in patients with CALD <18 years of age, and was conducted concurrently with ALD-102. This study did not involve the use of an investigational drug. The ALD-103 design was generally consistent with that of ALD-102, in terms of efficacy and safety assessments and their timing. Data derived from this study were used as a concurrent external comparator for outcomes after treatment with eli-cel and were collected up to 48 months after last allo-HSCT.

ALD-102, the pivotal Phase 2/3 clinical trial for eli-cel, was a multinational, multicenter, open-label, single-arm study to evaluate the safety and efficacy of eli-cel in 32 patients <18 years of age with early active CALD who did not have an available or willing MSD. Patients were followed for 24 months after eli-cel infusion. The primary efficacy endpoint was MFD-free survival after 24 months. The study is complete.

ALD-104 is a Phase 3 clinical trial with a design that closely parallels ALD-102, with similar enrollment criteria, efficacy assessments, and follow-up duration (24 months). A total of 35 patients received eli-cel in this study. Enrollment and treatment are complete and follow-up is ongoing.

LTF-304 is a long-term follow-up study, enrolling patients who completed the eli-cel parent studies (ALD-102 and ALD-104). This study monitors for long-term safety and continued efficacy through a total of 15 years after eli-cel infusion.

1.5. Efficacy

Results of the completed pivotal study, ALD-102, demonstrate that eli-cel treatment stabilizes neurologic and cognitive function in patients with early CALD (defined as a Loes score of 0.5-9 and NFS of 0 or 1) at high risk of progression at baseline, as indicated by contrast agent enhancement on brain MRI. eli-cel met the efficacy success criterion: 29/32 patients (90.6%, exact 95% CI: 75.0 to 98.0%) achieved Month 24 MFD-free survival, with the lower bound of the 2-sided 95% exact confidence interval well above the pre-specified benchmark of 50%. These findings indicate a compelling and statistically significant effect over the natural course of untreated CALD. Pooled results of ALD-102/104 support the above findings, and follow-up data suggest that eli-cel treatment provides a durable clinical benefit.

As pre-specified, the Transplant Population (TP; those who received eli-cel infusion) of ALD-102 (TP-102) is the population used to assess the primary efficacy endpoint. The pooled Transplant Population of ALD-102/104 (TP-102/104) is used for all other efficacy endpoints. In both ALD-102 and ALD-104, the TP is identical with the Intent-to-Treat (ITT) Population (see Section 5.1 for more explanation of study populations). Data from LTF-304 were integrated with data from each parent study for a given patient.

Primary endpoint analysis

The primary efficacy endpoint was the proportion of patients who achieved MFD-free survival at 24 months (i.e., Month 24 MFD-free survival). To qualify for the primary endpoint, patients must have been alive, MFD-free, had not received rescue cell administration or allo-HSCT, and had not withdrawn from the study or been lost to follow-up by 24 months post-infusion. The 32 patients treated with eli-cel in ALD-102 (hereafter referred to as TP-102) had baseline characteristics typical of early active CALD, indicating a poor prognosis and the potential for rapid disease progression without effective treatment.

The primary efficacy analysis was intended to establish that eli-cel was efficacious compared to a clinically meaningful benchmark based on findings from untreated patients in ALD-101 and published literature. Specifically, the Month 24 MFD-free survival in untreated GdE+ patients from ALD-101 was 21% (exact 95% confidence interval (CI) of 6.1% to 45.6%) therefore, the benchmark value of 50% is above the upper bound of the 95% CI for Month 24 MFD-free survival. A lower bound of the 2-sided 95% exact CI of Month 24 MFD-free survival above 50% was pre-defined as the success criterion for the primary efficacy endpoint in ALD-102.

The clinical benchmark and success criterion for the primary endpoint were agreed upon in discussions with FDA in 2018 and supported by the January 2021 Draft Guidance “Human Gene Therapy for Neurodegenerative Diseases” (FDA 2021), taking into consideration the ethical limitations of a randomized, placebo-controlled study in this pediatric rare-disease population.

Twenty-nine of 32 patients achieved Month 24 MFD-free survival (90.6%; exact 95% CI: 75.0, 98.0). Study ALD-102 (using the entire transplant population, TP-102), therefore met the success criterion for the primary efficacy endpoint of Month 24 MFD-free survival, with the lower bound

of the exact 95% CI surpassing the pre-specified benchmark of 50%. eli-cel therefore shows a compelling and statistically significant effect on Month 24 MFD-free survival when compared to a pre-specified benchmark that reflects the course of untreated CALD. Of the three patients who did not meet the primary endpoint, one experienced early and rapid disease progression with multiple MFDs and death and two patients were withdrawn at the investigator's discretion to receive allo-HSCT due to radiologic progression.

Event-free survival

After completion of Study ALD-102, myelodysplastic syndrome (MDS) was diagnosed in three eli-cel treated patients (refer to Section 6.6.5.1). These three patients include one patient in long-term follow up (LTF-304) after treatment in ALD-102, one patient in LTF-304 after treatment in ALD-104, and one patient in ALD-104 at the time of MDS diagnosis. To appropriately reflect these events in Kaplan-Meier analyses in the combined completed pivotal study ALD-102, the ongoing ALD-104, and the ongoing LTF-304 studies, the MFD-free survival analysis was broadened to an event-free survival analysis which includes all components of MFD-free survival and considers MDS as an additional event. The Kaplan-Meier analysis of event-free survival in the entire treated population estimated that 91.9% (95% CI: 79.8%, 96.9%) and 86.8% (95% CI: 72.7%, 93.9%) of TP-102/104 patients would be event-free at 24 and 36 months after eli-cel infusion, respectively.

Durable benefit of eli-cel treatment

eli-cel maintained an estimated event-free survival rate of 86.8% (95% CI: 72.7%, 93.9%) through 7 years of follow-up. Thus, despite enrollment of patients who would have a prognosis of rapid disease progression, MFDs have not developed in the majority of patients after eli-cel administration. Due to the occurrence of MDS in one patient at approximately 7.5 years after eli-cel and the small number of patients with follow-up visit after 7 years (n=3), event-free survival is considered not reliably characterized beyond 7 years.

In pooled analyses of the completed pivotal study, ALD-102, with the ongoing ALD-104 and LTF-304 studies, eli-cel maintained an estimated overall survival rate of 97.7% (95% CI: 84.6%, 99.7%) through 7 years of follow-up.

The persistence of eli-cel efficacy is also supported by biomarker data showing that the majority of TP-102/104 patients maintained detectable vector copies in peripheral blood leukocytes (PBL) and CD14+ cells at their latest assessment, demonstrating the long-term persistence of transduced repopulating HSCs. TP-102/104 patients also expressed ALDP in these cells at most visits, including the majority of patients at their latest assessment and several patients who had completed their Month 60 Visit. These results support the long-term expression of transgenic ALDP in the progeny of transduced HSCs.

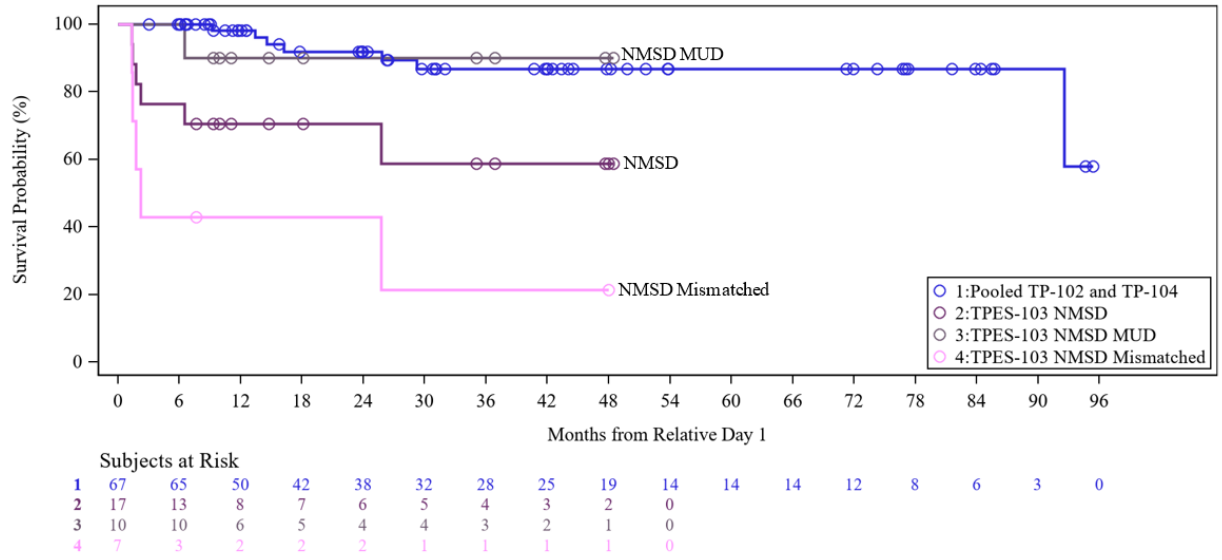
Comparison to external control study with allo-HSCT

Eligibility criteria for inclusion in the contemporaneous external control study, ALD-103, were broader than those for the eli-cel studies, ALD-102 and ALD-104. For interstudy efficacy comparisons, a subpopulation of patients in ALD-103 with baseline characteristics that closely matched the entry criteria for the eli-cel studies was used. These characteristics include a Loes score of 0.5-9 and NFS of 0 or 1, in combination reflecting early CALD, as well as the presence of white matter lesions with contrast agent enhancement on brain MRI, reflecting a population at

high risk of progression. This comparator subpopulation is identified by the prefix TPES (Strictly ALD-102 Eligible Transplant Population; i.e., TPES-103). Patients in TPES-103 (N=27) received allo-HSCT from either an MSD (N=10) or NMSD (N=17). Within the NMSD subgroup, patients received allo-HSCT from either a matched unrelated donor (MUD, N=10) or mismatched donor (Mismatched, N=7).

In pooled analyses of the completed pivotal study ALD-102 with the ongoing ALD-104 and LTF-304 studies, eli-cel maintained an estimated event-free survival rate of 86.8% (95% CI: 72.7%, 93.9%) through 7 years of follow-up. eli-cel (TP-102, TP-104 and pooled TP-102/104) compared favorably with allo-HSCT without MSD (TPES-103-NMSD; estimated event-free survival rate at Month 24 of 70.6% (95% CI: 43.1%, 86.6%)). Further analyses of the TPES-103-NMSD group by histocompatibility subgroups showed that the estimated Month 24 event-free survival rate for TP102/104 was similar to the rate of 90.0% (95% CI: 47.3%, 98.5%) for TPES-103-NMSD-MUD and higher than the rate of 42.9% (95% CI: 9.8%, 73.4%) for TPES103-NMSD-Mismatched (Figure A).

Figure A: Event-Free Survival Over Time for eli-cel and Allo-HSCT without Matched Sibling Donor, including Subgroups by Histocompatibility



Abbrev.: allo-HSCT, allogeneic hematopoietic stem cell transplantation; MFD, major functional disability; TP, Transplant Population; TPES, Strictly ALD 102 Eligible Transplant Population; MDS, myelodysplastic syndrome; MSD, matched sibling donor; NMSD, not a matched sibling donor. Kaplan-Meier method; events include deaths, MFDs, MDS, and rescue cell administration or second allo-HSCT. Patients who did not experience any event are censored at their date of last contact.
Rel. Day 1 is the day of eli-cel infusion for TP-102/104 and the day of allo-HSCT for TPES-103 populations.

Overall survival after eli-cel treatment was high. In pooled analyses of the completed pivotal study ALD-102 with the ongoing ALD-104 and LTF-304 studies, eli-cel maintained an estimated overall survival rate of 97.7% (95% CI: 84.6%, 99.7%) through 7 years of follow-up. The estimated Month 24 overall survival rate for TP-102/104 was higher than the rate of 86.3% (95% CI: 54.7%, 96.5%) for TPES-103-NMSD. Further analyses of the TPES-103-NMSD group by histocompatibility showed that the estimated Month 24 overall survival rate for TP-102/104

appeared higher than the rates of 85.7% (95% CI: 33.4%, 97.9%) for TPES-103-NMSD-MUD and of 85.7% (95% CI: 33.4%, 97.9%) for TPES-103-NMSD-Mismatched.

Supportive clinical and radiographic endpoints

Several additional direct clinical assessments of neurologic function and cognition, as well as radiographic endpoints support the primary efficacy analysis.

The NFS is a 25-point score used to evaluate the severity of gross neurologic dysfunction in CALD by scoring 15 symptoms across 6 categories (i.e., hearing, communication, vision, feeding, locomotion, and incontinence). A score of 0 denotes absence of clinical signs of cerebral disease (Moser et al. 2000; Miller et al. 2011). A baseline NFS of 0 or 1 was required for entry into ALD-102 and ALD-104, as well as for qualifying for the TPES-103. At Month 24 post-treatment, 89.2% of patients in TP-102/104 and 91.7% of patients in TPES-103 maintained their baseline NFS.

The MRI-based Loes score is a 34-point scale commonly used to quantify the extent of demyelinating brain lesions in CALD; higher scores indicate more severe disease (Loes et al. 1994). A Loes score of 0.5 to ≤ 9, indicating early disease, was required for entry into ALD-102 and ALD-104, as well as for qualifying for the TPES-103. Most patients treated with eli-cel showed an initial increase in Loes score which stabilized by Month 24; these findings were consistent with observations of disease stabilization after 24 months in the literature for allo-HSCT (Shapiro et al. 2000; Polgreen et al. 2011; Miller et al. 2011). At 24 Month post-treatment, 54.3% of patients in TP-102/104 and 53.8% of patients in TPES-103 showed an increase of < 6 from baseline. A higher percentage of patients in TP-102/104 had a cerebral MRI Loes score increase of ≥ 6 (8/35 [22.9%]) than in TPES-103 (1/13 [7.7%]); Table 14). In these patients, the Loes score appeared to stabilize between 24 and 36 months after eli-cel treatment.

One of the signs of cerebral inflammation in CALD is a compromised blood-brain barrier behind demyelinating lesions, which is shown by gadolinium enhancement (GdE positivity) on MRI. All patients in TP-102 and TP-104 were GdE+ at enrollment; all patients in TPES-103 were GdE+ prior to treatment. In TP-102/104, 31/35 (88.6%, 95% CI: 73.3%, 96.8%) patients treated with eli-cel were GdE- at Month 24. All evaluable patients in TPES-103 were GdE- after allo-HSCT at Month 24 (13/13 [100%, 95% CI: 75.3%, 100%]). Although the proportion of patients with GdE- for TPES-103 appears higher than for TP-102/104, the confidence intervals overlap, and the clinical significance is unknown. Patients with re-emergence of gadolinium enhancement did not show faster progression of neurologic function scores than patients without re-emergence after eli-cel treatment.

Patients underwent a panel of intelligence quotient (IQ) tests to assess age-appropriate cognitive ability. The results supported the other efficacy findings, providing additional evidence of meaningful neurologic disease stabilization in patients treated with eli-cel. The performance/ reasoning/visual IQ (PrvIQ) subscale was determined to be a meaningful subscale that is sensitive to CALD disease progression and unlikely to be biased with regard to the patient's primary language, as several patients were not native English speakers. Most TP-102/104 patients maintained a PrvIQ within or near the normal range after eli-cel treatment. While modest decreases in PrvIQ were observed especially at early timepoints following treatment, PrvIQ appeared stable after Month 24 through the last timepoint. The PrvIQ observed in TP-102/104 was comparable to TPES-103.

Supportive propensity score analysis

Since randomization of patients was not feasible in eli-cel trials, propensity score (PS) methods were used to allow for comparisons between eli-cel and allo-HSCT while controlling for potential differences between the treatment groups on pre-treatment patients' characteristics. If the propensity score approach achieves balance between the two groups (meaning that the background characteristics in both groups become comparable after adjustment for the propensity score), the estimation of the treatment effect in a non-randomized trial can be improved by mimicking some of the statistical properties of a randomized controlled trial.

The propensity score adjusted analyses were performed on selected efficacy endpoints comparing TP-102/104 and TPES-103 or TPES-103-NMSD by adjusting for pretreatment differences in background covariates and risk factors which were considered correlated with the clinical outcome. These additional propensity score adjusted analyses provided similar results to the pre-specified efficacy analyses, indicating that eli-cel has a benefit over allo-HSCT, particularly in patients without a matched sibling donor (see Section 10, Appendix B).

Efficacy conclusions

eli-cel stabilized neurologic and cognitive function in the majority of patients with early CALD based on direct clinical measures of neurologic function and cognition.

The completed, pivotal eli-cel study ALD-102 met the success criterion for the primary efficacy endpoint: eli-cel showed a compelling and statistically significant effect on Month 24 MFD-free survival when compared to a pre-specified benchmark that reflects the course of untreated CALD. Survival free of the major neurological disabilities that characterize this disease is a direct clinical assessment, and it is meaningful to patients, families, and clinicians. Therefore, eli-cel is considered superior to no treatment.

The findings of ALD-102 were supported by the pooled analysis of ALD-102, ALD-104, and LTF-304. The durability of the effect was demonstrated in clinical follow-up of > 7 years and in biomarker data showing persistence of transduced repopulating HSCs with ALDP expression in their progeny.

Event-free and overall survival after eli-cel compare favorably with allo-HSCT without MSD, particularly when using a mismatched donor.

Overall, the totality of the data demonstrates substantial evidence of eli-cel's efficacy in patients with early CALD.

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

1.6. Safety

The goal of the eli-cel program was to develop an autologous therapy that would allow for the treatment of early CALD without the immune-mediated complications of allo-HSCT (e.g., GVHD, graft rejection, TRM).

The safety analysis is derived from the 67 patients who received eli-cel in ALD-102 and ALD-104, with a median follow-up duration of 23.5 months. This population is referred to as TP-102/104 and includes data from the long-term follow-up study LTF-304. Safety data from

TP-102/104 are compared as relevant with data from all patients treated with allo-HSCT in ALD-103 (TP-103, N=59).

Primary safety endpoint: graft-versus-host disease

GVHD is a key cause of morbidity and mortality in allo-HSCT recipients, and avoidance of this complication impelled the eli-cel development program. Accordingly, the safety success criterion for ALD-102 was a statistically significant reduction ($p < 0.05$) in the proportion of patients who experienced either acute GVHD (≥ Grade II) or chronic GVHD by Month 24, compared with allo-HSCT in ALD-103.

This safety success criterion was met, with no eli-cel treated patient experiencing GVHD compared with 26/50 (52%) of evaluable patients receiving allo-HSCT in TP-103 ($p < 0.0001$). The majority of patients who experienced GVHD in TP-103 were in the NMSD subgroup.

Deaths

One death was reported among patients receiving eli-cel in TP-102/104; this patient had evidence of disease progression 2 weeks after treatment, developed multiple MFDs and died; his death was not considered related to drug product. In TP-103, 15/59 (25.4%) patients died, including 12 deaths after first allo-HSCT and 3 after second allo-HSCT. Five of these 15 patients were in the TPES subgroup (5/27, 18.5%); 3 of these died after first allo-HSCT and 2 after second allo-HSCT. Nine of the 15 deaths in TP-103 were classified as TRM, with 8 occurring within 1 year of infusion (all in the NMSD subgroup).

Engraftment

Initial hematopoietic reconstitution after transplant was assessed by engraftment. All eli-cel treated patients achieved neutrophil engraftment (NE). In TP-103, 53/59 (89.8%) patients achieved NE after first allo-HSCT, with primary or secondary graft failure observed in 10/38 (26.3%) evaluable patients, all of whom were in the NMSD subgroup.

Adverse events

Treatment with eli-cel (which occurs on Relative Study Day (Rel Day) 1, defined as the day of drug product infusion) is preceded by procedural and medical interventions, namely mobilization/apheresis with G-CSF with or without plerixafor, myeloablative conditioning using busulfan, and lymphodepletion using either cyclophosphamide (ALD-102) or fludarabine (ALD-104), that carry their own risks. The overall burden of eli-cel treatment derives from the entire course of therapy, and therefore this document describes the safety profile of the treatment regimen comprising mobilization/ apheresis, conditioning (myeloablation/ lymphodepletion), and treatment with eli-cel.

Thirty-one of 67 patients (46.3%) who underwent mobilization/apheresis had adverse events (AEs) attributed by the investigator to mobilization/apheresis. All of these were nonserious and resolved.

All patients treated with eli-cel in TP-102/104 (n=67) experienced at least 1 AE attributed to the conditioning regimen by the Investigator; most frequently thrombocytopenia, stomatitis, anemia, neutropenia, and nausea. Twenty patients (30%) had 31 serious AEs (SAEs) attributed to conditioning, all of which resolved.

Adverse drug reactions

Eight of 67 eli-cel treated patients (11.9%) experienced AEs related to eli-cel. Three patients were diagnosed with SAEs of MDS, 2 patients experienced SAEs of delayed hematopoietic reconstitution (1 was subsequently diagnosed with MDS), and 1 patient experienced an SAE of viral cystitis (due to BK virus). Three patients reported 3 nonserious AEs including vomiting and nausea that started and resolved on Rel Day 1 and were likely related to the cryopreservative dimethyl sulfoxide in the drug product.

Comparison of adverse events to allo-HSCT (TP-103)

As eli-cel is an autologous therapy, immunosuppression is not required after transplant, whereas prolonged immunosuppression to prevent or treat GVHD following allo-HSCT confers significant risk. Fewer eli-cel treated patients experienced severe infections in TP-102/104 (9/67 [13.4%]) than allo-HSCT recipients in TP-103 (34/59 [57.6%]) through Month 12, and the infections in TP-103 were frequently attributed to immunosuppression. No patients in TP-102/104 experienced severe hypertension, whereas this was reported in nearly half of the patients in TP-103 (28/59 [47.5%]). Additional therapies were required to manage the effects of prolonged immunosuppressant therapy associated with allo-HSCT in TP-103.

eli-cel risks

Insertional oncogenesis

Insertional oncogenesis has long been recognized as a potential safety concern for gene therapy products using viral vectors. Accordingly, patients in the eli-cel development program have been routinely monitored with hematologic assessments and measures of clonal dynamics. Integration site analysis (ISA) generally showed robust polyclonal reconstitution of the hematopoietic system. Three patients have been diagnosed with MDS, likely mediated by Lenti-D LVV insertion, following eli-cel. All three patients underwent allo-HSCT and 2 are in remission; the outcome of transplant in the third patient is pending (refer to Section 6.6.5.1 for details).

Prolonged cytopenias

Eighteen of 64 evaluable patients (28.1%) had Grade 3 or higher cytopenia of 1 or more cell lines on or after Rel Day 60, including decreased platelet count (14.1%), decreased neutrophil count (21.9%), and decreased hemoglobin (1.6%). On or after Rel Day 100, 7/54 patients (13.0%) had Grade 3 or higher cytopenia, including decreased platelet count (7.4%) or decreased neutrophil count (9.3%), and none had decreased hemoglobin (0%).

These cytopenias had limited clinical impact. Patients with thrombocytopenia had a similar incidence of bleeding as other patients, and those with neutropenia had a similar incidence of infection as other patients. Moreover, patients in long-term follow-up in LTF-304 generally demonstrate complete and stable hematopoietic reconstitution.

Hospitalizations

Patients treated with eli-cel (TP-102/104), spent fewer days in the hospital as compared to patients treated with allo-HSCT (TP-103), both before NE (median: 28 days for TP-102/104 vs. 51 days for TP-103) and after NE (median: 3 days for TP-102/104 vs. 14 days for TP-103).

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

1.7. Benefit-Risk Assessment

Hematopoietic stem cell transplantation is the only efficacious therapy for CALD, a rare but uniformly fatal disease.

Conventional treatment with allo-HSCT is effective, but many CALD patients do not have a suitable donor, establishing a need for autologous therapy. Accordingly, the benefit-risk of eli-cel is assessed in the context of allo-HSCT outcomes.

Chiefly, eli-cel treated patients are more likely to achieve both overall and event-free survival than allo-HSCT patients treated with an NMSD graft. This advantage is primarily driven by reduced transplant complications including graft failure and TRM within approximately 2 years of treatment. Event-free and overall survival following eli-cel treatment exceed 85% through 7 years of follow up, after which outcomes are not reliably characterized.

eli-cel treated patients do not require post-transplant immunosuppression and are not at risk of death due to GVHD. The occurrence of MDS is devastating but nonetheless compares favorably to fatalities following allo-HSCT in children with limited donor options.

The optimal use of eli-cel at this time is in those children with early CALD with only mismatched donors, but eli-cel is also an important option for those who do not have a suitable MUD.

Parents and treating physicians will need to consider treatment options on a case-by-case basis, considering the probability of rapid disease progression and the availability and histocompatibility of donors. They will also need to assess factors besides histocompatibility that play a role in transplant outcome, such as stem cell source, donor age and sex, donor-recipient CMV/EBV status and ABO compatibility, the barriers and delays to treatment, access to healthcare, the past experience and preferences of families, and the estimates of pros and cons provided by the transplant physician. The short-term reality of death due to immune complications will have to be balanced against the known short-term and unknown long-term risks of gene therapy.

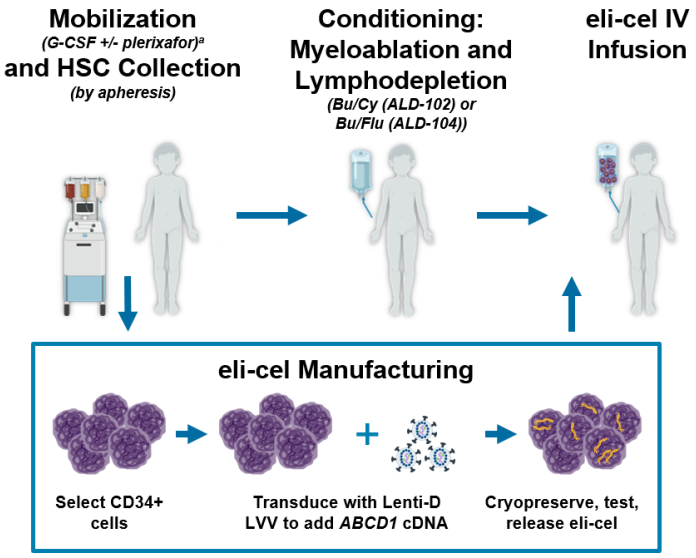
In consideration of the fatal nature of the disease and limitations of existing treatment, the benefit/risk of eli-cel is favorable in the indicated patient population.

2. DRUG PRODUCT

The drug product, eli-cel, consists of an autologous CD34+ cell-enriched population that contains the patient's HSCs transduced ex vivo with Lenti-D LVV encoding the *ABCD1* cDNA for ALDP. eli-cel is supplied frozen as a suspension in cryopreservation solution for intravenous infusion in 20 mL fluoro-ethylene-propylene bags. Each bag contains 2 to 30 × 10^6 CD34+ cells/mL, frozen in approximately 20 mL of solution. The minimum dose is 5.0 × 10^6 CD34+ cells/kg patient weight.

Each patient undergoes one HSC mobilization cycle with G-CSF with or without plerixafor in combination, followed by apheresis to harvest the cells. The collected cells are shipped to the manufacturing site where CD34+ cells are selected and then transduced with Lenti-D LVV to manufacture eli-cel drug product. The drug product is tested to demonstrate that it meets all product quality standards, after which it is released for patient administration. After myeloablative conditioning and eli-cel infusion, transduced HSCs engraft in the bone marrow and differentiate to reconstitute the hematopoietic system as well as provide ALDP to treat the patient's CALD (Figure 2).

Figure 2. Overview of eli-cel Treatment



Abbrev.: ALDP, adrenoleukodystrophy protein; Bu, busulfan; Cy, cytarabine; Flu, fludarabine; G-CSF, granulocyte colony-stimulating factor; HSC, hematopoietic stem cell.
[a] Plerixafor is required in ALD-104.

2.1. Lenti-D Lentiviral Vector

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

The structure of an LVV particle comprises an external lipid envelope and an internal protein core that includes 2 copies of the viral RNA genome complexed with nucleocapsid proteins and three viral enzymes: reverse transcriptase, integrase, and protease. All viral genes are absent

from the Lenti-D LVV genome, rendering the LVV incapable of replication. The Lenti-D LVV genome consists of a positive-strand RNA that carries key viral elements necessary for LVV function, as well as sequences encoding a functional ALDP. The only protein-coding element in the vector genome is the *ABCD1* transgene.

The expression of the transgene encoding ALDP, *ABCD1*, is controlled by a synthetic promoter called MNDU3 that is active in most cell types.

The function of Lenti-D LVV is to mediate integration of the therapeutic human *ABCD1* transgene encoding ALDP into the genome of the patient's own HSCs. Harvested CD34+ cells are transduced ex vivo with Lenti-D LVV, and during the transduction process, viral enzymes present in the LVV core reverse-transcribe the vector RNA into double-stranded DNA and facilitate the integration of the proviral DNA into the CD34+ cell genome. This integration step is critical because it allows the therapeutic transgene to be inherited by all daughter cells that derive from the transduced HSCs. The LVV itself is not directly administered to patients.

2.2. Quality and Control of Drug Product

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

A multivariate analysis of associations between drug product attributes and clinical parameters was performed to determine which of the former is predictive of clinical efficacy. Several drug product attributes were associated with clinical efficacy; the most predictive was vector copy number (VCN). Proposed commercial product specification acceptance criteria reflect levels which have been shown to result in positive clinical outcomes.

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

3. NONCLINICAL FINDINGS

Testing an autologous human gene therapy product in animal models poses significant challenges. It is acknowledged that there are limitations to the preclinical methods available for identifying and quantifying the oncogenic risk of gene therapy. The eli-cel nonclinical program was designed in acknowledgment of these intrinsic limitations. The nonclinical efficacy and safety profile that emerged supports the use of eli-cel in patients with CALD.

The pharmacology, toxicology, and genotoxicity of the Lenti-D LVV used for manufacturing eli-cel were evaluated in vitro and in vivo. In the in vitro studies, Lenti-D LVV transduction of fibroblasts from patients with CALD or mobilized peripheral blood CD34+ HSCs from patients with adrenomyeloneuropathy resulted in expression of high levels of ALDP and functional correction of VLCFA metabolism.

An in vitro immortalization-assay demonstrated a strongly reduced mutagenic potential compared with a positive control gamma retroviral vector and a positive control LVV containing the strong spleen focus-forming viral promoter. The Lenti-D LVV had the least genotoxic potential.

A pivotal Good Laboratory Practices-compliant, combined toxicity, genotoxicity, and biodistribution study of Lenti-D LVV-transduced healthy human donor mobilized peripheral blood CD34+ HSCs was conducted in myeloablated immunodeficient mice. The biodistribution of human cells and Lenti-D LVV sequences was consistent with the presence within blood and tissues of leukocytes derived from Lenti-D LVV-transduced human CD34+ HSCs.

There was no evidence of toxicity, genotoxicity, or oncogenesis (tumorigenicity) related to Lenti-D LVV integration and no toxicity related to production of ALDP transgenic protein. Integration site analysis of post-transplantation bone marrow cells demonstrated no preferred integration in the proximity of or within genes associated clinically with either clonal dominance or leukemia, and no evidence was observed of clonal dominance.

An additional biodistribution and general safety study with Lenti-D LVV-transduced human CD34+ HSCs administered to myeloablated, immunodeficient mice demonstrated engraftment of human-origin microglial cells within brain tissues with no toxicity or tumorigenicity.

Overall, Lenti-D LVV-transduced human CD34+ HSCs demonstrated the desired pharmacologic properties of stable *ABCD1* transgene expression, ALDP production, and improvement or correction of VLCFA metabolism, coupled with long-term engraftment in blood, bone marrow, brain, and other tissues in mice. There was no evidence of toxicity, genotoxicity (insertional mutagenesis resulting in oncogenic mutations), or oncogenesis (tumorigenicity).

These findings support the hypothesized mechanism of action by which transplantation of eli-cel into patients with CALD may be an effective gene therapy.

4. CLINICAL DEVELOPMENT PROGRAM

In the eli-cel clinical development program, consideration was given to the rarity of CALD, the severity and rapidly progressive nature of the disease, the pediatric population, the lack of approved treatment options, and the risks associated with the current therapeutic option (allo-HSCT).

Comparisons were made to allo-HSCT to contextualize the eli-cel benefits and risks.

4.1. Clinical Studies

The clinical development program demonstrates the efficacy and safety of eli-cel in pediatric patients with early CALD. The program comprises a completed historical data-collection study of the natural course of untreated CALD and outcomes of allo-HSCT (ALD-101), a completed contemporaneous comparator study of allo-HSCT (ALD-103); the completed pivotal eli-cel study (ALD-102); the supportive ongoing eli-cel study (ALD-104; enrollment complete); and the long-term follow-up study LTF-304 (Table 1). Of 67 patients treated in ALD-102 and ALD-104, 36 patients have reached their Month 24 follow-up visits and have enrolled in LTF-304.

The clinical studies of eli-cel treatment, ALD-102 and ALD-104, are single-arm, open label trials. Therefore, an external control approach was used as agreed with the FDA. ALD-101 primarily provided data for development of endpoints used in subsequent studies. ALD-103 provided contemporaneous allo-HSCT data for comparison with results obtained in the eli-cel studies.

The schedule of events and all endpoints for Study ALD-102 can be found on clinicaltrials.gov (NCT01896102).

Table 1. Clinical Studies Evaluating eli-cel and Allo-HSCT in Patients With CALD

Study Identifier (Status) NCT#	**Study Title**	**Age, Number of Patients, and Treatment Performed**	**Conditioning Regimen**	**Primary Efficacy Endpoint**	**Data Cut-Off Date for Ongoing Studies**
eli-cel Treatment and Long-Term Follow-up					
ALD-102 (completed 26 Mar 2021) NCT01896102	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 (CALD)	Males < 18 years: 30 planned/ 32 treated with eli-cel	busulfan (IV) and cyclophosphamide (IV)	Proportion of patients who are alive and have none of the 6 MFDs at Month 24 (i.e., Month 24 MFD-free survival)[a]	NA; study complete
ALD-104 (enrollment complete, follow-up ongoing) NCT03852498	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 (CALD)	Males < 18 years.: 35 planned/ 35 treated with eli-cel	busulfan (IV) and fludarabine (IV)	Proportion of patients who are alive, and have none of the 6 MFDs at Month 24[a]	Data cut: 18 August 2021, with select updates 7 January 2022
LTF-304 (ongoing) NCT02698579	Long-term Follow-Up of Subjects With Cerebral Adrenoleukodystrophy Who Were Treated With Lenti-D Drug Product	Long-term follow-up for all patients with CALD who received eli-cel in parent studies: approximately 60 planned/ 36 enrolled (29 from ALD-102 and 7 from ALD-104)	Not applicable (patients are not treated with eli-cel in this long-term follow-up study)	MFD-free survival	Data cut: 18 August 2021, with select updates 7 January 2022

Study Identifier (Status) NCT#	**Study Title**	**Age, Number of Patients, and Treatment Performed**	**Conditioning Regimen**	**Primary Efficacy Endpoint**	**Data Cut-Off Date for Ongoing Studies**
Allo-HSCT					
ALD-103 (completed 06 Dec 2019) (Sponsor terminated study after 59 patients were enrolled and analyzed) NCT02204904	A Prospective and Retrospective Data Collection Study to Evaluate Outcomes in Males ≤17 Years of Age Undergoing Allogeneic Hematopoietic Stem Cell Transplantation for the Treatment of Cerebral Adrenoleukodystrophy	Males < 18 years. 60 planned/ 59 treated with allo-HSCT Of these, 27 would have met ALD-102 entry criteria i.e. having early active CALD (TPES)	Investigator determined as per institutional guidelines	Primary efficacy endpoints were not specified; however, an objective of the study was to evaluate the efficacy of allo-HSCT in patients with CALD	NA; study complete
Allo-HSCT and Untreated Patients					
ALD-101 (completed 27 Mar 2012)	A Retrospective Study to Characterize the Natural History of Childhood Cerebral X-linked Adrenoleukodystrophy and to Investigate the Influence of Allogeneic Transplantation on Affected Subjects	Inclusion criterion: Males > 3 and < 15 years. Enrolled: Males > 1 to < 15 years 137 patients: 72 untreated, 65 treated with allo-HSCT	Not applicable (untreated) or Investigator determined as per institutional guidelines	Primary efficacy endpoints were not specified; however, the primary efficacy objectives of the study were to characterize the natural history of disease in untreated patients with CALD and to characterize the efficacy and safety outcomes of patients with CALD who are treated with bone marrow or cord blood stem cell transplants	NA; study complete

Abbrev.: Allo-HSCT, allogeneic hematopoietic stem cell transplantation; CALD, cerebral adrenoleukodystrophy; IV, intravenous; LSLV, last subject last visit; MFD, major functional disability; NA, not applicable; NCT, National Clinical Trial number; TPES, strictly eligible for ALD-102 transplant population.
[a] Patients must not have received rescue cell administration or allo-HSCT, and not have withdrawn or been lost to follow-up at Month 24 visit.

ALD-101 was a retrospective data collection study. It was conducted to characterize the natural history of disease in untreated patients with CALD, as well as the efficacy and safety outcomes of patients who were treated with allo-HSCT, to define efficacy and safety endpoints that could prove useful in the design of clinical studies. In ALD-101, data were collected on patients 1-15 years of age who had follow-up of ≥ 2 years after diagnosis (Untreated Cohort), allogeneic transplant (Allo-HSCT Cohort), or until death. Long-term follow-up data were also collected when available. The data from ALD-101 helped define the efficacy endpoints for studies ALD-102, ALD-103, and ALD-104, through identification of the 6 MFDs that are unambiguous indicators of neurologic disease progression: loss of communication, cortical blindness, tube feeding, total incontinence, wheelchair dependence, and complete loss of voluntary movement.

The results of ALD-101 confirmed earlier literature findings that the majority of untreated patients with CALD who were GdE+ on brain MRI developed MFDs within 2 years of GdE detection. These patients had a Month 24 MFD-free survival rate of 21% (exact 95% confidence interval [CI]: 6.1, 45.6; (Raymond et al. 2019)). Most patients with early CALD who received allo-HSCT did not develop MFDs within 2 years of treatment. It has been observed that survival varies by donor, with the best outcomes observed in patients who received a transplant from a matched sibling donor. The Month 24 MFD-free survival rate in patients with NFS ≤ 1, cerebral MRI Loes score 0.5 to ≤ 9, who received cells from a donor who was not a matched sibling, was 77% (exact 95% CI: 50.1, 93.2). Together these data helped inform the choice of the primary endpoint and benchmark to define the success criterion for ALD-102.

ALD-103 was a multi-site, global, prospective and retrospective study designed to evaluate outcomes of allo-HSCT in patients with CALD <18 years of age that was conducted concurrently with ALD-102 and did not involve the use of an investigational drug. The design of ALD-103 (prospective assessments) was largely consistent with ALD-102 with respect to efficacy and safety assessments and their timing. Data from ALD-103 are used as a contemporaneous external comparator for outcomes after treatment with eli-cel and were collected up to 48 months after last allo-HSCT.

ALD-102, the pivotal eli-cel clinical study, was a Phase 2/3 multinational, multicenter, open-label, single-arm study to evaluate the safety and efficacy of eli-cel in 32 patients <18 years of age with CALD who did not have an available or willing 10/10 HLA-matched sibling donor (MSD). Enrolled patients were followed for 24 months after eli-cel infusion. The primary efficacy endpoint for ALD-102 was the proportion of patients who were alive and who had not developed any of the 6 MFDs, had not received rescue cell administration or allo-HSCT, and had not withdrawn or been lost to follow-up at the Month 24 visit. The primary safety endpoint was the proportion of patients with acute GVHD (Grade ≥II) or chronic GVHD by Month 24 after drug product infusion.

ALD-104 is an ongoing Phase 3 international, multicenter, open-label, single-arm study in 35 patients <18 years of age with CALD with the same primary efficacy endpoint and same follow-up duration of 24 months as ALD-102. The conditioning regimen for ALD-104 uses busulfan and fludarabine rather than busulfan and cyclophosphamide used in ALD-102, and the primary safety endpoint is the proportion of patients with neutrophil engraftment after drug product infusion. Enrollment is complete and all 35 patients have been treated.

LTF-304, the long-term follow-up study, enrolls patients treated with eli-cel from ALD-102 and ALD-104. This study monitors patients for long-term safety and continued efficacy for 15 years after eli-cel infusion including 2 years in the parent study and 13 years in the long-term-follow-up study. This study has enrolled 36 patients from the 2 parent studies (ALD-102 and ALD-104).

4.2. Rationale for the Single-Arm Study Design of the Interventional Studies

The severity of CALD, the rarity of the disease, the lack of FDA-approved treatment options, the inability of such transplants to be blinded, and the potential impact of time required to conduct a donor match on cerebral disease progression precluded the conduct of a randomized controlled trial in the target patient population. Untreated patients who are GdE+, an inclusion criterion for ALD-102 and ALD-104, experience rapid neurological decline and development of MFDs, resulting in either a vegetative state or death within a short period of symptom onset of approximately 1.9 ± 2 years (Moser et al. 1987). Therefore, a single-arm, 24-month study design for pivotal ALD-102 was deemed appropriate for generating sufficient evidence to establish effectiveness of eli-cel for the treatment of CALD compared with the well-defined natural history of the disease. Specifically, to establish eli-cel benefit, a clinically meaningful benchmark for the primary endpoint of 24-Month MFD-free survival was defined, supported by results from untreated patients in natural history study ALD-101 and data from disease specific literature that reports on overall survival (Baumann et al. 2003; Peters et al. 2004; Beam et al. 2007; Miller et al. 2011; van den Broek et al. 2018). To provide additional supportive evidence and confirm the treatment benefit of eli-cel, analyses were defined to make comparisons with concurrently-collected data from allo-HSCT treated patients in ALD-103. ALD-104 also provides supportive evidence for the conclusions from pivotal study ALD-102.

4.3. Clinical Pharmacology

eli-cel adds functional copies of *ABCD1* cDNA into patients' HSCs through ex vivo transduction of autologous CD34+ cells with Lenti-D LVV to address the underlying genetic cause of the disease. After eli-cel infusion, transduced CD34+ HSCs engraft in the bone marrow. Conventional pharmacokinetics methods based on absorption, distribution, metabolism, and excretion cannot be used to monitor the presence of the drug product because of the nature of eli-cel.

The proposed mechanism of eli-cel is that these transduced HSCs differentiate into various cell types following engraftment, including monocytes, that migrate to the brain where these cells further differentiate into macrophages and cerebral microglia that can produce functional ALDP (Varvel et al. 2012; Sevenich 2018). Thus, successful treatment with eli-cel is hypothesized to require transgene presence and expression in cerebral macrophages and/or microglial cells. Functional ALDP expressed in the brain would enable the local degradation of VLCFAs there, which in turn could stabilize the disease by preventing further inflammation and demyelination. Following successful engraftment, the expression of ALDP is expected to be lifelong.

Although the presence or expression of the transgene in cerebral cells cannot be measured directly in patients, these properties can be measured in peripheral blood leukocytes, including

CD14+ monocytes that are of the same lineage as macrophages and dendritic cells. Presence of vector sequences in peripheral CD14+ cells suggests that these sequences may also be present in derived macrophages and microglial cells in the brain. The extent to which ALDP+ cells in peripheral blood represent cerebral ALDP+ microglial cells or macrophages is not known.

4.3.1. Methodology

The presence of vector sequences in the genomic DNA of differentiated cells is detected using quantitative polymerase chain reaction and the results are expressed as VCN (vector copies per diploid genome [c/dg]). VCN measured in drug product, which is comprised largely of CD34+ cells, is referred to as drug product (DP) VCN; VCN measured in peripheral blood leukocytes (PBL) is referred to as PB VCN; and VCN measured in CD14+ monocytes is referred to as CD14+ VCN. PB VCN and CD14+ VCN were measured in patients with results shown in Section 5.3.5.

The MNDU3 promoter controls *ABCD1* transgene expression in all transduced HSCs and their progeny, including those in the macrophage/microglia cell lineage. The presence of ALDP is detected via intracytoplasmic immunostaining using anti-ALDP antibodies, fluorescently labelled secondary antibodies, and flow cytometry. %ALDP+ Cells is determined by calculating the percentage of cells that stained above background levels from endogenous ALDP in non-transduced cells.

The principal biochemical abnormality in CALD is the accumulation of saturated VLCFA, particularly hexacosanoic (C26:0) and tetracosanoic (C24:0) fatty acids, because of the impaired capacity to degrade these substances. Degradation of VLCFA normally occurs in the peroxisome facilitated by the peroxisomal transporter ALDP. Analysis of VLCFA in fasting serum is an accepted method of diagnosis of ALD and this assay depends on demonstration of increased levels of C26:0 in a screening assay, followed by further discrimination of positive results by considering the C26:0 and docosanoic (C22:0) fatty acid and C24:0/C22:0 ratios (Moser et al. 1999). Additionally, VLCFAs are components of more complex lipids, including lysophosphatidylcholines (LysoPCs); C26:0 LysoPC is a potential diagnostic marker of ALD (Hubbard et al. 2006; Sandlers et al. 2012).

Production of functional ALDP in HSC progeny after transplantation potentially could result in decreased levels of VLCFA in fasting serum, and allo-HSCT with myeloablative conditioning has previously been reported to result in decreased VLCFA in serum (Kato et al. 2019). The extent to which changes in measured VLCFA metabolism are representative of changes within central nervous system or brain tissues is not known.

4.3.2. Dosing

eli-cel drug product is administered as a single intravenous dose of $\geq 5.0 \times 10^6$ CD34+ cells/kg. This dose was chosen based on literature review that indicates that this dose is sufficient to provide robust hematopoietic reconstitution of myeloablated patients. The minimum CD34+ dose accepted as safe practice and associated with favorable engraftment kinetics in autologous hematopoietic stem cell transplantation (HSCT) is approximately 1.5 to 3.0×10^6 cells/kg (Jillella and Ustun 2004). Initially, the dose selected for ALD-102 for patients with CALD using mobilization/apheresis as a cell source was $\geq 3.0 \times 10^6$ CD34+ cells/kg. However, reports that

optimal neutrophil and platelet engraftment in either allogeneic or autologous HSCT occurs at doses around 5.0×10^6 cells/kg (Weaver et al. 1995; Hatzimichael and Tuthill 2010; Duong et al. 2014) resulted in a subsequent dose increase to $\geq 5.0 \times 10^6$ CD34+ cells/kg. All patients treated with eli-cel in the clinical program have received a dose of $\geq 5.0 \times 10^6$ CD34+ cells/kg. Specifically, the median (min, max) total cell dose administered to patients in the pooled ALD-102 and ALD-104 population was 12.0×10^6 (5.0, 38.2) CD34+ cells/kg. There is no ceiling on dose.

This dose was obtainable using HSCs collected after a single mobilization cycle for each patient. Mobilization was accomplished using G-CSF in all patients in ALD-102; a subset of patients (11/32) also received plerixafor, which was recommended to augment mobilization if required. Mobilization was accomplished using both G-CSF and plerixafor for all patients in ALD-104.

All treated patients (N=67) in ALD-102 and ALD-104 achieved NE. No correlations were observed between cell dose and day of NE or platelet engraftment (PE).

5. EFFICACY

eli-cel gene therapy stabilizes neurologic function in patients with early CALD and may provide a durable long-term beneficial effect on clinical indices.

5.1. Populations Analyzed in the eli-cel Clinical Program

The Transplant Population (TP) of each study included patients who received an infusion of eli-cel or allo-HSCT. The TPs are abbreviated as TP-102, TP-103, and TP-104, for studies ALD-102, ALD-103, and ALD-104, respectively. Importantly, data collected in LTF-304, the long-term follow-up study, are merged with the corresponding parent study information and are included in all relevant analyses; these data are referred to by their parent study.

The Strictly ALD-102-Eligible Transplant Population (TPES) is a subset of the TP population, which consists of allo-HSCT treated patients who have baseline characteristics that would have allowed strict eligibility for ALD-102 and ALD-104: NFS ≤ 1, cerebral MRI Loes score ≥ 0.5 to ≤ 9, and GdE+; therefore, providing the best comparator to TP-102 and TP-104. The TPES is abbreviated as TPES-103 for study ALD-103. Patients in TPES-103 received allo-HSCT from either an MSD (TPES-103-MSD) or NMSD (TPES-103-NMSD). Within the NMSD subgroup, patients received allo-HSCT from either a matched unrelated donor (TPES-103-NMSD-MUD) or mismatched donor (TPES-103-NMSD-Mismatched). (See also Figure 3)

The Intent-to-Treat (ITT) Population comprises patients who met the inclusion criteria, were enrolled in an interventional study and initiated study procedures, regardless of whether the patient received an eli-cel infusion. For pivotal study ALD-102, the ITT and TP populations were identical (N=32). The cohort of all patients in the study is the primary population analyzed for the primary endpoints. For ongoing study ALD-104, enrollment is complete (N=35) and the ITT and TP populations are also identical. ALD-104 provides supportive data for pivotal study ALD-102.

The TP-102 population is the primary population analyzed for the primary efficacy and safety endpoints. The pooled TP-102/104 population is analyzed for secondary and additional endpoints, including efficacy comparisons with TPES-103 and its subgroups.

ALD-103 was contemporaneous with ALD-102, reflecting the current standard of care for allo-HSCT practice. TPES-103 is used for efficacy comparisons because all patients in TPES-103 would have satisfied the NFS, cerebral MRI Loes score, and GdE status criteria for entry into ALD-102 and ALD-104. In contrast, safety comparisons focus primarily on the TP-103 population in order to include the broadest set of patients, as summarized in Section 6.

5.2. Efficacy Endpoints

5.2.1. Efficacy Assessments

5.2.1.1. Major Functional Disabilities

Based on a comprehensive review of data from ALD-101, a group of CALD experts identified 6 MFDs that were chosen based on their clinical significance and impact on independent

functioning. Prevention of the development of MFDs, which represent significant neurologic deterioration, has been recognized as an important clinical benefit and is expected to significantly reduce the burden on the patient, the patients' families, and healthcare resources (Raymond et al. 2019). The 6 identified MFDs are defined in Table 2. The inclusion criteria in ALD-102 limited eligibility to patients with early-stage disease and prohibited patients from entering the trial with a pre-existing MFD or with a neurological function score indicating significant cerebral disease.

Table 2. Major Functional Disabilities in Patients With CALD

MFD	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. Permanent alternative method of feeding required; this does not include transient tube feeding in the case of mucositis
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

Evaluation of patients for MFDs has been used outside of studies of eli-cel in patients with CALD in a case series (Kuhl et al. 2018).

5.2.1.2. Neurologic Function Score

The NFS is calculated on a 25-point composite scale that assesses both minor and major functional disabilities (Moser et al. 2000) and was designed by Dr. Gerald Raymond and colleagues, specifically for the consistent and reproducible clinical evaluation of the severity of gross neurologic dysfunction in patients with CALD. The NFS is the most common clinical evaluation tool used by clinical specialists caring for these patients and assesses 15 functional domains affected by the disease across 6 categories: hearing, communication, vision, feeding, locomotion, and incontinence (Moser et al. 2000; Miller et al. 2011). A score of 0 denotes absence of clinical signs of cerebral disease. The NFS is widely accepted, and interobserver reliability of > 95% has been reported (Moser et al. 2000; Miller et al. 2011).

5.2.1.3. Cerebral MRI Assessment: Loes Score

Brain MRI was used to determine the cerebral MRI Loes score and pattern. All MRIs were assessed by an independent central reader who was blinded to patient identification and time point. The consistency of cerebral MRI Loes score reads was evaluated and found to be high. The 34-point Loes scoring scale measures the extent and location of brain abnormalities such as the presence of white matter changes, degree of demyelination, and the presence of focal or global atrophy (Loes et al. 1994). The maximum severity score is 34; any score ≥ 0.5 is considered abnormal (Loes et al. 2003). Early CALD has been described as patients with Loes scores > 0 to 9 in the literature (Peters et al. 2004; Mahmood et al. 2007; Kuhl et al. 2018).

5.2.1.4. Contrast Agent Enhancement on MRI

Contrast agent enhancement, most frequently gadolinium, thus termed "GdE+" on brain MRI, represents a clinically important radiographic biomarker of active neuroinflammatory disease and poor prognosis in untreated patients (Melhem et al. 2000; Raymond et al. 2019). Brain MRI was used to determine GdE status of enrolled patients. All MRIs were assessed by one independent central reader who was blinded to patient identification and time point.

5.2.1.5. Neuropsychological Testing

Patients underwent a panel of age-appropriate IQ tests to measure intelligence and cognitive ability. Wechsler tests measure intelligence and cognitive ability, and the multiple tests are designed to be age appropriate. For the eli-cel clinical program, the main focus of IQ assessment was the PrvIQ subset of the Wechsler analysis, subdomains selected due to their relevance to the population of patients with CALD.

5.2.1.6. Pediatric Quality of Life

The Pediatric Quality of Life Inventory (PedsQL™) assesses physical, emotional, social, and school functioning domains on a 100-point scale, with higher scores indicating better health-related quality of life. The accepted minimal clinically important difference for an individual reported in the literature is 4.5 points (Varni et al. 2003; Seid et al. 2006; Seid et al. 2010; Vetter et al. 2012).

5.2.2. Primary Efficacy Endpoint, Clinical Benchmark, and Success Criterion

The primary efficacy endpoint was the proportion of patients who had not developed any of the 6 MFDs, had not died nor received rescue cell administration or allo-HSCT, and had not withdrawn or been lost to follow-up at the Month 24 visit. The success of the primary endpoint for pivotal study ALD-102 was determined by meeting a predefined clinically meaningful benchmark of 50% that was supported by results from ALD-101 as well as from data from literature that reported on overall survival for patients with CALD at various timepoints of approximately 50 to 90% with allo-HSCT (Baumann et al. 2003; Peters et al. 2004; Beam et al. 2007; Miller et al. 2011; van den Broek et al. 2018). The success of eli-cel for the treatment of CALD is based on a comparison of the ALD-102 primary efficacy endpoint of Month 24 MFD-free survival to the clinically meaningful benchmark, such that the lower bound of the 2-sided 95% exact CI of the proportion of patients with Month 24 MFD-free survival must be > 50%. This external comparator approach was agreed in discussions with the FDA and is

supported by the January 2021 FDA Draft Guidance "Human Gene Therapy for Neurodegenerative Diseases," regarding the ethical limitations of a randomized, placebo-controlled study in a pediatric population (FDA 2021).

The rationale for the choice of the untreated GdE+ population to define the success criterion for the primary efficacy analysis in ALD-102 is 2-fold: GdE+ status in an untreated patient is a critical characteristic associated with rapid, catastrophic cerebral disease progression (Melhem et al. 2000; Moser et al. 2000; Loes et al. 2003; Miller et al. 2016) and the presence of contrast enhancement was previously and is still often used to determine treatment in medical practice.

5.2.3. Secondary Efficacy Endpoints

MFD-free survival was also assessed over time by Kaplan-Meier analysis as a secondary endpoint. After cases of myelodysplastic syndrome (MDS) were reported in eli-cel treated patients (refer to Section 6.6.5.1), the Kaplan-Meier analysis of MFD-free survival was broadened to an event-free survival analysis which includes all components of MFD-free survival but also considers MDS as an event. This briefing document presents the Kaplan-Meier analysis of event-free survival to capture all events of MDS observed in the clinical program. Further, overall survival (OS) estimated by Kaplan-Meier analysis is a standard endpoint for the outcomes of clinical studies evaluating the efficacy of transplantation procedures and was requested by the FDA. The Hazard Ratio (HR) estimated from the Cox-proportional hazard model was used to compare the risk of failing the survival endpoints of eli-cel treated patients with that of allo-HSCT-treated patients in TPES-103 and donor subgroups. An HR < 1 indicates a reduced risk of failing over time.

Other secondary efficacy endpoints included change in total NFS from Baseline to Month 24, proportion of patients who demonstrated resolution of gadolinium positivity (i.e., GdE-) on MRI at Month 24, and time to sustained GdE- in ALD-102, with sustained GdE- defined as gadolinium resolution without a subsequent evaluation indicating gadolinium positivity.

5.2.4. Criteria for Participation in ALD-102 and ALD-104

For inclusion in the eli-cel studies, patients had to be age <18 years at the time of parental/guardian consent or patient assent, with active early CALD defined by elevated VLCFA values, a cerebral MRI Loes score between 0.5 and 9 (inclusive), and GdE+ on MRI of demyelinating lesions; as well as having an NFS ≤ 1. These same criteria are used by most centers to select patients who are appropriate for allo-HSCT.

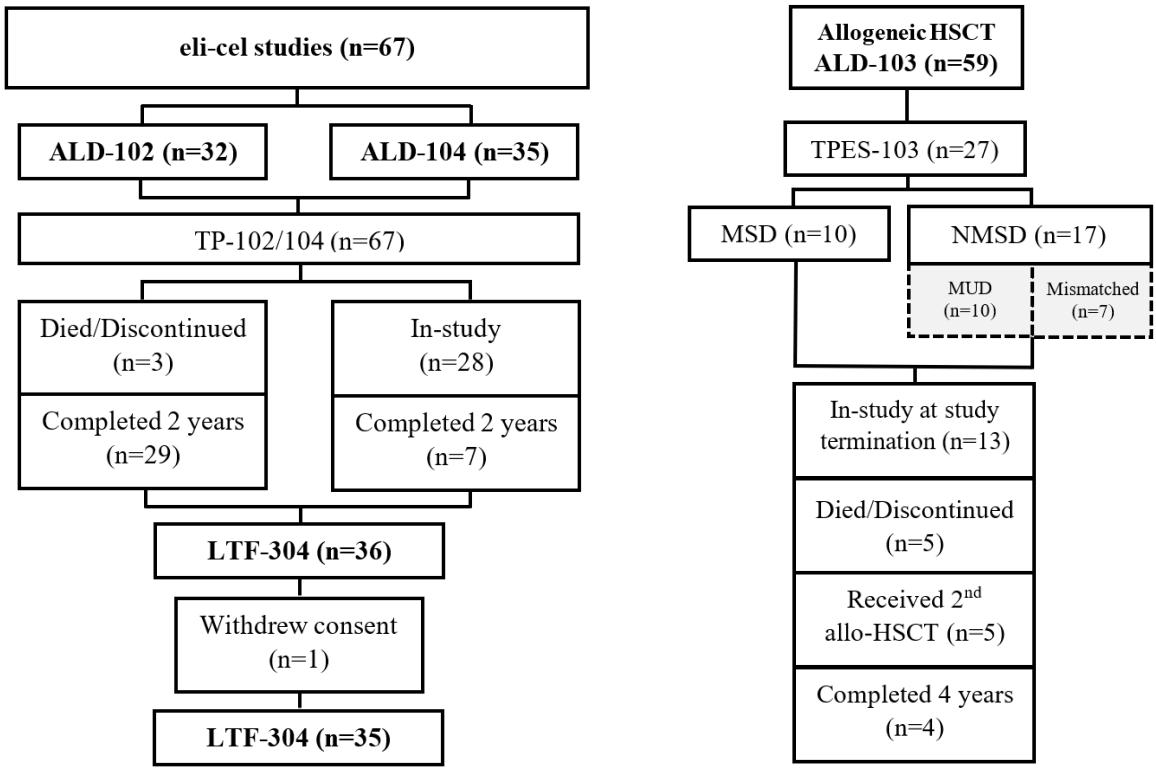
Exclusion criteria were prior allogeneic transplant or gene therapy; concurrent use of statins, Lorenzo's Oil, or dietary regimens to lower VLCFA levels; use of another investigational drug or procedure within 3 months before Screening that might confound study outcomes; and hematological, hepatic, renal, or cardiac compromise or any clinically significant disease or condition that would be contraindicated for any of the study procedures. Patients with availability of an HLA-MSD were excluded from enrollment in ALD-102, but not in ALD-104.

5.3. Efficacy Results

5.3.1. Disposition in TP-102/104 and TPES-103

Figure 3 shows the disposition of patients in TP-102/104 and TPES-103. All patients who completed ALD-102 or ALD-104 enrolled in LTF-304. One patient refused further follow up and withdrew from LTF-304.

Figure 3. Disposition in TP-102/104 and TPES-103



Abbrev.: MUD, matched unrelated donor; MSD, matched sibling donor; NMSD, not a matched sibling donor

The disposition of the subgroups in TPES-103 are shown in Table 3. Of the 17 patients without a matched sibling donor (NMSD) in TPES-103, 10 had an MUD and 7 had a mismatched donor.

Table 3. Disposition in ALD-103 (TPES-103, TPES-103-MSD, TPES-103-NMSD)

	TPES-103 (N=27)	TPES-103 MSD (N=10)	TPES-103 NMSD (N=17)
Underwent 1st allo-HSCT, n (%)	27 (100)	10 (100)	17 (100)
Completed study to Month 48, n (%)	4 (14.8)	1 (10.0)	3 (17.6)
Discontinued study, n (%)	23 (85.2)	9 (90.0)	14 (82.4)
Unable to comply with protocol-defined visits	1 (3.7)	1 (10.0)	0
Lost to follow-up	1 (3.7)	1 (10.0)	0
Death	3 (11.1)	2 (20.0)	1 (5.9)
Study terminated by sponsor	13 (48.1)	5 (50.0)	8 (47.1)

	TPES-103 (N=27)	TPES-103 MSD (N=10)	TPES-103 NMSD (N=17)
Subject to receive another allo-HSCT	5 (18.5)	0	5 (29.4)
Median (min, max) duration of follow-up, months	24.3 (0.9, 48.5)	34.7 (4.1, 48.3)	11.1 (0.9, 48.5)

Abbrev.: MSD, matched sibling donor; NMSD, not a matched sibling donor; TPES, strictly ALD-102 eligible population.

5.3.2. Baseline Demographics and Disease Characteristics

As shown in Table 4, patients enrolled in ALD-102/104 were a median (min, max) age of 6 (4, 14) years at the time of infusion, were all male, and were predominately White (54%), although race was not reported for 30% of patients. Age and race demographics were generally consistent across studies.

See Table 18 in Section 6.4.1 for baseline demographics in other populations.

Table 4. Baseline Demographics (TP-102/104, TPES-103, TPES-103-MSD, TPES-103-NMSD)

	eli-cel	allo-HSCT		
	TP-102/104 N=67	TPES-103 N=27	TPES-103 MSD N=10	TPES-103 NMSD N=17
Sex, n (%)				
Male	67 (100)	27 (100)	10 (100)	17 (100)
Age at first HSC infusion, years				
Median	6	8	7.5	8.0
Min, max	4, 14	5, 11	6, 9	5, 11
Age at first HSC infusion category, n (%)				
≥ 2 to < 6	21 (31.3)	3 (11.1)	0	3 (17.6)
≥ 6 to < 12	43 (64.2)	24 (88.9)	10 (100)	14 (82.4)
≥ 12 to < 18	3 (4.5)	0	0	0
Age at CALD diagnosis, years				
Median	6	7	7	7
Min, max	1, 13	0, 11	6, 9	0,11
Race, n (%)				
White	36 (54)	25 (93)	10 (100)	15 (88)
Black or African American	3 (4)	0	0	0
Asian	1 (1)	0	0	0
Other	7 (10)	2 (7)	0	2 (12)
Not provided or reported	20 (30)	0	0	0
Ethnicity				
Hispanic	17 (25)	7 (26)	3 (30)	4 (24)
Non-Hispanic	41 (61)	11 (41)	5 (50)	6 (35)
Not reported or unknown	9 (13)	9 (33)	2 (20)	7 (41)

	eli-cel	allo-HSCT		
	TP-102/104 N=67	TPES-103 N=27	TPES-103 MSD N=10	TPES-103 NMSD N=17
Time from CALD diagnosis to Rel Day 1, months				
Mean (SD)	8.0 (8.01)	12.6 (21.98)	5.7 (7.23)	16.6 (26.63)
Median	5.8	3.5	3.3	3.6
Min, max	2.5, 49.9	0.6, 78.0	1.3, 25.4	0.6, 78.0

Abbrev.: CALD, cerebral adrenoleukodystrophy; GdE, gadolinium enhancement; HSC, hematopoietic stem cell; HSCT, hematopoietic stem cell transplant; NFS, neurologic function score; SD, standard deviation.

Consistent with the eligibility criteria, patients had baseline characteristics typical of early CALD with a prognosis of rapid disease progression (Table 5); the median (min, max) cerebral MRI Loes score was 2.00 (1.0, 9.0) and the baseline NFS was 0 in 64/67 (95.5%) patients in TP-102/104.

Table 5. Baseline Disease Characteristics (TP-102/104, TPES-103, TPES-103-MSD, TPES-103-NMSD)

	eli-cel	allo-HSCT		
	TP-102/104 N=67	TPES-103 N=27	TPES-103 MSD N=10	TPES-103 NMSD N=17
Baseline NFS, n (%)				
0	64 (95.5)	26 (96.3)	10 (100)	16 (94.1)
1	3 (4.5)	1 (3.7)	0	1 (5.9)
Baseline Loes score				
n	67	27	10	17
Median	2	3	3.5	2.0
Min, max	1, 9	1, 9	1, 9	1, 9
Baseline GdE status, n (%)				
GdE+	66 (98.5)	27 (100)	10 (100)	17 (100)
GdE-	1 (1.5)[a]	0	0	0
Baseline Loes pattern [b]				
1, 2, or 5	55 (82.1)	24 (88.9)	10 (100)	14 (82.4)
3 and/or 4	11 (16.4)	2 (7.4)	0	2 (11.8)
Other or missing	1 (1.5)	1 (3.7)	0	1 (5.9)

Abbrev.: CALD, cerebral adrenoleukodystrophy; GdE, gadolinium enhancement; HSC, hematopoietic stem cell; HSCT, hematopoietic stem cell transplant; NFS, neurologic function score.

[a] One patient was GdE+ at enrollment in ALD-104 and GdE- at a subsequent MRI prior to conditioning that is considered baseline. Available literature describes that GdE+ can resolve in untreated patients, and that these patients maintain a high risk of disease progression (Melhem et al. 2000). The pertinent patient is included in the presented analyses of TP-102/104, but contributes less than 2 years of follow-up data.

[b] Patterns 1, 2, or 5 have been associated with a faster progression compared to patterns 3 or 4.

5.3.3. Primary Endpoint in ALD-102 and Comparison to Benchmark

eli-cel met the efficacy success criterion: 29/32 patients (90.6%, exact 95% CI: 75.0% to 98.0%) achieved Month 24 MFD-free survival, with the lower bound of the 2-sided 95% exact confidence interval well above the pre-specified benchmark of 50%. These findings indicate a compelling and statistically significant effect over the natural course of untreated CALD, i.e. the

Month 24 MFD-free survival rate in untreated GdE+ patients from ALD-101 of 21% (exact 95% CI; 6.1% to 45.6%; (Raymond et al. 2019)). The one patient who developed MFDs early during the trial and two patients who withdrew to receive allo-HSCT are described further in Section 5.3.4.1.

An interim analysis of ongoing Study ALD-104 showed similar results. Thirteen patients have reached 24 months and are evaluable for this endpoint. All but one evaluable subject (12/13 [92.3%]) met the primary efficacy endpoint of Month 24 MFD-free survival (exact 95% CI: 64.0%, 99.8%).

5.3.4. Durability of Treatment Effect

5.3.4.1. Event-free survival

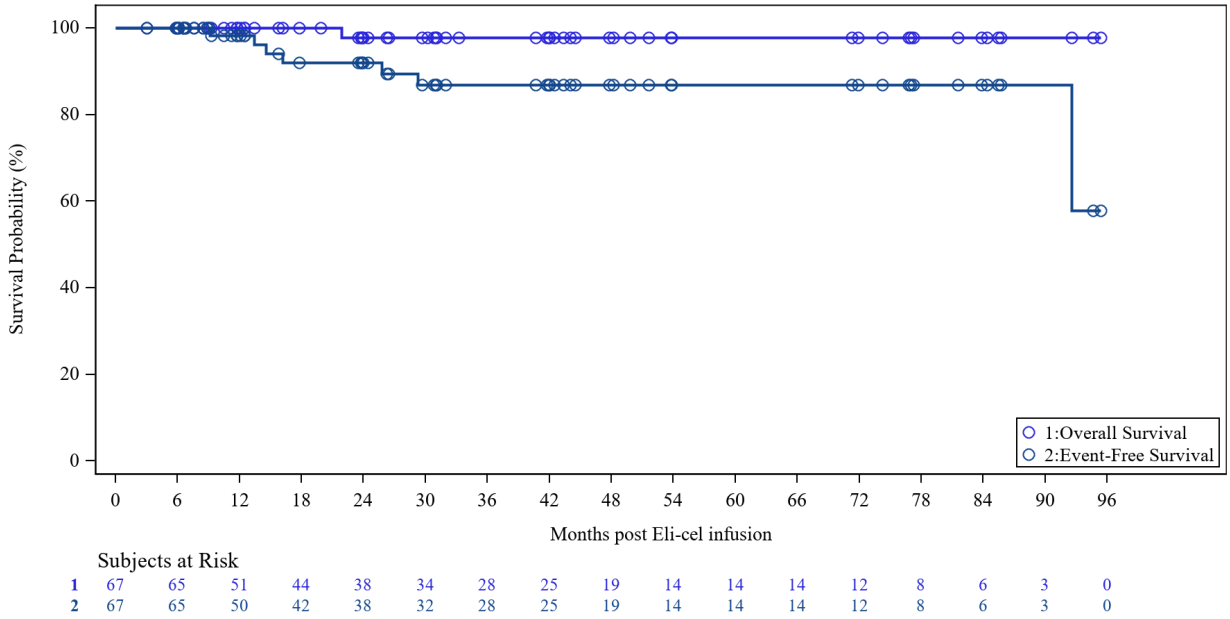
Kaplan-Meier analysis of event-free survival estimated that 91.9% (95% CI: 79.8%, 96.9%) of TP-102/104 patients would be event-free at 24 months after eli-cel infusion (Figure 4, Table 6). This analysis was based on data from 67 treated patients (TP-102/104), 60 of whom did not experience an event and were censored at their date of last follow-up on the study. Event-free survival was not achieved for seven patients including: two patients who developed MFDs, two patients who withdrew to undergo allo-HSCT, and three patients who developed MDS at 14 months, 26 months, and approximately 7.5 years following infusion, respectively (see Section 6.6.5.1 Treatment-Emergent Malignancies). The two patients who developed MFDs include one patient who progressed rapidly, developed multiple MFDs and died while enrolled in ALD-102 (see Section 6.6.7.1 Death) and one patient who experienced an SAE of transverse myelitis and developed an MFD of total incontinence approximately 29 months after treatment while enrolled in ALD-104 (see Section 6.8 Late-Breaking Update). The two patients who withdrew at investigator's discretion to undergo allo-HSCT are described below:

- One patient was withdrawn from Study ALD-102 at approximately 13 months after drug product infusion because his MRI showed increased white matter involvement compared with the assessment 6 months prior with associated contrast enhancement. At Baseline, this patient had a cerebral MRI Loes score of 2.0, an NFS of 0, and was GdE+ with a Loes Pattern 3 (associated with slower progression). At his last evaluation in ALD-102 before allo-HSCT (Rel Day 407), his PB VCN was low at 0.124 c/dg (Month 12), he had a cerebral MRI Loes score of 9.0, an NFS of 1, and was GdE+. At time of withdrawal, this patient had not developed any MFDs but had an increase of 1 in his NFS due to episodes of incontinence.
- One patient was withdrawn from Study ALD-102 approximately 16 months after drug product infusion because there had been a steady increase in cerebral MRI Loes score over time. At Baseline, this patient had a cerebral MRI Loes score of 1.0, an NFS of 0, and was GdE+ with a Loes Pattern 1 (associated with faster progression). At the time of withdrawal, his PB VCN was 0.397 c/dg, and his cerebral MRI Loes score had increased to 10.0 (Rel Day 438), although he was GdE- (Rel Day 438), and his NFS remained 0 (Rel Day 342).

eli-cel maintained an estimated event-free survival rate of 86.8% (95% CI: 72.7%, 93.9%) through 7 years of follow-up. Due to the occurrence of MDS at approximately 7.5 years after

eli-cel treatment and the low number of patients (n=3) with follow-up over 7 years, the event-free survival is considered not reliably characterized beyond 7 years.

Figure 4. Event-free and Overall Survival Over Time (TP-102/104)



Kaplan Meier method; events include deaths, MFDs, MDS, and rescue cell administration or second allo-HSCT (for event-free survival) and death (for overall survival). Patients who did not experience any event are censored at their date of last contact. Rel. Day 1 is the day of eli-cel infusion for TP-102/104.

Table 6. Kaplan-Meier Event-free, and Overall Survival Analyses (TP-102/104)

	TP-102/104 N=67	
	Event-free survival	**Overall survival**
Survival (months)		
Median (95% CI)	- (92.6, -)	- (-, -)
25th percentile (95% CI)	92.6 (29.2, -)	- (-, -)
75th percentile (95% CI)	- (92.6, -)	- (-, -)
Survival rate (95% CI)		
12 months after HSCT	98.2 (88.0, 99.7)	100.0 (100.0, 100.0)
24 months after HSCT	91.9 (79.8, 96.9)	97.7 (84.6, 99.7)
36 months after HSCT	86.8 (72.7, 93.9)	97.7 (84.6, 99.7)
48 months after HSCT	86.8 (72.7, 93.9)	97.7 (84.6, 99.7)
60 months after HSCT	86.8 (72.7, 93.9)	97.7 (84.6, 99.7)
72 months after HSCT	86.8 (72.7, 93.9)	97.7 (84.6, 99.7)
84 months after HSCT	86.8 (72.7, 93.9)	97.7 (84.6, 99.7)
94 months after HSCT	57.9 (9.1, 88.3)	97.7 (84.6, 99.7)

	TP-102/104 N=67	
	Event-free survival	**Overall survival**
Events, n (%)	7 (10.4)	1 (1.5)
Death	0	1 (1.5)
MFD	2 (3.0)	NA
Second HSCT	2 (3.0)	NA
MDS	3 (4.5)	NA
Censoring, n (%)	60 (89.6)	66 (98.5)
Withdrawal or lost to follow-up	1 (1.5)	1 (1.5)
Second allo-HSCT	NA	2 (3.0)
Completed study	0	0
Ongoing at time of data cut	59 (88.1)	63 (94.0)
Study termination by sponsor	0	0

Abbrev.: CI, confidence interval; HSCT, hematopoietic stem cell transplantation; TP, Transplant Population
Kaplan-Meier method, events include deaths, MFDs, MDS, and rescue cell administration or second allo-HSCT (for event-free survival) and death (for overall survival). Patients who did not experience any event are censored at their date of last contact.
Rel. Day 1 is the day of eli-cel infusion for TP-102/104

5.3.4.2. Overall Survival

Kaplan-Meier analysis of overall survival estimated that 97.7% (95% CI: 84.6%, 99.7%) of TP-102/104 patients would be alive at 24 months after eli-cel infusion (Figure 7, Table 6). This analysis was based on data from 67 treated patients (TP-102/104), 66 of whom were censored, including one patient who withdrew consent during long-term follow-up, two patients who withdrew from ALD-102 to undergo allo-HSCT, and 63 patients who were alive at their date of last follow-up on the study. Survival was not achieved by one ALD-102 patient who experienced rapid CALD disease progression within weeks of eli-cel treatment, developed multiple MFDs and died while enrolled in ALD-102 (see Section 6.6.7.1 Death).

No patient died after 24 months, supporting the durability of eli-cel's treatment effect. eli-cel maintained an estimated overall survival rate of 97.7% (95% CI: 84.6%, 99.7%) beyond 7 years of follow-up (N=6).

5.3.4.3. Biomarkers

The persistence of eli-cel efficacy is also supported by biomarker data showing that the majority of patients in TP-102/104 maintained the VCN in PBL and CD14+ cells at their latest assessment, demonstrating the long-term persistence of transduced repopulating HSCs. TP-102/104 patients also expressed ALDP in these cells at most visits, including several patients who had completed their Month 60 Visit, supporting the long-term expression of transgenic ALDP in the progeny of transduced HSCs.

The extent to which changes in biomarkers measured in PBL or CD14+ cells are representative of changes within the brain tissues is not known.

Peripheral Blood Vector Copy Number Over Time

All 67 patients had detectable PB VCN after engraftment, indicating transduced HSCs containing vector sequences were successfully engrafted in all patients.

By Month 1 after receiving eli-cel in ALD-102/104, median (min, max) VCN was 0.81 (0.10, 1.88) c/dg (based on values available for N=66 subjects at this timepoint) for PBLs and 1.20 (0.11, 2.81) c/dg (N=61) for CD14+ cells, demonstrating the early presence of transduced cells (Table 7). By Month 6, VCN had declined slightly and stabilized. The kinetics were similar for median PB VCN and CD14+ VCN. Note that most PBLs including CD14+ monocytes generally have a short lifetime in peripheral blood, and the cells used to determine CD14+ VCN have been recently derived from precursor cells. Thus, stable VCN levels in CD14+ cells over time demonstrate long-term persistence of transduced repopulating HSCs. One ALD-104 patient with an SAE of disease progression showed rapid decline of VCN in PBL and CD14+ cells and is planned to undergo allo-HSCT (see Section 6.8 Late-Breaking Update).

Table 7. PB VCN and CD14+ VCN Over Time (TP-102/104)

	VCN Over Time (c/dg)								
Cell Type	**M1**	**M6**	**M12**	**M24**	**M36**	**M48**	**M60**	**Year 6**	**Year 7**
PBLs									
n	66	52	46	36	26	15	14	10	3
Median	0.81	0.68	0.53	0.48	0.42	0.50	0.45	0.44	0.47
Min, max	0.10, 1.88	0.07, 3.13	0.07, 3.24	0.08, 2.77	0.05, 1.67	0.06, 1.49	0.05, 1.51	0.25, 1.05	0.28, 0.52
CD14+									
n	61	47	45	35	25	15	14	1	-
Median	1.20	0.77	0.55	0.50	0.55	0.56	0.48	1.96	-
Min, max	0.11, 2.81	0.07, 3.96	0.08, 4.00	0.06, 4.26	0.06, 1.91	0.06, 1.69	0.06, 1.57	1.96, 1.96	-

Abbrev.: M, month; PB, peripheral blood; PBL, peripheral blood leukocytes; TP, transplant population; VCN, vector copy number.

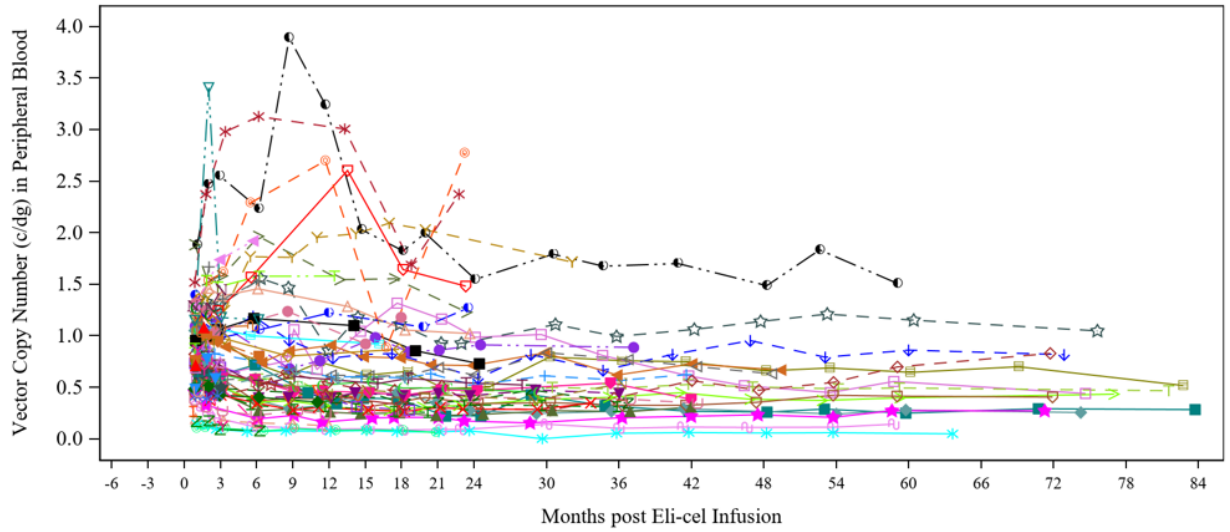
During engraftment, the contribution of late progenitor/short-term engrafting cells (with higher VCN values) to PB VCN and CD14+ VCN values will generally peak early, while lower but stable VCN values are achieved later once the blood cells are predominantly derived from the early progenitor/long-term repopulating HSCs.

Figure 5 shows PB VCN results over time by patient for TP-102/104. PB VCN at any time point was variable between patients, but with similar kinetics between patients, with most patients achieving relatively stable levels by 6 months after eli-cel infusion. These results indicate stable persistence of the inserted genetic material over time in each patient, with the longest follow-up out to Year 7. The persistence of PB VCN detection is consistent with those obtained from other studies using similar LVVs in other indications, where vector sequences in peripheral blood cells have been demonstrated up to at least 12 years post-drug product infusion (Negre et al. 2016), supporting long-term stable engraftment of transduced HSCs.

Some patients took longer than 6 months post-drug product infusion to plateau. An early peak is most likely due to a larger proportion of highly transduced short-term engrafting cells, which are

subsequently replaced by long-term engrafting cells derived from HSCs with a lower average VCN.

Figure 5. PB VCN Over Time (TP-102/104)



Each line represents the values for one patient over time.
Abbrev.: PB, peripheral blood; TP, transplant population; VCN, vector copy number.

%ALDP+ Cells Over Time

Median baseline values of PB %ALDP+ Cells were below the limit of quantitation (BLQ). By Month 1 after receiving eli-cel, median (min, max) PB %ALDP+ Cells was 20.20% (1.50%, 43.56%) (N=61), demonstrating early expression of the transgene. By Month 6, PB %ALDP+ Cells declined slightly and stabilized, with kinetics similar to PB VCN. At some visits, the lowest values were BLQ (and shown as 2.00 or 1.5 which represent 50% of the limits of quantification in the two assays employed).

Similar kinetics for median values were observed for CD14+ cells. By Month 1 after receiving eli-cel, median (min, max) CD14+ %ALDP+ Cells was 24.55% (1.50%, 57.54%; N=56), demonstrating early expression of the transgene. By Month 6, CD14+ %ALDP+ Cells had stabilized at median (min, max) of 23.30% (1.50%, 86.15%) (N=43). Similar kinetics were observed for CD14+ VCN, and the lowest values were BLQ at some visits.

Median CD14+ %ALDP+ Cells values were higher than median PB %ALDP+ Cells at all time points (Table 8). A greater proportion of CD14+ monocytes were ALDP+ relative to PB that contains all other leukocytes. These observations are consistent with the fact that the cytoplasm of CD14+ monocytes contain abundant lysosomes and peroxisomes, the latter of which contain ALDP. Thus, CD14+ monocytes may inherently contain more cytoplasmic ALDP, which may facilitate detection with the utilized method for quantifying ALDP+ cells.

Table 8. PB %ALDP+ Cells and CD14+ %ALDP+ Cells Over Time (TP-102/104)

	%ALDP+ Cells						
Cell Type	**M1**	**M6**	**M12**	**M24**	**M36**	**M48**	**M60**
PBLs							
n	61	47	44	36	22	15	12
Median	20.2	16.3	15.9	10.53	10.8	7.95	4.95
Min, Max	1.50, 43.56	1.50, 46.77	1.50, 40.88	2.00, 26.60	1.50, 42.40	3.14, 40.02	1.50, 27.54
Mean	20.59	16.43	15.74	11.76	13.03	10.35	7.10
SD	10.880	10.435	9.633	6.837	10.135	9.206	7.508
CD14+							
n	56	43	43	36	22	14	12
Median	24.55	23.3	19.3	17.2	16.21	12.43	16.1
Min, Max	1.50, 57.54	1.50, 86.15	1.50, 66.34	5.73, 45.00	1.50, 73.96	4.39, 47.08	1.50, 40.92
Mean	24.90	27.81	24.39	19.87	21.97	17.34	17.60
SD	12.770	19.738	16.436	11.531	18.919	11.516	15.152

Abbrev.: %ALDP+ Cells, percentage of cells expressing ALDP; M, month; PB, peripheral blood; PBL, peripheral blood leukocyte; SD, standard deviation; VCN, vector copy number (c/dg).
The below quantitation limit (BQL) values are set to 50% of the lower limit of quantification (i.e., reported as 2.00% during study ALD-102 and 1.50% during study ALD-104 and LTF-304).
Baseline is defined as the assessment closest but prior to conditioning in ALD-102 study.

5.3.5. Comparison to Contemporaneous External Control Study ALD-103

Results of the Kaplan-Meier analyses used to compare the pooled eli-cel treated population TP-102/104 with the allo-HSCT treated population TPES-103 (the strictly ALD-102 eligible TP) and its subgroups are summarized below.

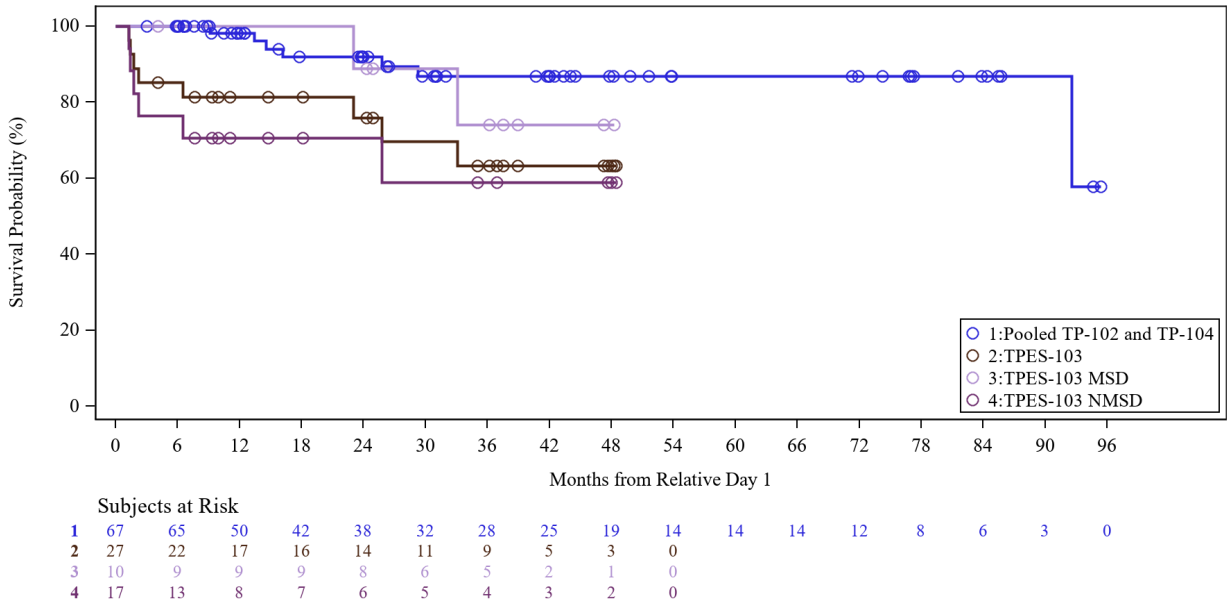
5.3.5.1. Event-Free Survival

Kaplan-Meier analysis of event-free survival estimated that 91.9% (95% CI: 79.8%, 96.9%) of TP-102/104 patients would be event-free at 24 months after eli-cel infusion, which is higher than the corresponding estimate of 75.9% (95% CI: 53.4%, 88.6%) for the allo-HSCT treated TPES-103 population. The estimated Month 24 event-free survival rate for TP-102/104 was similar to the rate of 88.9% (95% CI: 43.3%, 98.4%) estimated for TPES-103-MSD and higher than the rate of 70.6% (95% CI: 43.1%, 86.6%) for TPES-103-NMSD. Based on an observed hazard ratio (HR) of 0.268 (95% CI: 0.093, 0.773), eli-cel reduces the risk of failing the endpoint of event-free survival by 73.2% compared with allo-HSCT treated patient in TPES-103. The risk reduction with eli-cel is 43.2% in the subset of TPES-103 patients with an MSD and 81.4% in those without an MSD (Figure 6 and Table 9). Events in TPES-103 included death and second HSCT, the latter occurring particularly early following first allo-HSCT due to graft failure.

Further analyses of the TPES-103-NMSD group by histocompatibility subgroups showed that the estimated Month 24 event-free survival rate for TP-102/104 was higher than the rate of 42.9% (9.8%, 73.4%) for TPES-103-NMSD-Mismatched. It was similar to the rate of 90.0% (47.3%, 98.5%) seen in the TPES-103-NMSD-MUD subgroup.

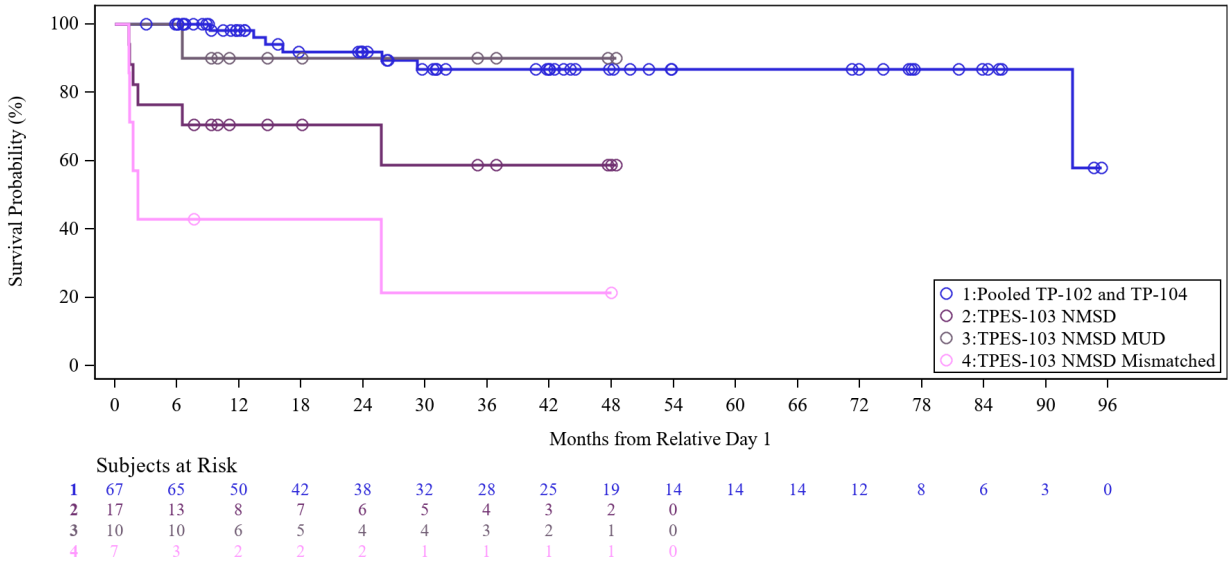
Based on observed hazard ratios (HR) of 0.061 (95% CI: 0.018, 0.205), eli-cel reduces the risk of failing the endpoint of event-free survival by 93.9% as compared to allo-HSCT treated patients in the TPES-103-NMSD-Mismatched subgroup, and may reduce it by 21.7% (HR: 0.783 (95% CI: 0.094, 6.524)) in TPES-103-NMSD-MUD subgroup (Figure 7 and Table 9).

Figure 6. Event-Free Survival Over Time by Donor Subgroups (TP-102/104, TPES-103, TPES-103-MSD, TPES-103-NMSD)



Abbrev.: allo-HSCT, allogeneic hematopoietic stem cell transplantation; MFD, major functional disability; TP, Transplant Population; TPES, Strictly ALD-102 Eligible Transplant Population; MDS, myelodysplastic syndrome; MSD, matched sibling donor; NMSD, not a matched sibling donor.
Kaplan-Meier method; events include deaths, MFDs, MDS, and rescue cell administration or second allo-HSCT. Patients who did not experience any event are censored at their date of last contact.
Rel. Day 1 is the day of eli-cel infusion for TP-102/104 and the day of allo-HSCT for TPES-103 populations.

Figure 7. Event-Free Survival Over Time by Histocompatibility (TP-102/104, TPES-103-NMSD, TPES-103-NMSD-MUD, TPES-103-NMSD-Mismatched)



Abbrev.: allo-HSCT, allogeneic hematopoietic stem cell transplantation; MFD, major functional disability; TP, Transplant Population; TPES, Strictly ALD 102 Eligible Transplant Population; MDS, myelodysplastic syndrome; MSD, matched sibling donor; NMSD, not a matched sibling donor. Kaplan-Meier method; events include deaths, MFDs, MDS, and rescue cell administration or second allo-HSCT. Patients who did not experience any event are censored at their date of last contact.
Rel. Day 1 is the day of eli-cel infusion for TP-102/104 and the day of allo-HSCT for TPES-103 populations.

Table 9. Kaplan-Meier Event-Free Survival Analysis of eli-cel and Allogeneic-HSCT by Donor Subgroups (TP-102/104, TPES-103, TPES-103-MSD, TPES-103-NMSD, TPES-103-NMSD-MUD, TPES-103-NMSD-Mismatched)

	eli-cel	allo-HSCT				
	TP-102/104 N=67	TPES-103 N=27	TPES-103 MSD N=10	TPES-103 NMSD N=17	TPES-103 NMSD-MUD N=10	TPES-103 NMSD-Mismatched N=7
Event-free Survival (months)						
Median (95% CI)	- (92.6, -)	- (25.8, -)	- (23.0, -)	- (2.2, -)	- (6.5, -)	2.2 (1.3, -)
25th percentile (95% CI)	92.6 (29.2, -)	25.8 (1.4, -)	33.1 (23.0, -)	6.5 (1.3, -)	- (6.5, -)	1.4 (1.3, 2.2)
75th percentile (95% CI)	- (92.6, -)	- (-, -)	- (-, -)	- (-, -)	- (-, -)	25.8 (1.7, -)
Hazard ratio (95% CI)[a]		0.268 (0.093, 0.773)	0.568 (0.114, 2.820)	0.186 (0.060, 0.580)	0.783 (0.094, 6.524)	0.061 (0.018, 0.205)
Event-free survival rate						
12 months after HSCT	98.2 (88.0, 99.7)	81.3 (60.8, 91.8)	100.0 (100.0, 100.0)	70.6 (43.1, 86.6)	90.0 (47.3, 98.5)	42.9 (9.8, 73.4)
24 months after HSCT	91.9 (79.8, 96.9)	75.9 (53.4, 88.6)	88.9 (43.3, 98.4)	70.6 (43.1, 86.6)	90.0 (47.3, 98.5)	42.9 (9.8, 73.4)
36 months after HSCT	86.8 (72.7, 93.9)	63.2 (38.2, 80.4)	74.1 (28.9, 93.0)	58.8 (27.5, 80.4)	90.0 (47.3, 98.5)	21.4 (1.2, 58.6)
48 months after HSCT	86.8 (72.7, 93.9)	63.2 (38.2, 80.4)	74.1 (28.9, 93.0)	58.8 (27.5, 80.4)	90.0 (47.3, 98.5)	21.4 (1.2, 58.6)
60 months after HSCT	86.8 (72.7, 93.9)	- (-, -)	- (-, -)	- (-, -)	- (-, -)	- (-, -)
72 months after HSCT	86.8 (72.7, 93.9)	- (-, -)	- (-, -)	- (-, -)	- (-, -)	- (-, -)
84 months after HSCT	86.8 (72.7, 93.9)	- (-, -)	- (-, -)	- (-, -)	- (-, -)	- (-, -)
94 months after HSCT	57.9 (9.1, 88.3)	- (-, -)	- (-, -)	- (-, -)	- (-, -)	- (-, -)
Events, n (%)	7 (10.4)	8 (29.6)	2 (20.0)	6 (35.3)	1 (10.0)	5 (71.4)
Death	0	3 (11.1)	2 (20.0)	1 (5.9)	0	1 (14.3)
MFD	2 (3.0)	0	0	0	0	0
Second HSCT	2 (3.0)	5 (18.5)	0	5 (29.4)	1 (10.0)	4 (57.1)
MDS	3 (4.5)	0	0	0	0	0

	eli-cel	allo-HSCT				
	TP-102/104 N=67	**TPES-103 N=27**	**TPES-103 MSD N=10**	**TPES-103 NMSD N=17**	**TPES-103 NMSD-MUD N=10**	**TPES-103 NMSD-Mismatched N=7**
Censoring, n (%)	60 (89.6)	19 (70.4)	8 (80.0)	11 (64.7)	9 (90.0)	2 (28.6)
Withdrawal/lost to follow-up	1 (1.5)	2 (7.4)	2 (20.0)	0	0	0
Completed study	0	4 (14.8)	1 (10.0)	3 (17.6)	2 (20.0)	1 (14.3)
Study ongoing at data cut	59 (88.1)	0	0	0	0	0
Study termination by sponsor	0	13 (48.1)	5 (50.0)	8 (47.1)	7 (70.0)	1 (14.3)

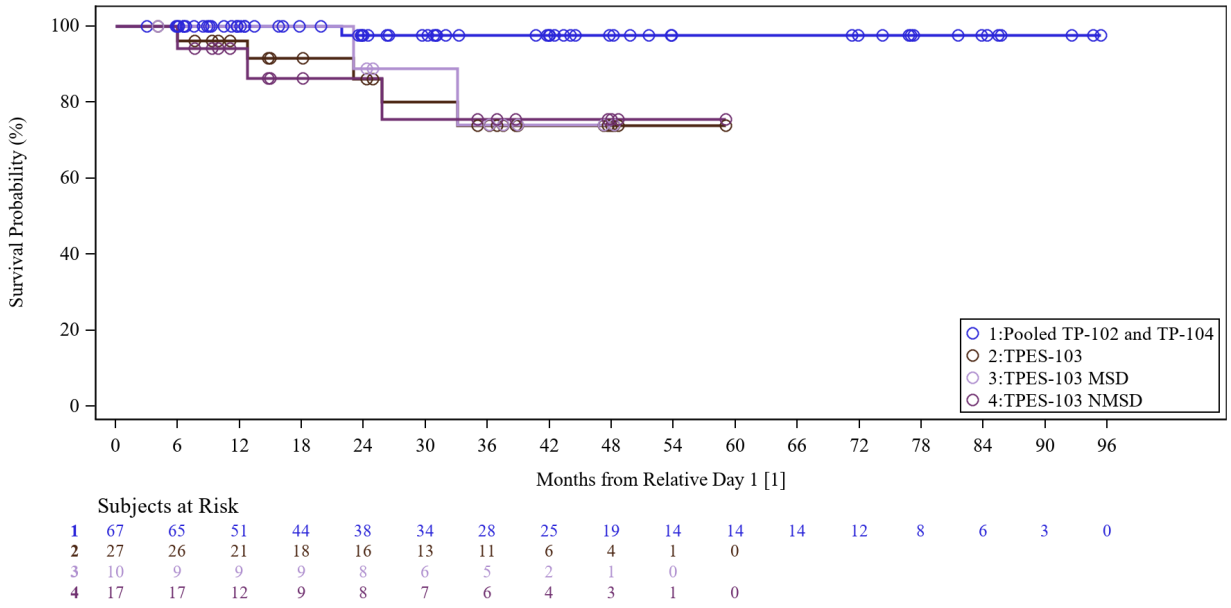
Abbrev.: MFD, major functional disability; MDS, myelodysplastic syndrome; MSD, matched sibling donor; NMSD, not a matched sibling donor; MUD, matched unrelated donor; CI, confidence interval; HSCT, hematopoietic stem cell transplantation; TP, Transplant Population; TPES, Strictly ALD-102 Eligible Transplant Population.
Estimates of Event-free survival are obtained using the Kaplan-Meier method, where events include deaths, MFDs, MDS, and rescue cell administration or second allo-HSCT. Patients who did not experience any event are censored at their date of last contact.
Rel. Day 1 is the day of eli-cel infusion for TP-102/104 and the day of allo-HSCT for TPES-103 populations.
[a] The hazard ratio of TP-102/104 vs. the other analysis population is based on an univariate Cox regression model with treatment group as the predictor.

5.3.5.2. Overall Survival

Kaplan-Meier analysis estimated an overall survival rate of 97.7% (95% CI: 84.6%, 99.7%) for TP-102/104 at 24 months after eli-cel infusion, which is higher than the corresponding estimate of 86.2% (95% CI: 62.6%, 95.4%) for the allo-HSCT treated TPES-103 population. The estimated Month 24 overall survival rate for TP-102/104 appeared higher than the rate of 88.9% (95% CI: 43.3%, 98.4%) estimated for TPES-103-MSD and was higher than the rate of 86.3% (95% CI: 54.7%, 96.5%) for TPES-103-NMSD. Based on an observed HRs of 0.082 (0.010, 0.701), eli-cel reduces the risk of death by 91.8% compared with allo-HSCT treated patient in TPES-103. The risk reduction with eli-cel is 89.5% in the subset of TPES-103 patients with an MSD and 92.5% in those without an MSD (Figure 8 and Table 10).

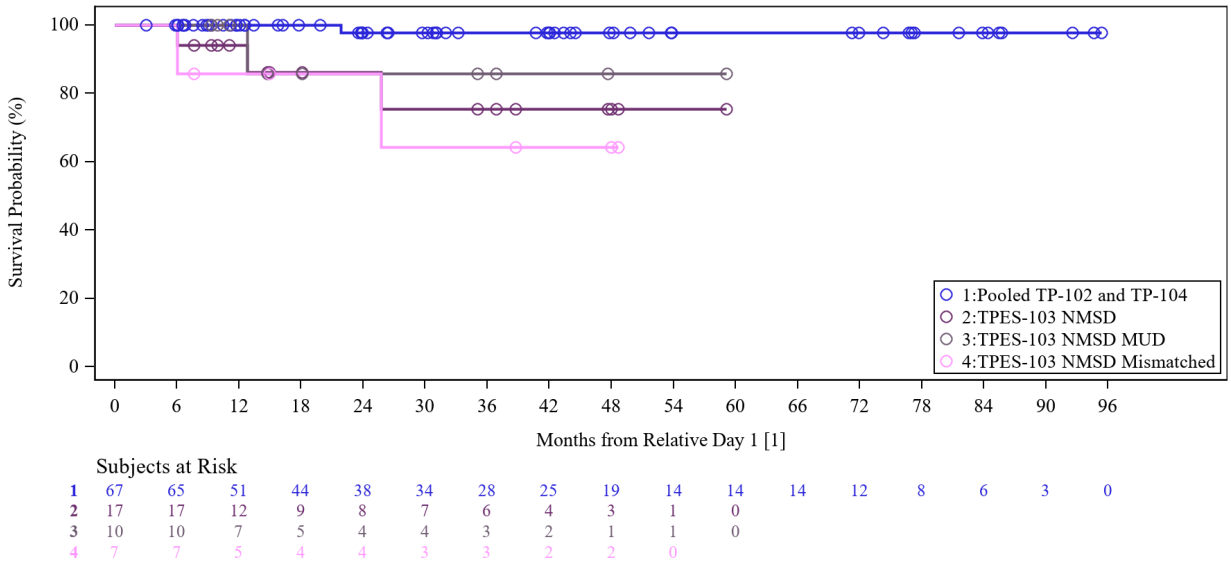
Further analyses of the TPES-103-NMSD group by histocompatibility showed that the estimated Month 24 overall survival rate for TP-102/104 appeared higher than the rates of 85.7% (33.4%, 97.9%) for TPES-103-NMSD-MUD and of 85.7% (33.4%, 97.9%) for TPES-103-NMSD-Mismatched. Based on observed HRs of 0.051 (0.005, 0.567) and 0.116 (0.007, 1.895), eli-cel reduces the risk of failing the endpoint of overall survival by 94.9% compared with TPES-103-NMSD-Mismatched and may reduce the risk of failing the endpoint of overall survival by 88.4% compared with allo-HSCT treated patient in TPES-103-NMSD-MUD, respectively (Figure 9 and Table 10).

Figure 8. Overall Survival by Donor Subgroups (TP-102/104, TPES-103, TPES-103-MSD, TPES-103-NMSD)



Abbrev.: TPES, Strictly ALD-102 Eligible Transplant Population; MSD, matched sibling donor; NMSD, not a matched sibling donor.
Kaplan-Meier method; event is death of all causes. TP-102/104 patients who withdrew to receive allo-HSCT were censored at their end of study visit; all other patients who are alive are censored at their last contact date.
Rel. Day 1 is the day of eli-cel infusion for TP-102/104 and the day of allo-HSCT for TPES-103 populations.

Figure 9. Overall Survival by Histocompatibility (TP-102/104, TPES-103-NMSD, TPES-103-NMSD-MUD, TPES-103-NMSD-Mismatched)



Abbrev.: TPES, Strictly ALD-102 Eligible Transplant Population; MSD, matched sibling donor; NMSD, not a matched sibling donor; MUD, Matched Unrelated Donor.
Kaplan-Meier method; event is death of all causes. TP 102/104 patients who withdrew to receive allo-HSCT were censored at their end of study visit; all other patients who are alive are censored at their last contact date.
Rel. Day 1 is the day of eli-cel infusion for TP-102/104 and the day of allo-HSCT for TPES-103 populations.

Table 10. Kaplan-Meier Overall Survival Analysis of eli-cel and Allogeneic-HSCT by Donor and Histocompatibility Subgroups (TP-102/104, TPES-103, TPES-103-MSD, TPES-103-NMSD, TPES-103-NMSD-MUD, TPES-103-NMSD-Mismatched)

	eli-cel	allo-HSCT				
	TP-102/104 N=67	**TPES-103 N=27**	**TPES-103 MSD N=10**	**TPES-103 NMSD N=17**	**TPES-103 NMSD-MUD N=10**	**TPES-103 NMSD-Mismatched N=7**
Overall survival, months						
Median (95% CI)	- (-, -)	- (33.1, -)	- (23.0, -)	- (25.8, -)	- (12.8, -)	- (6.0, -)
25th percentile (95% CI)	- (-, -)	33.1 (12.8, -)	33.1 (23.0, -)	- (6.0, -)	- (12.8, -)	25.8 (6.0, -)
75th percentile (95% CI)	- (-, -)	- (-, -)	- (-, -)	- (-, -)	- (-, -)	- (25.8, -)
Hazard ratio (95% CI)[a]		0.082 (0.010, 0.701)	0.105 (0.010, 1.159)	0.075 (0.008, 0.725)	0.116 (0.007, 1.895)	0.051 (0.005, 0.567)
Overall survival rate (95% CI)						
12 months after HSCT	100.0 (100.0, 100.0)	96.2 (75.7, 99.4)	100.0 (100.0, 100.0)	94.1 (65.0, 99.1)	100.0 (100.0, 100.0)	85.7 (33.4, 97.9)
24 months after HSCT	97.7 (84.6, 99.7)	86.2 (62.6, 95.4)	88.9 (43.3, 98.4)	86.3 (54.7, 96.5)	85.7 (33.4, 97.9)	85.7 (33.4, 97.9)
36 months after HSCT	97.7 (84.6, 99.7)	73.9 (47.3, 88.5)	74.1 (28.9, 93.0)	75.5 (39.7, 91.8)	85.7 (33.4, 97.9)	64.3 (15.1, 90.2)
48 months after HSCT	97.7 (84.6, 99.7)	73.9 (47.3, 88.5)	74.1 (28.9, 93.0)	75.5 (39.7, 91.8)	85.7 (33.4, 97.9)	64.3 (15.1, 90.2)
60 months after HSCT	97.7 (84.6, 99.7)	- (-, -)	- (-, -)	- (-, -)	- (-, -)	- (-, -)
72 months after HSCT	97.7 (84.6, 99.7)	- (-, -)	- (-, -)	- (-, -)	- (-, -)	- (-, -)
84 months after HSCT	97.7 (84.6, 99.7)	- (-, -)	- (-, -)	- (-, -)	- (-, -)	- (-, -)
94 months after HSCT	97.7 (84.6, 99.7)	- (-, -)	- (-, -)	- (-, -)	- (-, -)	- (-, -)
Events, n (%)	1 (1.5)	5 (18.5)	2 (20.0)	3 (17.6)	1 (10.0)	2 (28.6)
Censoring, n (%)	66 (98.5)	22 (81.5)	8 (80.0)	14 (82.4)	9 (90.0)	5 (71.4)
Withdrawal or lost to follow-up	1 (1.5)	2 (7.4)	2 (20.0)	0	0	0
Second HSCT	2 (3.0)	0	0	0	0	0
Completed study	0	4 (14.8)	1 (10.0)	3 (17.6)	1 (10.0)	2 (28.6)

	eli-cel	allo-HSCT				
	TP-102/104 N=67	**TPES-103 N=27**	**TPES-103 MSD N=10**	**TPES-103 NMSD N=17**	**TPES-103 NMSD-MUD N=10**	**TPES-103 NMSD-Mismatched N=7**
Ongoing at time of data cut	63 (94.0)	0	0	0	0	0
Study termination by sponsor	0	16 (59.3)	5 (50.0)	11 (64.7)	8 (80.0)	3 (42.9)

Abbrev.: allo-HSCT, allogeneic hematopoietic stem cell transplantation; CI, confidence interval; MSD, matched sibling donor; NMSD, not a Matched Sibling Donor; TP, Transplant Population; TPES, Strictly ALD-102 Eligible Transplant Population.
Estimates of overall survival rates are obtained using the Kaplan-Meier method, where the event is death of all causes. TP-102/104 patients who withdrew to receive allo-HSCT were censored at their end of study visit; all other patients who are alive are censored at their last contact date.
[a] The hazard ratio of TP-102/104 vs. other analysis population is based on an univariate Cox regression model with treatment group as the predictor.

5.3.5.3. Supportive Propensity Score Adjusted Analyses for Selected Efficacy Endpoints

Since randomization of patients is not feasible in the eli-cel trials due to rare disease nature and devastating disease characteristics, confounding may occur if one or more covariates are related to the treatment assignment and/or the outcome. Consequently, there can be systematic differences between the patients treated with eli-cel and those treated with allo-HSCT. The propensity score (PS) was defined by (Rosenbaum and Rubin Donald B 1983) as the probability of assignment to treatment conditional on a set of observed baseline covariates. Propensity score analysis can minimize the effect of confounding and provide some of the advantages of a randomized trial. Propensity score adjusted analyses were performed on the efficacy endpoints of event-free survival and overall survival comparing TP-102/104 versus TPES-103. Additionally, because eli-cel is proposed for patients who do not have an available and willing HLA-MSD, analyses were also performed using the subset of TPES-103 patients who had a transplant from a donor that was not from a matched sibling donor, the TPES-103-NMSD.

Based on input from clinicians and informed by the HSCT and ALD literature, baseline disease characteristic variables that are considered independently correlated with CALD prognosis were identified and used in the PS analyses, including age at CALD diagnosis (years), age at infusion (years), months from CALD diagnosis to Rel Day 1, baseline Loes score, baseline NFS score, baseline Loes pattern group, and presence of co-morbid conditions at baseline.

The results of the PS-adjusted analyses support the overall conclusions regarding comparisons of TP-102/104 to TPES-103, suggesting that eli-cel confers a clinical benefit over patients treated with allo-HSCT, even after comprehensive adjustment of multiple baseline factors.

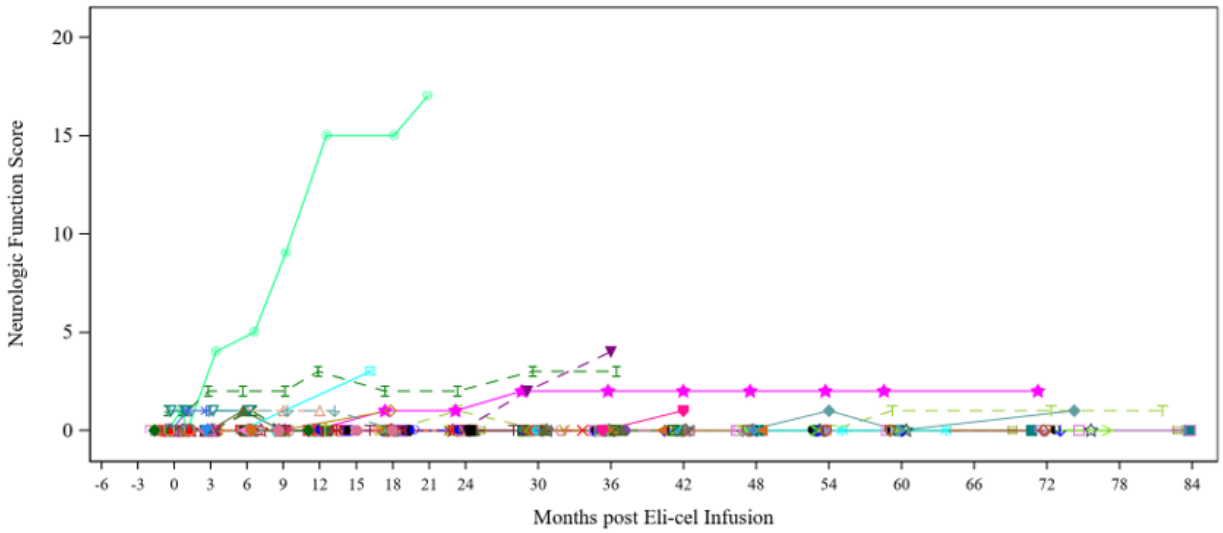
See Section 10, Appendix B for detailed information on the propensity score-adjusted analyses.

5.3.6. Supportive Clinical, Neuropsychological, and Quality of Life Endpoints

5.3.6.1. Neurologic Function Score

Overall, the TP-102/104 and TPES-103 populations had similar results for NFS. At Month 24 post-treatment, 89.2% of patients in TP-102/104 and 91.7% of patients in TPES-103 maintained their baseline NFS; an increase of ≤ 3 from baseline has been observed in 8.1% and 8.3% of TP-102/104 and TPES-103 patients, respectively. A single TP-102/104 patient (2.7%) experienced rapid disease progression within 24 months post-treatment, having an increase to an NFS of 17 at 22 months with 4 MFDs and subsequently died. Of patients with assessment after 24 months, one patient experienced an increase from NFS of 0 (at Month 24) to 4 (at Months 36 through 42) based on running difficulties, walking difficulties, vison impairment, and seizure. (Figure 10 and Table 11).

Figure 10. Neurologic Function Score Over Time (TP-102/104)



Each line represents the values for one patient over time.

Table 11. Neurologic Function Score at Month 24 (TP-102/104, TPES-103)

	TP-102/104 N=67	TPES-103 N=27
Change from Baseline at Month 24, n (%)		
n	37	12
No change	33 (89.2)	11 (91.7)
Increased ≤ 3	3 (8.1)	1 (8.3)
Increased > 3	1 (2.7)	0
NFS at Month 24, n (%)		
n	37	12
0	33 (89.2)	11 (91.7)
1	2 (5.4)	1 (8.3)
>1 to ≤ 4	1 (2.7)	0
>4	1 (2.7)	0

Abbrev.: CI, confidence interval; NFS, neurologic function score; TP, Transplant Population; TPES, Strictly ALD-102 Eligible Transplant Population.
The analysis is based on patients who have non-missing Baseline and Month 24 assessments.

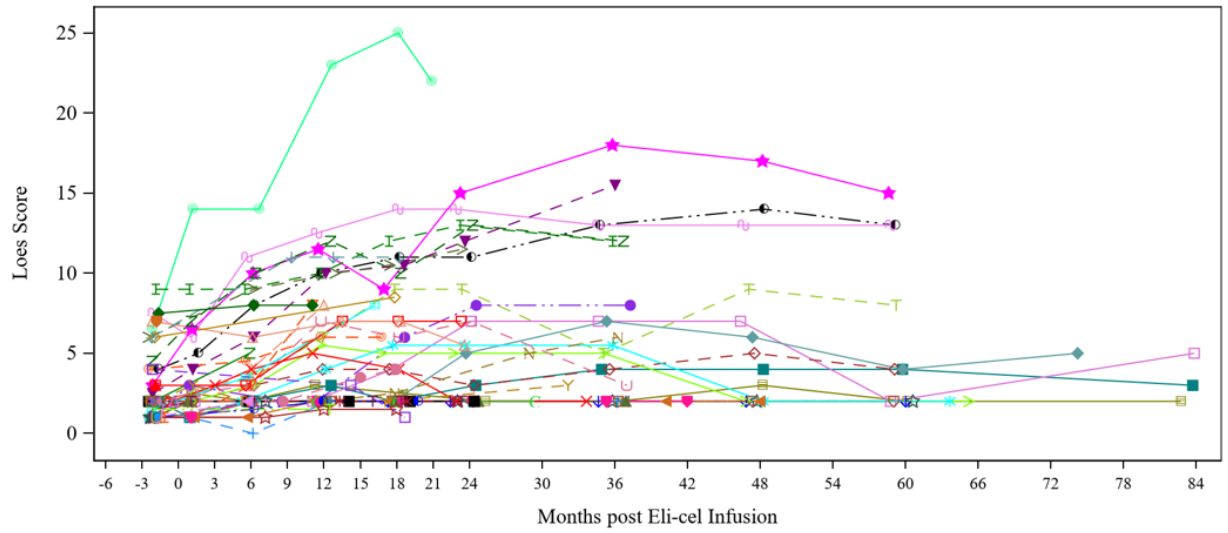
5.3.6.2. Cerebral MRI: Loes Score

Most patients treated with eli-cel showed an initial increase in cerebral MRI Loes score that stabilized by Month 24, suggesting stabilization of white matter disease occurs generally between 12 and 24 months after eli-cel infusion (Figure 11 and Table 12). The observed trend of an initially increasing cerebral MRI Loes score followed by stabilization is consistent with data from the literature that shows disease stabilization 1 to 2 years after allo-HSCT (Shapiro et al. 2000; Polgreen et al. 2011; Miller et al. 2011).

At Month 24 post-treatment, 54.3% of patients in TP-102/104 and 53.8% of patients in TPES-103 showed an increase of < 6 from baseline. A higher percentage of patients in TP-102/104 had a cerebral MRI Loes score increase of ≥ 6 (8/35 [22.9%]) than in TPES-103

(1/13 [7.7%]); Table 12). In these patients, the Loes score appeared to stabilize between 24 and 36 months after eli-cel treatment.

Figure 11. Loes Score Over Time (TP-102/104)



Each line represents the values for one patient over time.

Table 12. Cerebral MRI Loes Score at Month 24 (TP-102/104, TPES-103)

	TP-102/104 N=67	TPES-103 N=27
Stable Loes score at Month 24[a]		
Evaluable patients	35	13
n (%)	29 (82.9)	12 (92.3)
Exact 95% CI	66.4, 93.4	64.0, 99.8
Loes score at Month 24		
n	35	13
Mean (SD)	6.07 (5.031)	4.50 (4.082)
Median (25%, 75%)	5.00 (2.00, 9.00)	2.00 (2.00, 6.00)
Minimum, maximum	2.0, 22.0	0.0, 15.0
Change from Baseline, n (%)		
Decreased	1 (2.9)	4 (30.8)
No change	7 (20.0)	1 (7.7)
Increased < 6	19 (54.3)	7 (53.8)
Increased ≥ 6	8 (22.9)	1 (7.7)

Abbrev.: CI, confidence interval; TP, Transplant Population; TPES, Strictly ALD-102 Eligible Transplant Population.
[a] defined as maintaining a Loes score ≤9 or not increasing by ≥6 points from Baseline
Evaluable patients are defined as those who have completed the baseline and Month 24 MRI assessment.

5.3.6.3. Contrast Agent Enhancement on MRI

All patients in TP-102 and TP-104 were GdE+ at enrollment; all patients in TPES-103 were GdE+ prior to treatment as required by protocol. In TP-102/104, 31/35 (88.6%, 95% CI: 73.3%, 96.8%) patients treated with eli-cel were GdE- at Month 24 (Table 13). All evaluable patients in TPES-103 were GdE- after allo-HSCT at Month 24 (13/13 [100%]). Although this proportion of GdE- at Month 24 is higher than for TP-102, the confidence intervals overlap, and the clinical significance is unknown. Patients with re-emergence of gadolinium enhancement did not show faster progression of neurologic function scores than patients without re-emergence after eli-cel treatment.

Table 13. GdE- Status at Month 24 (TP-102/104, TPES-103)

	TP-102/104 N=67	TPES-103 N=27
Patients who are GdE- at Month 24		
Evaluable patients[a]	35	13
n (%)	31 (88.6)	13 (100)
Exact 95% CI	73.3, 96.8	75.3, 100

Abbrev.: CI, confidence interval; GdE, gadolinium enhancement (on cerebral MRI); TP, Transplant Population; TPES, Strictly ALD-102 Eligible Transplant Population

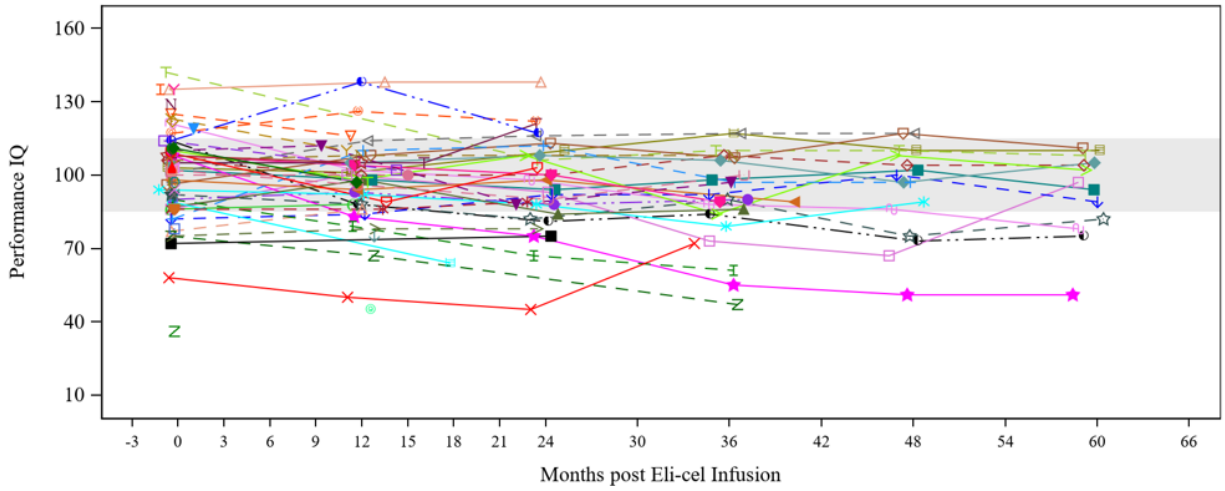
[a] Evaluable patients are defined as those who have completed the Month◦24 MRI assessment.

5.3.6.4. Neuropsychological Testing

The neuropsychological test results support the other efficacy findings and provide additional evidence of meaningful neurologic disease stabilization, including stabilization of cognitive function, in patients treated with eli-cel.

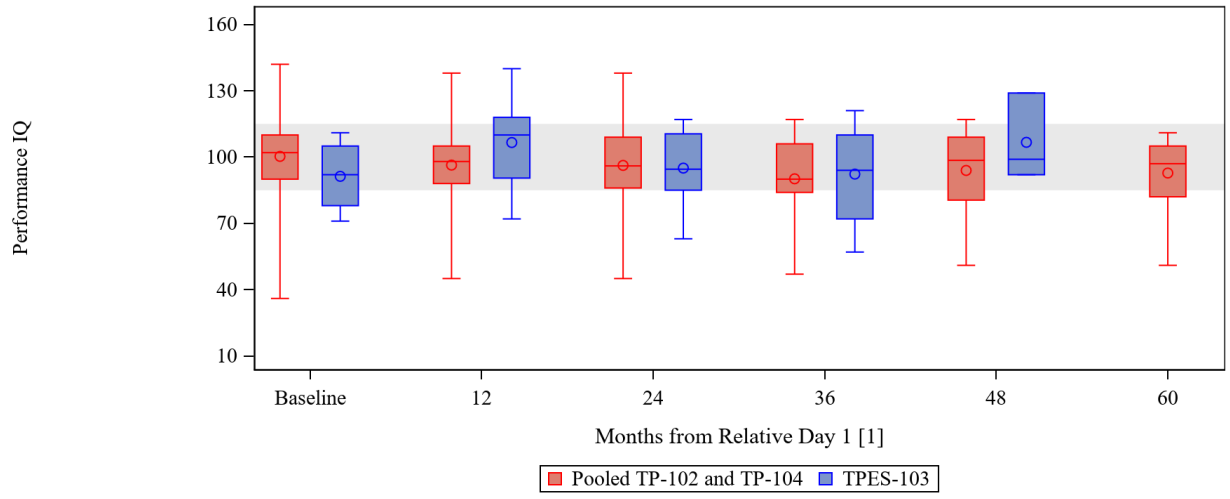
The PrvIQ (Performance/Reasoning/Visual IQ subset) subscale is sensitive to CALD disease progression and less biased than Verbal and Full-Scale IQ with regards to a patient's primary language, as several patients were not native English speakers. Most TP-102/104 patients maintained a PrvIQ within or near the normal range after eli-cel treatment (Figure 12). While modest decreases in PrvIQ (Figure 12) were observed especially at early timepoints following treatment, PrvIQ appeared generally stable after Month 24 through the last timepoint in the majority of patients. The PrvIQ observed in TP-102/104 was comparable to TPES-103 (Figure 13) and closely aligned to results reported for allo-HSCT by (Pierpont et al. 2017).

Figure 12. Performance/Reasoning/Visual Intelligence Quotient Subset (PrvIQ) Over Time by Patient (TP-102/104)



Each line represents the values for one patient over time.
Abbrev.: PrvIQ, performance/reasoning/visual intelligence quotient.
Gray area marks the normal range (i.e., 100 ± 15) for PrvIQ
One patient's Month 48 value is excluded due to a confirmed data entry error.

Figure 13. Performance/Reasoning/Visual Intelligence Quotient Subset (PrvIQ) Over Time in eli-cel compared to allo-HSCT (TP-102/104, TPES-103)



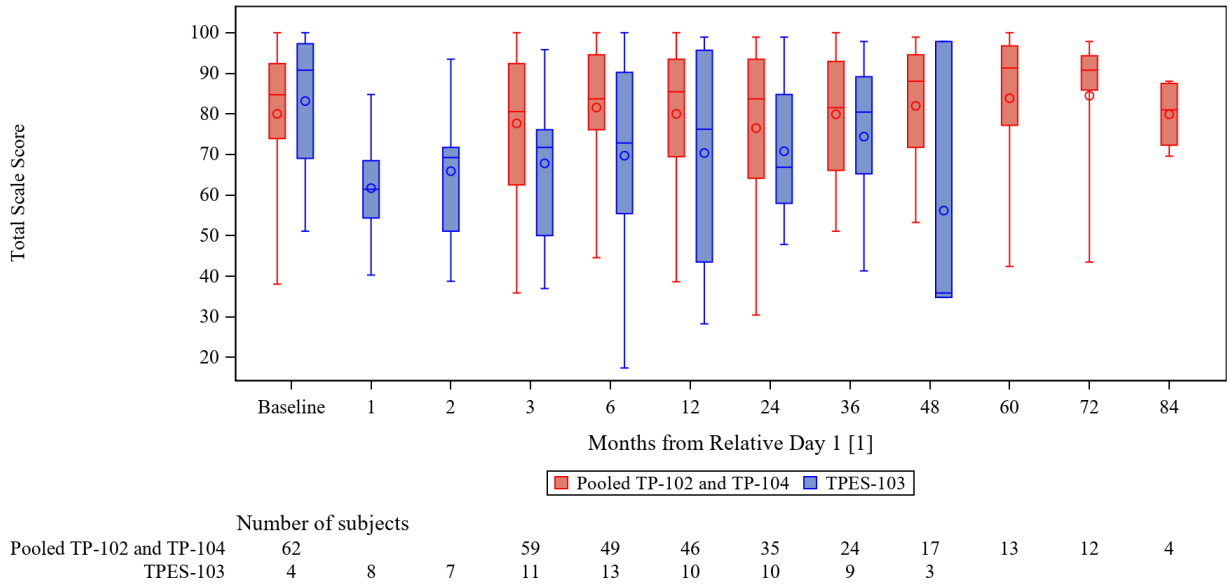
	Number of subjects					
Pooled TP-102 and TP-104	62	45	32	26	16	13
TPES-103	11	12	8	7	3	

[1] Relative Day 1 is the day of HSCT or eli-cel administration
Abbrev.: PrvIQ, performance/reasoning/visual intelligence quotient from Wechsler testing.
Gray area marks the normal range (i.e., 100 ± 15) for PrvIQ
One patient's Month 48 value is excluded due to a confirmed data entry error.
Circle indicates mean, line inside the box indicates median, box indicates 25th-75th percentile, and whiskers indicate range.

5.3.6.5. Quality of Life Assessment

Overall, the PedsQL scores support the treatment benefit of eli-cel. The scores for the Total PedsQL scale, that encompasses physical, emotional, social, and school functioning domains, albeit demonstrating substantial variability, trended higher than scores available for the allo-HSCT treated patients in TPES-103 (Figure 14).

Figure 14. Pediatric Quality of Life Total Score Over Time (TP-102/104, TPES-103)



[1] Relative Day 1 is the day of HSCT or eli-cel administration
[2] Only scores collected from Caregive Forms are included
[3] Only assessments in the 1st allo-HSCT period are included in TPES-103

5.4. Efficacy Summary

Results from the eli-cel clinical development program indicate that eli-cel stabilized neurologic disease and preserved cognitive function in the majority of patients with early CALD. eli-cel is unequivocally superior to no treatment and provided comparable neurologic disease stabilization to allo-HSCT. Direct clinical observations such as the NFS and neuropsychological testing support these findings.

MFD-free survival, reflecting the absence of major events such as death, major functional disability, or requirement for a second stem cell transplantation, showed a compelling and statistically significant effect of eli-cel when compared to the pre-specified benchmark of 50%, i.e., compared to no treatment. Among the 32 patients in the pivotal study ALD-102, 29 achieved the primary efficacy endpoint of Month 24 MFD-free survival (90.6%; exact 95% CI: 75.0%, 98.0%), indicating that the vast majority of patients were MFD-free at 24 months after eli-cel infusion. Similar interim results have been observed in evaluable patients in the ongoing supportive study ALD-104.

Event-free survival after eli-cel treatment was high, especially when compared with allo-HSCT patients with a mismatched donor. In pooled analyses of the completed pivotal study ALD-102 with the ongoing ALD-104 and LTF-304 studies, eli-cel maintained an estimated event-free

survival rate of 86.8% (95% CI: 72.7%, 93.9%) through 7 years of follow-up. eli-cel compared favorably with allo-HSCT without an MSD (estimated rate at Month 24 of 70.6% [95% CI: 43.1%, 86.6%]). Further analyses by histocompatibility showed that the estimated Month 24 event-free survival rate for eli-cel was similar to the rate of 90.0% (95% CI: 47.3%, 98.5%) for allo-HSCT with MUD, and notably higher than the rate of 42.9% (95% CI: 9.8%, 73.4%) for allo-HSCT with mismatched donor. Based on observed hazard ratios (HR) of 0.783 (95% CI: 0.094, 6.524) and 0.061 (95% CI: 0.018, 0.205), eli-cel may reduce the risk of failing the endpoint of event free survival by 21.7% compared to allo-HSCT patients with MUD, and reduces the risk by 93.9% compared with allo-HSCT patients with a mismatched donor.

Overall survival after eli-cel treatment was high. In pooled analyses of the completed pivotal study ALD-102 with the ongoing ALD-104 and LTF-304 studies, eli-cel maintained an estimated overall survival rate of 97.7% (95% CI: 84.6%, 99.7%) from Month 24 through 7 years of follow-up. The estimated Month 24 overall survival rate for TP-102/104 compared favorably with allo-HSCT without MSD (estimated rate at Month 24 of 86.3% [95% CI: 54.7%, 96.5%]). Further analyses by histocompatibility showed that the estimated Month 24 overall survival rate for eli-cel appeared higher than the rates of 85.7% (95% CI: 33.4%, 97.9%) for allo-HSCT with MUD and of 85.7% (95% CI: 33.4%, 97.9%) for allo-HSCT with mismatched donor. Based on observed HRs of 0.116 (0.007, 1.895) and 0.051 (0.005, 0.567), eli-cel may reduce the risk of death by 88.4% compared to allo-HSCT with MUD and reduces the risk of death by 94.9% compared with allo-HSCT with mismatched donor.

Propensity score adjusted analyses accounting for potential baseline distribution differences support the conclusions of the primary analysis.

Overall, the totality of the data demonstrates substantial evidence of eli-cel’s efficacy in patients with early CALD.

6. SAFETY

As an autologous therapy, eli-cel allows for treatment without the immune-mediated complications of allogenic hematopoietic stem cell transplant (e.g., GVHD, graft rejection, TRM). However, gene therapy carries unique risks, such as insertional oncogenesis, which must be balanced against the known risks of allo-HSCT.

The safety assessment derives from completed study ALD-102 and ongoing studies ALD-104 and LTF-304 from the eli-cel development program. Sixty-seven patients were treated with eli-cel. Enrollment and treatment are complete; thus, 67 treated patients constitute the final clinical trial population for this program. A contemporaneous external control study of allo-HSCT (ALD-103) is presented for comparison. The safety of eli-cel is primarily assessed from the pooled TP-102/104 and compared with CALD patients treated with allo-HSCT in TP-103 as relevant.

6.1. Analysis Populations and Subgroups Analyzed for Safety

The populations used in safety analyses include:

- The **Intent-to-treat population (ITT)** consists of patients who initiated any study procedures, beginning with mobilization by G-CSF. All patients enrolled in ALD-102 and ALD-104 underwent autologous transplant with eli-cel, and therefore the ITT and TP are identical.
- The **Transplant Population (TP)** consists of patients who underwent an eli-cel infusion or allo-HSCT; abbreviated as TP-102, TP-104, TP-102/104, and TP-103, as relevant. Data from LTF-304 were integrated with data from each parent study for a given patient. The primary populations used for comparison are the TPs, with the primary between-study safety evaluations comparing the TP-102/104 pool and TP-103. In selected analyses, specific subgroups of TP-103 are used for comparative purposes, such as patients who received allo-HSCT from an MSD, an NMSD, or the NMSD subgroups (MUD or Mismatched).
- The **Strictly ALD-102-Eligible Transplant Population (TPES)** consists of patients in TP-103 who are comparable to patients in ALD-102 (and thus ALD-104) based on baseline characteristics. These patients have baseline NFS, cerebral MRI Loes score, and GdE status that would have made them strictly eligible for studies ALD-102 and ALD-104, abbreviated as TPES-103. Of note, the TPES-103 population includes a higher proportion of MSD patients than TP-103 and thus the assessment of safety in TPES-103 is confounded by donor category. A medical review of the safety profiles of TP-103 and TPES-103 identified limited differences; therefore, TP-103 is the primary population for comparison. The TPES population is only utilized in the discussion of AEs in the Nervous system disorders System Organ Class (SOC) and in the discussion of deaths.
- The **Successful Neutrophil Engraftment Population (NEP)** consists of patients who achieved NE [defined as 3 consecutive absolute neutrophil count (ANC) laboratory values of $\geq 0.5\times10^9$ cells/L obtained on different days by 42 days (Rel Day 43)] after first eli-cel infusion or allo-HSCT; abbreviated as NEP-102/104 and NEP-103. This population is only utilized in the discussion of hospitalization after NE.

6.2. Primary Safety Assessment

The primary safety endpoint, the proportion of patients who experience either acute GVHD (≥ Grade II) or chronic GVHD by Month 24, was statistically tested in TP-102 for superiority over TP-103 as the primary safety success criterion. For all other safety analyses, p-values are provided as descriptive measures.

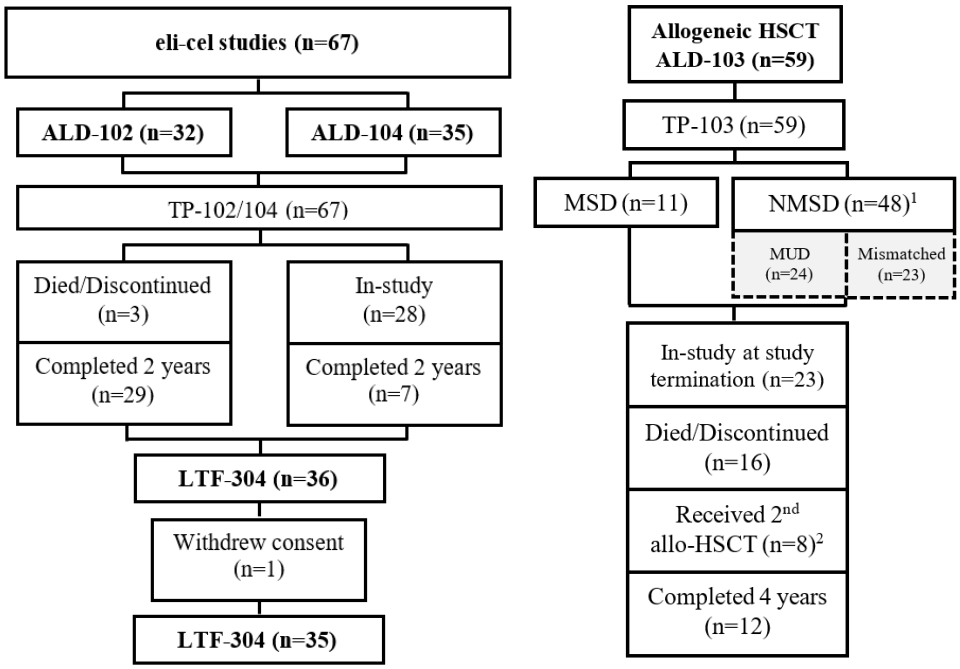
6.3. Extent of Exposure

Treatment with eli-cel is preceded by the procedural and medical interventions of mobilization/apheresis with G-CSF, with or without plerixafor, followed by myeloablative and lymphodepleting conditioning before transplantation. These procedures carry their own risks and are therefore included in this exposure section.

6.3.1. Disposition

Figure 15 shows the disposition of patients in TP-102/104 and TP-103.

Figure 15. Disposition of patients in TP-102/104 and TP-103



[1] One patient is a matched related donor under NMSD (not pictured in the figure)
[2] An additional patient required a subsequent transplant following Month 48

Sixty-seven patients were treated in ALD-102 (N=32) and ALD-104 (N=35). The TP-102/104 population therefore includes 67 patients (Table 14).

All 32 patients enrolled in ALD-102 received eli-cel (Table 14). One patient died (1/32; 3.1%) and 2 patients (2/32; 6.3%) discontinued the study to receive allo-HSCT. Twenty-nine patients completed their Month 24 visits and all 29 subsequently enrolled in LTF-304.

All 35 patients enrolled in ALD-104 received eli-cel treatment (Table 14). Seven patients have completed the study and subsequently enrolled in LTF-304 and 28 patients were still followed in ALD-104.

Table 14. Disposition of Patients by Study (TP-102, TP-104, TP-102/104)

Parameter	TP-102 N=32 n (%)	TP-104 N=35 n (%)	TP-102/104 N=67 n (%)
Initiated mobilization	32 (100)	35 (100)	67 (100)
Initiated conditioning	32 (100)	35 (100)	67 (100)
Received eli-cel[a]	32 (100)	35 (100)	67 (100)
In study by data cut	28 (87.5)	35 (100)	63 (94.0)
Discontinued parent or follow-up study[b]	4 (12.5)	0	4 (6.0)
Completed Study ALD-102 or ALD-104	29 (90.6)	7 (20.0)	36 (53.7)
Reasons for Discontinuation			
Death	1 (3.1)	0	1 (1.5)
Received allo-HSCT	2 (6.3)	0	2 (3.0)
Refused further follow-up	1 (3.1)	0	1 (1.5)

Abbrev.: allo, allogeneic; HSCT, hematopoietic stem cell transplantation; TP, transplant population.
For ALD-102 and ALD-104 patients, the discontinuation reason from ALD-102 or ALD-104 is presented if the patient discontinued in the parent study, otherwise the discontinuation from LTF-304 is presented.
[a] The TP consists of patients who received an HSCT, including those who received eli-cel in studies ALD-102 and ALD-104 (TP-102 and TP-104, respectively).
[b] One patient died, 2 patients discontinued the parent study to receive allo-HSCT, 1 patient withdrew from LTF-304.

Following first allo-HSCT in ALD-103, 12 of the 59 enrolled patients discontinued due to death (20.3%) and 8/59 patients needed a subsequent allo-HSCT (13.6%) by Month 48 (Table 15). One additional patient received a subsequent transplant following Month 48.

The Sponsor terminated ALD-103 because the objective of collecting contemporaneous data on allo-HSCT for the treatment of CALD was met. Therefore, the primary reason for patient discontinuation in ALD-103 is 'Study terminated by Sponsor'.

Table 15. Disposition of Patients In ALD-103 by Donor Subgroup (TP-103)

Parameter	TP-103		
	Overall N=59 n (%)	MSD N=11 n (%)	NMSD N=48 n (%)
Initiated conditioning	59 (100)	11 (100)	48 (100)
Received an HSCT (TP)	59 (100)	11 (100)	48 (100)
Completed Month 48 in study	12 (20.3)	1 (9.1)	11 (22.9)
Discontinued study	47 (79.7)	10 (90.9)	37 (77.1)

Parameter	TP-103 Overall N=59 n (%)	TP-103 MSD N=11 n (%)	TP-103 NMSD N=48 n (%)
Reasons for Discontinuation[a]			
Protocol deviation[b]	2 (3.4)	1 (9.1)	1 (2.1)
Lost to follow-up	2 (3.4)	1 (9.1)	1 (2.1)
Death	12 (20.3)	2 (18.2)	10 (20.8)
Receive allo-HSCT[c]	8 (13.6)	0	8 (16.7)
Study terminated by Sponsor	23 (39.0)	6 (54.5)	17 (35.4)

Abbrev.: allo, allogeneic; HSCT, hematopoietic stem cell transplantation; MSD, matched sibling donor; NMSD, not a matched sibling donor; TP, transplant population.
[a] For ALD-103 patients who had multiple allo-HSCTs, the discontinuation reason for the initial allo-HSCT is presented.
[b] Stated in clinical study report as patients who were unable to comply with protocol-defined visits.
[c] 1 patient had a second allo-HSC infusion but is not included in this count because the second allo-HSC infusion occurred after completion of the Month 48 Visit of the first allo-HSCT period.

6.3.2. Exposure in eli-cel-Treated Patients

6.3.2.1. Mobilization: Exposure to G-CSF and Plerixafor

All patients treated with eli-cel underwent mobilization and apheresis for collection of autologous cells for drug product manufacture. In ALD-102, all patients received G-CSF, and 11/32 (34.4%) patients also received plerixafor for mobilization. All patients in ALD-104 received both G-CSF and plerixafor. Dosing of G-CSF and plerixafor was performed according to the relevant approved prescribing information for each agent. All patients underwent a single cycle of mobilization to meet the minimum drug product cell dose of $\geq 5.0 \times 10^6$ CD34+ cells/kg.

In ALD-102, each mobilization cycle ranged from 5 to 9 days, with cell collection beginning 3 to 5 days after initiation of mobilization. Patients received average daily doses of G-CSF of 8.9 to 12.5 μg/kg/day, and daily doses of plerixafor 0.24 mg/kg/day.

In ALD-104, each mobilization cycle ranged from 5 to 7 days, with cell collection beginning 5 to 7 days after initiation of mobilization. Patients received average daily doses of G-CSF of 10.0 to 11.0 μg/kg/day, and daily doses of plerixafor of 0.24 mg/kg/day.

6.3.2.2. Conditioning: Exposure to Conditioning Agents

All patients treated with eli-cel underwent myeloablation with busulfan and lymphodepletion with either cyclophosphamide (ALD-102) or fludarabine (ALD-104). In both studies, there was a minimum of 48 hours of washout between conditioning and eli-cel infusion.

In ALD-102, all 32 patients underwent myeloablation with busulfan and lymphodepletion with cyclophosphamide. Busulfan was administered over 4 days, and the median (min, max) estimated average daily busulfan area under the curve (AUC) was 4717.5 (4039, 5041) μM*min. Cyclophosphamide was administered at 50 mg/kg/day for 4 days (totaling 200 mg/kg). The median (min, max) total dose of cyclophosphamide was 199.15 (150.6, 212.9) mg/kg.

In ALD-104, all 35 patients underwent myeloablation with busulfan and lymphodepletion with fludarabine. Busulfan was administered over 4 days, and the median (min, max) estimated average daily busulfan AUC was 5303.0 (3478, 5695) μM*min. Initially, fludarabine dosing was 30 mg/m^2 for 6 days, but was later adjusted to 40 mg/m^2 for 4 days. The majority of patients received fludarabine 160 mg/m^2 (15 patients) or 180 mg/m^2 (11 patients).

6.3.2.3. Exposure to eli-cel

All eli-cel-treated patients received a single lot of eli-cel with a dose of ≥ 5.0 × 10^6 CD34+ cells/kg and met the minimum cell dose requirement per protocol (Table 16).

Table 16. eli-cel Dosing Information (TP-102, TP-104, TP-102/104)

Parameter	TP-102 N=32	TP-104 N=35	TP-102/104 N=67
Cell dose (× 10^6 CD34+ cells/kg)			
n	32	35	67
Median	11.4	12.8	12.0
Min, Max	5.0, 20.1	5.1, 38.2	5.0, 38.2
Drug product VCN (c/dg)			
n	32	35	67
Median	1.2	1.3	1.3
Min, Max	0.5, 2.7	0.7, 3.1	0.5, 3.1

Abbrev.: c/dg; copies per diploid genome; max, maximum; min, minimum; TP, transplant population; VCN, vector copy number.

6.3.3. Exposure to Allo-HSCT

In ALD-103, patients were exposed to a variety of conditioning regimens according to institutional guidelines, including busulfan (96.6%) with either cyclophosphamide (47.5%) and/or fludarabine (64.4%) for lymphodepletion. Some patients received other conditioning agents such as anti-thymocyte globulin (47.5%) or alemtuzumab (23.7%) for enhanced lymphodepletion.

Patients in ALD-103 (N=59) received allo-HSCs from either an MSD (11/59) or NMSD (48/59). Safety outcomes were anticipated to be better for those with an MSD (Raymond et al. 2019). Within the NMSD subgroup, patients received allo-HSCs from either an MUD (24/48) or mismatched donor (23/48). Of note, 1 patient had a matched related non-sibling donor and therefore is not included in the MUD or Mismatched subgroups.

Eight of 59 (13.6%) patients in ALD-103 required a second allo-HSCT by Month 48; 1 of these patients subsequently underwent a third allo-HSCT. One additional patient received a second allo-HSCT infusion after Month 48.

6.3.4. Duration of Follow-Up

Follow up after transplant [median (min, max)] was 23.5 (1.4, 88.1) months for TP-102/104 and 23.0 (0.9, 49.5) months for TP-103 (Table 17).

Table 17. Duration of Follow-up (TP-102, TP-104, TP-102/104, TP-103)

Parameter	eli-cel			allo-HSCT
	TP-102 N=32	TP-104 N=35	TP-102/104 N=67	TP-103 N=59
Duration of follow-up, months post HSCT[a]				
n	32	35	67	59
Median	49.0	6.3	23.5	23.0
Min, Max	13.4, 88.1	1.4, 26.9	1.4, 88.1	0.9, 49.5
Patient-years of follow-up[b]				
Patient years	145.4	33.1	178.6	115.6

Abbrev.: allo, allogeneic; HSCT, hematopoietic stem cell transplantation; max, maximum; min, minimum; TP, transplant population.
[a] Duration of follow-up is the time from day of eli-cel infusion or day of initial allo-HSCT to day of last contact in the first allo-HSCT period. Includes follow-up time from Study LTF-304.
[b] Patient-years of follow-up is the sum over all patients' duration of follow-up.

6.4. Demographics, Baseline Disease Characteristics, and Concomitant Medication Use

6.4.1. Demographics and Baseline Disease Characteristics

Patients treated with eli-cel were all male, age 4 to 14 years at the time of HSCT, as is typical of the CALD population (Table 18). Most of the patients who reported race were White (36/67 [54%]). Baseline neurologic assessments were consistent with early-stage disease at high risk for progression. Demographics were similar between TP-102/104 and TP-103, and disease characteristics were similar between TP-102/104 and TPES-103.

Table 18. Baseline Demographics and Disease Characteristics (TP-102/104, TP-103, TPES-103)

	eli-cel	allo-HSCT			
	TP-102/104 N=67	TP-103 Overall N=59	TP-103 MSD N=11	TP-103 NMSD N=48	TPES-103 N=27
Sex					
Male	67 (100)	59 (100)	11(100)	48 (100)	27 (100)
Race, n (%)					
White	36 (54)	51 (86)	10 (91)	41 (85)	25 (93)
Black/African American	3 (4)	2 (3)	0	2 (4)	0
Asian	1 (1)	1 (2)	1 (9)	0	0
Other	7 (10)	3 (5)	0	3 (6)	2 (7)
Not provided, known, or reported	20 (30)	2 (3)	0	2(4)	0
Ethnicity, n (%)					
Hispanic	17 (25)	12 (20)	3 (27)	9 (19)	7 (26)
Non-Hispanic	41 (61)	32 (54)	5 (45)	27 (56)	11 (41)

	eli-cel	allo-HSCT			
	TP-102/104 N=67	**TP-103 Overall N=59**	**TP-103 MSD N=11**	**TP-103 NMSD N=48**	**TPES-103 N=27**
Not provided, known, or reported	9 (13)	15 (25)	3 (27)	12 (25)	9 (33)
Baseline NFS, n (%)[a]					
0	64 (95.5)	43 (72.9)	10 (90.9)	33 (68.8)	26 (96.3)
1	3 (4.5)	7 (11.9)	0	7 (14.6)	1 (3.7)
> 1 and ≤ 4	0	4 (6.8)	1 (9.1)	3 (6.3)	0
> 4	0	1 (1.7)	0	1 (2.1)	0
Missing	0	4 (6.8)	0	4 (8.3)	0
Baseline Loes score[b]					
n	67	56	10	46	27
Median	2.00	4.25	3.50	5.25	3.00
Min, max	1.0, 9.0	0.0, 18.5	1.0, 9.0	0.0, 18.5	1.0, 9.0
Age at diagnosis of CALD, years					
Median	6	7	7	8	7
Min, max	1, 13	0, 14	6, 9	0, 14	0, 11
Age at first HSCT, years					
Median	6	8	8	8	8
Min, max	4, 14	2, 14	6, 9	2, 14	5, 11
Age at first HSCT, n (%)[c]					
< 2 years	0	0	0	0	0
≥ 2 to < 6 years	21 (31.3)	7 (11.9)	0	7 (14.6)	3 (11.1)
≥ 6 to < 12 years	43 (64.2)	49 (83.1)	11 (100)	38 (79.2)	24 (88.9)
≥ 12 to < 18 years	3 (4.5)	3 (5.1)	0	3 (6.3)	0
≥ 18 years	0	0	0	0	0

Abbrev.: allo, allogeneic; CALD, cerebral adrenoleukodystrophy; HSCT, hematopoietic stem cell transplantation; max, maximum; min, minimum; MRI, magnetic resonance imaging; MSD, matched sibling donor; NFS, neurologic function score; NMSD, not a matched sibling donor; TP, transplant population; TPES, strictly ALD-102-eligible transplant population.

[a] NFS is a 25-point composite scale that assesses functional disabilities. The scoring ranges from 0 to 25, with 0 indicating normal functioning on all parameters (Moser et al. 2000).

[b] Brain MRI imaging is used to determine Loes score. The 34-point Loes scoring scale measures the extent and location of brain abnormalities such as the presence of white matter changes, degree of demyelination, and the presence of focal or global atrophy (Loes et al. 1994). A Loes score of 0 indicates a normal brain MRI image.

[c] HSCT refers to either eli-cel in ALD-102/104 and allo-HSCT in ALD-103.

6.4.2. Concomitant Medication Use

In the peri-transplant period, similar concomitant medications were used in eli-cel and allo-HSCT studies. For example, patients received antiemetics, blood/platelet transfusions, symptomatic treatment for side effects of conditioning, prophylaxis for infections, and steroids to treat adrenal insufficiency/prevent adrenal crises.

Meaningful differences in concomitant medications were seen after patients achieved NE and were discharged from hospital. In contrast to patients treated with eli-cel, patients in TP-103

required immunosuppressive drugs to prevent or treat allograft rejection and GVHD, in addition to therapy to manage the complications of immunosuppressants such as antihypertensives and anti-infectives.

6.5. Neutrophil and Platelet Engraftment

Initial hematopoietic reconstitution after transplant was assessed by NE and PE. Neutrophil engraftment was defined as 3 consecutive absolute neutrophil counts (ANC) $\geq 0.5 \times 10^9$ cells/L after initial post-infusion nadir obtained on different days by 42 days post-HSCT (Rel Day 43). The first day of the 3 different days with ANC $\geq 0.5 \times 10^9$ cells/L is considered the day of engraftment. A patient was considered to have primary engraftment failure if he did not achieve NE by Rel Day 43. A patient was considered to have secondary engraftment failure if he achieved and then subsequently lost NE by Month 24. Platelet engraftment was defined as 3 consecutive platelet counts $\geq 20 \times 10^9$ cells/L obtained on different days while no platelet transfusions were administered for 7 days immediately preceding and during the evaluation period.

6.5.1. Neutrophil Engraftment

All eli-cel treated patients (67/67, 100%) had successful primary NE, with median (min, max) NE occurring on Rel Day 13 (11, 41) post-transplant (Table 19). For the allo-HSCT patients in TP-103, 53/59 (89.8%) patients achieved primary NE, with a median (min, max) NE on Rel Day 17 (12, 36).

In TP-102/104, no evaluable patients experienced primary or secondary graft failure by Month 24 compared with 10/38 (26.3%) evaluable patients in TP-103 (Table 19). All patients who experienced graft failure were NMSD patients; 6 had primary engraftment failure and 4 had secondary engraftment failure. Nine of these patients received a second allo-HSCT, and 1 patient underwent a third allo-HSCT after experiencing primary engraftment failure after both first and second allo-HSCTs.

Table 19. Neutrophil Engraftment (TP-102/104, TP-103)

	eli-cel	allo-HSCT		
	TP-102/104 N=67	**TP-103 Overall N=59**	**TP-103 MSD N=11**	**TP-103 NMSD N=48**
NE by Rel Day 43				
Number of patients evaluable[a]	67	59	11	48
Patients with NE after first HSCT, n (%)	67 (100)	53 (89.8)	11 (100)	42 (87.5)
Exact 95% CI	94.6, 100	79.2, 96.2	71.5, 100	74.8, 95.3
TP-102/104 comparison p-value[b]		0.0091	-	0.0044
Primary NE failure				
n (%)	0	6 (10.2)	0	6 (12.5)
Exact 95% CI	0, 5.4	3.8, 20.8	0, 28.5	4.7, 25.2
TP-102/104 comparison p-value[b]		0.0091	-	0.0044

	eli-cel	allo-HSCT		
	TP-102/104 N=67	TP-103 Overall N=59	TP-103 MSD N=11	TP-103 NMSD N=48
Secondary NE failure by Month 24[c]				
Number of patients evaluable[d]	42	32	8	24
n (%)	0	4 (12.5)	0	4 (16.7)
Exact 95% CI	0, 8.4	3.5, 29.0	0, 36.9	4.7, 37.4
TP-102/104 comparison p-value[b]		0.0313	-	0.0147
Primary or secondary NE failure by Month 24[d]				
Number of patients evaluable[d]	42	38	8	30
n (%)	0	10 (26.3)	0	10 (33.3)
Exact 95% CI	0, 8.4	13.4, 43.1	0.0, 36.9	17.3, 52.8
TP-102/104 comparison p-value[b]		0.0003	-	< 0.0001
Rel Day of NE				
n	67	53	11	42
Median	13.0	17.0	17.0	17.5
Min, max	11, 41	12, 36	12, 23	12, 36
TP-102/104 comparison p-value[b]		< 0.0001	0.1295	< 0.0001

Abbrev.: allo, allogeneic; CI, confidence interval; HSCT, hematopoietic stem cell transplantation; max, maximum; min, minimum; MSD, matched sibling donor; NE, neutrophil engraftment; NMSD, not a matched sibling donor; Rel Day, Relative Study Day after infusion; TP, transplant population.
Parameters are only reported following first allo-HSCT for patients in ALD-103.
'-' indicates that the p-value was not calculable because patients in both groups either all had or did not have the applicable event.
[a] Evaluable patients include those who had NE or had been followed to at least Rel Day 43.
[b] P-value was based on Fisher's exact test comparing TP-102/104 versus other populations.
[c] Evaluable patients include those who achieved NE and either had secondary engraftment failure or had been followed for at least 24 months if no events.
[d] Evaluable patients include those who were evaluable for primary or secondary engraftment failure.

6.5.2. Platelet Engraftment

All eli-cel-treated patients (67/67, 100%) had successful PE (Table 20), with median (min, max) PE occurring on Rel Day 29 (14, 108). For the evaluable allo-HSCT patients in TP-103, 47/47 (100%) patients achieved PE, with a median (min, max) PE on Rel Day 26 (13, 67).

The majority of patients in TP-102/104 achieved PE by Day 60 (n=64), which is within the range of the Rel Day of PE in TP-103. Three patients experienced PE after Rel Day 100 in TP-102/104, achieving PE on Rel Days 104, 106, and 108, and each had significant underlying pathology that contributed to later PE; 2 patients were subsequently diagnosed with MDS (see Section 6.6.5.1) and 1 subject had concurrent parvovirus B19 infection (see Section 6.6.5.4).

Table 20. Platelet Engraftment (TP-102/104, TP-103)

	eli-cel	allo-HSCT		
	TP-102/104 N=67	TP-103 Overall N=59	TP-103 MSD N=11	TP-103 NMSD N=48
PE				
Number of patients evaluable[a]	67	47	11	36
Patients who achieved PE after first HSCT, n (%)	67 (100)	47 (100)	11 (100)	36 (100)
Exact 95% CI	94.6, 100	92.5, 100	71.5, 100	90.3, 100
TP-102/104 comparison p-value[b]		-	-	-
Rel Day of PE				
n	67	47	11	36
Median	29	26.0	26.0	27.5
Min, Max	14, 108	13, 67	20, 47	13, 67
TP-102/104 comparison p-value[c]		0.2319	0.3356	0.3326

Abbrev.: allo, allogeneic; CI, confidence interval; HSCT, hematopoietic stem cell transplantation; max, maximum; min, minimum; MSD, matched sibling donor; NMSD, not a matched sibling donor; PE, platelet engraftment; Rel Day, Relative Study Day after infusion; TP, transplant population.
Data only include PE in the first allo-HSCT period for patients in ALD-103.
'-' means the p-value was not calculable because patients in both groups either all had or did not have the applicable event.
[a] Evaluable patients include those who achieved PE by Month 24 or were followed for ≥ 24 months if no events.
[b] P-value is based on Fisher's exact test.
[c] P-value is based on Wilcoxon rank sum test.

6.6. Adverse Events

The following sections will focus primarily on AE analyses in TP-102/104. Because treatment with eli-cel is preceded by mobilization/apheresis and conditioning, which carry their own risks, Section 6.6.4.1 and Section 6.6.4.2 provide analyses of AEs attributed to mobilization and conditioning, respectively.

In addition, select comparative data between TP-102/104 and TP-103 (including subgroups as appropriate) will be presented based on the AE collection strategy (see Figure 16).

Key AE comparisons between TP-102/104 and TP-103 are:

- ≥ Grade 3 treatment emergent adverse events (TEAEs) through Month 12 (Section 6.6.7.3)
- ≥ Grade 3 TEAE infections through Month 12 (Section 6.6.7.3)
- Treatment emergent serious adverse events (TESAEs) through Month 48 (Section 6.6.7.4)

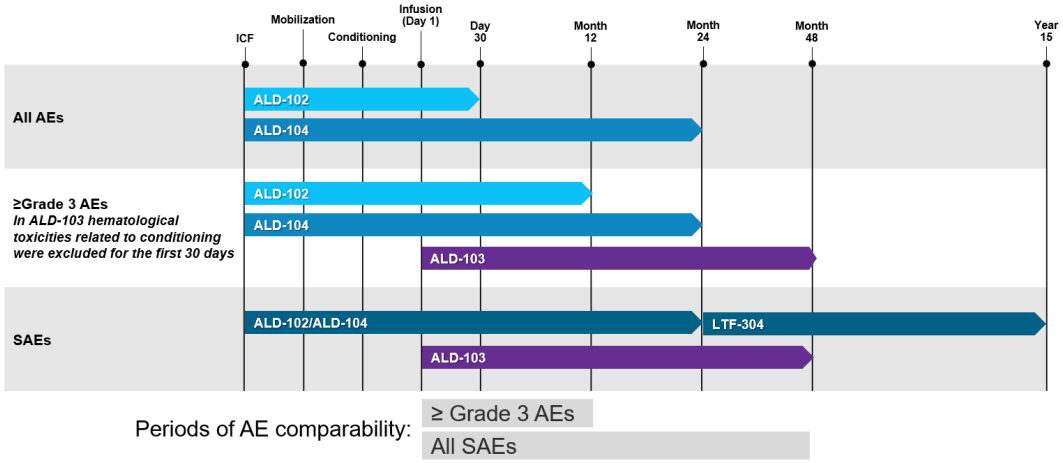
Unless otherwise specified (e.g., for analysis of GVHD), comparisons with TP-103 are limited to the first allo-HSCT.

In ALD-102 and ALD-104, investigators were required to provide their assessments of the causal relationship of all AEs to eli-cel. If an AE was assessed as not related to eli-cel, investigators were then required to determine the attribution of the AE using categories of mobilization, apheresis, conditioning, other study procedure, disease under study or disease progression, or other. In ALD-103, investigators also determined the attribution of AEs with the choices reflecting allo-HSCT study conduct.

6.6.1. Collection of Adverse Events

The timing and strategy of AE collection for ALD-102/ALD-104 and LTF-304, as well as for ALD-103, are shown in Figure 16.

Figure 16. Collection of Adverse Events



Abbrev.: AE, adverse event; ICF, informed consent form; SAE, serious adverse event.

6.6.2. Graft-Versus-Host Disease

The primary safety endpoint, the proportion of patients who experience either acute GVHD (≥ Grade II) or chronic GVHD by Month 24, was statistically tested in TP-102 for superiority over TP-103 as the primary safety success criterion. No patients (0/32) in TP-102 experienced either acute (≥ Grade II) or chronic GVHD by Month 24, compared with 26/50 (52%) patients in TP-103. This difference was statistically significant ($p < 0.0001$) and thus met the pre-specified success criterion.

The primary analysis and additional GVHD data including TP-103 subgroups (MSD and NMSD) are presented below (Table 21).

Table 21. Proportion of Patients with Either Acute GVHD (≥ Grade II) or Chronic GVHD by Month 24 (TP-102, TP-103, TP-103 MSD, TP-103 NMSD)

	eli-cel	allo-HSCT[a]		
	TP-102 N=32	TP-103 Overall N=59	TP-103 MSD N=11	TP-103 NMSD N=48
Patients with acute GVHD (≥ Grade II) by Month 24				
Evaluable patients	32	49	10	39
n (%)	0	15 (30.6)	1 (10.0)	14 (35.9)
Exact 95% CI	0, 10.9	18.3, 45.4	0.3, 44.5	21.2, 52.8
p-value[b]		0.0002	0.2381	0.0001
Patients with chronic GVHD by Month 24				
Evaluable patients	32	39	9	30
n (%)	0	14 (35.9)	2 (22.2)	12 (40.0)
Exact 95% CI	0, 10.9	21.2, 52.8	2.8, 60.0	22.7, 59.4
p-value[b]		0.0001	0.0439	< 0.0001
Patients with either acute GVHD (≥ Grade II) or chronic GVHD by Month 24				
Evaluable patients	32	50	10	40
n (%)	0	26 (52.0)	3 (30.0)	23 (57.5)
Exact 95% CI	0.0, 10.9	37.4, 66.3	6.7, 65.2	40.9, 73.0
p-value[b]		< 0.0001	0.0105	< 0.0001

Abbrev.: allo, allogeneic; CI, confidence interval; DLC, date of last contact; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; MSD, matched sibling donor; NMSD, not a matched sibling donor; TP, transplant population.
Evaluable patients defined as those who had the respective event by Month 24 (Rel Day 730) in any allo-HSCT period or had been followed for ≥ 12 months (DLC Rel Day ≥ 365 days) in the latest allo-HSCT period if no events.
[a] Analysis of GVHD includes data from patients who underwent ≥ 1 allo-HSCT. Undergoing rescue cell administration /subsequent allo-HSCT(s) was not considered as events in this analysis.
[b] P-values are based on Fisher's exact test comparing TP-102 versus other populations.

Of the 26 patients in TP-103 who had acute (≥ Grade II) or chronic GVHD, or both acute and chronic GVHD, 5 died, with 4 deaths attributed to ≥ Grade II acute GVHD (all in NMSD) and 1 death to chronic GVHD (MSD).

Of the 15 patients in TP-103 who experienced acute GVHD, 5 patients met Grade IV and 5 patients met Grade III criteria; the majority of these had gastrointestinal manifestations. The remaining 5 patients met Grade II; the majority of these involved the skin. In the 14 patients with chronic GVHD, disease was categorized as extensive in 4 and limited in 7 patients, with skin involvement in the majority; characterization in the remaining 3 patients was unknown.

To characterize the risk of GVHD in the proposed indicated population (NMSD), the primary safety success criterion was analyzed using the allo-HSCT subgroup from the proposed

indication (TP-103 NMSD [refer to Table 23], and its component subgroups, TP-103 NMSD-MUD and TP-103 NMSD-Mismatched populations). In TP-103 NMSD-MUD 10/21 (47.6%) and in NMSD-Mismatched 12/18 (66.7%) evaluable patients experienced acute or chronic GVHD.

6.6.3. Overview of Adverse Events

A summary of AEs occurring in eli-cel treated patients is presented in Table 22.

Table 22. Overview of Adverse Events in TP-102/104 (ICF to DLC)

Adverse Event Category[a]	TP-102/104 N=67 n (%)
≥1 AE	67 (100)
≥ 1 SAE	37 (55.2)
≥ 1 TEAE[b]	67 (100)
≥ 1 TESAE	35 (52.2)
≥ 1 Grade ≥ 3 AE	64 (95.5)
≥ 1 Grade ≥ 3 TEAE	64 (95.5)
≥ 1 AE related to mobilization/apheresis	31 (46.3)
≥ 1 AE related to conditioning	67 (100)
≥ 1 AE attributed to disease under study or disease progression	18 (26.9)
≥ 1 AE resulting in death[c]	1 (1.5)

Abbrev.: AE, adverse event; DLC, date of last contact; ICF, informed consent form; ITT, intent-to-treat; SAE, serious adverse event; TEAE, treatment-emergent adverse event; TESAE, treatment-emergent serious adverse event.
[a] AEs related to eli-cel were assessed using a later data cut and are described in Section 6.6.5.4
[b] TEAEs are defined as AEs starting on or after the initiation of eli-cel infusion.
[c] An additional death occurred that is not captured in this table; this patient died after receiving allo-HSCT off study, so this death is not included in the clinical database.

Adverse events occurring in ≥ 10% of patients in TP-102/104 by System Organ Class (SOC), Preferred Term (PT), and time period are shown in Table 34 in Section 11, Appendix C. Patients generally experienced adverse events that were consistent with the effects of conditioning. Patients most frequently experienced AEs of thrombocytopenia (65/67, 97.0%), stomatitis (57/67, 85.1%), neutropenia (55/67, 82.1%), and anemia (53/67, 75.1%).

6.6.4. Adverse Events Attributed to Mobilization, Apheresis, and Conditioning

6.6.4.1. Adverse Events Attributed to Mobilization and Apheresis

Thirty one of 67 patients (46.3%) in TP-102/104 experienced 55 AEs attributed to mobilization/apheresis by the investigator, and all occurred during the mobilization to conditioning time period. All were nonserious and Grade 1 or 2 in severity except for 1 Grade 3 AE of hypokalemia. All AEs resolved and were generally consistent with those expected due to mobilization and associated procedures (Mortzell Henriksson et al. 2016).

The most frequently reported AEs were in SOCs and PTs that included Metabolism and nutrition disorders (11/67 [16.4%]): hypokalemia in 8 patients and hypomagnesemia in 6 patients;

Gastrointestinal disorders (10/67 [14.9%]): nausea in 6 patients and vomiting in 5 patients; Musculoskeletal and connective tissue disorders (6/67 [9.0%]): bone pain in 4 patients; and Blood and lymphatic system disorders (6/67 [9.0%]): anemia in 5 patients.

6.6.4.2. Adverse Events Attributed to Conditioning

All eli-cel treated patients (67/67) experienced an AE attributed to conditioning by the investigator. The majority of all AEs in the eli-cel program were attributed to conditioning (Table 23 and Table 24).

Most conditioning-related events were non-serious. Twenty-one patients experienced 34 SAEs attributed to conditioning that included 12 events of febrile neutropenia, 10 events of pyrexia, 2 events of stomatitis and bacteremia, and 1 event each of otitis media, decreased appetite, vascular device infection, constipation, nausea, vomiting, Streptococcus, and Stenotrophomonas infection, all of which resolved.

Table 23. Adverse Events Attributed to Conditioning in ≥ 10% of Patients by SOC, PT, and Study Period (TP-102/104)

System Organ Class Preferred Term	**C to < NE N=67** n (%), Events	**NE to M12 N=67** n (%), Events	**> M12 to M24 N=46** n (%), Events	**D1 to DLC N=67** n (%), Events
Patients with at least 1 AE	**67 (100.0), 919**	**49 (73.1), 176**	**2 (4.3), 2**	**67 (100), 792**
Blood and lymphatic system disorders	**67 (100.0), 383**	**30 (44.8), 70**	**0, 0**	**67 (100), 399**
Thrombocytopenia	59 (88.1), 100	9 (13.4), 14	0, 0	64 (95.5), 113
Neutropenia	56 (83.6), 84	18 (26.9), 26	0, 0	54 (80.6), 102
Anemia	51 (76.1), 86	11 (16.4), 15	0, 0	51 (76.1), 82
Febrile neutropenia	48 (71.6), 53	0, 0	0, 0	48 (71.6), 52
Leukopenia	19 (28.4), 37	6 (9.0), 15	0, 0	17 (25.4), 40
Lymphopenia	14 (20.9), 18	0, 0	0, 0	4 (6.0), 5
Gastrointestinal disorders	**66 (98.5), 278**	**11 (16.4), 13**	**0, 0**	**65 (97.0), 151**
Stomatitis	60 (89.6), 73	0, 0	0, 0	57 (85.1), 69
Nausea	54 (80.6), 68	6 (9.0), 6	0, 0	15 (22.4), 18
Vomiting	50 (74.6), 67	2 (3.0), 2	0, 0	13 (19.4), 15
Abdominal pain	23 (34.3), 25	0, 0	0, 0	15 (22.4), 16
Diarrhea	14 (20.9), 15	1 (1.5), 1	0, 0	11 (16.4), 12
Constipation	13 (19.4), 13	2 (3.0), 2	0, 0	3 (4.5), 3
Metabolism and nutrition disorders	**48 (71.6), 95**	**8 (11.9), 10**	**0, 0**	**33 (49.3), 49**
Decreased appetite	42 (62.7), 52	3 (4.5), 3	0, 0	21 (31.3), 24
Hypokalemia	18 (26.9), 20	4 (6.0), 4	0, 0	11 (16.4), 11
Hypophosphatemia	8 (11.9), 8	2 (3.0), 2	0, 0	9 (13.4), 10
Skin and subcutaneous tissue disorders	**38 (56.7), 48**	**23 (34.3), 32**	**0, 0**	**52 (77.6), 74**
Alopecia	33 (49.3), 33	15 (22.4), 15	0, 0	48 (71.6), 48
Skin hyperpigmentation	3 (4.5), 3	9 (13.4), 9	0, 0	11 (16.4), 11
Investigations	**17 (25.4), 27**	**4 (6.0), 6**	**2 (4.3), 2**	**13 (19.4), 18**
Alanine aminotransferase increased	8 (11.9), 8	2 (3.0), 2	0, 0	5 (7.5), 5

System Organ Class Preferred Term	C to < NE N=67 n (%), Events	NE to M12 N=67 n (%), Events	> M12 to M24 N=46 n (%), Events	D1 to DLC N=67 n (%), Events
General disorders and administration site conditions	**16 (23.9), 18**	**9 (13.4), 11**	**0, 0**	**17 (25.4), 22**
Pyrexia	10 (14.9), 10	8 (11.9), 10	0, 0	12 (17.9), 16
Respiratory, thoracic and mediastinal disorders	**16 (23.9), 23**	**2 (3.0), 2**	**0, 0**	**18 (26.9), 23**
Epistaxis	8 (11.9), 11	1 (1.5), 1	0, 0	9 (13.4), 11
Oropharyngeal pain	7 (10.4), 7	0, 0	0, 0	6 (9.0), 6
Nervous system disorders	**9 (13.4), 9**	**3 (4.5), 5**	**0, 0**	**5 (7.5), 8**
Headache	8 (11.9), 8	0, 0	0, 0	2 (3.0), 2
Vascular disorders	**8 (11.9), 9**	**2 (3.0), 2**	**0, 0**	**5 (7.5), 6**
Hypertension	8 (11.9), 9	2 (3.0), 2	0, 0	5 (7.5), 6

Abbrev.: AE, adverse event; C, conditioning; DLC, date of last contact; NE, neutrophil engraftment; PT, preferred term; SOC, system organ class.
PTs (and their associated SOC) are included for AEs that were observed in ≥ 10% of patients (≥ 7 patients) in any shown study period and are sorted based on decreasing frequency by SOC and then PT per the C to < NE study period. For such PTs the frequency of AEs is shown even if they occurred in < 10% of patients in some study periods. The SOC values presented show the incidence of all patients /events that occurred under that SOC (not only those events meeting the ≥ 10% threshold).
Patients at risk for each period (N in column header) is defined to be the patients who entered the study period. For AEs with worsening severity in which the AE started in the first period and worsened in the next period, the patient was counted in both periods. Patients were counted once for each SOC and PT even if they had multiple instances of the event in 1 period. If an AE started in 1 reporting period and continued into the next reporting period, it was counted only in the first period. If an AE started and stopped in 1 reporting period and then recurred in the next reporting period, it was counted in both periods. All events reported in the database are counted in the number of events.
Hematologic abnormalities reported as AEs that were coded to PTs in the Investigations SOC (e.g., platelet count decreased) have been pooled with appropriate terms in the Blood and Lymphatic System SOC (e.g., thrombocytopenia) for tabulation.

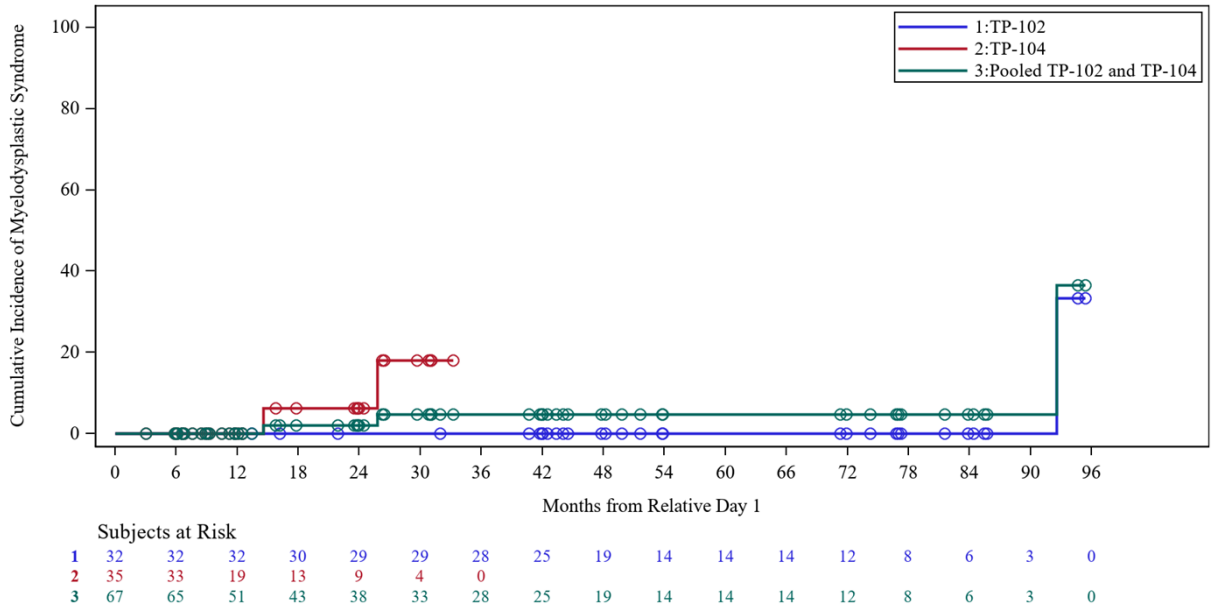
6.6.5. Treatment-Emergent Adverse Events

In TP-102/104, all 67 patients experienced at least 1 TEAE. Events occurring in ≥ 10% of patients are presented in Table 35 in Section 11, Appendix C in the Day 1 to Date of Last Contact (D1 to DLC) column. The TEAE profile was similar to the overall AE profile, reflective of the prolonged consequences of conditioning, including thrombocytopenia, anemia, neutropenia, stomatitis, and febrile neutropenia.

6.6.5.1. Treatment-Emergent Malignancies

In TP-102/104, 3 patients were diagnosed with myelodysplastic syndrome (MDS). Two of these patients received eli-cel in ALD-104 and were diagnosed within approximately 2 years of treatment. The third patient received eli-cel in ALD-102 and was diagnosed approximately 7.5 years after eli-cel infusion. A Kaplan-Meier (KM) analysis of time to MDS was performed to characterize the risk of malignancy. In TP-102/104, the Kaplan-Meier estimated cumulative incidence of MDS is 4.8% (95% CI: 1.2, 18.1%) from Month 36 to Month 84 (Figure 17). Few patients have been followed past this time; thus, the long-term risk of MDS is undefined.

Figure 17. Cumulative Incidence of Myelodysplastic Syndrome (TP-102/104)



The 2 patients treated in ALD-104 were diagnosed with unilineage MDS (affecting the megakaryocytes). Both patients achieved platelet engraftment more than 100 days after eli-cel infusion and platelet counts were consistently below baseline levels. In addition, starting at Month 6, each patient had a clone contributing at least 50% of cells to the analyzed cell population, with vector integrations in the *MECOM* gene. *MECOM* dysregulation shown by over-expression of the *EVI1* transcript was seen in both patients. The diagnosis of MDS in these patients was based on persistent unexplained thrombocytopenia and dysplastic megakaryocytes in the setting of clonal hematopoiesis. Both patients subsequently underwent treatment with allo-HSCT and in both patients the MDS is considered in remission.

MECOM encodes a number of transcripts and protein variants, including MDS1, transcription factor *EVI1*, and *MDS1-EVI1* (Hinai and Valk 2016). *EVI1* was found to play a role in controlling stem cell proliferation and fate and is considered a proto-oncogene; *MDS1-EVI1* has been proposed to function as a tumor suppressor (Hinai and Valk 2016; Ivanochko et al. 2019). Chromosomal aberrations involving the *MECOM* locus that dysregulate *EVI1* expression have been observed in acute myeloid leukemia (AML) and MDS, with increased expression of *EVI1* being linked to poor prognosis (Metais and Dunbar 2008; Lugthart et al. 2011).

Molecular testing did not identify known somatic leukemic mutations or chromosomal aberrations in either patient.

Based on the identification of the clones at Month 6 in the peripheral blood of both patients, with evidence of *EVI1* overexpression at the *MECOM* locus, which has been previously implicated in AML and MDS, and the absence of chromosomal abnormalities or mutations common in MDS, bluebird bio determined that these 2 cases of MDS are likely mediated by Lenti-D LVV insertion.

The third patient was diagnosed with MDS approximately 7.5 years after receiving eli-cel in ALD-102. At the last in-person study visit prior to diagnosis (approximately 2.5 years prior), the

patient had an unremarkable complete blood count and polyclonal reconstitution based on integration site analysis (ISA). He came to medical attention when he developed easy bruising and was found to be significantly thrombocytopenic. A bone marrow biopsy showed multilineage dysplasia and 15% myeloblasts and the patient was diagnosed with MDS with excess blasts 2 (MDS-EB-2). Molecular testing showed KRAS and NRAS mutations in a subset of these cells.

Bone marrow aspirate samples showed blast cells were positive for lentiviral vector. ISA on a peripheral blood sample obtained at MDS diagnosis identified a clone that is highly likely to contain 6 Lenti-D LVV insertion sites (IS) and contributes greater than 50% of cells to the population analyzed. Unlike the two cases of MDS described above, there was no evidence for a *MECOM* IS in the expanded clone. Instead the ISA showed the presence of an IS in *PRDM16*, a gene that is related to *MECOM*, with 63% sequence similarity (Duhoux et al. 2012). Both *PRDM16* and *MECOM* belong to the family of PR-domain proteins and are involved in chromosomal translocation in MDS/AML. Like *MECOM*, full-length *PRDM16* is thought to function as a tumor suppressor with expression of an oncogenic shorter isoform (Duhoux et al. 2012).

Based on the identification of the expanded clone with an IS in a known proto-oncogene that has previously been implicated in AML and MDS, this case of MDS is also considered as likely mediated by Lenti-D LVV insertion, albeit the specific mechanism of Lenti-D LVV insertion leading to insertional oncogenesis is not fully determined at this time due to the lack of established assays to clearly assess dysregulated expression of the short form of *PRDM16*.

The patient underwent chemotherapy and an allo-HSCT approximately 2 months following diagnosis. A bone marrow biopsy 5 weeks following allo-HSCT showed 5% cellularity with 0.15% myeloblasts. Testing for chimerism showed 100% donor cells. Molecular testing did not show KRAS or NRAS mutations.

The risk of insertional oncogenesis with lentiviral vectors across bluebird bio products is provided in Section 12, Appendix D. bluebird bio has 3 unique ex vivo LVV-transduced HSC products currently being used in clinical trials using 2 different LVVs. eli-cel uses the Lenti-D LVV, which is distinct from the other LVV, BB305 LVV. BB305 LVV is used in manufacturing of both betibeglogene autotemcel (beti-cel) for the treatment of patients with transfusion-dependent β-thalassemia and lovotibeglogene autotemcel (lovo-cel) for the treatment of patients with sickle cell disease. The malignancy events observed for eli-cel do not have a clear predictive value for the other products.

6.6.5.2. Treatment-Emergent Infections

In TP-102/104, 29/67 (43.3%) patients experienced 68 TEAEs in the Infections and infestations SOC, predominantly during the NE to Month 12 period (40 events in 19/67 [28.4%]) patients).

Infections that occurred in 2 or more patients from D1 to Last Follow-up were vascular device infection and viral upper respiratory tract infection (4/67 [6%]), gastroenteritis, otitis media, and sinusitis (3/67 [4.5%]) and anal candidiasis, device related infections, folliculitis, gastroenteritis viral, oral candidiasis, Pseudomonal bacteremia, rhinovirus infection, skin infection, and viral infection, each in 2/67 (3%) patients.

Eleven of 67 (16.4%) patients had 13 TESAEs in the Infections and infestations SOC. Serious infections that occurred in 2 or more patients from D1 to Last Follow-up were Pseudomonal bacteremia and vascular device infection, each in 2/67 (3%) patients. All other serious infections of cystitis viral, gastroenteritis, influenza, otitis media, sinusitis, viral infection, Stenotrophomonas infection, Streptococcal bacteremia and viral upper respiratory tract infection occurred in 1 patient each, 1/67 (1.5%).

A medical review identified opportunistic infections in 4 patients; 1 patient experienced a TESAE of Pseudomonal bacteremia with an opportunistic strain, 1 patient experienced a TESAE of Pseudomonal bacteremia and Stenotrophomonas infection, 1 patient experienced a TESAE of cystitis viral (BK virus) which was considered possibly related to eli-cel, and 1 patient experienced a nonserious TEAE of human herpesvirus 6.

The majority of infections in TP-102/104 were nonserious, not opportunistic, and either self-limited or resolved with standard therapy.

6.6.5.3. Treatment-Emergent Seizures

In TP-102/104, 7 patients experienced 15 TEAEs of new onset seizure. In 5 patients, the first event of seizure occurred approximately 2 years following eli-cel infusion and in the remaining 2 patients, seizures occurred more than 3 years following eli-cel. A time-to-first seizure KM analysis was performed to characterize the risk of seizure following eli-cel treatment. In TP-102/104, the seizure free survival rate was 80.6% (95% CI: 61.2-90.0%) at Month 48 and 74.4% (95% CI: 51.7-87.5%) from Month 60 to Month 72.

Of the 7 patients who experienced TEAEs of seizure, 4 patients experienced an isolated event whereas in 3 patients multiple AEs of seizure were reported. All 3 patients with recurrent seizures initiated anti-convulsant therapy. In addition, 2 patients with isolated episodes initiated anti-convulsant therapy.

Of the 7 patients, 5 did not maintain a stable Loes score, with stability defined as a Loes score $\leq$ 9 or not increased by $\geq$ 6 points from baseline; in 1 of these patients, a stable NFS was not maintained, with stability defined as an NFS score of $\leq$ 4 without an increase of 3 points from baseline. This patient experienced an increase in NFS from 0 to 4 due to seizure, running difficulties, walking difficulties, and vision impairment. Refer to Section 5.3.6 for further details on changes in NFS and Loes score.

6.6.5.4. Adverse Drug Reactions

In TP-102/104, 8 of 67 patients (11.9%) experienced AEs related to eli-cel. Five patients experienced serious events; these are described below. Three patients reported nonserious AEs including vomiting and nausea that started and resolved on Rel Day 1 and were likely related to the cryopreservative dimethyl sulfoxide in the drug product.

- A male patient, 5-years old at the time of consent, experienced hemorrhagic cystitis and was diagnosed with a Grade 3 SAE of **BK viral cystitis** from Rel Day 42 to 48. He was treated supportively, and the event resolved without sequelae.
- A male patient, 12-years old at the time of consent, experienced an event of **delayed hematopoietic reconstitution** on Rel Day 55 requiring prolonged support with G-CSF, eltrombopag, platelet transfusions, and packed red blood cell (pRBC) transfusions after

transplant. He achieved NE on Rel Day 12 and PE on Rel Day 104. This patient had a clone with vector integrations in the *MECOM* gene which comprised approximately 90% of CD15+ (myeloid) cells in peripheral blood. The event of delayed hematopoietic reconstitution resolved with sequelae on Day 784 when the patient was diagnosed with **MDS** based on persistent cytopenias and megakaryocyte dysplasia in the setting of a clonal process. The patient underwent allo-HSCT on Rel Day 896. On Rel Day 955, a repeat bone marrow biopsy showed hypocellular marrow with trilineage hematopoiesis which was expected for that time post-transplant. The MDS was considered in remission with no overt dysplasia or abnormal morphology of megakaryocytes. The event was considered resolved. Refer to Section 6.6.5.1 for details.

- A male patient, 9-years old at the time of consent, experienced **delayed hematopoietic reconstitution** on Rel Day 57 requiring prolonged support with G-CSF, eltrombopag, platelet transfusions, and pRBC transfusions after transplant. A bone marrow biopsy at the time of event onset revealed parvovirus B19 infection, which was assessed as a likely contributor to the event. Despite treatment with intravenous immunoglobulin, this patient has continued to have detectable parvovirus in bone marrow samples. A bone marrow biopsy from Rel Day 707 showed atypical megakaryocytes and next generation sequencing revealed a variant that leads to loss of function of the thrombopoietin receptor which was likely germline. Laboratory tests on the same day showed white blood count $5.1\times10^3/\mu L$, hemoglobin 14.6 g/dL, and platelets $100\times10^3/\mu L$. On Rel Day 944, laboratory tests showed white blood count $5.9\times10^3/\mu L$, hemoglobin 14.2 g/dL, platelets $142\times10^3/\mu L$, and absolute neutrophil count $3.1\times10^3/\mu L$. The event of delayed hematopoietic reconstitution was considered ongoing.
- A male patient, 11-years old at the time of consent, was diagnosed with **MDS** on Rel Day 444. This patient had a clone detected at Month 6 with vector integrations in the *MECOM* gene. At the Month 12 Visit, a bone marrow biopsy revealed dysplastic megakaryocytes. A subsequent bone marrow biopsy on Rel Day 435 revealed dysmegakaryopoiesis. The report concluded that this patient's findings met criteria for the diagnosis of MDS with unilineage dysplasia. The patient underwent allo-HSCT on Rel Day 582 and a bone marrow biopsy on Rel Day 685 showed no evidence of MDS. The event remains ongoing. Refer to Section 6.6.5.1 for more details.
- A male patient, 4-years old at the time of consent, was diagnosed with **MDS** approximately 7.5 years after receiving eli-cel in ALD-102. The patient presented with fatigue, pallor, soft tissue fullness on the posterior scalp, and petechiae. CBC showed white blood count 8.8×10^3 cells/µL, platelets 25×10^3 cells/µL, and hemoglobin 10.8 g/dL. A bone marrow biopsy and aspirate showed 15% myeloblasts, concurrent with 3% blasts in the peripheral blood. Molecular testing showed KRAS and NRAS mutations in a subset of these cells. The patient had a clone detected at the time of diagnosis with a vector integration in the *PRDM16* gene. The patient underwent chemotherapy and an allo-HSCT approximately 2 months following diagnosis. A bone marrow biopsy 5 weeks following allo-HSCT showed 5% cellularity with 0.15% myeloblasts. Testing for chimerism showed 100% donor cells. Molecular testing did not show KRAS or NRAS mutations.

See Section 6.6.5.1 for more information on malignancies.

6.6.6. Serious Adverse Events in TP-102/104

In TP-102/104, 35/67 patients (52.2%) experienced 83 SAEs (Table 24) and the overall SAE profile was largely consistent with the persistent effects of conditioning. The most frequently reported TESAEs were febrile neutropenia (12/67 [17.9%]) and pyrexia (12/67 [17.9%]). Most TESAEs resolved with exceptions of the SAEs ongoing at the time of death in 1 patient, the SAE of delayed hematopoietic reconstitution in 1 patient, and 3 SAEs of MDS.

Table 24. Serious Adverse Events by SOC, PT, and Study Period (TP-102/104)

System Organ Class Preferred Term	M to < C N=67 n (%), Events	C to < NE N=67 n (%), Events	NE to M12 N=67 n (%), Events	> M12 to M24 N=46 n (%), Events	D1 to DLC N=67 n (%), Events
Patients with ≥ 1 SAE	**2 (3.0), 2**	**12 (17.9), 15**	**23 (34.3), 40**	**5 (10.9), 13**	**35 (52.2), 83**
Blood and lymphatic system disorders	**0, 0**	**12 (17.9), 12**	**2 (3.0), 2**	**1 (2.2), 1**	**13 (19.4), 15**
Febrile neutropenia	0, 0	12 (17.9), 12	0, 0	0, 0	12 (17.9), 12
Pancytopenia	0, 0	0, 0	2 (3.0), 2	1 (2.2), 1	2 (3.0), 3
General disorders and administration site conditions	**0, 0**	**0, 0**	**11 (16.4), 14**	**0, 0**	**13 (19.4), 16**
Pyrexia	0, 0	0, 0	11 (16.4), 14	0, 0	12 (17.9), 15
Fatigue	0, 0	0, 0	0, 0	0, 0	1 (1.5), 1
Infections and infestations	**1 (1.5), 1**	**0, 0**	**9 (13.4), 11**	**2 (4.3), 2**	**11 (16.4), 13**
Vascular device infection	1 (1.5), 1	0, 0	2 (3.0), 2	0, 0	2 (3.0), 2
Pseudomonal bacteremia	0, 0	0, 0	2 (3.0), 2	0, 0	2 (3.0), 2
Cystitis viral	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Gastroenteritis	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Influenza	0, 0	0, 0	0, 0	1 (2.2), 1	1 (1.5), 1
Otitis media	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Sinusitis	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Stenotrophomonas infection	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Streptococcal bacteremia	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Viral infection	0, 0	0, 0	0, 0	1 (2.2), 1	1 (1.5), 1
Viral upper respiratory infection	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Nervous system disorders	**0, 0**	**1 (1.5), 1**	**2 (3.0), 2**	**2 (4.3), 4**	**7 (10.4), 16**
Seizure	0, 0	0, 0	0, 0	1 (2.2), 1	5 (7.5), 10
Dyskinesia	0, 0	1 (1.5), 1	0, 0	0, 0	1 (1.5), 1
Myelitis transverse	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Neurological decompensation	0, 0	0, 0	1 (1.5), 1	1 (2.2), 3	1 (1.5), 4

System Organ Class Preferred Term	M to < C N=67 n (%), Events	C to < NE N=67 n (%), Events	NE to M12 N=67 n (%), Events	> M12 to M24 N=46 n (%), Events	D1 to DLC N=67 n (%), Events
Gastrointestinal disorders	**0, 0**	**2 (3.0), 2**	**3 (4.5), 4**	**0, 0**	**6 (9.0), 7**
Stomatitis	0, 0	2 (3.0), 2	0, 0	0, 0	2 (3.0), 2
Vomiting	0, 0	0, 0	2 (3.0), 2	0, 0	2 (3.0), 2
Abdominal pain	0, 0	0, 0	0, 0	0, 0	1 (1.5), 1
Constipation	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Nausea	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Psychiatric disorders	**1 (1.5), 1**	**0, 0**	**2 (3.0), 2**	**0, 0**	**3 (4.5), 4**
Aversion	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Depression	0, 0	0, 0	0, 0	0, 0	1 (1.5), 1
Suicidal ideation	0, 0	0, 0	0, 0	0, 0	1 (1.5), 1
Tic	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Autism spectrum disorder	1 (1.5), 1	0, 0	0, 0	0, 0	0, 0
Injury, poisoning and procedural complications	**0, 0**	**0, 0**	**3 (4.5), 3**	**0, 0**	**3 (4.5), 3**
Anaphylactic transfusion reaction	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Head injury	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Spinal fracture	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Cardiac disorders	**0, 0**	**0, 0**	**0, 0**	**1 (2.2), 1**	**2 (3.0), 2**
Cardio-respiratory arrest	0, 0	0, 0	0, 0	1 (2.2), 1	1 (1.5), 1
Sinus bradycardia	0, 0	0, 0	0, 0	0, 0	1 (1.5), 1
Hepatobiliary disorders	**0, 0**	**0, 0**	**0, 0**	**1 (2.2), 1**	**1 (1.5), 1**
Acute hepatic failure	0, 0	0, 0	0, 0	1 (2.2), 1	1 (1.5), 1
Investigations	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Transaminases increased	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Metabolism and nutrition disorders	**0, 0**	**0, 0**	**1 (1.5), 1**	**0, 0**	**1 (1.5), 1**
Decreased appetite	0, 0	0, 0	1 (1.5), 1	0, 0	1 (1.5), 1
Musculoskeletal and connective tissue disorders	**0, 0**	**0, 0**	**0, 0**	**1 (2.2), 1**	**1 (1.5), 1**
Rhabdomyolysis	0, 0	0, 0	0, 0	1 (2.2), 1	1 (1.5), 1
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	**0, 0**	**0, 0**	**0, 0**	**1 (2.2), 1**	**1 (1.5), 1**
Myelodysplastic syndrome	0, 0	0, 0	0, 0	1 (2.2), 1	1 (1.5), 1
Renal and urinary disorders	**0, 0**	**0, 0**	**0, 0**	**1 (2.2), 1**	**1 (1.5), 1**
Acute kidney injury	0, 0	0, 0	0, 0	1 (2.2), 1	1 (1.5), 1

System Organ Class Preferred Term	M to < C N=67 n (%), Events	C to < NE N=67 n (%), Events	NE to M12 N=67 n (%), Events	> M12 to M24 N=46 n (%), Events	D1 to DLC N=67 n (%), Events
Respiratory, thoracic and mediastinal disorders	**0, 0**	**0, 0**	**0, 0**	**1 (2.2), 1**	**1 (1.5), 1**
Respiratory distress	0, 0	0, 0	0, 0	1 (2.2), 1	1 (1.5), 1

Abbrev.: AE, adverse event; C, conditioning; DLC, date of last contact; M, mobilization; NE, neutrophil engraftment; PT, preferred term; SAE, serious adverse event; SOC, system organ class.
PTs (and their associated SOC) are included for SAEs that were observed in any shown study period and are sorted based on decreasing frequency by SOC and then PT per the D1 to DLC study period. Data are not presented for 2/67 (3%) patients in ICF to < M who experienced SAEs of adrenal insufficiency and 1/67 (1.5%) who experienced procedural pain.
Patients at risk for each period (N in column header) is defined to be the patients who entered the study period. If an SAE started in 1 reporting period and continued into the next reporting period, it was counted only in the first period. If an SAE started and stopped in 1 reporting period and then recurred in the next reporting period, it was counted in both periods. Patients were counted once for each SOC and PT even if they had multiple instances of the event in 1 period. For SAEs with worsening severity in which the SAE started in the first period and worsened in the next period, the patient was counted in both periods. All events reported in the database are counted in the number of events.
Hematologic abnormalities reported as AEs that were coded to PTs in the Investigations SOC (e.g., platelet count decreased) have been pooled with appropriate terms in the Blood and Lymphatic System SOC (e.g., thrombocytopenia) for tabulation.

Of note, 1 patient experienced an SAE of transverse myelitis. Approximately 7 months after eli-cel therapy, he presented with progressive left lower extremity weakness and episodic incontinence; laboratory tests showed transaminitis. Spinal MRI showed multilevel cord edema. His nasal swab was positive for adenovirus and entero/rhinovirus. The patient was treated with steroids and plasmapheresis and transferred to acute care rehab for physical therapy. The event was considered resolved with sequelae. The patient subsequently developed increased episodes of urinary incontinence and new onset episodes of bowel incontinence. Approximately 29 months after eli-cel, the patient was diagnosed with total incontinence, an MFD, which was considered to be a consequence of the transverse myelitis rather than CALD (refer to Section 5.3.4.1 for a discussion of Event-free survival). Two months later, the patient experienced a 20 second seizure and was treated with levetiracetam. Refer to Section 6.8 Late-Breaking Update for more information on the SAEs of total incontinence and seizure.

6.6.7. TP-102/104 and TP-103 Comparative Safety Data

6.6.7.1. Death

One death (1/67, 1.5%) was reported in TP-102/104 and was not considered related to drug product. This patient experienced rapid disease progression starting 2 weeks after treatment with eli-cel, with an increase in cerebral MRI Loes score from 6.5 at Baseline to 13.5 at Rel Day 14. He developed 4 SAEs of neurological decompensation (total incontinence by Month 9, cortical blindness and loss of communication by Month 12, and wheelchair dependence by Month 21) followed by respiratory distress, acute hepatic failure, acute kidney injury, rhabdomyolysis, viral infection, and cardio-respiratory arrest on Rel Day 666.

In contrast, 15/59 (25.4%) patients died in TP-103, 12 after first allo-HSCT and 3 after second allo-HSCT (Table 25). Five of these 15 patients were in the TPES subgroup (5/27, 18.5%), 3 died after first allo-HSCT and 2 after second allo-HSCT.

Table 25. Deaths in ALD-103 (TP-103)

Patient	Period	Cause of Death	GVHD (Y/N)	Population/Donor
1	First allo-HSCT	Transplant-related	Yes	TP/NMSD
2	First allo-HSCT	Unknown	Yes	TP/NMSD
3	First allo-HSCT	Cardiac arrest	Yes	TPES/NMSD
4	First allo-HSCT	Transplant-related	Yes	TP/NMSD
5	First allo-HSCT	Transplant-related	Yes	TP/NMSD
6	First allo-HSCT	Progressive disease	No	TP/NMSD
7	First allo-HSCT	Transplant-related	Yes	TPES/MSD
8	First allo-HSCT	Transplant-related	Yes	TP/NMSD
9	First allo-HSCT	Septic shock (abdominal focus)	No	TPES/MSD
10	First allo-HSCT	Transplant-related	Yes	TP/NMSD
11	First allo-HSCT	Transplant-related	Yes	TP/NMSD
12	First allo-HSCT	Progressive disease	No	TP/NMSD
13	Second allo-HSCT	Transplant-related	No	TP/NMSD
14	Second allo-HSCT	Transplant-related	Yes	TPES/NMSD
15	Second allo-HSCT	Progressive disease	Yes	TPES/NMSD

Abbrev.: allo-HSCT, allogeneic hematopoietic stem cell transplantation; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; MSD, matched sibling donor; NMSD, not a matched sibling donor; TP, transplant population.

6.6.7.2. Transplant-Related Mortality

Categorization of events of TRM is at the discretion of the investigators. No TRM events occurred in evaluable patients in TP-102/104 by Rel Day 101 (within first 100 days) or by Rel Day 366 (within 1 year) after eli-cel infusion (Table 26). In TP-103, 2 events of TRM occurred by Rel Day 101, and another 6 events of TRM occurred by Rel Day 366, totaling 8 TRM events (8/45 [17.8%]). Of these, 6 occurred after first allo-HSCT, and 2 occurred after second allo-HSCT. One additional death was classified as TRM in an MSD patient that occurred after Rel Day 366. All patients with TRM had experienced GVHD and/or engraftment failure and all TRM events by Rel Day 366 occurred in NMSD patients.

Table 26. Transplant-Related Mortality (TP-102/104, TP-103, TP-103 MSD, TP-103 NMSD)

	eli-cel	TP-103		
	TP-102/104 N=67	Overall N=59	MSD N=11	NMSD N=48
Transplant-related mortality (Rel Day 1 to Rel Day 101)				
Evaluable patients	60	57	10	47
n (%)	0	2 (3.5)	0	2 (4.3)
Exact 95% CI	0.0, 6.0	0.4, 12.1	0.0, 30.8	0.5, 14.5
p-value[a]		0.2352	-	0.1906
Transplant-related mortality (Rel Day 1 to Rel Day 366)				
Evaluable patients	46	45	9	36
n (%)	0	8 (17.8)	0	8 (22.2)
Exact 95% CI	0.0, 7.7	8.0, 32.1	0.0, 33.6	10.1, 39.2
p-value[a]		0.0025	-	0.0008

Abbrev.: CI, confidence interval; HSCT, hematopoietic stem cell transplantation; MSD, matched sibling donor; NMSD, not a matched sibling donor; TP, transplant population.
Analysis of TRM includes data from patients who underwent ≥ 1 allo-HSCT.
'-' means the p-value was not calculable because no patients in either group had the applicable event.
If a patient received rescue cell therapy in ALD-102 or ALD-104 or subsequent allo-HSCT(s) in ALD-103, the patient is considered to have an event if the patient died due to transplant-related causes within 100 days (or 365 days) post any HSCT.
[a] P-value is based on Fisher's exact test comparing TP-103 (overall and subgroups) with pooled TP-102/104.

6.6.7.3. Treatment-Emergent Adverse Events

As described earlier, due to the differences in collection of AE data between studies, only select comparisons can be made between adverse events in TP-102/104 and those in TP-103.

≥ Grade 3 TEAEs

Grade 3 or higher TEAEs that occurred in at least 5% of patients through Month 12 in TP-102/104 compared with TP-103 are presented Table 27 with SOCs and PTs listed in decreasing frequency based on TP-103. Of note, ≥ Grade 3 hematological TEAEs are excluded from this table due to the limited collection of these events in ALD-103.

Key differences in the overall ≥ Grade 3 non-hematological TEAE (D1 to Month 12) profiles of TP-102/104 and TP-103 generally result from the need for post-transplant immunosuppression in allo-HSCT recipients as opposed to autologous eli-cel recipients. Specifically:

- In the Vascular disorders SOC, no patients in TP-102/104 experienced an event, while 28/59 patients (47.5%) in TP-103 experienced 34 ≥ Grade 3 TEAEs. Twenty-nine of these 34 events were hypertension, which was experienced by 28/59 (47.5%) patients. Most required antihypertensive medication. Hypertension is most likely a side effect of immunosuppressant therapy (Puschel et al. 2012).
- In the Infections and infestations SOC, 9 of 67 patients (13.4%) in TP-102/104 experienced 12 ≥ Grade 3 TEAEs, whereas 34/59 patients (57.6%) in TP-103 experienced 81 events. Infections following HSCT are associated with significant

morbidity and mortality (Marr 2012; Sahin et al. 2016; Cho et al. 2018) and as such a comparison of ≥ Grade 3 infections in eli-cel and allo-HSCT treated patients is presented in more detail below.

- In the Immune system SOC, no patients in TP-102/104 experienced an event, while 7/59 (11.9%) patients in TP-103 experienced 8 ≥ Grade 3 TEAEs, including engraftment syndrome and transplant rejection.

Table 27. ≥ Grade 3 Treatment-Emergent Adverse Events Occurring in ≥ 5% Patients by SOC and PT From Day 1 to Month 12 (TP-102/104, TP-103)

System Organ Class Preferred Term	TP-102/104 N=67 n (%), E	TP-103 Overall N=59 n (%), E	TP-103 MSD N=11 n (%), E	TP-103 NMSD N=48 n (%), E
Patients with ≥ 1 Grade ≥ 3 TEAE	**64 (95.5), 425**	**54 (91.5), 454**	**11 (100.0), 62**	**43 (89.6), 392**
Infections and infestations	**9 (13.4), 12**	**34 (57.6), 81**	**6 (54.5), 9**	**28 (58.3), 72**
Clostridium difficile infection	0	7 (11.9), 7	0	7 (14.6), 7
Device related infection	1 (1.5), 1	6 (10.2), 6	3 (27.3), 3	3 (6.3), 3
Epstein-Barr viremia	0	4 (6.8), 4	0	4 (8.3), 4
Pneumonia	1 (1.5), 1	4 (6.8), 4	2 (18.2), 2	2 (4.2), 2
BK virus infection	0	3 (5.1), 4	0	3 (6.3), 4
Cytomegalovirus viremia	0	3 (5.1), 4	0	3 (6.3), 4
Human herpes virus 6 infection	0	3 (5.1), 3	0	3 (6.3), 3
Staphylococcal bacteremia	0	3 (5.1), 3	1 (9.1), 1	2 (4.2), 2
Upper respiratory tract infection	0	3 (5.1), 3	1 (9.1), 1	2 (4.2), 2
Gastrointestinal disorders	**36 (53.7), 48**	**33 (55.9), 64**	**9 (81.8), 16**	**24 (50.0), 48**
Stomatitis	31 (46.3), 31	30 (50.8), 30	8 (72.7), 8	22 (45.8), 22
Nausea	7 (10.4), 7	11 (18.6), 13	4 (36.4), 4	7 (14.6), 9
Abdominal pain	2 (3.0), 2	4 (6.8), 4	0	4 (8.3), 4
Diarrhea	1 (1.5), 1	4 (6.8), 4	1 (9.1), 1	3 (6.3), 3
Vomiting	4 (6.0), 4	4 (6.8), 4	2 (18.2), 2	2 (4.2), 2
Metabolism and nutrition disorders	**21 (31.3), 23**	**28 (47.5), 52**	**7 (63.6), 11**	**21 (43.8), 41**
Decreased appetite	16 (23.9), 16	24 (40.7), 28	6 (54.5), 6	18 (37.5), 22
Hypokalemia	5 (7.5), 5	10 (16.9), 10	2 (18.2), 2	8 (16.7), 8
Dehydration	0	3 (5.1), 4	0	3 (6.3), 4
Hyperkalemia	0	3 (5.1), 3	0	3 (6.3), 3
Vascular disorders	**0**	**28 (47.5), 34**	**4 (36.4), 5**	**24 (50.0), 29**
Hypertension	0	28 (47.5), 29	4 (36.4), 5	24 (50.0), 24
Respiratory, thoracic and mediastinal disorders	**7 (10.4), 7**	**13 (22.0), 20**	**3 (27.3), 4**	**10 (20.8), 16**
Hypoxia	1 (1.5), 1	5 (8.5), 5	0	5 (10.4), 5
Epistaxis	5 (7.5), 5	3 (5.1), 3	2 (18.2), 2	1 (2.1), 1
Respiratory failure	0	3 (5.1), 4	0	3 (6.3), 4
General disorders and administration site conditions	**4 (6.0), 4**	**12 (20.3), 19**	**2 (18.2), 2**	**10 (20.8), 17**
Pyrexia	3 (4.5), 3	7 (11.9), 7	2 (18.2), 2	5 (10.4), 5
Nervous system disorders	**3 (4.5), 5**	**10 (16.9), 26**	**2 (18.2), 3**	**8 (16.7), 23**
Neurological decompensation	1 (1.5), 1	6 (10.2), 17	0	6 (12.5), 17
Headache	0	3 (5.1), 3	2 (18.2), 2	1 (2.1), 1
Psychiatric disorders	**1 (1.5), 1**	**8 (13.6), 10**	**0**	**8 (16.7), 10**
Agitation	0	4 (6.8), 4	0	4 (8.3), 4

System Organ Class Preferred Term	TP-102/104 N=67 n (%), E	TP-103 Overall N=59 n (%), E	TP-103 MSD N=11 n (%), E	TP-103 NMSD N=48 n (%), E
Renal and urinary disorders	**1 (1.5), 1**	**8 (13.6), 10**	**0**	**8 (16.7), 10**
Cystitis hemorrhagic	0	3 (5.1), 3	0	3 (6.3), 3
Immune system disorders	**0**	**7 (11.9), 8**	**1 (9.1), 1**	**6 (12.5), 7**
Engraftment syndrome	0	3 (5.1), 3	0	3 (6.3), 3
Investigations	**3 (4.5), 5**	**5 (8.5), 7**	**0**	**5 (10.4), 8**
Alanine aminotransferase increased	3 (4.5), 3	1 (1.7), 1	0	1 (2.1), 1

Abbrev.: AE, adverse event; D, day; E, event; M, month; MSD, matched sibling donor; NMSD, not matched sibling donor; PT, preferred term; SOC, system organ class; TP, transplant population.
PTs (and their associated SOC) are included for AEs that were observed in ≥ 5% of patients during D1 to M12 study period in either TP-102/104 pool or TP-103 (overall and in donor category subgroups) and are sorted based on decreasing frequency by SOC and then PT, and then alphabetically based upon the TP-103 overall column. In TP-103, the PTs presented are based on the ≥ 5% threshold in TP-103 overall (and not based on percentages in the donor category subgroups). The SOC values presented show the incidence of all patients/events that occurred under that SOC (not only those events meeting the ≥ 5% threshold).
Patients at risk for each period (N in column header) is defined to be the patients who entered the study period. If an AE started in 1 reporting period and continued into the next reporting period, it was counted only in the first period. If an AE started and stopped in 1 reporting period and then recurred in the next reporting period, it was counted in both periods. Patient were counted once for each SOC and PT even if they had multiple instances of the event in 1 period. For AEs with worsening severity in which the AE started in the first period and worsened in the next period, the patient was counted in both periods. All events reported in the database are counted in the number of events.

≥ Grade 3 Treatment-Emergent Infections

A summary of all Grade 3 or higher TEAEs that occurred in the Infections and infestations SOC through Month 12 for TP-102/104 and TP-103 is provided in Table 28.

Table 28. ≥ Grade 3 Infection and Infestation Treatment-Emergent Adverse Events by PT From Day 1 to Month 12 (TP-102/104, TP-103)

Preferred Term	TP-102/104 N=67 n (%), E	TP-103 Overall N=59 n (%), E	TP-103 MSD N=11 n (%), E	TP-103 NMSD N=48 n (%), E
Patients with ≥ 1 TEAE ≥ Grade 3 Infections and infestations SOC	**9 (13.4), 12**	**34 (57.6), 81**	**6 (54.5), 9**	**28 (58.3, 72)**
Clostridium difficile infection	0	7 (11.9), 7	0	7 (14.6), 7
Device related infection	1 (1.5), 1	6 (10.2), 6	3 (27.3), 3	3 (6.3), 3
Epstein-Barr viremia	0	4 (6.8), 4	0	4 (8.3), 4
Pneumonia	1 (1.5), 1	4 (6.8), 4	2 (18.2), 2	2 (4.2), 2
Human herpes virus 6 infection	0	3 (5.1), 3	0	3 (6.3), 3
Staphylococcal bacteremia	0	3 (5.1), 3	1 (9.1), 1	2 (4.2), 2
BK virus infection	0	3 (5.1), 4	0	3 (6.3), 4
Cytomegalovirus viremia	0	3 (5.1), 4	0	3 (6.3), 4
Upper respiratory tract infection	0	3 (5.1), 3	1 (9.1), 1	2 (4.2), 2
Adenovirus infection	0	2 (3.4), 4	0	2 (4.2), 2
Enterococcal bacteremia	0	2 (3.4), 2	0	2 (4.2), 2
Sepsis	0	2 (3.4), 2	0	2 (4.2), 2
Sinusitis	1 (1.5), 1	2 (3.4), 2	1 (9.1), 1	1 (2.1), 1

Preferred Term	TP-102/104 N=67 n (%), E	TP-103		
		Overall N=59 n (%), E	MSD N=11 n (%), E	NMSD N=48 n (%), E
Gastroenteritis adenovirus	0	1 (1.7), 1	0	1 (2.1), 1
Rhinovirus infection	0	1 (1.7), 1	0	1 (2.1), 1
Bacillus bacteremia	0	1 (1.7), 1	0	1 (2.1), 1
Bacteremia	0	1 (1.7), 1	0	1 (2.1), 1
Bronchiolitis	0	1 (1.7), 1	0	1 (2.1), 1
Clostridium difficile colitis	0	1 (1.7), 2	0	1 (2.1), 2
Coxsackie viral infection	0	1 (1.7), 1	0	1 (2.1), 1
Cystitis	0	1 (1.7), 1	0	1 (2.1), 1
Cytomegalovirus infection	0	1 (1.7), 1	1 (9.1), 1	0
Acute sinusitis	0	1 (1.7), 1	0	1 (2.1), 1
Atypical pneumonia	0	1 (1.7), 1	0	1 (2.1), 1
Enterococcal infection	0	1 (1.7), 1	0	1 (2.1), 1
Epstein-Barr virus infection	0	1 (1.7), 1	0	1 (2.1), 1
Fungal infection	0	1 (1.7), 1	0	1 (2.1), 1
Gastroenteritis adenovirus	0	1 (1.7), 1	0	1 (2.1), 1
Gastroenteritis astroviral	0	1 (1.7), 1	0	1 (2.1), 1
Gastrointestinal viral infection	0	1 (1.7), 1	0	1 (2.1), 1
Human herpesvirus 6 encephalitis	0	1 (1.7), 1	0	1 (2.1), 1
Kidney infection	0	1 (1.7), 1	0	1 (2.1), 1
Klebsiella bacteremia	0	1 (1.7), 1	0	1 (2.1), 1
Lip infection	0	1 (1.7), 1	0	1 (2.1), 1
Lower respiratory tract infection	0	1 (1.7), 1	0	1 (2.1), 1
Oral candidiasis	0	1 (1.7), 1	0	1 (2.1), 1
Otitis media	1 (1.5), 1	1 (1.7), 1	0	1 (2.1), 1
Parvovirus B19 infection	0	1 (1.7), 1	0	1 (2.1), 1
Parvovirus infection	0	1 (1.7), 1	0	1 (2.1), 1
Rhinovirus infection	0	1 (1.7), 1	0	1 (2.1), 1
Septic shock	0	1 (1.7), 1	0	1 (2.1), 1
Serratia infection	0	1 (1.7), 1	0	1 (2.1), 1
Staphylococcal infection	0	1 (1.7), 1	0	1 (2.1), 1
Tooth abscess	0	1 (1.7), 1	0	1 (2.1), 1
Urinary tract infection	0	1 (1.7), 1	0	1 (2.1), 1
Viral infection	0	1 (1.7), 1	0	1 (2.1), 1
Viral upper respiratory infection	0	1 (1.7), 1	0	1 (2.1), 1
Cystitis viral	1 (1.5), 1	0	0	0
Pseudomonal bacteremia	2 (3.0), 2	0	0	0
Soft tissue infection	1 (1.5), 1	0	0	0
Stenotrophomonas infection	1 (1.5), 1	0	0	0
Streptococcal bacteremia	1 (1.5), 1	0	0	0
Vascular device infection	2 (3.0), 2	0	0	0

Abbrev.: AE, adverse event; D, day; E, event; M, month; MSD, matched sibling donor; NMSD, not matched sibling donor; PT, preferred term; SOC, system organ class; TEAE, treatment-emergent adverse event; TP, transplant population.
Note: PTs are included for all Grade 3 or higher TEAEs that occurred in the Infections and infestations SOC from D1 to M12 in either TP-102/104 pool or TP-103 (overall and in donor category subgroups). PTs are sorted based on decreasing frequency and then alphabetically based upon the TP-103 overall column.
Patients at risk for each period (N in column header) is defined as the patients who entered the study period. If an AE started in 1 reporting period and continued into the next reporting period, it was counted only in the first period. If an AE started and stopped in one reporting period and then recurred in the next reporting period, it was counted in both periods. Patients were counted once for each SOC and PT even if they had multiple instances of the event in one 1 period. For AEs with worsening severity in which the AE started in the first period and worsened in the next period, the patient was counted in both periods. All events reported in the database are counted in the number of events.

Nine of 67 (13.4%) eli-cel-treated patients experienced 12 ≥ Grade 3 TEAEs compared with 34/59 (57.6%) TP-103 patients who experienced 81 events. The majority of these severe infections (72/81 events) in TP-103 were in 28 patients in the NMSD subgroup.

A medical review determined that 16 patients in TP-103 experienced 26 opportunistic infections after the first allo-HSCT, including 14 that were SAEs. In contrast, in TP-102/104, 3 patients experienced 3 ≥ Grade 3 TEAEs that were considered opportunistic: cystitis viral (BK virus), Pseudomonal bacteremia, Pseudomonal bacteremia and concurrent Stenotrophomonas infection. The remaining ≥ Grade 3 infection TEAEs in TP-102/104 were considered typical of the interventions and populations under study.

6.6.7.4. Treatment-Emergent Serious Adverse Events

Treatment-emergent SAEs reported from D1 to M48 are compared between TP-102/104 and TP-103 in Table 35 in Section 11, Appendix C, with SOCs and PTs listed in decreasing frequency based on TP-103.

Key differences in the TESAE profiles of TP-102/104 and TP-103 generally resulted from the need for post-transplant immunosuppression in allo-HSCT recipients versus autologous eli-cel recipients.

- The proportion of patients with serious infections was more than 2-fold higher in TP-103 than in TP-102/104 (22/59 [37.3%] vs. 11/67 [16.4%], respectively). Many serious infections (14 events) in TP-103 were opportunistic.
- There were no TESAEs reported in the Vascular disorders SOC for TP-102/104; however, 5/59 (8.5%) patients in TP-103 reported a TESAE in this SOC. Two patients had serious events of hypertension, a known consequence of immunosuppressants, and 2 patients had events that were thrombotic (deep vein thrombosis and thrombosis) that may be linked to the endothelial effects of immunosuppression (Puschel et al. 2012).
- There were no TESAEs related to autoimmunity in TP-102/104; however, 2 patients in TP-103 experienced 3 TESAEs attributed to autoimmunity by the investigator. One patient in the NMSD subgroup experienced a TESAE of autoimmune hemolytic anemia and 1 patient in the MSD subgroup experienced 2 TESAEs attributed to autoimmunity by the investigator: hemolytic anemia and encephalopathy.

TESAEs through Month 48 in the Nervous system disorders SOC were compared between TPES-103 and TP-102/104. The TPES subgroup of TP-103 was used for this comparison given the similarities in the baseline neurologic status between TPES-103 and TP-102/104. Six of 67 (9.0%) patients in TP-102/104 compared with 3/27 (11.1%) in TPES-103 had events. As noted above, many of these events in TP-102/104 were seizures (refer to Section 6.6.5.3) compared with no patients in TPES-103. Differential follow up and small sample size limits this comparison.

6.6.8. Duration of Hospitalization

Patients treated with eli-cel in TP-102/104 spent fewer days hospitalized, both before and after NE, when compared with patients treated with allo-HSCT in TP-103. Overall, patients who received eli-cel were observed to have significantly shorter in-patient hospitalizations before NE (median [min, max] of 28 [15, 59] days) compared with patients who received allo-HSCT (median [min, max] of 51 [25, 240] days), $p < 0.0001$.

Analysis of the population of patients who successfully achieved NE (NEP) showed that patients in NEP-102/104 had fewer incidences of in-patient hospitalization after NE compared with patients in NEP-103, with 24/67 (35.8%) and 27/53 (50.9%) patients hospitalized during the post-NE to Month 24 period, respectively. The median (min, max) duration of hospitalization was shorter in this time period for patients in NEP-102/104 (3 [2, 33] days) than for patients in NEP-103 (14 [3, 308] days), $p < 0.0001$.

6.7. Clinical Laboratory Evaluations

6.7.1. Hematology Results

Severe depletion of neutrophils and platelets was observed in patients during and after conditioning, as intended. After eli-cel infusion, all evaluable patients (67/67) in TP-102/104 achieved both NE and PE. From Month 12 to Month 24, 13/46 (28.3%) patients had a hematology parameter below the low threshold for potential clinical significance (PCS), whereas from Month 24 to DLC, all evaluable patients had neutrophils and platelets above the low threshold for PCS, except for 1 patient with a single PCS low value for platelets and 1 patient with PCS low value for leukocytes in TP-102. At the most recent visit, neither of these patients met PCS criteria.

Patients in LTF-304 generally continue to demonstrate complete and stable hematopoietic reconstitution.

6.7.1.1. Prolonged Cytopenias

Prolonged cytopenia following transplant is considered a risk of treatment with eli-cel, given that a substantial minority of patients had persistent cytopenias of 1 or more cell lineages after transplant. However, these cytopenias had limited clinical impact; patients with cytopenia had comparable bleeding and infection to all other patients.

Individual patient hematology data over time were reviewed to assess the recovery of neutrophils, hemoglobin, and platelets to values above the Common Terminology Criteria for Adverse Events (CTCAE; Version 4.03) Grade 3 threshold. This analysis showed that

18/64 (28.1%) of patients in TP-102/104 had any ≥ Grade 3 cytopenia on or after Rel Day 60, including decreased platelet count (14.1%), decreased neutrophil count (21.9%), and decreased hemoglobin (1.6%). On or after Rel Day 100, 13.0% of patients had any ≥ Grade 3 or higher cytopenia, including decreased platelet count (7.4%) or decreased neutrophil count (9.3%), and none had decreased hemoglobin (0%).

Of note, this is a conservative analysis in that it includes patients who had only isolated values meeting the prespecified criteria. A review revealed that approximately half of these patients had a single Grade 3 or higher low platelet or neutrophil count on or after Rel Day 60, rather than persistent cytopenia(s).

None of the patients with prolonged thrombocytopenia had a bleeding AE on or after Rel Day 60. Five of 14 patients with prolonged neutropenia had 9 AEs of infection on or after Rel Day 60; 2 of these infections were considered opportunistic (Pseudomonas bacteremia and human herpesvirus 6 infection) and all were self-limited or resolved except for the ongoing non-serious human herpesvirus 6 infection.

6.7.2. eli-cel-Specific Clinical Laboratory Evaluations

6.7.2.1. Replication-Competent Lentivirus

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

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

6.7.2.2. Integration Site Analysis

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

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

Although ISA can be used to provide an indication of oligoclonality by assessing the relative frequency of individual IS, it cannot provide a determination of whether an IS that is present at high frequency is a representation of benign clonal expansion or is associated with malignancy. Hematologic assessments remain the standard means by which patients are evaluated for

hematologic malignancy. Clinical data are required to inform treatment decisions. There are several reports of high frequency clones that have not resulted in adverse clinical consequences (Negre et al. 2016; Thompson et al. 2018; De Ravin et al.).

Patients are monitored routinely by ISA through 15 years post eli-cel treatment.

After eli-cel treatment, ISA generally showed robust polyclonal reconstitution of the hematopoietic cell system, based on the identification of hundreds to thousands of unique mappable IS in all patients, with the highest total number of unique mappable IS at any single time point ranging from 552 to 15683 per patient. Relative IS frequencies commonly fluctuate over time, and thus, individual IS may increase in frequency but then plateau or decline. This variance likely reflects the dynamics of HSC clones and subsequent progenitors cycling through periods of expansion and quiescence in the bone marrow.

Clonal contribution above 50%

Four patients had ISA results that showed the presence of a clone contributing at least 50% of analyzed cells at one or more timepoint. In two patients, the clone was identified at the first ISA assessment (Month 6) and both patients were later diagnosed with MDS. In one patient, the clone was identified approximately 7.5 years after eli-cel infusion; the patient had been diagnosed with MDS. Refer to Section 6.6.5.1 for details on the patients diagnosed with MDS. In the fourth patient, the clone was identified in the myeloid CD15+ population approximately 5 years after eli-cel infusion and has remained above 50% in the CD15+ population through the patient's most recent visit at Year 6.5, when the clone contributed more than 50% of cells in the whole blood as well. As of the last visit, his CBCs are within normal range and bone marrow aspirates and biopsies have shown no morphology, flow cytometry, cytogenetic, or molecular evidence of malignancy. Thus, due to the absence of malignancy, this finding appears to represent benign clonal expansion.

These patients have multiple IS in the relevant clones. Importantly, 3 patients (2 with MDS and 1 with apparent benign clonal expansion) have an IS in *MECOM*. *MECOM* dysregulation is evidenced by increased expression of *EVI1* transcript in these 3 patients. In the fourth patient (diagnosed with MDS), ISA showed the presence of an IS in *PRDM16*, a gene that is related to *MECOM*, with 63% sequence similarity. Both *PRDM16* and *MECOM* are proto-oncogenes and belong to the family of PR-domain proteins that are involved in chromosomal translocation in MDS/AML. Like *MECOM*, full-length *PRDM16* is thought to function as a tumor suppressor with expression of an oncogenic shorter isoform. The other IS identified in these clones are not known proto-oncogenes.

Oligoclonality: IS with a Relative Frequency ≥10%

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

Eleven patients met these criteria at their last timepoint. Of these, 4 patients have been described above (3 with MDS and 1 with benign clonal expansion). In addition, 1 patient met these criteria

based on an ISA method no longer in use and withdrew from study in 2018 to receive allo-HSCT due to concerns of radiographic CALD progression, so no follow up ISA data are available.

The remaining 6 patients (4 with persistent oligoclonality) are described in Table 29 which provides detail on timing and location of IS with a Relative Frequency ≥10%, as well as complete blood count and bone marrow biopsy/molecular testing when performed. Further, bluebird bio's interpretation of the available data is provided, i.e, none of these patients have evidence of hematologic malignancy as of their last assessments.

Of note, 2 additional patients met the criteria of an IS with a Relative Frequency ≥10% at a previous timepoint but no longer did at the last assessment, indicating the potential for this finding to be transient, consistent with expected clonal dynamics.

Table 29. Patients with an IS with a Relative Frequency ≥10%

Patient	Study visit ISA first above ≥10% Rel Frequency /Insertion Site	Study Visit of last CBC	Most recent CBC results				Time since last CBC (in months) (0-2, 2-4, 4-6)	Most recent bone marrow and molecular testing	Interpretation
			ANC (10^9/L)	WBC (10^9/L)	Plt (10^9/L)	Hb (g/dL)			
102-13[a]	M54/*MECOM*	M84	4.2	7.3	188	15.7	0-2	Not done	No evidence of malignancy
102-23[a]	M24/*SMG6*	M54	2.8	8.0	306	13.4	2-4	Not done	No evidence of malignancy
102-31[a]	Clone 1[b]: M18/*PLAG1, SECISBP2* Clone 2[b]: M42/*MECOM, EVI5*	M48	3.5	5.1	184	11.2	0-2	(M48) 40-50% cellularity with maturing trilineage hematopoiesis, no dysplasia Normal karyotype; no abnormalities on FISH; no pathologic variants on next generation sequencing.	No evidence of malignancy
104-09[a]	M24/*LINC00982*	M30	3.1	5.9	149	14.2	0-2	(M26) 30% cellularity with maturing trilineage hematopoiesis, atypical megakaryopoiesis. Parvovirus detected Normal karyotype; next generation sequencing revealed likely pathogenic loss of function heterozygous variant in the MPL gene[c]; no abnormalities on flow cytometry.	No evidence of malignancy. Megakaryocyte atypia may be attributed to parvovirus
104-22	M12/*MPL*	M18	2.7	4.9	118	14.5	2-4	Not done	No evidence of malignancy

104-27	M12/*SMG6, ACSF3, PDE3A*	M12	1.5	4.2	232	12.6	4-6	Not done	No evidence of malignancy

Abbrev.: allo-HSCT, allogeneic hematopoietic stem cell transplant; ANC, absolute neutrophil count; CBC, complete blood count; FISH, fluorescence in-situ hybridization; Hb, hemoglobin; M, month; Plt, platelet; WBC, white blood cell.
[a] Indicates patient met persistent oligoclonality criteria
[b] Integration sites are presumed to be present in the same clone, based on RelFreq values that are tracking together over time
[c] This variant, seen at variant allele frequencies suggestive of germline origin, is different from the activating variants seen in the myeloproliferative neoplasms, as it leads to a loss of function.

6.8. Late-Breaking Update

A late-breaking data cut from the safety database was performed on 29-Apr-2022 to provide the most up-to-date SAE information. The following additional SAEs were reported; one of these (disease progression) was considered related to eli-cel:

Total incontinence: A 6-year-old male at the time of consent developed an MFD of Grade 3 total incontinence approximately 29 months after eli-cel treatment in ALD-104. The patient was previously diagnosed with myelitis (transverse) on Rel Day 201. The event of total incontinence was not considered related to eli-cel, and, according to the investigator, is unlikely to be due to CALD, but rather to the previously reported SAE of transverse myelitis.

Seizure: The same patient who developed total incontinence also experienced a Grade 3 SAE of seizure (new onset) approximately 30 months after treatment with eli-cel in ALD-104. The patient had a 20-second seizure, was hospitalized and received levetiracetam. The following day the event of seizure was considered resolved. The patient was discharged from the hospital and was noted to have an intercurrent urinary tract infection. The event was assessed by the investigator as not related to eli-cel and likely triggered by an underlying condition in addition to his old lesion.

COVID-19: A 13-year-old male at the time of consent experienced a Grade 1 SAE of COVID-19 infection approximately 1 year after eli-cel treatment in ALD-104 and was hospitalized overnight for treatment and observation. The following day the patient was hemodynamically stable and discharged from the hospital with event resolution. The investigator assessed the event as not related to eli-cel.

Disease progression: A 9-year-old male at the time of consent experienced a Grade 3 SAE of disease progression approximately 6 months after eli-cel treatment in ALD-104. This patient was unique in the eli-cel program in that he had a full deletion of the *ABCD1* gene. Following treatment, he had persistent gadolinium enhancement, increasing Loes score, and declining peripheral blood (PB) VCN. A therapeutic trial of immunosuppression was administered based on the hypothesis that the disease progression was the result of a potential immune response to ALDP protein. The PB VCN increased numerically but remained low. The patient remained clinically stable with no symptoms of neurologic decline. However, the investigator determined the patient should undergo allo-HSCT in an attempt to halt disease progression. The patient underwent a 6/8 umbilical cord blood transplant on Rel Day 352. On Rel Day 368 neutrophil engraftment was achieved and on Rel Day 373 engraftment results in the peripheral blood showed expected mixed donor engraftment, 42% donor in the lymphoid and 100% donor in the myeloid fraction. The investigator assessed the event of disease progression as possibly related to eli-cel.

Septic shock: A 4-year-old male at the time of consent experienced a Grade 4 SAE of septic shock following allo-HSCT for treatment of MDS. This patient was diagnosed with MDS approximately 7.5 years after eli-cel treatment in ALD-102 and underwent allo-HSCT (refer to Section 6.6.5.1). Five days after transplant, the patient experienced septic shock due to a central line infection. He was transferred to the intensive care unit and intubated. The patient was extubated 5 days later and maintained on antibiotics to manage his two infections (Streptococcus mitis/oralis and Clostridium perfringens) in addition to post-transplant support medication which

included daily filgrastim and frequent platelet transfusions. The event was considered resolved with discontinuation of antibiotics approximately one month after onset. The investigator assessed the event as not related to eli-cel but due to the central line placed for allo-HSCT.

Hyperglycemia: A 11-year-old male at the time of consent experienced a Grade 3 SAE of hyperglycemia believed to be due to tacrolimus following allo-HSCT for treatment for MDS. The patient was diagnosed with MDS approximately 14 months after eli-cel treatment in ALD-104 (refer to Section 6.6.5.1). Approximately 3 months after transplant, the patient was hospitalized to manage hyperglycemia (458 mg/dL). The patient's condition improved three days later, and he was discharged with initiation of insulin and metformin. The final diagnosis was post-transplant diabetes probably secondary to the use of tacrolimus. The event was ongoing. The investigator assessed the event as not related to eli-cel.

Complication associated with device and catheter site haemorrhage: A 6-year-old male at the time of consent experienced a Grade 3 SAE of a damaged lumen of the central line with bleeding approximately 8 months after eli-cel treatment in ALD-104 and was hospitalized overnight for monitoring and sedated line removal. The following day the patient was clinically stable and discharged from the hospital with event resolution. The investigator assessed the event as not related to eli-cel.

Worsening of visual impairment: A 6-year old male at the time of consent developed a Grade 3 SAE of worsening visual impairment approximately 4 years after eli-cel treatment in ALD-102. The patient previously experienced nonserious adverse events of Grade 2 visual acuity reduced and visual field defect 18 months and 36 months respectively after eli-cel treatment. At the Year 4 study visit, the patient's visual function had worsened since the last visit and his ability to carry out daily activities was limited. His visual acuity was assessed as 20/70 as measured using a bedside pocket vision screening card. The event was ongoing. The investigator assessed the event as not related to eli-cel.

6.9. Safety Summary

The safety success criterion for the eli-cel development program was based on achieving a statistically significant reduction ($p < 0.05$) in the proportion of patients who experienced either ≥ Grade II acute GVHD or chronic GVHD following eli-cel treatment compared with those who received allo-HSCT. As autologous therapy, eli-cel was not anticipated to result in GVHD, whereas this is a key cause of morbidity and mortality in allo-HSCT recipients. The safety success criterion was met with no patient in TP-102 experiencing GVHD compared with 26/50 (52%) patients in TP-103 ($p < 0.0001$).

One death (1/55 [1.8%]) was reported in TP-102/104 compared with 15 deaths (15/59, 25.4%) in TP-103; 12 patients died after their first allo-HSCT and 3 after their second allo-HSCT. Five of these 15 patients were in the TPES subgroup (5/27 [18.5%]); 3 of these died after first allo-HSCT and 2 after second allo-HSCT. Nine of the deaths in TP-103 were classified as TRM, with 8 occurring within 1 year of infusion, all in the NMSD subgroup. There was no TRM within 1 year after eli-cel treatment based on the evaluable patients in TP-102/104.

In TP-102/104, all evaluable patients had successful NE (67/67 [100%]), with no observed secondary graft failure. In TP-103, 53/59 (89.8%) patients achieved NE after their first

allo-HSCT, with primary or secondary engraftment failure observed in 10/38 (26.3%) evaluable patients, all of whom were in the NMSD subgroup.

As eli-cel is an autologous therapy, immunosuppression is not needed after transplant, whereas prolonged immunosuppression to prevent or treat GVHD following allo-HSCT confers significant risk. Through Month 12, fewer patients experienced severe infections in TP-102/104 (9/67 [13.4%]) than in TP-103 (34/59 [57.6%]), and the infections in TP-103 were often attributed to immunosuppression. In addition, no patients in TP-102/104 experienced severe hypertension compared with almost half of all patients in TP-103 (28/59 [47.5%]). Additional concomitant therapies were required to prevent and/or treat the effects of prolonged immunosuppressant therapy in TP-103.

Safety of eli-cel

The use of eli-cel is preceded by procedural and medical interventions that carry their own risks. The majority of AEs in this program were consistent with the known side effects of the procedures and pharmacotherapy entailed in this pretreatment and were predominately non-serious, time-limited, and managed with standard of care treatment.

Adverse events related to eli-cel included 3 serious events of MDS, 2 serious events of delayed hematopoietic reconstitution (1 in a patient later diagnosed with MDS), 1 serious event of BK viral cystitis, and 2 nonserious events of vomiting and 1 nonserious event of nausea which were likely related to the cryopreservative dimethyl sulfoxide in the drug product.

Insertional oncogenesis has long been recognized as a potential safety concern for ex vivo gene addition-modified HSC products using retroviral vectors, including LVV-transduced HSCs. In the eli-cel program, 3 patients have been diagnosed with MDS, all of which are considered likely mediated by Lenti-D LVV insertion.

A substantial minority of patients had persistent cytopenias of 1 or more cell lineages after transplant; these cytopenias had limited clinical impact. Patients in long-term follow-up study LTF-304 generally demonstrate complete and stable hematopoietic reconstitution.

Vector-derived RCL is a theoretical risk that has not been detected in any eli-cel treated patient.

6.10. Safety Conclusions

In this development program, eli-cel treated patients experienced significantly less GVHD than allo-HSCT treated patients (0 vs 52%, $p < 0.0001$), thus meeting the primary safety success criterion. As an autologous therapy, eli-cel allows for treatment without the immune mediated complications of allogeneic HSCT, including GVHD, graft rejection, and TRM. No eli-cel-treated patients experienced engraftment failure or TRM, and eli-cel-treated patients experienced fewer serious and opportunistic infections than allo-HSCT-treated patients. Risks associated with eli-cel include insertional oncogenesis and prolonged cytopenias.

eli-cel treated patients will continue to be followed in the clinical setting in ongoing ALD-104 and the long term follow up study LTF-304 up to 15 years post-eli-cel. In the post-marketing setting, a voluntary registry study, REG-502, is planned which will follow patients for 15 years after eli-cel treatment. Details about post-marketing pharmacovigilance are included in Section 13, Appendix E.

7. BENEFIT-RISK

The assessment of the eli-cel benefit-risk profile is complex. The development program provides clear evidence of a clinically meaningful and durable treatment effect. The majority of adverse events are reflective of the known effects of the conditioning agents. The morbidity and mortality associated with allo-HSCT are entirely avoided, but the gene-therapy-specific risk of insertional oncogenesis is incompletely characterized.

As CALD results in progressive loss of neurologic function and death, all eligible patients will be treated. The implementation of newborn screening will likely result in the identification of more affected patients prior to the onset of symptoms, thereby increasing the number of patients eligible for treatment.

Allo-HSCT is currently the only available therapeutic option for children with CALD, but the availability and success of treatment are heterogeneous. Outcomes following allo-HSCT depend on donor availability and type, varying according to whether a graft is from an MSD or NMSD. Historically, recipients of an allo-HSCT from an MSD have good outcomes. The ready availability of an MSD generally means the child with CALD is treated without delay, and thus without significant concern for disease progression while awaiting transplant. Unfortunately, only a minority of affected children have an MSD. Recipients of an NMSD graft have higher rates of immune-incompatibility than MSD recipients, and outcomes differ further according to whether the NMSD graft is from a MUD or a mismatched donor. Beyond donor-recipient histocompatibility, other factors such as stem cell source, donor age and sex, donor-recipient CMV/EBV status, and ABO compatibility may play a role in transplant outcome.

The morbidity and mortality of allo-HSCT derived from an NMSD are chiefly the result of immune-incompatibility, including GVHD, graft failure, and ultimately TRM. Some data (Kuhl et al. 2018) suggest that immune events may also be linked to an increased risk of future neurologic deterioration, perhaps due to limited exchange of bone marrow-derived donor macrophages in the brain. Further, all allo-HSCT recipients, regardless of donor type, are treated with immunosuppressants for approximately a year after transplant, with attendant risks including infection and hypertension. Recent advances in HLA matching have improved outcomes in recipients of a MUD allograft, but the identification of a well-matched donor may take months. Those who are transplanted with a graft from a mismatched donor have the highest rates of immune complications and death (Mallhi et al. 2017).

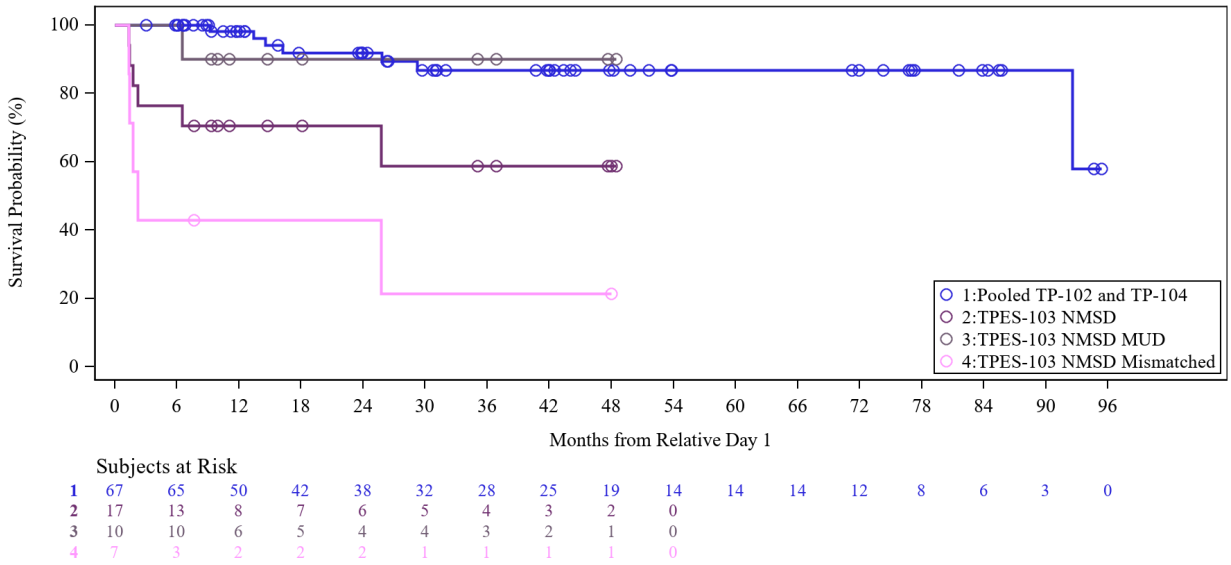
The potential to stabilize neurologic disease without the risk of immune complications and without the delay of searching for an appropriate HLA matched donor was the basis for the development of an autologous treatment option with eli-cel. The proposed indication is the treatment of pediatric patients with early CALD who do not have an available and willing MSD.

7.1. Benefits

Efficacy data from the eli-cel development program show consistent neurologic disease stabilization across endpoints, including direct measures of neurologic function and cognition. These are supported by data from radiologic assessments and pharmacodynamic bioassays. The clinical effect size is substantial and meaningful compared to the natural history of untreated disease and is comparable to that of allo-HSCT in patients with similar disease severity at baseline. In pivotal study ALD-102, 90.6% of patients treated with eli-cel achieved MFD-free survival at Month 24. Neurologic disease stabilization following eli-cel is durable in the majority of patients, with the longest follow-up being > 95 months.

The analysis of event-free survival encompasses the key events related to underlying disease and treatment, including death, MFDs, MDS, and need for second HSCT. Figure 18 and Table 30 demonstrate that the event-free survival rate after eli-cel treatment is similar or higher to allo-HSCT with an NMSD MUD or Mismatched donor, respectively, through 4 years of available post-treatment follow-up. Both eli-cel and the TPES-103-NMSD-MUD population confer significantly higher event-free survival rates than the TPES-103-NMSD-Mismatched population. eli-cel maintains an estimated event-free survival of 86.8% (95% CI: 72.7%, 93.9%) through 7 years of follow-up, after which the event-free survival is not reliably characterized.

Figure 18. Event-Free Survival Over Time by Histocompatibility (TP-102/104, TPES-103-NMSD, TPES-103-NMSD-MUD, TPES-103-NMSD-Mismatched)



Abbrev.: MFD, major functional disability; TPES, Strictly ALD-102 Eligible Transplant Population; NMSD, not a matched sibling donor; MUD, HLA-matched unrelated donor
Kaplan-Meier method; events include deaths, MFDs, MDS, and rescue cell administration or second allo-HSCT. Patients who did not experience any event are censored at their date of last contact.
Rel. Day 1 is the day of eli-cel infusion for TP-102/104 and the day of allo-HSCT for TPES-103 populations.

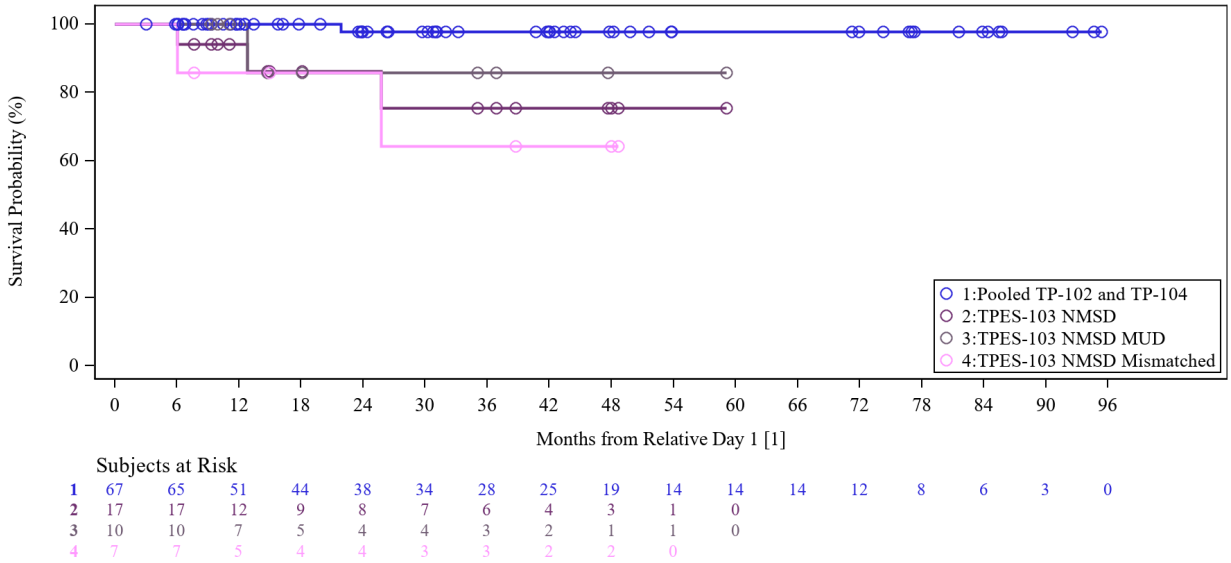
Table 30. Kaplan-Meier Event-Free Survival Analysis by Histocompatibility (TP-102/104, TPES-103-NMSD populations)

	eli-cel	allo-HSCT		
	TP-102/104 N=67	TPES-103 NMSD N=17	TPES-103 NMSD-MUD N=10	TPES-103 NMSD-Mismatched N=7
Survival rates, % (95% CI)				
Month 12	98.2 (88.0, 99.7)	70.6 (43.1, 86.6)	90.0 (47.3, 98.5)	42.9 (9.8, 73.4)
Month 24	91.9 (79.8, 96.9)	70.6 (43.1, 86.6)	90.0 (47.3, 98.5)	42.9 (9.8, 73.4)
Month 36	86.8 (72.7, 93.9)	58.8 (27.5, 80.4)	90.0 (47.3, 98.5)	21.4 (1.2, 58.6)
Month 48	86.8 (72.7, 93.9)	58.8 (27.5, 80.4)	90.0 (47.3, 98.5)	21.4 (1.2, 58.6)
Month 60	86.8 (72.7, 93.9)	- (-, -)	- (-, -)	- (-, -)
Month 72	86.8 (72.7, 93.9)	- (-, -)	- (-, -)	- (-, -)
Month 84	86.8 (72.7, 93.9)	- (-, -)	- (-, -)	- (-, -)
Month 94	57.9 (9.1, 88.3)	- (-, -)	- (-, -)	- (-, -)
Events, n (%)	7 (10.4)	6 (35.3)	1 (10.0)	5 (71.4)
Death	0	1 (5.9)	0	1 (14.3)
MFD	2 (3.0)	0	0	0
Second HSCT	2 (3.0)	5 (29.4)	1 (10.0)	4 (57.1)
MDS	3 (4.5)	0	0	0

Abbrev.: MFD, major functional disability; CI, confidence interval; HSCT, hematopoietic stem cell transplantation; TPES, Strictly ALD-102 Eligible Transplant Population; TPES, Strictly ALD-102 Eligible Transplant Population; NMSD, not a matched sibling donor; MUD, HLA-matched unrelated donor; MDS, myelodysplastic syndrome
Estimates of Event-free survival are obtained using the Kaplan-Meier method, where events include deaths, MFDs, MDS, and rescue cell administration or second allo-HSCT. Patients who did not experience any event are censored at their date of last contact.
Rel. Day 1 is the day of eli-cel infusion for TP-102/104 and the day of allo-HSCT for TPES-103 populations.

Overall survival is higher after eli-cel treatment as compared to allo-HSCT with an NMSD, particularly with a mismatched donor, through 4 years of available post-treatment follow-up (Figure 19 and Table 31). eli-cel maintains an estimated overall survival of 97.7% (95% CI: 84.6%, 99.7%) through 7 years of follow-up.

Figure 19. Overall Survival Over Time by Histocompatibility (TP-102/104, TPES-103-NMSD, TPES-103-NMSD-MUD, TPES-103-NMSD-Mismatched)



Abbrev.: TPES, Strictly ALD-102 Eligible Transplant Population; MSD, matched sibling donor; NMSD, not a matched sibling donor; MUD, matched unrelated donor.
Kaplan-Meier method; event is death of all causes. TP 102/104 patients who withdrew to receive allo-HSCT were censored at their end of study visit; all other patients who are alive are censored at their last contact date.
Rel. Day 1 is the day of eli-cel infusion for TP-102/104 and the day of allo-HSCT for TPES-103 populations.

Table 31. Kaplan-Meier Analysis of Overall Survival by Histocompatibility (TP-102/104, TPES-103-NMSD populations)

	eli-cel	allo-HSCT		
	TP-102/104 N=67	**TPES-103 NMSD N=17**	**TPES-103 NMSD-MUD N=10**	**TPES-103 NMSD-Mismatched N=7**
Survival rates, % (95% CI)				
Month 12	100.0 (100.0, 100.0)	94.1 (65.0, 99.1)	100.0 (100.0, 100.0)	85.7 (33.4, 97.9)
Month 24	97.7 (84.6, 99.7)	86.3 (54.7, 96.5)	85.7 (33.4, 97.9)	85.7 (33.4, 97.9)
Month 36	97.7 (84.6, 99.7)	75.5 (39.7, 91.8)	85.7 (33.4, 97.9)	64.3 (15.1, 90.2)
Month 48	97.7 (84.6, 99.7)	75.5 (39.7, 91.8)	85.7 (33.4, 97.9)	64.3 (15.1, 90.2)
Month 60	97.7 (84.6, 99.7)	- (-, -)	- (-, -)	- (-, -)
Month 72	97.7 (84.6, 99.7)	- (-, -)	- (-, -)	- (-, -)
Month 84	97.7 (84.6, 99.7)	- (-, -)	- (-, -)	- (-, -)
Month 94	97.7 (84.6, 99.7)	- (-, -)	- (-, -)	- (-, -)
Death, n (%)	1 (1.5)	3 (17.6)	1 (10.0)	2 (28.6)

Abbrev.: allo-HSCT, allogeneic hematopoietic stem cell transplantation; CI, confidence interval; TP, Transplant Population; TPES, Strictly ALD-102 Eligible Transplant Population; MUD, matched unrelated donor
Estimates of overall survival rates are obtained using the Kaplan-Meier method, where the event is death of all causes. Patients who are alive are censored at their last contact date.
TP 102/104 patients who withdrew to receive allo-HSCT were censored at their end of study visit; all other patients who are alive are censored at their last contact date.

The eli-cel treatment effect is profound, and consistency across multiple endpoints substantiates the finding of neurologic disease stabilization. Although the long-term benefits are not yet well characterized, both LTF-304 and a planned, post-approval Registry Study will further monitor long-term efficacy and provide an improved understanding of eli-cel over time.

7.2. Risks

The eli-cel treatment regimen, comprising mobilization/apheresis, conditioning, and infusion of eli-cel drug product, has a safety profile dominated by the known effects of G-CSF, plerixafor, and conditioning. All patients treated with eli-cel experienced an AE attributed to conditioning, and most AEs were associated with conditioning. These events were generally nonserious, consistent with the expected safety risks of the conditioning agents used, and resolved with standard management. The gene-therapy-specific risk of insertional oncogenesis is reflected in the event-free survival analysis, presented above, and is further discussed in the benefit-risk analysis below.

There was 1 death among patients treated with eli-cel in the clinical program. The patient had early and rapid progression of CALD, which was not attributed to treatment.

Eight patients experienced 10 drug product-related AEs (1 event worsened in severity and thus was counted as 2 events). Three patients were diagnosed with SAEs of MDS, 2 patients experienced SAEs of delayed hematopoietic reconstitution (1 of whom was subsequently diagnosed with MDS), and 1 patient experienced an SAE of viral cystitis (due to BK virus). Three patients reported 3 nonserious AEs that started and resolved on Rel Day 1 that were likely related to the cryopreservative dimethyl sulfoxide in the drug product: 2 events of vomiting and 1 event of nausea.

eli-cel clinical risks include insertional oncogenesis and prolonged cytopenias.

7.3. Benefit-Risk Assessment

Hematopoietic stem cell transplantation is the only efficacious therapy for CALD, a rare, but uniformly fatal disease.

Conventional treatment with allo-HSCT is effective, but many CALD patients do not have a suitable donor, establishing a need for autologous therapy. Accordingly, the benefit-risk of eli-cel is assessed in the context of allo-HSCT outcomes.

Chiefly, eli-cel treated patients are more likely to achieve both overall and event-free survival than allo-HSCT patients treated with an NMSD graft, with risk reductions of more than 80%. This advantage is primarily driven by reduced transplant complications including graft failure and TRM within approximately 2 years of treatment. Event-free and overall survival following eli-cel treatment exceed 85% through 7 years of follow up, after which outcomes are not reliably characterized.

eli-cel treated patients do not require immunosuppression and are not at risk of death due to immune complications. The occurrence of MDS is devastating but nonetheless compares favorably to fatalities following allo-HSCT in children with limited donor options.

The optimal use of eli-cel at this time is in those children with early CALD with only mismatched donors, but eli-cel is also an important option for those who do not have a suitable MUD.

Parents and treating physicians will need to consider treatment options on a case-by-case basis, considering the probability of rapid disease progression and the availability and histocompatibility of donors. They will also need to assess factors besides histocompatibility that play a role in transplant outcome, such as stem cell source, donor age and sex, donor-recipient CMV/EBV status and ABO compatibility, the barriers and delays to treatment, access to healthcare, the past experience and preferences of families, and the estimates of pros and cons provided by the transplant physician. The short-term reality of death due to immune complications will have to be balanced against the known short-term and unknown long-term risks of eli-cel. Those decisions are nuanced and personal, and cannot be guided solely by clinical data.

In consideration of the fatal nature of the disease and limitations of existing treatment, the benefit/risk of eli-cel is favorable in the indicated patient population.

The understanding of the long-term benefits and risks of eli-cel will become more precise over years of follow-up. Until that time, the community of families and health care providers overseeing the care of these vulnerable children will make the best decisions possible, and in that realm, eli-cel represents a critically important treatment option.

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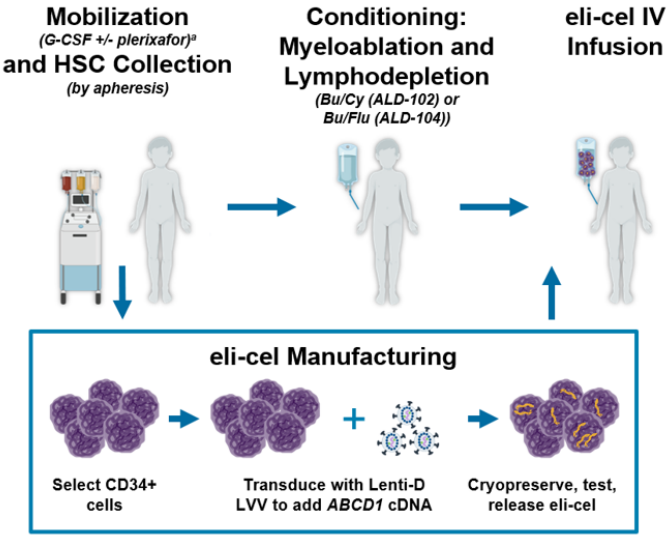
9. APPENDIX A: MANUFACTURING AND PRODUCT UNDERSTANDING

Elivaldogene autotemcel (eli-cel) is a genetically modified autologous CD34+ cell-enriched population that contains hematopoietic stem cells (HSCs) transduced ex vivo with Lenti-D lentiviral vector (LVV) encoding *ABCD1* cDNA for adrenoleukodystrophy protein (ALDP). It is a suspension for intravenous infusion; the cells are suspended in a cryopreservation solution.

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

Each patient undergoes HSC mobilization with granulocyte colony-stimulating factor (G-CSF) with or without plerixafor in combination, followed by apheresis to harvest the cells. The collected cells are shipped to the manufacturing site where CD34+ cells are selected and then transduced with Lenti-D LVV to manufacture eli-cel drug product. The drug product is tested to demonstrate that it meets all product quality standards after which it is released for patient administration. After conditioning and eli-cel infusion, transduced HSCs engraft in the bone marrow and differentiate to reconstitute the hematopoietic system as well as provide ALDP to treat the patient's CALD (Figure 20).

Figure 20. Overview of eli-cel Treatment



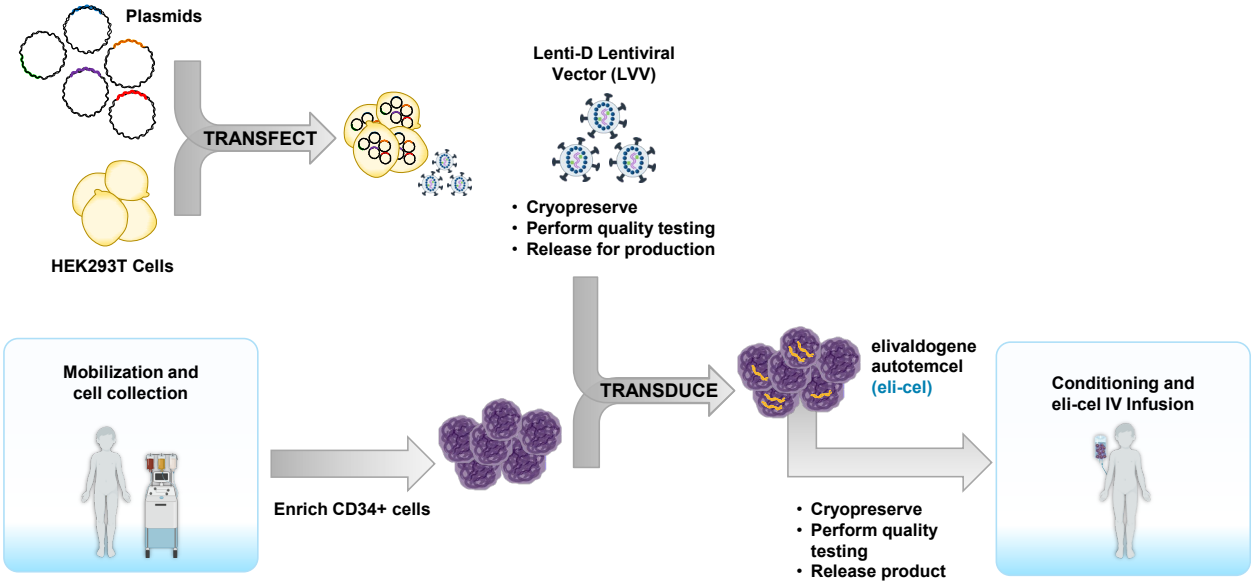
Abbrev.: Bu, busulfan; Cy, cytarabine; Flu, fludarabine; G-CSF, granulocyte colony-stimulating factor; HSC, hematopoietic stem cell.
[a] Plerixafor is required in ALD-104.

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

9.1. Materials and Critical Components

The cellular starting material used to manufacture eli-cel drug substance is autologous hematopoietic progenitor cells collected by apheresis (HPC-A). The Lenti-D LVV critical component, used to transduce the CD34+ cells enriched from HPC-A, is produced using plasmids and HEK293T cells. A schematic overview of the manufacturing process illustrating how these materials are used to manufacture the LVV and drug product is provided in Figure 21.

Figure 21. Overview of Materials and Use in eli-cel Manufacturing Process



9.1.1. Plasmids

A multi-plasmid system, consisting of a plasmid transfer vector (pLBP100) containing the *ABCD1* "therapeutic" transgene, and 4 packaging plasmids containing viral packaging genes, including HIV-1-derived gag/pol, tat, rev, and the vesicular stomatitis virus derived glycoprotein G (VSV-G) envelope, are used to produce Lenti-D LVV. The multi-plasmid system was designed to prevent recombination and emergence of replication competent lentivirus (RCL).

Importantly, the viral packaging genes encoding these viral proteins are only present on the plasmids. No viral packaging genes are included in the Lenti-D LVV particle and thus it is replication incompetent. In HEK293T cells the viral protein components produced from the plasmids lead to LVV particle formation and the packaging of the viral RNA genome, which is encoded by the pLBP100 transfer vector. HIV-1 viral genes that are dispensable were removed from the plasmid system, and include those that encode HIV envelope, vpr, vpu, and nef proteins. Notably, all these deleted genes are required for HIV pathogenesis.

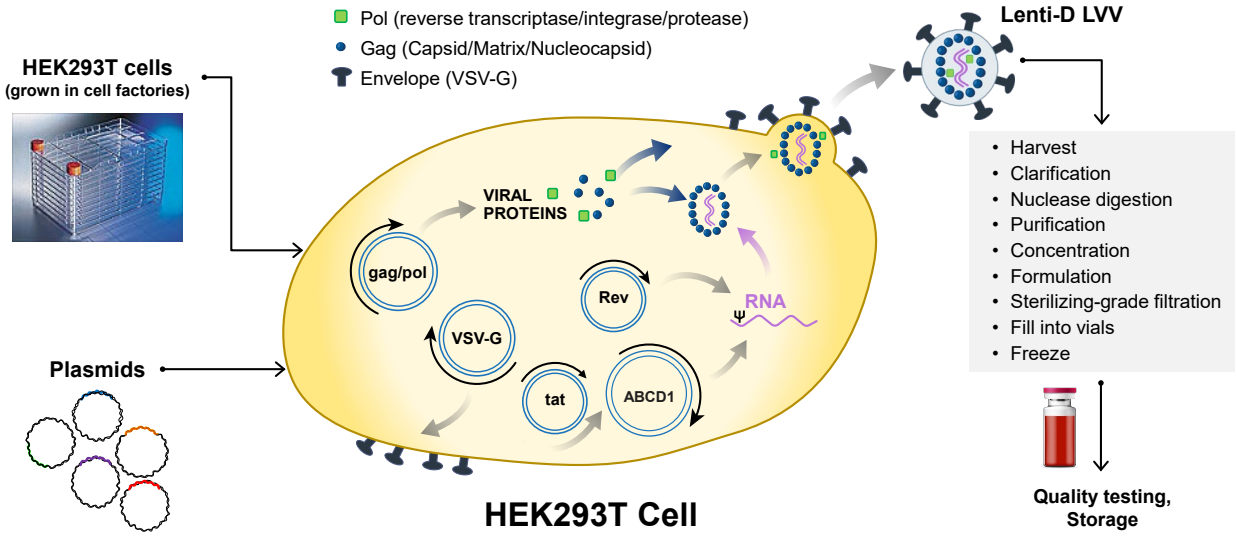
9.1.2. Lenti-D LVV

Lenti-D LVV is a replication defective, self-inactivating, third generation (LVV components are encoded on separate plasmids), HIV-1 based lentiviral vector with the VSV-G envelope protein, carrying the human *ABCD1* gene under the transcriptional control of an internal enhancer/promoter derived from the unique 3' region of the murine myeloproliferative sarcoma virus with a negative control region deletion (MNDU3) (see Section 12, Appendix D for more information on the MNDU3 promoter) (Robbins et al. 1997). The self-inactivating feature of the Lenti-D LVV is due to the transcriptional enhancer and promoter having been deleted from the HIV-derived viral long terminal repeat; this greatly limits the potential for generating new viral RNA genomes in transduced cells and also limits the ability of the integrated provirus to influence the transcription of nearby genes.

Lenti-D LVV is produced using a split-plasmid system that is common to all third-generation lentiviral vectors and is generated by transient transfection of HEK293T cells with the plasmid transfer vector pLBP100 and the 4 packaging plasmids. Lenti-D LVV produced in the HEK293T cells (as shown in Figure 22) is harvested, purified via chromatography, formulated, and filled into vials prior to storage at ≤ -65°C. HEK293T is a modified human embryonic kidney cell line. A HEK293T Master Cell Bank and Working Cell Banks have been established and tested in accordance with ICH and FDA guidance documents. The HEK293T cell lines have been demonstrated to be free of adventitious contaminants, including HIV-1 and HIV-2.

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

Figure 22. Production of Lenti-D LVV by Transfection of HEK293T Cells



Lenti-D LVV manufacturing process consistency is mainly controlled by (1) raw material and reagent qualification programs, (2) in-process monitoring, (3) in-process control testing, (4) lot release and stability tests, and (5) validation of the manufacturing process and continuous process verification.

Lenti-D LVV lot release tests include assays for quality, identity, safety (including a test to detect RCL), purity, and potency. One potency assay is used to quantify the concentration of LVV infectious particles (transducing units/mL), which informs the amount of Lenti-D LVV used in the eli-cel manufacturing process. The other potency assay measures the ability of the LVV transgene to encode functional ALDP protein that can metabolize very long chain fatty acids.

9.1.3. Autologous Cells

eli-cel manufacture starts with autologous cells collected from the patient by apheresis after mobilization with G-CSF with or without plerixafor in combination. These cells are identified as autologous hematopoietic progenitor cells collected by apheresis, or HPC-A.

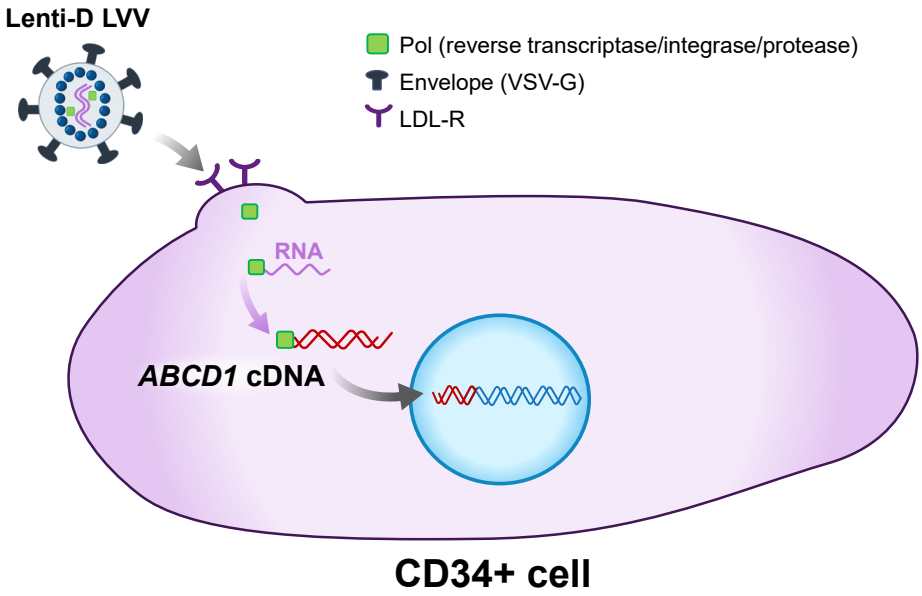
The mobilized cells contain mature blood-derived mononuclear cells, such as macrophages, B-cells, T-cells, and neutrophils. Approximately 1% of the mobilized cells express high levels of the surface transmembrane protein CD34, which is considered a marker for hematopoietic stem and progenitor cells.

9.2. eli-cel (Drug Product)

9.2.1. Manufacturing Process

The autologous cells are shipped from the apheresis collection center to the drug product manufacturing facility, where they are processed using a device cleared by FDA for separation of hematopoietic stem cells to enrich for cells expressing CD34. The CD34+ enriched cell population is stimulated ex vivo with a mixture of recombinant human cytokines and then transduced ex vivo with Lenti-D LVV as shown in Figure 23. Transduced cells will carry at least one copy of the *ABCD1* transgene.

Figure 23. Transduction of Autologous Cells



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

The eli-cel manufacturing process is designed to maximize the quantity of CD34+ cells recovered from the collected autologous cells while maintaining their stem cell characteristics. Culture time following transduction cannot be extensive as it is necessary to minimize the potential for any HSC differentiation. In this respect, the eli-cel manufacturing approach differs from that of chimeric antigen receptor T-cell (CAR-T cell) products that frequently use a culture step following genetic modification to increase the number of cells to achieve clinical dosing requirements.

9.2.2. Manufacturing Control Strategy

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

The drug product release testing includes assays for identity, safety, purity, and potency and strength. The suite of potency assays includes those that quantify DNA insertions, confirm the ability of the transduced CD34+ cells to form diverse hematopoietic colonies, quantify ALDP expression, and confirm the ability of the drug product cells to terminally differentiate into cells that can metabolize very long chain fatty acids, mimicking the mechanism of action needed to treat the disease. Further details of the specifications are not disclosed here as this information is considered to be proprietary (note: this information was shared with FDA as part of the BLA).

9.2.3. Chain of Identity

bluebird bio has implemented a drug product COI strategy based on the requirements of donor-to-recipient bi-directional product tracking. In this strategy, all operations from patient enrollment to delivery of drug product to the Qualified Treatment Center (QTC) for administration are controlled, assuring each individual patient is infused with drug product manufactured with their own HSCs. Policies and procedures govern practices at the QTC, Drug Product Manufacturer, and bluebird bio to assure the bluebird bio COI system functionality and expectations. Applicable training exists and is required for appropriate personnel at QTC, Drug Product Manufacturer, and at bluebird bio.

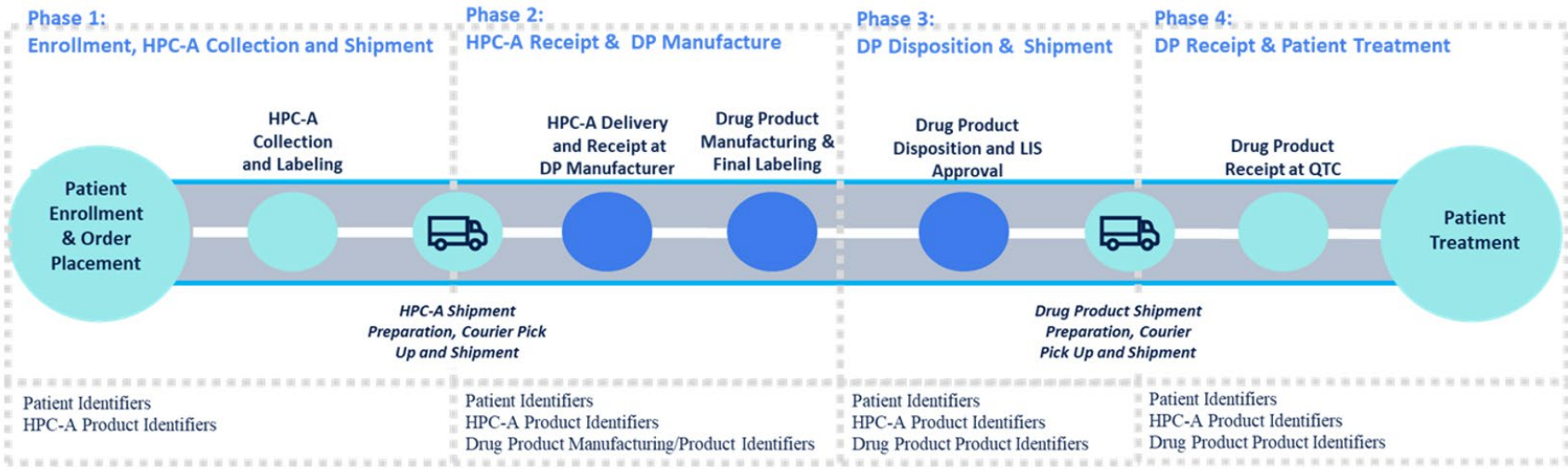
bluebird bio's COI system leverages a combination of physical labeling and procedural controls based on three COI unique traceability identifier categories, each subsequently comprised of specific COI data elements. The 3 categories of COI unique traceability identifiers are: Patient Identifier(s), HPC-A Product Identifier(s), and Drug Product Manufacturing/Product Identifier(s). The combination of these identifiers enables donor-to-recipient tracking and traceability and assures the HPC-A collected from a patient and used to make drug product is returned to that same patient.

The COI process contains 4 distinct phases (Figure 24) in which defined COI data elements are assigned and/or verified by bluebird bio, the QTC, and the Drug Product Manufacturer. The 4 phases include:

- Enrollment, HPC-A Collection and Shipment
- HPC-A Receipt and Drug Product Manufacturing
- Drug Product Disposition and Shipment
- Drug Product Receipt at QTC and Patient Treatment

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

Figure 24. Chain of Identity Summary



10. APPENDIX B: PROPENSITY SCORE ANALYSIS

10.1. Propensity Score Adjusted Analyses

10.1.1. Demonstration of Efficacy versus Benchmark

In order to demonstrate the benefit of eli-cel, Month 24 major functional disability (MFD)-free survival (the primary efficacy endpoint in Study ALD-102) was compared to a pre-specified clinically meaningful benchmark. In order to demonstrate success, the lower bound of the two-sided 95% exact confidence interval of Month 24 MFD-free survival must be > 50% to show benefit. The study results established that treatment with eli-cel had a significant clinical benefit over no treatment/supportive care when administered at an early stage of cerebral disease. The effect is large, with 90.6% of patients in ALD-102 meeting the Month 24 MFD-free survival criteria, with the lower bound of the exact 95% CI at 75.0%, which is well above the pre-established benchmark.

10.1.2. Demonstration of Relative Efficacy vs Allo-HSCT

Since randomization of patients was not feasible in eli-cel trials, in order to demonstrate the relative benefit of eli-cel vs. allo-HSCT, the selected efficacy results observed for patients treated with eli-cel in ALD-102 and ALD-104 (TP-102/104) were compared to the subset of patients who were treated with allo-HSCT in the contemporaneous external comparator study ALD-103 (using the population TPES-103). This subset of patients from Study ALD-103 was selected according to baseline characteristics matching inclusion criteria for Studies ALD-102 and ALD-104. Thus, comparative efficacy analyses between TP-102/104 and TPES-103 already considered the inclusion criteria/baseline characteristics.

Propensity score (PS)-adjusted analyses are designed to adjust for remaining minor differences in the baseline characteristic variable distributions between TP-102/104 and TPES-103 patients. Additionally, because eli-cel is proposed for patients who do not have an available and willing HLA-MSD, analyses were also performed including the subset of TPES-103 patients who did not have an MSD (i.e., TPES-103-NMSD cohort).

The PS-adjusted analyses on the efficacy endpoints of event-free survival and overall survival are provided below.

10.1.3. Methods for Propensity Score Adjusted Analysis

The propensity score is the probability of treatment assignment conditional on observed baseline characteristics. The propensity score allows one to analyze a nonrandomized study in a way mimics some of the particular characteristics of a randomized controlled trial (Austin 2011). Conditioning on the propensity score, the distribution of measured baseline covariates is similar between treatment groups.

Two weighting methods and one regression method were used to provide PS adjustments. Specifically:

1. Inverse probability weighting (IPTW; weight being normalized) (Austin 2011)
2. Overlap weighting (OW; weight being normalized) (Li et al. 2019)
3. Use PS as a covariate in the regression model (PS in regression model) (Austin 2011)

IPTW is the most popular method by far; OW based on the PS estimated from a logistic model leads to exact balance between treatment groups for all covariates (Li et al. 2019); PS as a covariate in the regression model is easy to implement. These 3 PS methods make use of all observed data, each method has its own limitation. Simulation studies show that PS methods perform better with larger sample sizes. For studies with limited sample size, such as TP-102/104 and TPES-103, confidence on the adjusted outcome can be strengthened if results from the 3 PS methods are similar and consistent.

Appropriate diagnostics exist for each of the above 3 PS methods to assess whether the PS model had been adequately specified. For IPTW and OW, the standardized mean difference (SMD) before and after the PS adjustment can be used to compare the mean of continuous and binary variables between treatment groups. The recommend criteria for upper limit of SMD is 0.25 (Stuart et al. 2013) (Rubin, 2001). However, many authors use an upper limit of 0.10 (Austin 2011). For regression model using the propensity score as a covariate, the model fit can be assessed by goodness-of-fit diagnostic statistics.

10.1.4. Baseline Covariates in Propensity Score Analysis

Studies ALD-102, ALD-104 and ALD-103 were designed with input from investigators and key opinion leaders (KOLs), and important baseline disease variables had been discussed and identified for the data collection. Based on input from clinicians and the cerebral adrenoleukodystrophy (CALD) study team and informed by the HSCT and ALD literatures, baseline disease characteristic variables that are considered independently correlated with CALD prognosis were identified and used in the PS analyses. However, it is noted that there may be unmeasurable variables which related to either treatment assignment or the CALD prognostic or both missing in the PS adjustment.

The baseline variables that were considered independently correlated with CALD prognosis that were used in the PS analyses were as follows:

1. Age at CALD diagnosis, years
2. Age at first HSC infusion, years
3. Time from CALD diagnosis to Relative Day 1 (day of HSC infusion, either eli-cel for TP-102/104 or allo-HSCT for TPES-103), months
4. Baseline Loes score
5. Baseline NFS
6. Baseline Loes pattern group (Patterns 1, 2, or 5 versus Patterns 3 or 4)
7. Presence of co-morbid condition at Baseline

Baseline GdE status was not used in the PS derivation as their distribution was balanced between the two cohorts (the entry criteria required positive baseline GdE status for TP-102/104 and TPES-103; Table 32).

For event-free survival and overall survival, all 7 baseline variables were included in the PS analyses comparing TP-102/104 versus TPES-103 or TPES-103-NMSD.

Table 32. Baseline Disease Characteristics (TP-102/104, TPES-103, TPES-103-NMSD)

	eli-cel	allo-HSCT	
	TP-102/104 N=67	**TPES-103 N=27**	**TPES-103 NMSD N=17**
Age at CALD diagnostic, (year)			
Median	6	7	7
Min, Max	1, 13	0, 11	0, 11
Age at HSC infusion, (year)			
Median	6	8	8
Min, Max	4, 14	5, 11	5, 11
Time from CALD diagnosis to Rel Day 1, months			
Median	5.8	3.5	3.6
Min, Max	2.5, 49.9	0.6, 78.0	0.6, 78.0
Baseline NFS, n (%)			
0	64 (95.5)	26 (96.3)	16 (94.1)
1	3 (4.5)	1 (3.7)	1 (5.9)
Baseline Loes score			
Median	2	3	2
Min, Max	1, 9	1, 9	1, 9
Baseline Loes pattern, n (%)			
1, 2, or 5	55 (82.1)	24 (88.9)	14 (82.4)
3 and/or 4	11 (16.4)	2 (7.4)	2 (11.8)
Other or missing	1 (1.5)	1 (3.7)	1 (5.9)
Baseline GdE status, n (%)			
GdE+	66 (98.5)	27 (100)	17 (100)
GdE-	1 (1.5)[a]	0	0
Presence of any significant co-morbid conditions, n (%)			
Yes	63 (94.0)	24 (88.9)	15 (88.2)
No	4 (6.0)	3 (11.1)	2 (11.8)

Abbrev.: Allo-HSCT, allogeneic hematopoietic stem cell transplantation; CALD, cerebral adrenoleukodystrophy; GdE, gadolinium enhancement; MSD, matched sibling donor; NFS, neurologic function score; NMSD, not a matched sibling donor; TP, transplant population; TPES, strictly ALD-102 eligible transplant population.

[a] One subject was GdE+ at enrollment in ALD-104 and GdE- at a subsequent MRI prior to conditioning that is considered baseline. Available literature describes that GdE+ can resolve in untreated patients, and that these patients maintain a high risk of disease progression (Melhem et al. 2000). The pertinent subject is included in the presented analyses of TP-102/104, but contributes less than 2 years of follow-up data.

10.1.5. Propensity Score Results

10.1.5.1. Part I: Baseline Variable Distribution Differences Before and After PS Adjustment

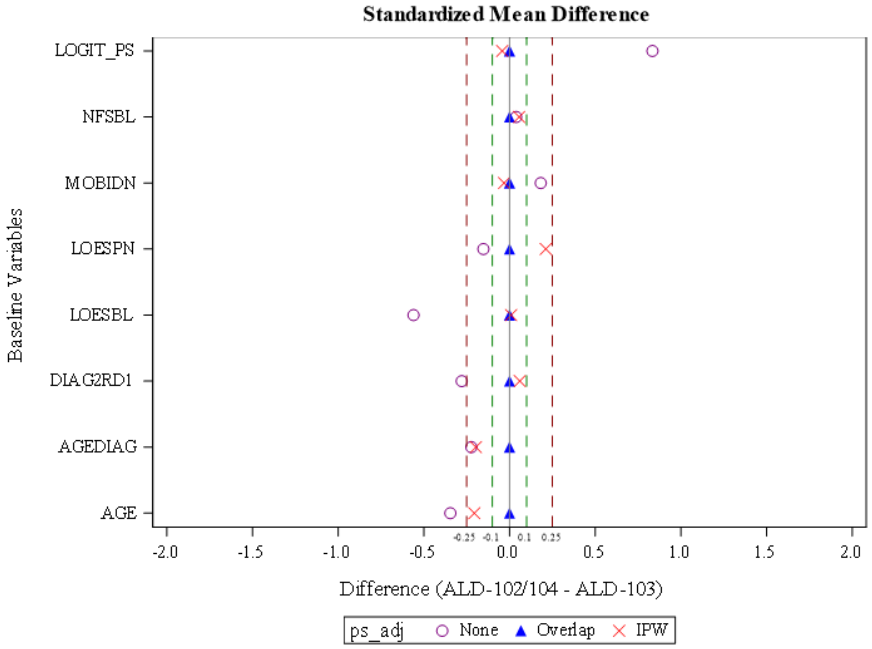
Table 33 and Figure 25 present standardized mean difference (mean difference divided by standard deviation) before and after OW and IPTW adjustments. After PS weighted adjustment through OW, mean difference between TP-102/104 and TPES-103 became zero for all 7 baseline variables, as predicted by the OW method theory (Li et al. 2019). After PS weighted adjustments through IPTW, the baseline differences between TP-102/104 and TPES-103 decreased for 5 out of 7 variables included in the PS analyses, with absolute value of SMDs for two baseline variables (Baseline Loes pattern and Baseline NFS) increased after the PS adjustment. After IPTW, absolute SMDs are below the upper limit of 0.10 for 4 out of 7 baseline variables, a desirable standard for the PS analysis by Austin, 2011, the rest 3 baseline variables had absolute SMDs less than 0.25, and variance ratios between the two groups after the IPTW are between 0.35-2.89. The linear propensity (logit propensity score) decreased from 0.83394 to -0.04396 with 94.73% reduction after IPTW.

Table 33. Standardized Mean Difference (TP-102/104 – TPES-103) Before and After OW and IPTW, TP-102/104 (N=67) vs. TPES-103 (N=27)

Standardized Mean Differences (ALD-102/104 – ALD-103)						
Variable		**Mean Difference**	**Standard Deviation**	**Standardized Difference**	**Percent Reduction**	**Variance Ratio**
Logit Propensity Score	Before PS adj.	0.75965	0.91091	0.83394		0.6566
	OW adj.	0		0	100	
	IPW adj.	-0.04005		-0.04396	94.73	0.7596
Age at first HSCT infusion (years)	Before PS adj.	-0.71144	2.05961	-0.34543		1.7573
	OW adj.	0		0	100	
	IPW adj.	-0.39884		-0.19365	43.94	2.8929
Age at CALD diagnosis (years)	Before PS adj.	-0.50912	2.28417	-0.22289		1.1419
	OW adj.	0		0	100	
	IPW adj.	-0.40460		-0.17713	20.53	2.0458
Baseline Loes score	Before PS adj.	-1.37811	2.45955	-0.56031		0.4838
	OW adj.	0		0	100	
	IPW adj.	0.02201		0.00895	98.40	0.8236

Standardized Mean Differences (ALD-102/104 – ALD-103)						
Variable		**Mean Difference**	**Standard Deviation**	**Standardized Difference**	**Percent Reduction**	**Variance Ratio**
Time from CALD diagnosis to Relative Day 1 (months)	Before PS adj.	-4.61688	16.54413	-0.27906		0.1327
	OW adj.	0		0	100	
	IPW adj.	0.75926		0.04589	83.55	0.3579
Baseline Loes pattern (1,2, or 5 vs. 3, 4)	Before PS adj.	0.05307	0.34350	0.15449		1.3894
	OW adj.	0		0	100	
	IPW adj.	-0.08583		-0.24987	0.00	0.7253
Presence of co-morbid condition at Baseline	Before PS adj.	0.05141	0.28245	0.18202		0.5556
	OW adj.	0		0	100	
	IPW adj.	-0.00764		-0.02706	85.13	1.1282
Baseline NFS	Before PS adj.	-0.00774	0.19804	-0.03908		1.1992
	OW adj.	0		0	100	
	IPW adj.	-0.01086		-0.05484	0.00	1.3196

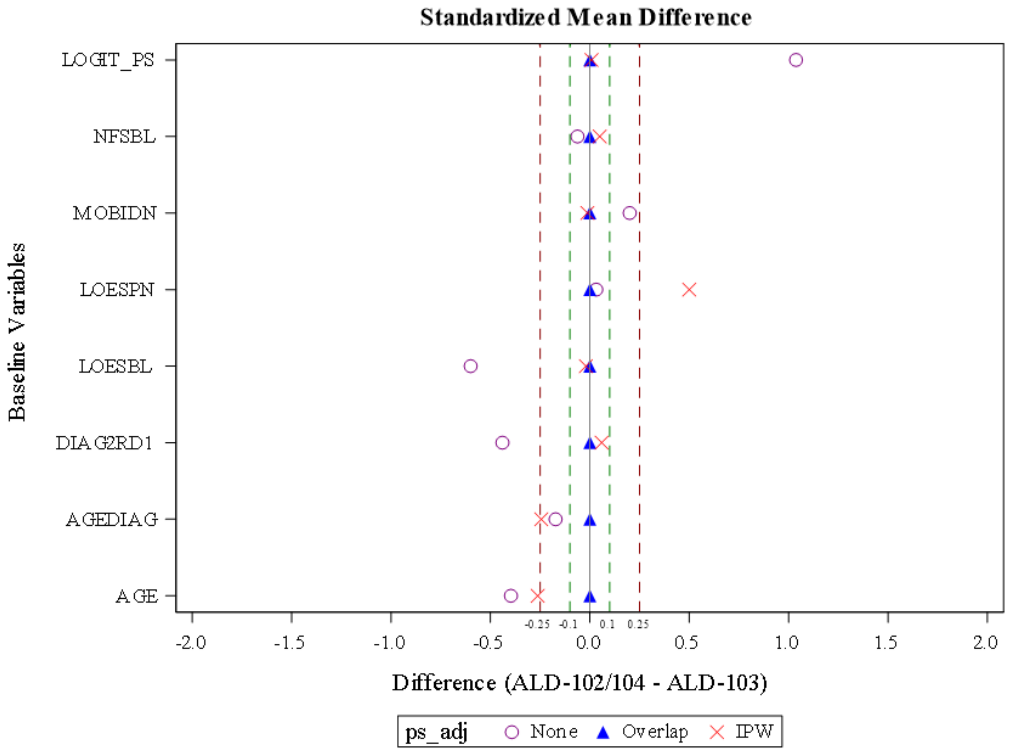
Figure 25. Baseline Variable Distribution Standardized Mean Difference Before and After Adjustment, TP-102/104 (N=67) Versus TPES-103 (N=27)



Abbrev.: LOGIT_PS, logit of propensity score; NFSBL, Baseline NFS; MOBIDN, Presence of co-morbid condition at Baseline; LOESPN, Baseline Loes pattern; LOESBL, Baseline Loes score; DIAG2RD1, Time from CALD diagnosis to Relative Day 1 (months); AGEDIAG, Age at CALD diagnosis (years); AGE, Age at first HSCT infusion (years).

Figure 26 presents SMDs before and after OW and IPTW adjustment comparing TP-102/104 and TPES-103-NMSD. After OW adjustment, SMDs between TP-102/104 and TPES-103-NMSD became zero for all 7 baseline variables; after IPTW, absolute SMDs are below the upper limit of 0.10 for 4 out of 7 baseline variables, a desirable standard for the PS analysis (Austin 2011), 1 baseline variables had absolute SMDs less than 0.25, two baseline variables (Baseline Loes pattern and Age at CALD Diagnosis) had SMD increased after the PS adjustment. The variance ratios between the two groups after the IPTW are between 0.34-2.44.

Figure 26. Baseline Variable Distribution Standardized Mean Difference Before and After Adjustment, TP-102/104 (N=67) Versus TPES-103-NMSD (N=17)



Abbrev.: LOGIT_PS, logit of propensity score; NFSBL, Baseline NFS; MOBIDN, Presence of co-morbid condition at Baseline; LOESPN, Baseline Loes pattern; LOESBL, Baseline Loes score; DIAG2RD1, Time from CALD diagnosis to Relative Day 1 (months); AGEDIAG, Age at CALD diagnosis (years); AGE, Age at first HSCT infusion (years).

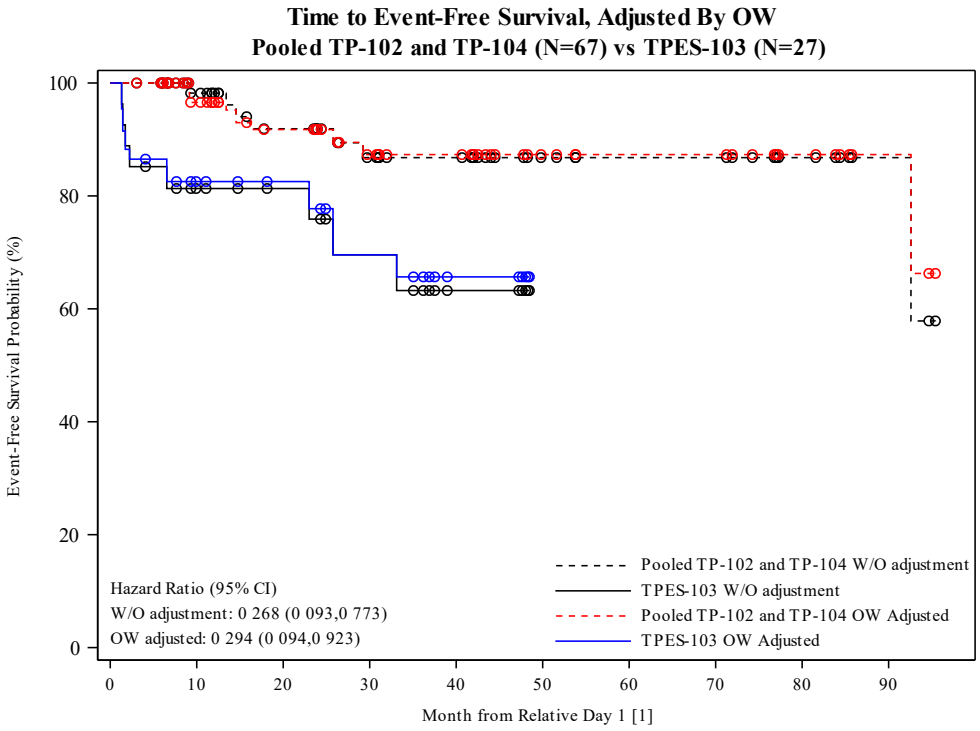
10.1.5.2. Part II: Selected Efficacy Endpoints Results Before and After PS Adjustment

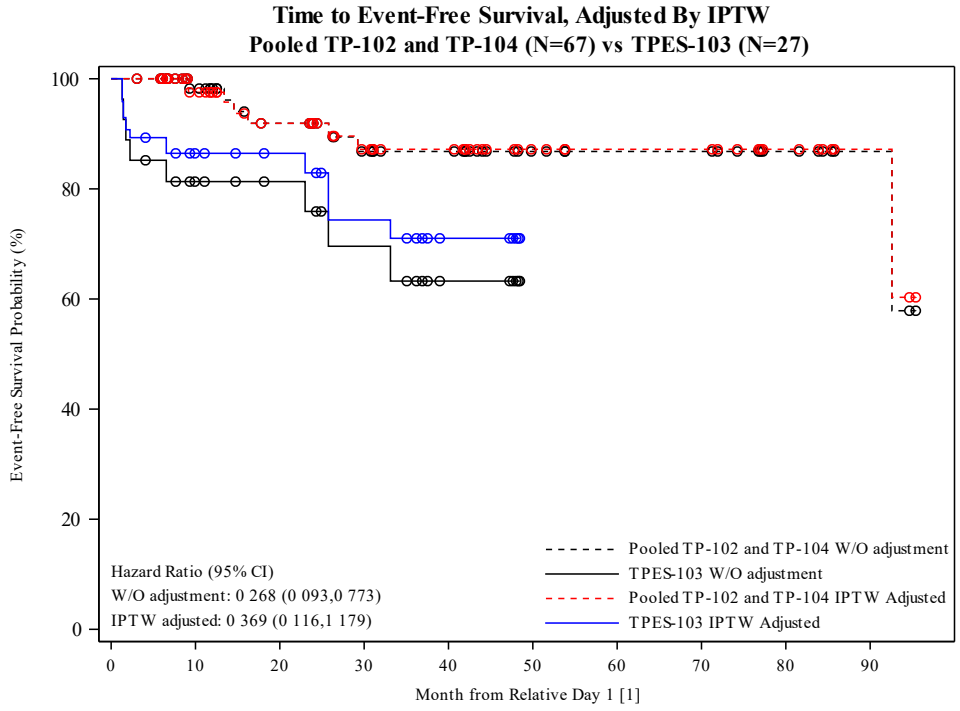
10.1.5.2.1. Event-free Survival Over Time After PS Adjustment

Figure 27 presents effect of PS adjustment on event-free survival over time comparing TP-102/104 to TPES-103. The black lines show the unadjusted data, the colored lines are adjusted.

After the PS adjustment of baseline distribution differences using the 3 different PS methods, the hazard ratio (HR) changed from 0.268 to a range of 0.294 to 0.369 for TP-102/104 versus TPES-103. While the effect size slightly decreases, these HRs suggest that eli-cel confers a clinical benefit over patients treated with allo-HSCT, even after comprehensive adjustment of multiple baseline factors.

Figure 27: Event-Free Survival Over Time Before and After Propensity Score Adjustment, TP-102/104 Versus TPES-103



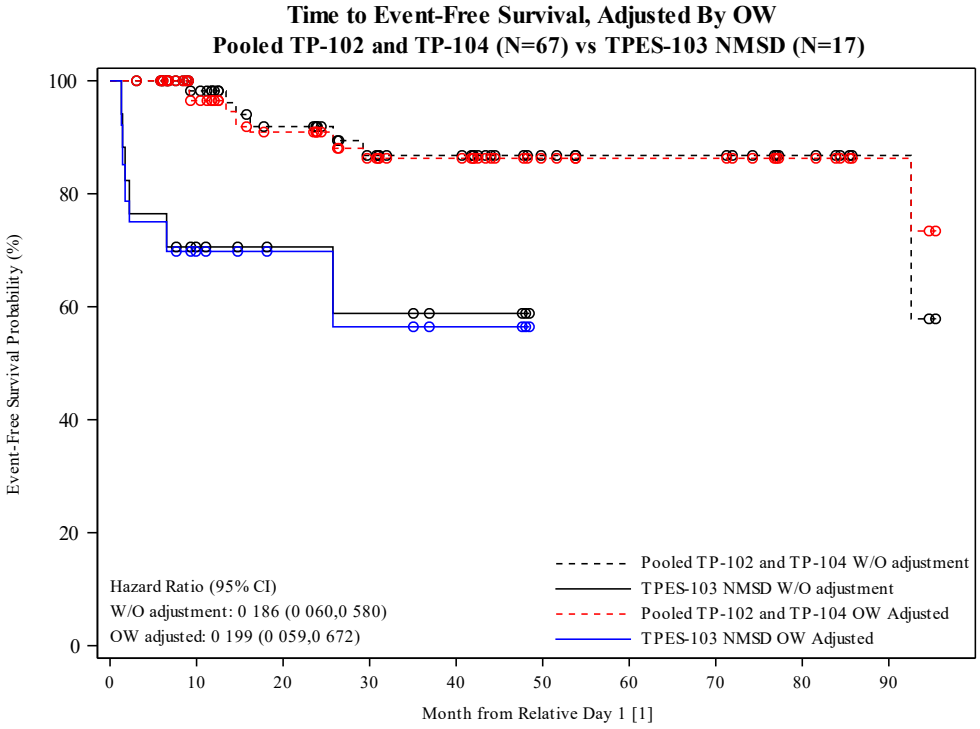


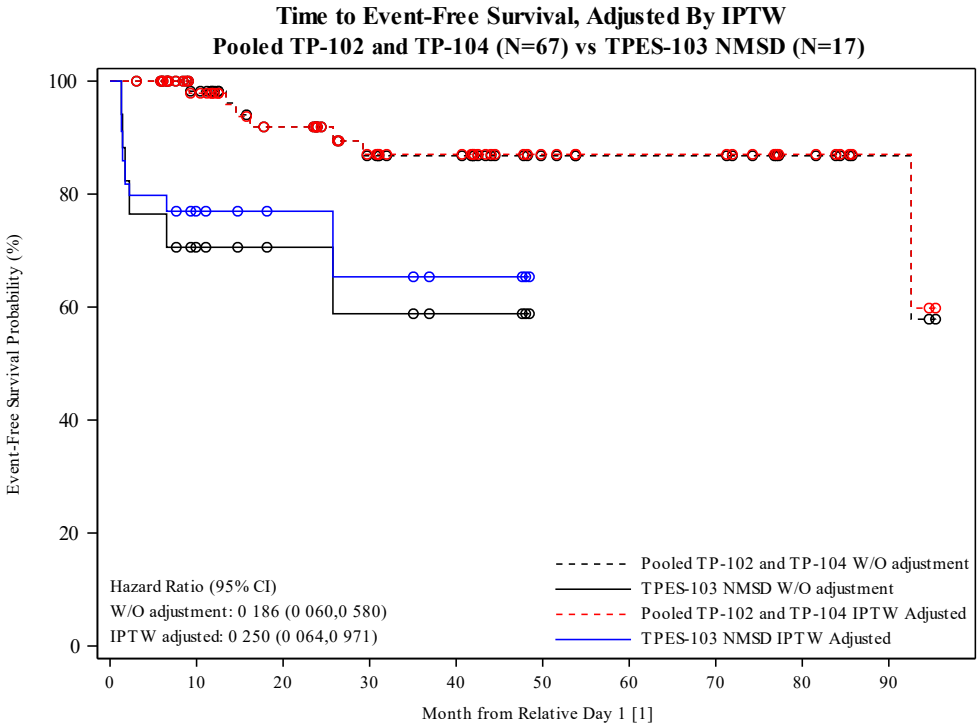
Abbrev.: IPTW, inverse probability weighting; OW, overlap weighting; PS, propensity score; TP, transplant population; TPES, strictly ALD 102 eligible transplant population.
In the PS as a covariate model (the 3rd plot), the curves (colored lines) are generated by a Cox model, and are not Kaplan-Meier curves.
[1]. For TP-102/104, Rel Day 1 is the day of eli-cel drug product infusion, and for TPES-103, Rel Day 1 is the day of the allo-HSC infusion.

Figure 28 presents the effect of PS adjustment on event-free survival over time comparing TP-102/104 to TPES-103-NMSD.

After the PS adjustment of baseline distribution differences, the hazard ratio (HR) changed from 0.186 to a range of 0.175 to 0.250 for TP-102/104 versus TPES-103-NMSD. When PS as a covariate included in the Cox proportional hazard model, the estimated HR decreased to 0.175, while the estimated HR increased after the PS adjustment with the other two methods. The upper limit of 95% CIs of these HRs are less than 1, suggesting that eli-cel confers a clinical benefit over patients treated with allo-HSCT without an MSD, even after comprehensive adjustment of multiple baseline factors.

Figure 28. Event-Free Survival Over Time Before and After Propensity Score Adjustment, TP-102/104 Versus TPES-103-NMSD



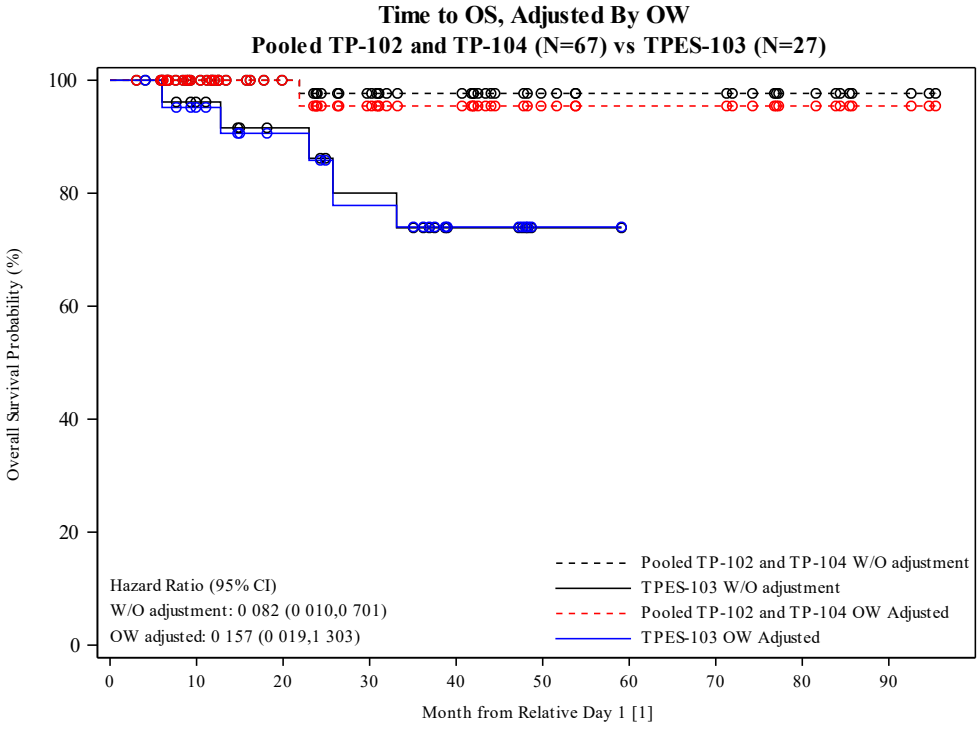


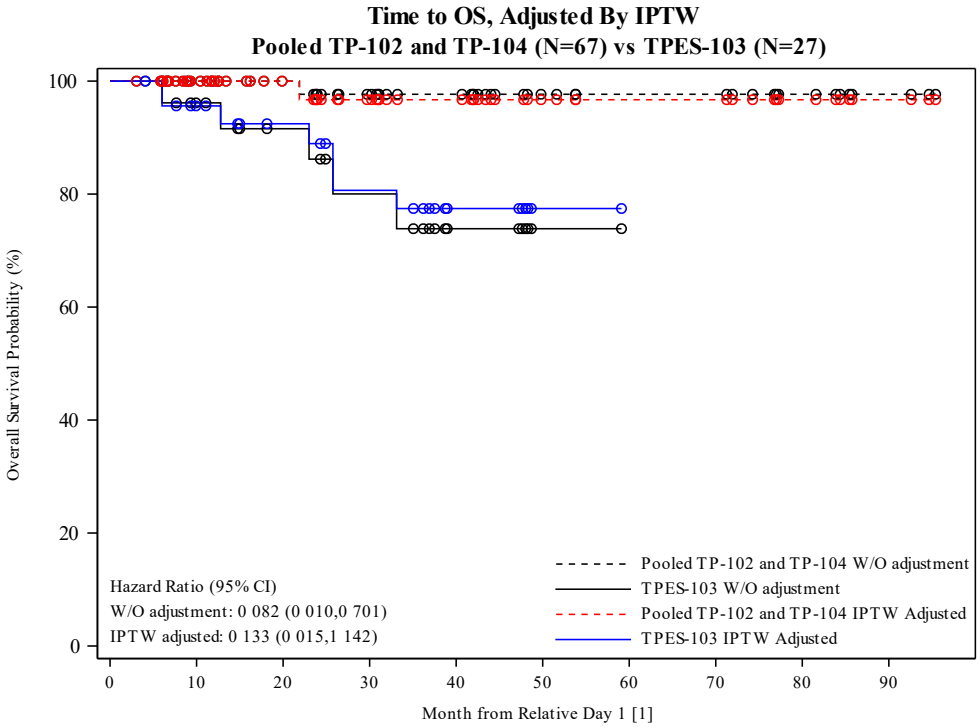
Abbrev.: IPTW, inverse probability weighting; OW, overlap weighting; PS, propensity score; TP, transplant population; TPES, strictly ALD 102 eligible transplant population; NMSD, not a matched sibling donor
In the PS as a covariate model (the 3rd plot), the curves (colored lines) are generated by a Cox model, and are not Kaplan-Meier curves.
[1]. For TP-102/104, Rel Day 1 is the day of eli-cel drug product infusion, and for TPES-103, Rel Day 1 is the day of the allo-HSC infusion.

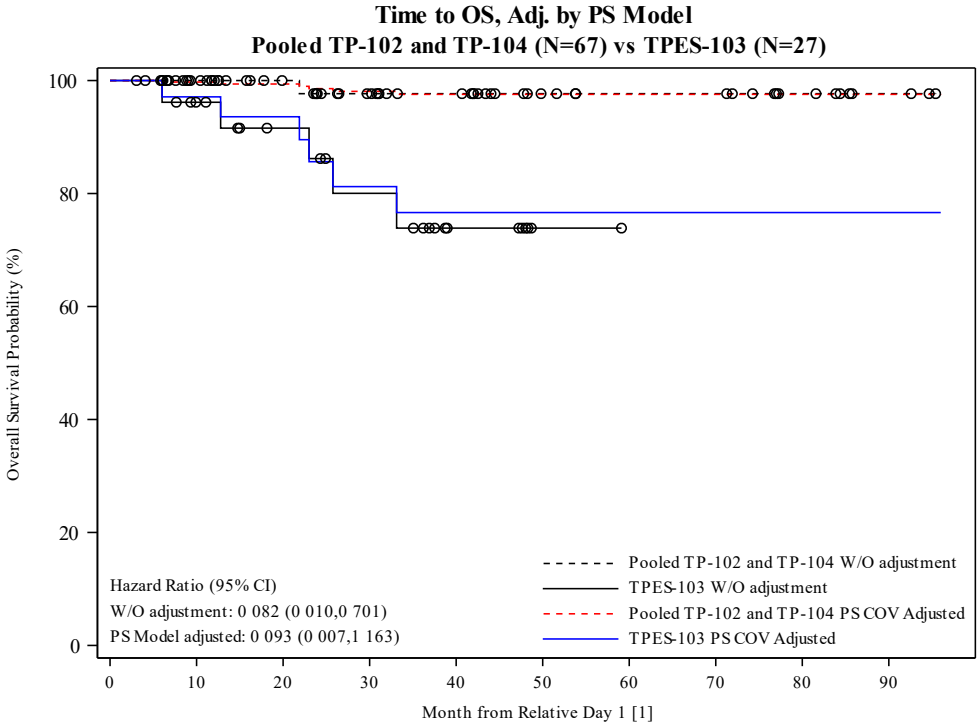
10.1.5.2.2. Overall Survival

Figure 29 presents effect of PS adjustment on overall survival over time comparing TP-102/104 to TPES-103. After PS adjustment of baseline distribution differences, the HR changed from 0.082 to within the range of 0.093 to 0.157 for TP-102/104 versus TPES-103. This suggests that while effect size is slightly decreased, eli-cel may reduce the risk of death over patients treated with allo-HSCT after PS adjustment for baseline characteristics.

Figure 29. Overall Survival Before and After Propensity Score Adjustment, TP-102/104 Versus TPES-103





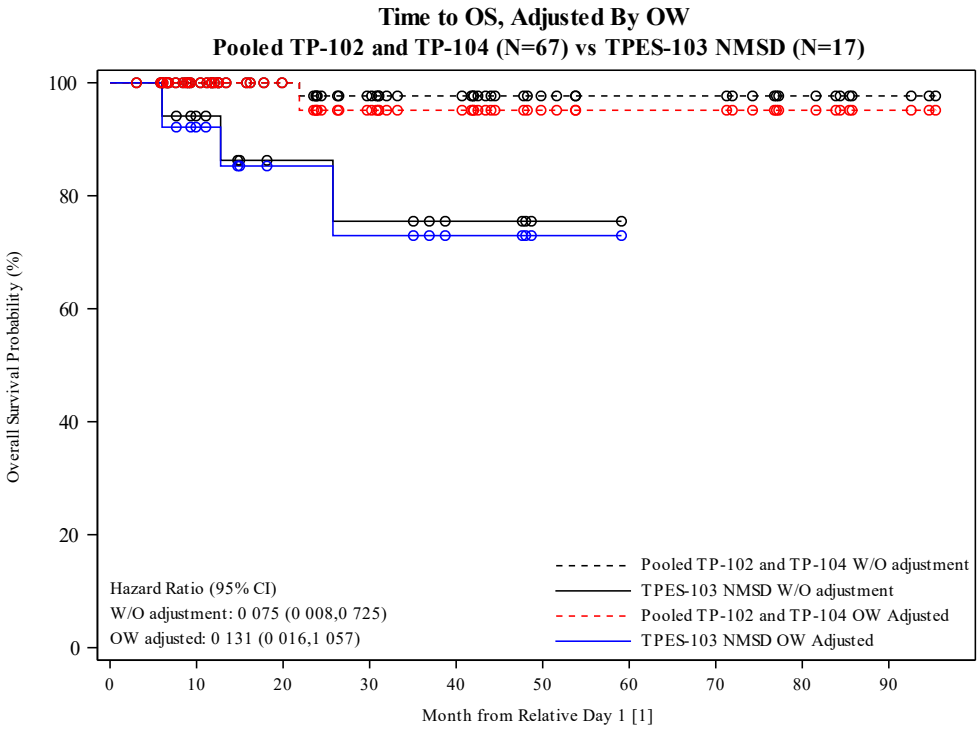


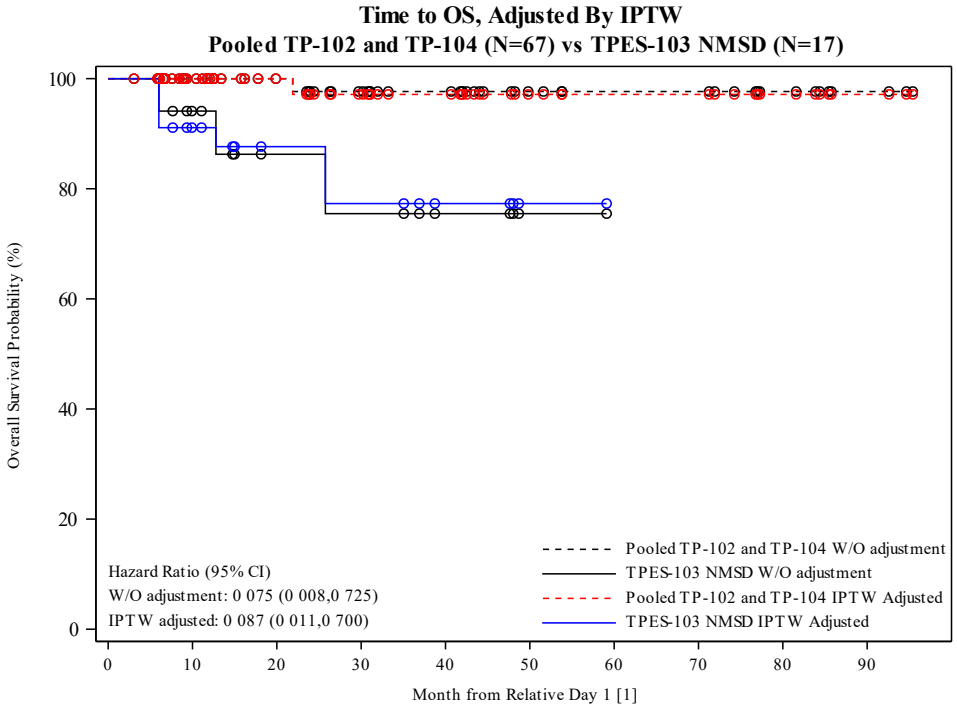
Abbrev.: IPTW, inverse probability weighting; OW, overlap weighting; PS, propensity score; TP, transplant population; TPES, strictly ALD 102 eligible transplant population.
In the PS as a covariate model (the 3rd plot), the curves (colored lines) are generated by a Cox model, and are not Kaplan-Meier curves.
[1]. For TP-102/104, Rel Day 1 is the day of eli-cel drug product infusion, and for TPES-103, Rel Day 1 is the day of the allo-HSC infusion.

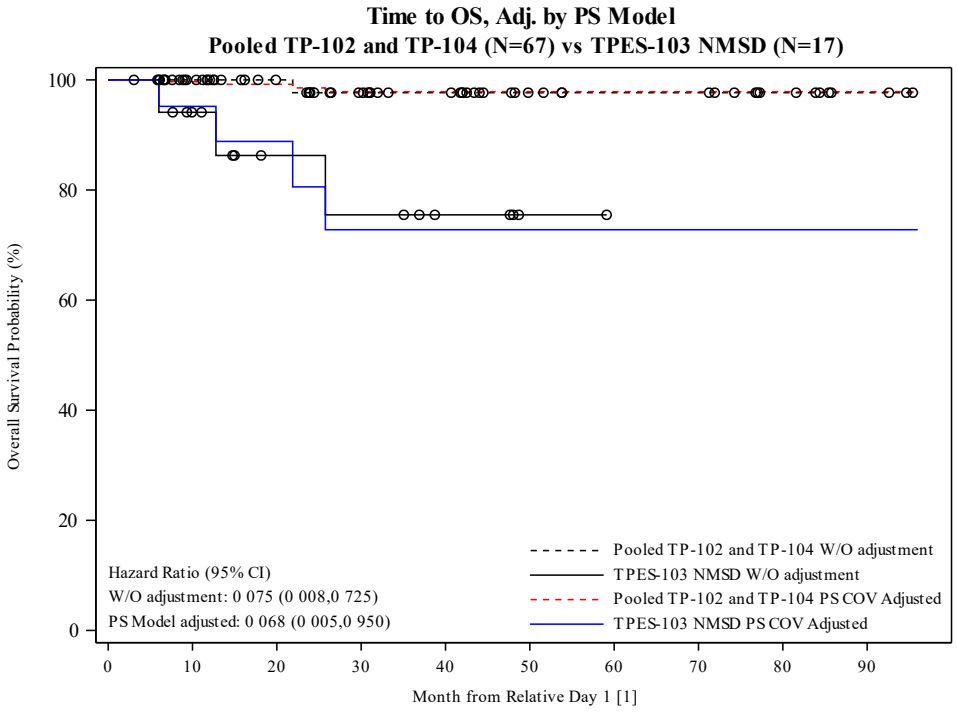
Figure 30 presents effect of PS adjustment on overall survival over time comparing TP-102/104 to TPES-103-NMSD subpopulation.

After PS adjustment of baseline distribution differences, the HR changed from 0.075 to within the range of 0.068 to 0.131 for TP-102/104 versus TPES-103-NMSD. This suggests that eli-cel may reduce the risk of death over patients without an MSD treated with allo-HSCT after PS adjustment for baseline characteristics.

Figure 30. Overall Survival Before and After Propensity Score Adjustment, TP 102/104 Versus TPES 103-NMSD







Abbrev.: IPTW, inverse probability weighting; OW, overlap weighting; PS, propensity score; TP, transplant population; TPES, strictly ALD 102 eligible transplant population; NMSD, not a matched sibling donor.
In the PS as a covariate model (the 3rd plot), the curves (colored lines) are generated by a Cox model, and are not Kaplan-Meier curves.
[1]. For TP-102/104, Rel Day 1 is the day of eli-cel drug product infusion, and for TPES-103, Rel Day 1 is the day of the allo-HSC infusion.

10.1.6. Overall Conclusions

PS-adjusted analyses of selected efficacy endpoints have been performed. These analyses are designed to account for differences in the baseline characteristics between TP-102/104 and TPES-103. These additional analyses, using 3 different PS methods, support the original conclusions comparing ALD-102/ALD-104 with the ALD-103 TPES and TPES-NMSD populations; however, in most analyses the effect size was slightly reduced after the PS adjustment.

Since the PS analysis was not pre-planned in the study protocol nor statistical analysis plan, it is possible that some baseline confounding variables were not included in the adjusted analyses and the PS analysis does not adjust for unmeasured confounding effect. However, with this data set of identified and collected major baseline disease characteristic variables, the analyses continue to suggest that eli-cel has a benefit over allo-HSCT, particularly in patients without a matched sibling donor, based on similar and consistent results from the 3 different propensity score methods. These results support the pre-specified study analyses.

11. APPENDIX C: ADDITIONAL SAFETY INFORMATION

Table 34. Adverse Events Occurring in ≥ 10% of Patients by SOC, PT, and Study Term (ITT-102/104)

System Organ Class Preferred Term	**M to < C (N=67)** n (%), Events	**C to < NE (N=67)** n (%), Events	**NE to M24 (N=67)** n (%), Events	**> M12 to M24 (N=46)** n (%), Events	**D1 to M12 (N=67)** n, (%) Events	**D1 to DLC (N=67)** n (%), Events
Patients with at least 1 AE	**57 (85.1), 186**	**67 (100), 1135**	**61 (91.0), 375**	**18 (39.1), 48**	**67 (100), 1086**	**67 (100), 1163**
Blood and lymphatic system disorders	**9 (13.4), 14**	**67 (100), 387**	**32 (47.8), 74**	**1 (2.2), 1**	**67 (100), 406**	**67 (100), 407**
Thrombocytopenia	3 (4.5), 3	60 (89.6), 101	9 (13.4), 14	0, 0	65 (97.0), 114	65 (97.0), 114
Neutropenia	0, 0	56 (83.6), 84	19 (28.4), 27	0, 0	55 (82.1), 103	55 (82.1), 103
Anemia	6 (9.0), 8	53 (79.1), 88	11 (16.4), 15	0, 0	53 (79.1), 84	53 (79.1), 84
Febrile neutropenia	0, 0	48 (71.6), 54	0, 0	0, 0	48 (71.6), 53	48 (71.6), 53
Leukopenia	2 (3.0), 3	19 (28.4), 37	6 (9.0), 15	0, 0	17 (25.4), 40	17 (25.4), 40
Lymphopenia	0, 0	14 (20.9), 18	0, 0	0, 0	4 (6.0), 5	4 (6.0), 5
Gastrointestinal disorders	**23 (34.3), 35**	**66 (98.5), 317**	**28 (41.8), 43**	**2 (4.3), 3**	**66 (98.5), 203**	**66 (98.5), 208**
Stomatitis	0, 0	60 (89.6), 73	0, 0	0, 0	57 (85.1), 69	57 (85.1), 69
Vomiting	9 (13.4), 9	50 (74.6), 72	10 (14.9), 12	1 (2.2), 1	22 (32.8), 29	23 (34.3), 31
Abdominal pain	1 (1.5), 1	25 (37.3), 29	3 (4.5), 3	0, 0	18 (26.9), 23	19 (28.4), 24
Nausea	11 (16.4), 12	55 (82.1), 71	7 (10.4), 8	0, 0	18 (26.9), 22	18 (26.9), 22
Constipation	3 (4.5), 4	24 (35.8), 27	7 (10.4), 7	0, 0	14 (20.9), 15	14 (20.9), 15
Diarrhea	1 (1.5), 1	17 (25.4), 18	2 (3.0), 2	0, 0	14 (20.9), 15	14 (20.9), 15
Skin and subcutaneous tissue disorders	**9 (13.4), 11**	**48 (71.6), 67**	**25 (37.3), 40**	**2 (4.3), 2**	**56 (83.6), 95**	**56 (83.6), 97**
Alopecia	0, 0	33 (49.3), 33	15 (22.4), 15	0, 0	48 (71.6), 48	48 (71.6), 48
Skin hyperpigmentation	0, 0	3 (4.5), 3	9 (13.4), 9	0, 0	11 (16.4), 11	11 (16.4), 11
Pruritus	3 (4.5), 3	11 (16.4), 11	0, 0	0, 0	9 (13.4), 9	9 (13.4), 9
Rash	2 (3.0), 2	7 (10.4), 7	1 (1.5), 1	0, 0	4 (6.0), 4	4 (6.0), 4
Metabolism and nutrition disorders	**12 (17.9), 16**	**49 (73.1), 112**	**12 (17.9), 14**	**1 (2.2), 1**	**37 (55.2), 68**	**38 (56.7), 69**
Decreased appetite	0, 0	42 (62.7), 52	3 (4.5), 3	0, 0	21 (31.3), 24	21 (31.3), 24
Hypokalemia	8 (11.9), 8	23 (34.3), 27	4 (6.0), 4	0, 0	16 (23.9), 18	16 (23.9), 18
Hypophosphatemia	0, 0	8 (11.9), 8	2 (3.0), 2	0, 0	9 (13.4), 10	9 (13.4), 10

System Organ Class Preferred Term	**M to < C (N=67)** n (%), Events	**C to < NE (N=67)** n (%), Events	**NE to M24 (N=67)** n (%), Events	**> M12 to M24 (N=46)** n (%), Events	**D1 to M12 (N=67)** n, (%) Events	**D1 to DLC (N=67)** n (%), Events
General disorders and administration site conditions	**32 (47.8), 43**	**23 (34.3), 30**	**17 (25.4), 22**	**1 (2.2), 1**	**29 (43.3), 39**	**30 (44.8), 43**
Pyrexia	4 (6.0), 4	14 (20.9), 14	15 (22.4), 20	1 (2.2), 1	22 (32.8), 28	23 (34.3), 31
Catheter site pain	22 (32.8), 31	3 (4.5), 3	0, 0	0, 0	0, 0	0, 0
Nervous system disorders	**7 (10.4), 9**	**20 (29.9), 23**	**20 (29.9), 34**	**7 (15.2), 9**	**22 (32.8), 37**	**29 (43.3), 63**
Headache	3 (4.5), 4	14 (20.9), 15	5 (7.5), 5	1 (2.2), 1	11 (16.4), 11	12 (17.9), 12
Seizure	0, 0	0, 0	2 (3.0), 2	2 (4.3), 2	0, 0	7 (10.4), 15
Respiratory, thoracic and mediastinal disorders	**3 (4.5), 3**	**23 (34.3), 33**	**8 (11.9), 9**	**1 (2.2), 1**	**28 (41.8), 38**	**28 (41.8), 39**
Epistaxis	0, 0	12 (17.9), 15	1 (1.5), 1	0, 0	13 (19.4), 15	13 (19.4), 15
Cough	0, 0	5 (7.5), 5	3 (4.5), 3	0, 0	7 (10.4), 7	7 (10.4), 7
Oropharyngeal pain	1 (1.5), 1	7 (10.4), 7	0, 0	0, 0	6 (9.0), 6	6 (9.0), 6
Injury, poisoning and procedural complications	**9 (13.4), 9**	**13 (19.4), 15**	**14 (20.9), 20**	**4 (8.7), 5**	**19 (28.4), 24**	**21 (31.3), 29**
Allergic transfusion reaction	0, 0	4 (6.0), 4	3 (4.5), 3	0, 0	7 (10.4), 7	7 (10.4), 7
Investigations	**5 (7.5), 5**	**22 (32.8), 49**	**8 (11.9), 11**	**2 (4.3), 2**	**14 (20.9), 28**	**16 (23.9), 30**
Alanine aminotransferase increased	0, 0	11 (16.4), 12	2 (3.0), 2	0, 0	7 (10.4), 7	7 (10.4), 7
Aspartate aminotransferase increased	0, 0	8 (11.9), 10	2 (3.0), 2	1 (2.2), 1	4 (6.0), 5	5 (7.5), 6
Cardiac disorders	**0, 0**	**13 (19.4), 16**	**2 (3.0), 2**	**1 (2.2), 1**	**9 (13.4), 12**	**11 (16.4), 14**
Sinus tachycardia	0, 0	7 (10.4), 9	1 (1.5), 1	0,0	6 (9.0), 9	6 (9.0), 9

System Organ Class Preferred Term	**M to < C (N=67)** n (%), Events	**C to < NE (N=67)** n (%), Events	**NE to M24 (N=67)** n (%), Events	**> M12 to M24 (N=46)** n (%), Events	**D1 to M12 (N=67)** n, (%) Events	**D1 to DLC (N=67)** n (%), Events
Vascular disorders	**2 (3.0), 3**	**11 (16.4), 13**	**3 (4.5), 5**	**1 (2.2), 1**	**9 (13.4), 12**	**9 (13.4), 13**
Hypertension	2 (3.0), 3	8 (11.9), 9	2 (3.0), 3	1 (2.2), 1	5 (7.5), 6	5 (7.5), 7

Abbrev.: AE, adverse event; C, conditioning; DLC, date of last contact; M, mobilization; NE, neutrophil engraftment; PT, preferred term; SOC, system organ class.

PTs (and their associated SOC) are included for AEs that were observed in ≥ 10% of patients (≥ 7 patients) in any shown study period and are sorted based on decreasing frequency by SOC and then PT per the **D1 to DLC** study period. For such PTs the frequency of AEs is shown even if they occurred in < 10% of patients in some study periods. The SOC values presented show the incidence of all patients/events that occurred under that SOC (not only those events meeting the ≥ 10% threshold).

Patients at risk for each period (N in column header) is defined as the patients who entered the study period. For AEs with worsening severity in which the AE started in the first period and worsened in the next period, the patient was counted in both periods. Patients were counted once for each SOC and PT even if they had multiple instances of the event in 1 period. If an AE started in 1 reporting period and continued into the next reporting period, it was counted only in the first period. If an AE started and stopped in 1 reporting period and then recurred in the next reporting period, it was counted in both periods. All events reported in the database are counted in the number of events.

Hematologic abnormalities reported as AEs that were coded to PTs in the Investigations SOC (e.g., platelet count decreased) have been pooled with appropriate terms in the Blood and Lymphatic System SOC (e.g., thrombocytopenia) for tabulation.

Table 35. Treatment-Emergent Serious Adverse Events From D1 to M48 (TP-102/104, TP-103)

System Organ Class Preferred Term	TP-102/104 N=67 n (%)	TP-103 Overall N=59 n (%)	TP-103 MSD N=11 n (%)	TP-103 NMSD N=48 n (%)
Patients with ≥ 1 TESAE to M48	35 (52.2)	43 (72.9)	8 (72.7)	35 (72.9)
Infections and infestations	11 (16.4)	22 (37.3)	6 (54.5)	16 (33.3)
Device related infection	0	5 (8.5)	3 (27.3)	2 (4.2)
BK virus infection	0	3 (5.1)	0	3 (6.3)
Clostridium difficile infection	0	3 (5.1)	0	3 (6.3),
Pneumonia	0	3 (5.1)	2 (18.2)	1 (2.1)
Staphylococcal bacteremia	0	3 (5.1)	1 (9.1)	2 (4.2)
Epstein-Barr viremia	0	2 (3.4)	0	2 (4.2)
Sepsis	0	2 (3.4)	0	2 (4.2)
Adenovirus infection	0	1 (1.7)	0	1 (2.1)
Atypical pneumonia	0	1 (1.7)	0	1 (2.1)
Bacillus bacteremia	0	1 (1.7)	0	1 (2.1)
Bacteremia	0	1 (1.7)	0	1 (2.1)
Bronchiolitis	0	1 (1.7)	0	1 (2.1)
Coxsackie viral infection	0	1 (1.7)	0	1 (2.1)
Cytomegalovirus infection	0	1 (1.7)	1 (9.1)	0
Cytomegalovirus viremia	0	1 (1.7)	0	1 (2.1)
Enterococcal bacteremia	0	1 (1.7)	0	1 (2.1)
Enterococcal infection	0	1 (1.7)	0	1 (2.1)
Gastroenteritis adenovirus	0	1 (1.7)	0	1 (2.1)
Gastroenteritis astroviral	0	1 (1.7)	0	1 (2.1)
Human herpesvirus 6 encephalitis	0	1 (1.7)	0	1 (2.1)
Human herpesvirus 6 infection	0	1 (1.7)	0	1 (2.1)
Kidney infection	0	1 (1.7)	0	1 (2.1)
Klebsiella bacteremia	0	1 (1.7)	0	1 (2.1)
Parvovirus infection	0	1 (1.7)	0	1 (2.1)
Pneumonia viral	0	1 (1.7)	0	1 (2.1)
Tooth abscess	0	1 (1.7)	0	1 (2.1)
Septic shock	0	2 (3.4)	1 (9.1)	1 (2.1)
Sinusitis	1 (1.5)	1 (1.7)	0	1 (2.1)
Upper respiratory tract infection	0	1 (1.7)	1 (9.1)	0
Urinary tract infection	0	1 (1.7)	0	1 (2.1)
Viral infection	1 (1.5)	1 (1.7)	0	1 (2.1)
Viral upper respiratory tract infection	1 (1.5)	0	0	0
Cystitis viral	2 (3.0)	0	0	0
Gastroenteritis	1 (1.5)	0	0	0
Influenza	1 (1.5)	0	0	0
Otitis media	1 (1.5)	0	0	0
Pseudomonal bacteremia	2 (3.0)	0	0	0
Stenotrophomonas infection	1 (1.5)	0	0	0
Streptococcal bacteremia	1 (1.5)	0	0	0
Vascular device infection	2 (3.0)	0	0	0

System Organ Class Preferred Term	TP-102/104 N=67 n (%)	TP-103 Overall N=59 n (%)	TP-103 MSD N=11 n (%)	TP-103 NMSD N=48 n (%)
Blood and lymphatic system disorders	13 (19.4)	12 (20.3)	2 (18.2)	10 (20.8)
Febrile neutropenia	12 (17.9)	4 (6.8)	1 (9.1)	3 (6.3)
Thrombocytopenia	0	4 (6.8)	0	4 (8.3)
Anemia	0	2 (3.4)	0	2 (4.2)
Bone marrow failure	0	2 (3.4)	0	2 (4.2)
Hemolytic anemia	0	2 (3.4)	1 (9.1)	1 (2.1)
Leukopenia	0	2 (3.4)	0	2 (4.2)
Neutropenia	0	2 (3.4)	0	2 (4.2)
Autoimmune hemolytic anemia	0	1 (1.7)	0	1 (2.1)
Cytopenia	0	1 (1.7)	0	1 (2.1)
Pancytopenia	2 (3.6)	0	0	0
Nervous system disorders	6 (9.0)	11 (18.6)	2 (18.2)	9 (18.8)
Neurological decompensation	1 (1.5)	6 (10.2)	0	6 (12.5)
Seizure	4 (6.0)	2 (3.4)	0	2 (4.2)
Aphasia	0	2 (3.4)	0	2 (4.2)
Encephalopathy	0	1 (1.7)	1 (9.1)	0
Intracranial pressure increased	0	1 (1.7)	1 (9.1)	0
Ischemic cerebral infarction	0	1 (1.7)	0	1 (2.1)
Dyskinesia	1 (1.5)	0	0	0
Myelitis transverse	1 (1.5)	0	0	0
General disorders and administration site conditions	11 (16.4)	8 (13.6)	2 (18.2)	6 (12.5)
Pyrexia	11 (16.4)	3 (5.1)	2 (18.2)	1 (2.1)
Death	0	2 (3.4)	0	2 (4.2)
Disease progression	0	2 (3.4)	0	2 (4.2)
Multiple organ dysfunction syndrome	0	1 (1.7)	0	1 (2.1)
Vascular disorders	0	5 (8.5)	1 (9.1)	4 (8.3)
Hypertension	0	2 (3.4)	1 (9.1)	1 (2.1)
Deep vein thrombosis	0	1 (1.7)	0	1 (2.1)
Hypotension	0	1 (1.7)	0	1 (2.1)
Thrombosis	0	1 (1.7)	0	1 (2.1)
Gastrointestinal disorders	6 (9.0)	6 (10.2)	3 (27.3)	3 (6.3)
Diarrhea	0	2 (3.4)	0	2 (4.2)
Abdominal pain	1 (1.5)	1 (1.7)	1 (9.1)	0
Gastritis	0	1 (1.7)	0	1 (2.1)
Hematemesis	0	1 (1.7)	1 (9.1)	0
Hematochezia	0	1 (1.7)	0	1 (2.1)
Intestinal obstruction	0	1 (1.7)	1 (9.1)	0
Vomiting	2 (3.0)	1 (1.7)	1 (9.1)	0
Constipation	1 (1.5)	0	0	0
Stomatitis	2 (3.0)	0	0	0
Immune system disorders	0	4 (6.8)	0	4 (8.3)
Anaphylactic reaction	0	2 (3.4)	0	2 (4.2)
Transplant rejection	0	2 (3.4)	0	2 (4.2)

System Organ Class Preferred Term	TP-102/104 N=67 n (%)	TP-103 Overall N=59 n (%)	TP-103 MSD N=11 n (%)	TP-103 NMSD N=48 n (%)
Metabolism and nutrition disorders	1 (1.5)	4 (6.8)	0	4 (8.3)
Feeding intolerance	0	2 (3.4)	0	2 (4.2)
Decreased appetite	1 (1.5)	1 (1.7)	0	1 (2.1)
Dehydration	0	1 (1.7)	0	1 (2.1)
Malnutrition	0	1 (1.7)	0	1 (2.1)
Respiratory, thoracic and mediastinal disorders	1 (1.5)	4 (6.8)	0	4 (8.3)
Hemothorax	0	2 (3.4)	0	2 (4.2)
Respiratory failure	0	2 (3.4)	0	2 (4.2)
Hypoxia	0	1 (1.7)	0	1 (2.1)
Pleural effusion	0	1 (1.7)	0	1 (2.1)
Pulmonary hemorrhage	0	1 (1.7)	0	1 (2.1)
Respiratory distress	1 (1.5)	0	0	0
Ear and labyrinth disorders	0	3 (5.1)	1 (9.1)	2 (4.2)
Hypoacusis	0	2 (3.4)	1 (9.1)	1 (2.1)
Auditory disorder	0	1 (1.7)	0	1 (2.1)
Injury, poisoning and procedural complications	3 (4.5)	3 (5.1)	0	3 (6.3)
Transplant failure	0	2 (3.4)	0	2 (4.2)
Graft loss	0	1 (1.7)	0	1 (2.1)
Anaphylactic transfusion	1 (1.5)	0	0	0
Head injury	1 (1.5)	0	0	0
Spinal fracture	1 (1.5)	0	0	0
Renal and urinary disorders	1 (1.5)	3 (5.1)	0	3 (6.3)
Acute kidney injury	1 (1.5)	2 (3.4)	0	2 (4.2)
Chronic kidney disease	0	1 (1.7)	0	1 (2.1)
Dysuria	0	1 (1.7)	0	1 (2.1)
Urinary tract obstruction	0	1 (1.7)	0	1 (2.1)
Endocrine disorders	0	2 (3.4)	0	2 (4.2)
Adrenocortical insufficiency acute	0	2 (3.4)	0	2 (4.2)
Cardiac disorders	2 (3.0)	1 (1.7)	0	1 (2.1)
Acute myocardial infarction	0	1 (1.7)	0	1 (2.1)
Cardiac arrest	0	1 (1.7)	0	1 (2.1)
Coronary artery disease	0	1 (1.7)	0	1 (2.1)
Cardio-respiratory arrest	1 (1.5)	0	0	0
Sinus bradycardia	1 (1.5)	0	0	0
Eye disorders	0	1 (1.7)	0	1 (2.1)
Visual impairment	0	1 (1.7)	0	1 (2.1)
Hepatobiliary disorders	1 (1.5)	1 (1.7)	0	1 (2.1)
Acute hepatic failure	1 (1.5)	0	0	0
Venoocclusive liver disease	0	1 (1.7)	0	1 (2.1)
Investigations	1 (1.5)	1 (1.7)	0	1 (2.1)
Weight decreased	0	1 (1.7)	0	1 (2.1)
Transaminases increased	1 (1.5)	0	0	0

System Organ Class Preferred Term	TP-102/104 N=67 n (%)	TP-103 Overall N=59 n (%)	TP-103 MSD N=11 n (%)	TP-103 NMSD N=48 n (%)
Musculoskeletal and connective tissue disorders	1 (1.5)	0	0	0
Rhabdomyolysis	1 (1.5)	0	0	0
Psychiatric disorders	3 (4.5)	1 (1.7)	0	1 (2.1)
Agitation	0	1 (1.7)	0	1 (2.1)
Aversion	1 (1.5)	0	0	0
Depression	1 (1.5)	0	0	0
Suicidal ideation	1 (1.5)	0	0	0
Tic	1 (1.5)	0	0	0

Abbrev.: AE, adverse event; MSD, matched sibling donor; NMSD, no matched sibling donor; PT, preferred term; SAE, serious adverse event; SOC, System Organ Class; TESAE, treatment-emergent serious adverse event; TP, transplant population.
PTs and their associated SOC are included for all SAEs that were observed in the D1 to M48 study period and are sorted based on decreasing frequency by SOC and then PT and then alphabetically based on TP-103 overall. Patients at risk for each period (N in column header) is defined as the patients who entered the study period. If an SAE started in 1 reporting period and continued into the next period, it was counted only in the first period. If an SAE started and stopped in 1 reporting period and then recurred in the next period, it was counted in both periods. Patients were counted once for each SOC and PT even if they had multiple instances of the event in 1 period. For SAEs with worsening severity in which the SAE started in the first period and worsened in the next period, the patient was counted in both periods. All events reported in the database are counted in the number of events.
Hematologic abnormalities reported as AEs that were coded to PTs in the Investigations SOC (e.g., platelet count decreased) have been pooled with appropriate terms in the Blood and Lymphatic System SOC (e.g., thrombocytopenia) for tabulation.

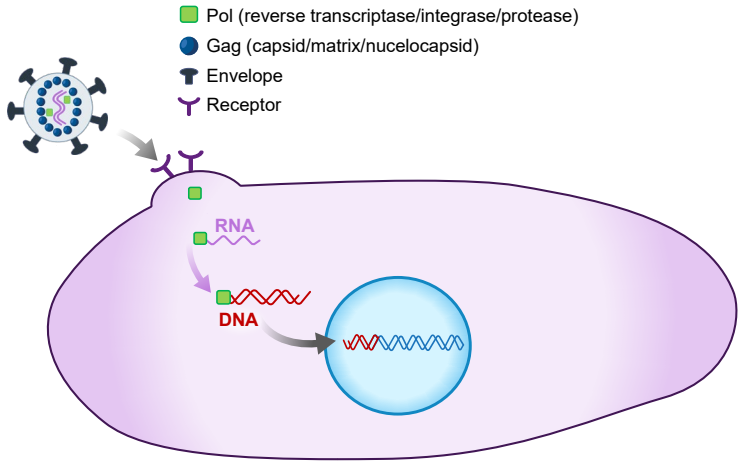
12. APPENDIX D: LENTIVIRAL VECTOR SAFETY

12.1. Introduction to Retroviral Vector Integration

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

Retroviral vectors are modified retroviruses in which the viral genes that encode viral proteins are replaced with a therapeutic transgene. Due to the absence of viral genes, the integrated vector provirus is incapable of replication and further propagation; hence retroviral vectors are replication incompetent. Integration of the transgene into the host genome is permanent and expression of the transgene depends on the presence of regulatory elements that control production of the therapeutic protein. A diagrammatic representation of transduction is shown in Figure 31.

Figure 31. Diagrammatic Representation of a Retroviral Vector Transducing a Cell



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

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

Each human gene has a promoter which serves as the "on" switch for the gene, typically adjacent to the transcriptional start site (TSS). When the gene is "turned on", the gene sequences that encode the protein (exons) are copied, or transcribed, into RNA along with the non-coding regions between each exon (introns). The introns are intervening sequences that are frequently larger than the exons and are removed during RNA processing (called "splicing") before export from the nucleus and translation into proteins.

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

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

- 1 case of lymphoid T-cell leukemia out of 33 patients with adenosine deaminase-severe combined immunodeficiency
- 5 cases of T-cell acute lymphoblastic leukemia out of 20 patients with X-linked severe combined immunodeficiency
- 4 cases of myeloblastic syndromes out of 5 patients with chronic granulomatous disease
- 9 cases of acute leukemia out of 10 patients with Wiskott-Aldrich syndrome

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

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

12.2. Lentiviral Vectors in bluebird bio Programs

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

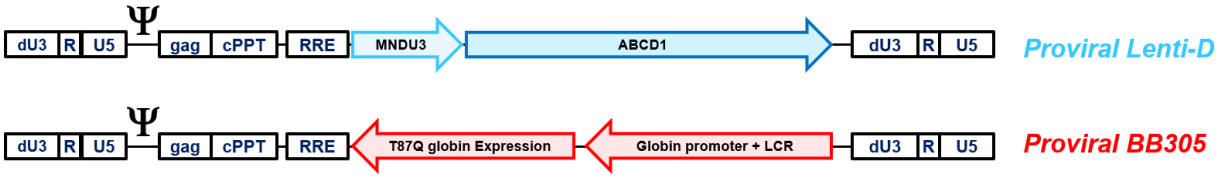
- **beti-cel**
  - **BB305 LVV**
  - CD34+ HSCs from patients with **transfusion-dependent β-thalassemia (TDT)**

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

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

BB305 LVV and Lenti-D LVV are illustrated in **Figure 32**.

Figure 32. LVV Proviral Structures



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

- **Transgenes differ, tailored to disease-specific genetic defect**
  - TDT and SCD are both caused by defects in the β-globin gene, and so the BB305 LVV encodes a functional β-globin ($\beta^{A\text{-}T87Q}$-globin).
  - CALD is caused by lack of the peroxisomal transmembrane adrenoleukodystrophy protein (ALDP), and so Lenti-D encodes a functional ALDP (encoded by an *ABCD1* cDNA).
- **Transgene structure**
  - $\beta^{A\text{-}T87Q}$-globin is expressed using the natural intron/exon configuration since intron 2 is known to be required for maximal β-globin production (Collis et al. 1990).
  - ALDP is expressed from a cDNA derived from the *ABCD1* gene, without introns, as adequate protein production is not dependent on splicing.
- **Transcriptional controls differ, tailored to cell-specific expression needed**
  - BB305 uses the human β-globin promoter and enhancer which drives high levels of gene expression, but only in the erythroid lineage (Grosveld et al. 1987). Thus, human β-globin production is restricted to this lineage.
  - Lenti-D uses the synthetic MNDU3 promoter which drives high levels of gene expression in multiple cell lineages (Challita et al. 1995; Haas et al. 2003). ALDP is thought to be produced in cerebral macrophages and microglial cells to stop progression of CALD, and so a ubiquitous promoter was chosen to ensure

appropriate expression of ALDP in all hematopoietic cells, including those engrafting in the central nervous system.

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

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

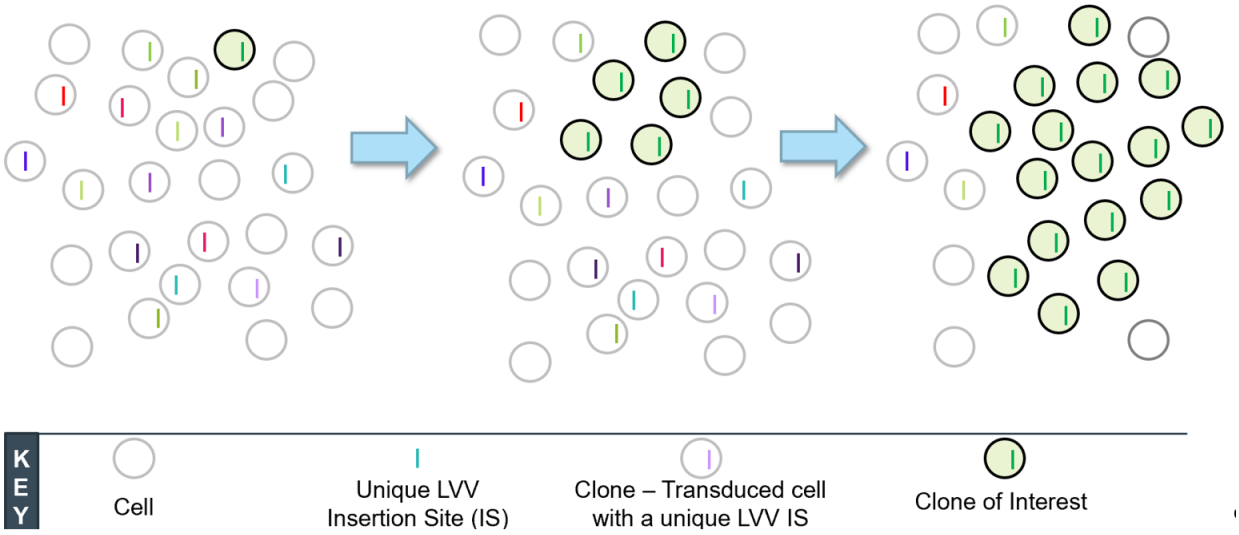
Thus, differences in LVV structure, inherent cell properties, and manufacturing may impact LVV integration profiles, as well as expansion of transduced cells after engraftment in treated patients.

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

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

Tracking the relative frequency over time of an IS allows for inferences into clonal dynamics of the cells that contain that IS, i.e., clones derived from the same progenitor cell, like an HSC. Due to normal clonal dynamics, relative contribution of clones can fluctuate over time; however, sometimes expansion of one or a few clones can occur to such a degree that one or a few clones dominate the peripheral blood cell population. Figure 33 is a diagrammatic representation of nucleated blood cells present in a patient after hematopoietic reconstitution, where one clone containing a unique IS preferentially expands compared to all other clones that contain different unique IS. ISA allows for the quantitation of the relative frequency of unique IS over time providing insight into clonal dynamics.

Figure 33. Diagrammatic Representation of Clonal Expansion



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

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

bluebird bio recommends regular ISA monitoring for all patients in our clinical studies. This approach has been modified over time as our understanding of clonal dynamics has matured, along with the improved methodology of ISA that has increased the accuracy of relative frequency (RelFreq) estimates of insertion sites (IS). ISA is currently performed using the quantitative S-EPTS/LM-PCR method (Schmidt et al. 2001). Patients in bluebird bio clinical studies receive routine ISA every 6 months for the first 5 years post-treatment and then annually through year 15 after treatment, coupled with CBC analysis every 6 months for the entirety of a 15-year follow-up period. Depending on RelFreq results, enhanced monitoring is recommended for patients whose results suggest oligoclonality, which bluebird bio defines as any IS with a RelFreq ≥10% with a VCN of ≥0.1 c/dg. Should there be any increased concern for malignancy, based on clinical signs and symptoms as well as the location of IS of interest and its rate of expansion, additional clinical and molecular work-ups are undertaken to further investigate the potential presence of malignancy. bluebird bio has been in an ongoing dialogue with the FDA on ISA monitoring for several years.

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

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

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

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

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

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

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

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

It should be noted that IS are frequently detected in proto-oncogenes, including *MECOM,* without any observation of oligoclonality or malignancy (e.g. (Reinhardt et al. 2021)). Thus, additional factors play a role in the development of malignancy in treated patients. A summary of results of other genetic tests in these patients is presented in Table 36. Some other mutations

have been identified in 2 of the 3 patients, but their significance in contributing to the development of MDS is not clear.

Table 36. Genetic Findings in Patients with MDS after Treatment with eli-cel

Time Period	Patient 104-18	Patient 104-08	Patient 102-03
First observation of ≥ 10% RelFreq of relevant IS	Month 6	Month 6	Month 92; not observed at previous visit at Month 60[b]
Location of IS currently at ≥ 10% RelFreq [a]	MECOM, SLC6A16	MECOM, ACTR3, RAP2C-AS1, ST3GAL6-AS1	PRDM16, GAB3, CAMK2A, TYK2, SNX12, MIRI06A
Mutation analyses at or after diagnosis			
Karyotype	46XY, Chr.14 aberration; germline	46XY normal	46XY normal
NGS	CDKN2A c.168C>G, germline	None detected	KRAS c.35G>C, 13.6%VAF NRAS c.35G>C, 2.5% VAF
IS associated with increased expression of adjacent gene?	Yes, MECOM. Other IS not tested	Yes, MECOM Other IS not tested	Yes

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

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

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

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

Table 37 summarizes the number of patients that currently satisfy the criteria for oligoclonality and persistent oligoclonality (i.e. those meeting the criteria for oligoclonality at two consecutive determinations) and Figure 34 provides ISA profiles for the IS of interest in those patients treated with eli-cel.

Table 37. Summary of Patients Currently with an IS ≥10% RelFreq

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

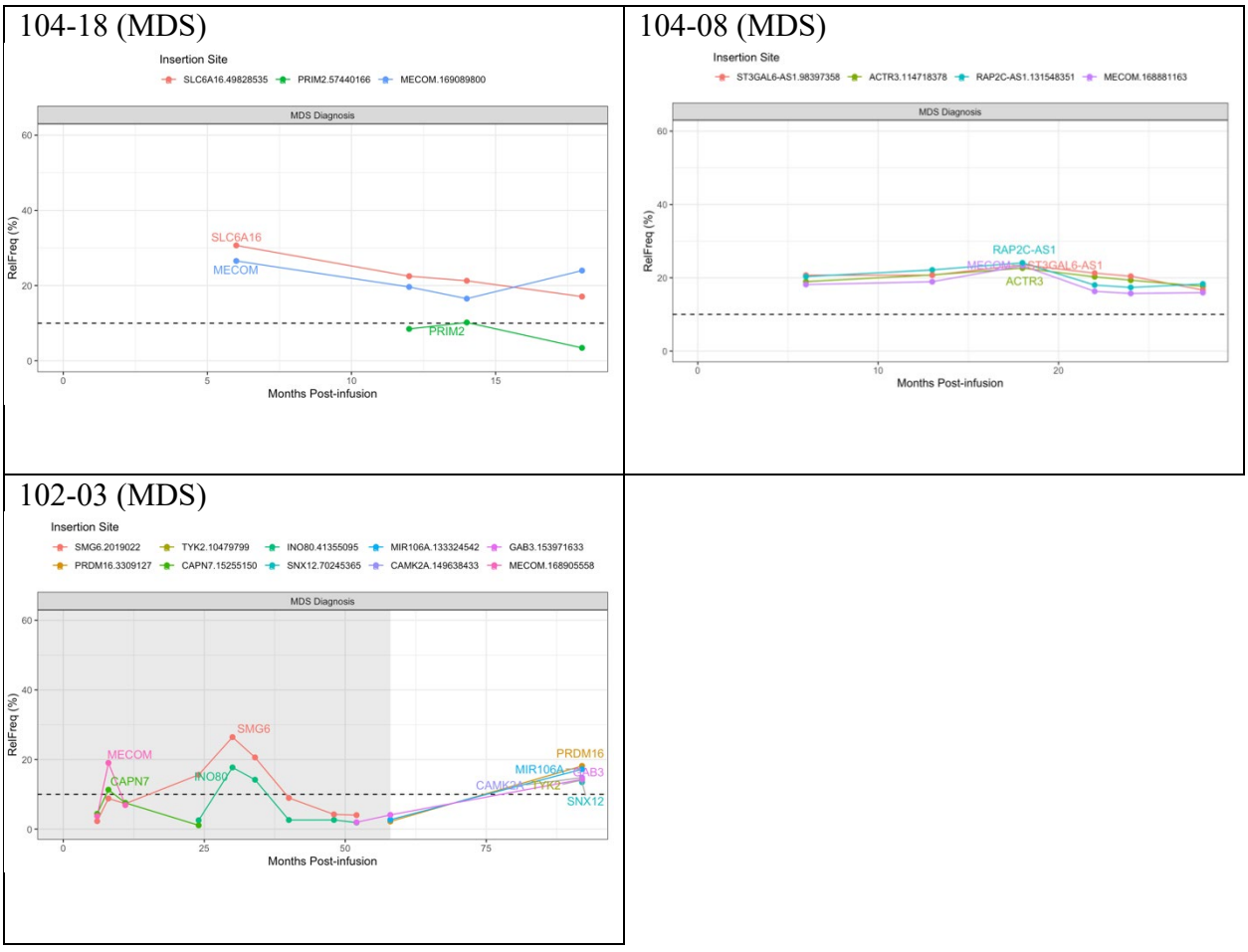
Data as of 29Apr2022 for all patients, except for 2 patients indicated by an asterisk, which included late breaking data. n defined as patients treated with gene therapy with available ISA data.

[a] one patient withdrew from study

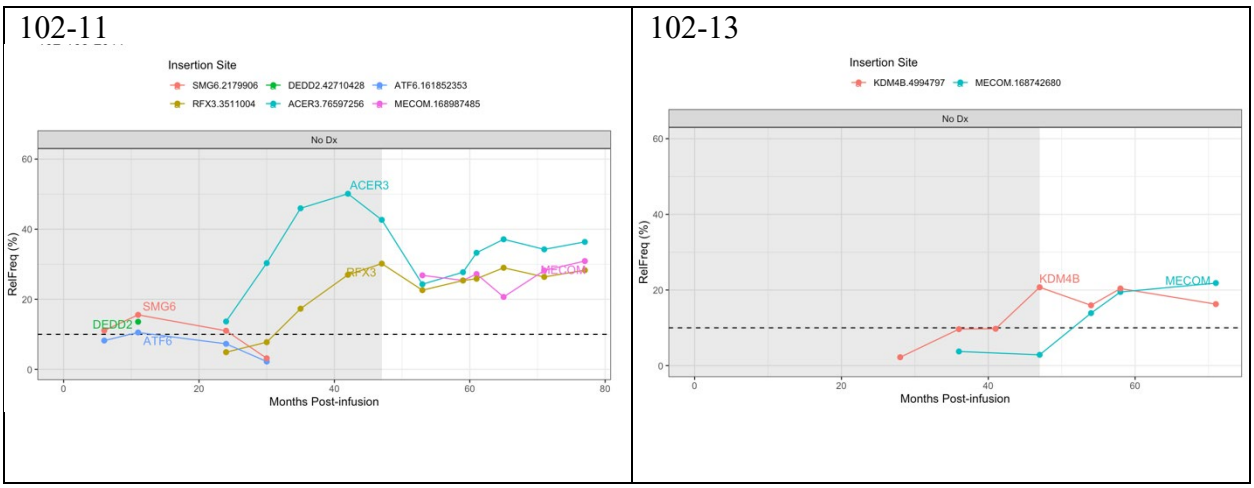
[b] one patient deceased

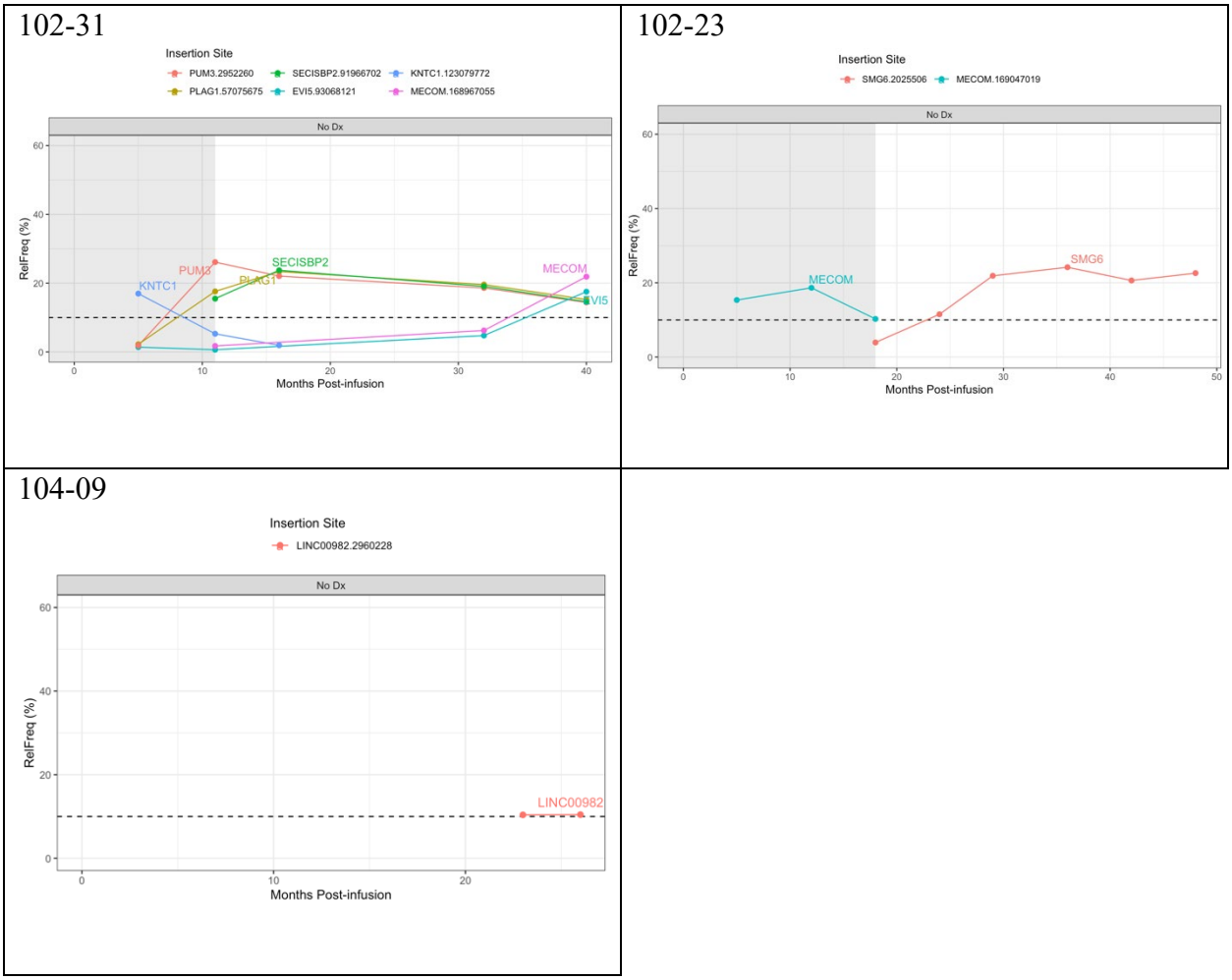
Figure 34. Oligoclonality Observed in Patients Treated with eli-cel

A. Persistent Oligoclonality in Patients with a Malignancy

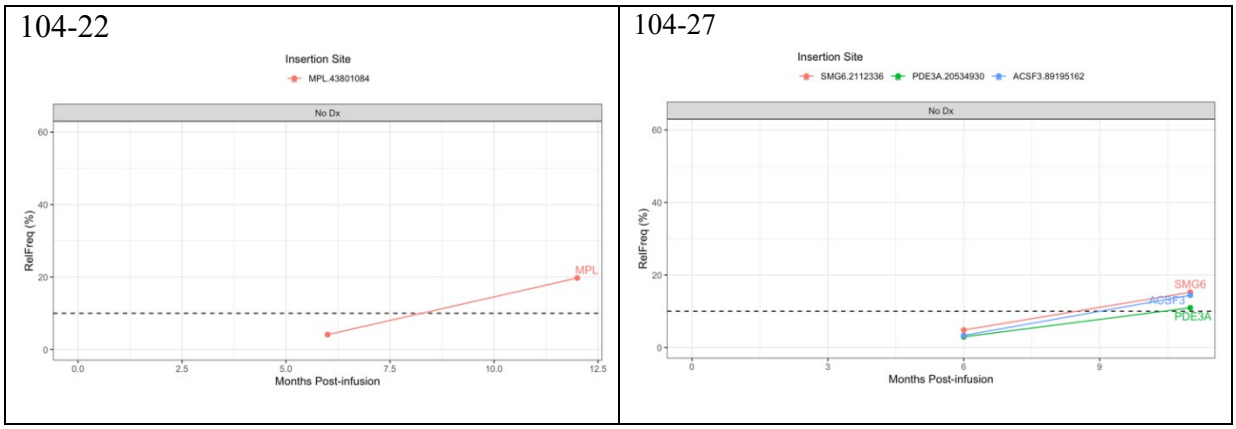


B. Ongoing Persistent Oligoclonality in Patients Without Evidence of a Malignancy





C. Other Patients with Oligoclonality at Latest Visit



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

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

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

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

The second case also had blasts with similar hallmark AML mutations, but in addition contained an LVV IS in a gene called VAMP4. ISA showed that the relative frequency of this IS increased markedly during the period in which clinical signs and symptoms indicated malignancy (Figure 36A). Molecular analyses indicated that the LVV IS was a passenger mutation that played no role in the development of AML (see below).

A summary of these key findings is presented in Table 38.

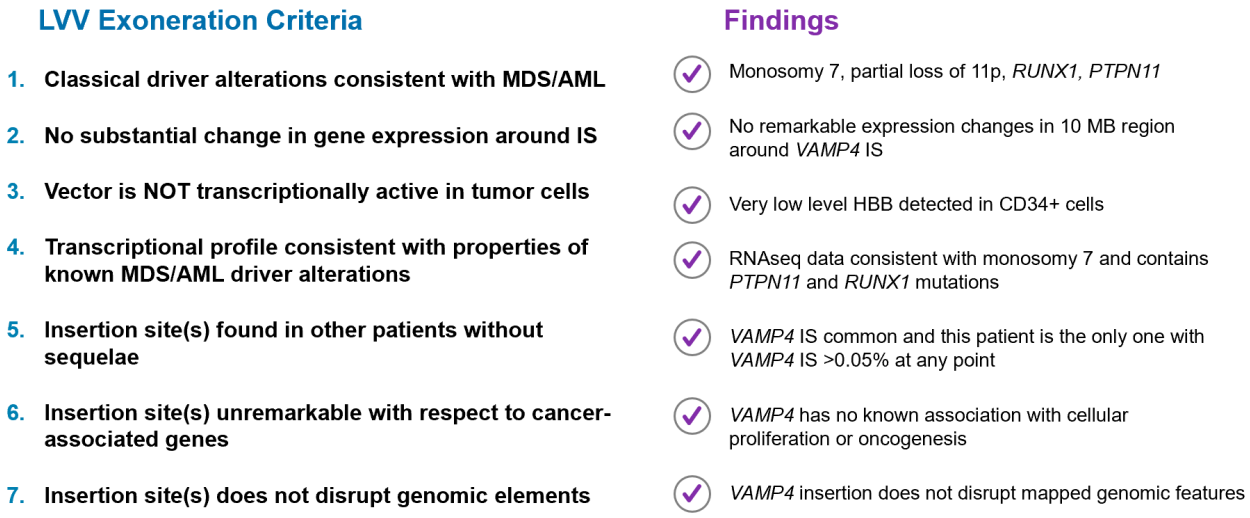
Table 38. Genetic Findings in Patients with MDS/AML after Treatment with lovo-cel

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

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

The role of the IS in VAMP4 was robustly evaluated after bluebird bio sought expert guidance and alignment with numerous key opinion leaders in the field of cell and gene therapy. After evaluating all established criteria for determining LVV involvement in development of AML, which are summarized in Figure 35, the totality of evidence supported that the IS in VAMP4 is a passenger, non-causative insertion.

Figure 35. Evidence Supporting a Benign Passenger Role for IS in VAMP4 Gene



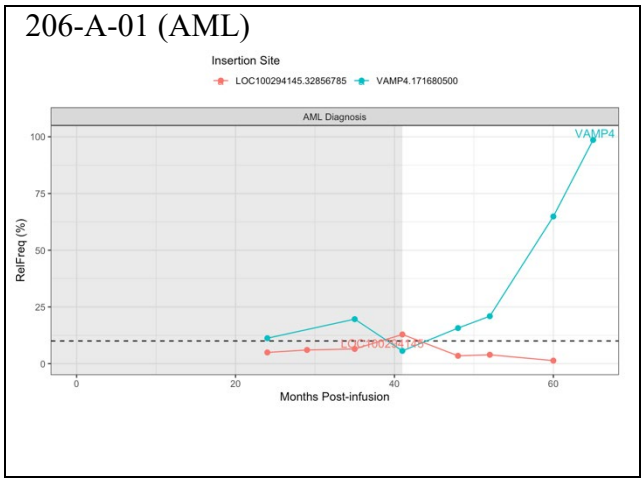
A parallel root cause investigation identified both lovo-cel and disease-specific risk factors for development of malignancy. Patients with sickle cell disease have been determined to be at an increased risk of hematological malignancy (Seminog et al. 2016; Brunson et al. 2017). To address this risk,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

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

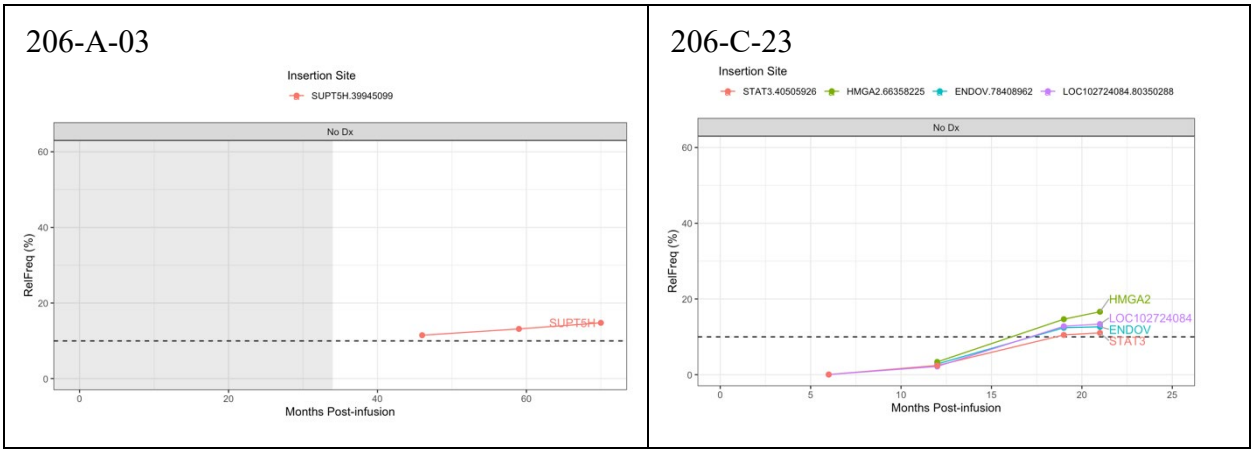
In addition to the patient who had AML, 2 additional patients have an IS present persistently at ≥ 10% RelFreq, without evidence of malignancy (Figure 36B).

Figure 36. Oligoclonality Observed in Patients Treated with lovo-cel

A. Persistent Oligoclonality in Patient with a Malignancy



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



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

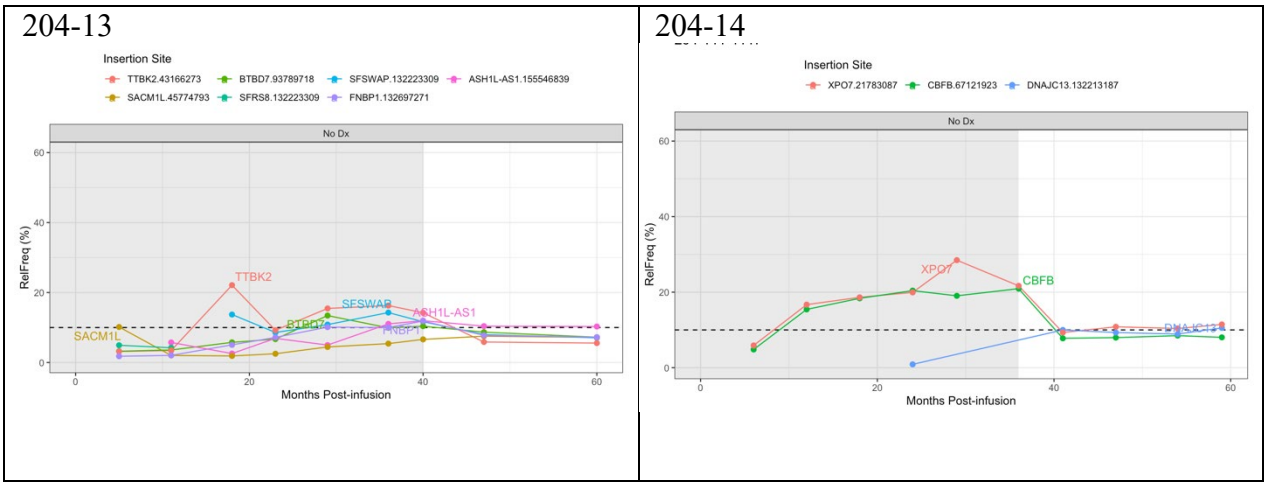
12.4.3. No malignancies in patients treated with beti-cel

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

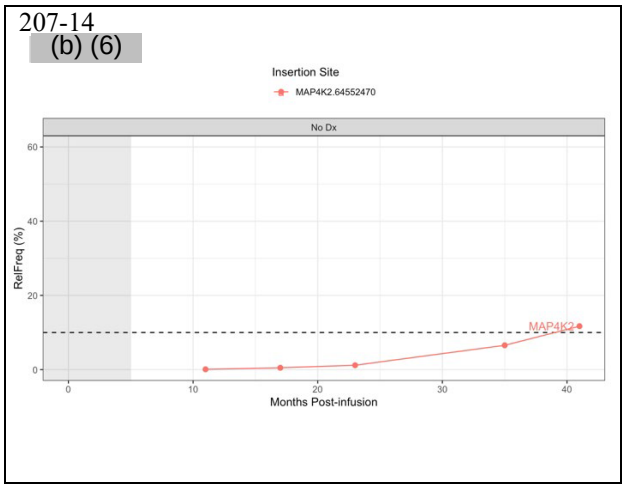
Two patients treated with beti-cel have an IS present persistently ≥ 10% RelFreq, without evidence of malignancy. Thus, the presence of persistent oligoclonality is not predictive of malignancy and can be present stably over several years (Figure 37A). One additional patient has an IS at >10% RelFreq at the latest ISA evaluation (Figure 37B).

Figure 37. Oligoclonality in Patients Treated with beti-cel

A. Ongoing Persistent Oligoclonality Without Evidence of Malignancy



B. Other Patients with Oligoclonality at Latest Visit



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

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

Oncogenesis is a known hazard for all myeloablative therapies that involve the transplantation of hematopoietic stem cells, even in the absence of gene therapy, due to the need for toxic myeloablative agents such as busulfan. All bluebird bio gene therapy programs have this in common. However, there appear to be differences in the rates of malignancy that occur in the different programs. These are potentially due to:

Different vector design. The ubiquitous promoter present in the Lenti-D LVV compared to the cell-lineage restricted BB305 LVV is likely to increase the risk of dysregulation of nearby genes

in multiple cell types, including HSCs. The demonstration of MECOM dysregulation in 2 patients with MDS who were previously treated with eli-cel is consistent with this.

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

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

12.6. Conclusions

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

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

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

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

13. APPENDIX E: POST-MARKETING SURVEILLANCE

bluebird bio has developed post-marketing plans to enable continued characterization of the benefit/risk of eli-cel as well as monitoring for long-term efficacy and safety per FDA Guidance for Industry on Long Term Follow Up After Administration of Human Gene Therapy Products (January 2020). The post-marketing monitoring will be multipronged. Patients treated with eli-cel in the clinical trials will continue to be followed in the ongoing clinical studies: ALD-104 and the long term follow up study LTF-304 for up to 15 years after eli-cel infusion. In addition, a voluntary registry study, REG-502, is planned which will follow patients treated in the post-marketing setting for 15 years after receiving eli-cel. In the post marketing setting, eli-cel will only be distributed through a limited and targeted number of Qualified Treatment Centers (QTCs) to manage administration and ensure that the chain of identity is maintained.

13.1. LTF-304

Longterm Follow-up of Subjects with Cerebral Adrenoleukodystrophy Who Were Treated with elivaldogene autotemcel

As described previously, patients treated in ALD-102 and ALD-104 are followed in the long-term follow-up study LTF-304 for a total of 15 years after eli-cel infusion, which includes 2 years in the parent study and an additional 13 years in LTF-304. Follow-up assessments are performed every 6 months through 5 years post-drug product infusion and then annually from 5 years through 15 years post-drug product infusion. Safety evaluations include laboratory assessments (i.e., CBC and ISA) to monitor for hematopoietic reconstitution, clonal dynamics, and malignancy.

13.2. REG-502

A Prospective, Multicenter, Observational, Long-Term Safety and Effectiveness Registry Study of Patients with Cerebral Adrenoleukodystrophy (CALD) treated with Elivaldogene Autotemcel

bluebird bio will initiate an observational registry study (REG-502) to characterize the safety and effectiveness of eli-cel in patients with CALD treated in the post-marketing setting. All patients treated with eli-cel in the 5-year enrollment period will be offered participation in REG-502. Given the patient population and the engagement of the CALD patient community, a high proportion of patients treated with eli-cel are expected to enroll in the registry study. Based on the number of transplants performed in the United States in the last 5 years, and donor type availability, it is estimated that 8-12 patients/year will be treated with eli-cel in the United States.

REG-502 will use the CIBMTR[1] registry platform for clinical data collection, consistent with data collection for allogeneic stem cell transplants in the United States. Clinical outcomes, gene-therapy specific data, and long-term safety outcomes (including malignancy) will be collected. In REG-502, all enrolled patients will be followed for up to 15 years after infusion with eli-cel which will allow for collection of comprehensive and long-term safety and effectiveness data. Efforts will be made to ensure long term follow up in the registry study. These efforts will include, but are not limited to: education on participation in the registry study,

[1] Center for International Blood and Marrow Transplant Research

outreach from the QTC to patients and/or patient-identified follow-up care providers, patient-friendly registry study updates, and HCP-focused registry study updates.

The clinical management of each patient will be at the discretion of the healthcare provider; the registry study will record safety and effectiveness assessments in accordance with routine clinical care including at least annual CBCs. Gene therapy-specific laboratory assessments such as integration site analysis (ISA) will be offered at least annually in the context of routine blood draws and more often if requested in the context of relevant clinical workup. For all enrolled patients, the registry study will collect and report information on all SAEs, AEs of interest, and eli-cel related AEs. In addition, in the case of a newly diagnosed malignancy, bluebird bio will attempt to collect follow up samples including ISA as clinically feasible from patients regardless of enrollment in REG-502.

13.3. Qualified treatment centers (QTCs)

In the post-marketing setting, eli-cel will be made available only at QTCs to manage administration and ensure that the chain of identity is maintained, given the complexity of autologous transplant. The limited and targeted QTC network includes clinical trial sites and sites with deep transplant and gene and cell therapy expertise. All planned QTCs are FACT[2] accredited. A QTC is a treatment center (including transplant center, apheresis collection center, and cell therapy lab) that has been qualified by bluebird bio to conduct specific activities related to the collection of cells, handling and administration of eli-cel. This will ensure chain of identity of the patient's cells and transfer of the patient's cells to the manufacturing site for drug product manufacturing are conducted properly. Released drug product will be shipped back to the treatment center for patient infusion. These qualified treatment centers are well versed in follow-up post-transplant and will be further trained on the approved US prescribing information and patient labeling, the reporting of AEs following treatment with eli-cel, and on participation in the post-marketing registry study. The registry study will be offered at all QTCs administering eli-cel, and, as stated above, enrollment will be offered to all patients treated during the 5-year study enrollment period.

13.4. Labeling

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

[2] Foundation for the Accreditation of Cellular Therapy