

Clinical Pharmacology BLA Review
Division of Clinical Evaluation and Pharmacology/Toxicology
Office of Tissues and Advanced Therapy

BLA	125755/0
Product	SKYSONA (elivaldogene autotemcel, eli-cel) Suspension for Intravenous Infusion, 4 – 30 million cells/mL
Sponsor	Bluebird Bio, Inc.
Indication	To slow the progression of neurologic dysfunction in boys 4 – 17 years of age with early, active cerebral adrenoleukodystrophy (CALD). Early, active CALD refers to asymptomatic or mildly symptomatic (neurologic function score, NFS \leq 1) boys who have gadolinium enhancement on brain magnetic resonance imaging (MRI) and Loes scores of 0.5-9.
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1 EXECUTIVE SUMMARY

Bluebird Bio, Inc. seeks approval of its BLA for SKYSONA (elivaldogene autotemcel, eli-cel, Lenti-D) for the treatment of patients with less than 18 years of age with early cerebral adrenoleukodystrophy who do not have an available and willing HLA-matched sibling hematopoietic stem cell (HSC) donor.

SKYSONA is genetically modified autologous CD34+ cell-enriched population that contains hematopoietic stem cells (HSCs) transduced ex vivo with lentiviral vector (LVV) encoding adenosine triphosphate [ATP]-binding cassette subfamily D member 1 (*ABCD1*) complementary deoxyribonucleic acid (cDNA) for human adrenoleukodystrophy protein (ALDP). SKYSONA is a cryopreserved cell suspension for one time treatment with a single dose intravenous infusion. The proposed minimum dose of SKYSONA is 5.0×10^6 CD34+ cells/kg.

The clinical pharmacology section of this biologics license application (BLA) consists of one completed Phase 2/3 study (Study ALD-102), one ongoing Phase 3 study (Study ALD-104), and one long-term follow-up study (Study LTF-304) in boys (less than 18 years of age) with early, active cerebral adrenoleukodystrophy (CALD). After infusion of SKYSONA, transduced CD34+ HSCs engraft in the bone marrow and differentiate into various cell types, including peripheral blood monocytes (CD14+) producing functional ALDP that are believed to migrate to the brain where they further differentiate into macrophages and cerebral microglia. Functional ALDP can then participate in the local degradation of very long chain fatty acids (VLCFAs) in the brain,

which is believed to slow or possibly prevent further inflammation and demyelination. All subjects who received SKYSONA with at least 1 month follow-up produced ALDP in CD14+ cells, demonstrating early expression of the transgene. In general, the %ALDP+ cell counts stabilized at 6 months after SKYSONA infusion and remained stable through Month 24. In Study ALD-102, ALDP expression was detectable in 4 of the 7 subjects who had last follow-up through Month 60, indicating long-term expression of transgenic ALDP in the progeny of hematopoietic stem cells.

The proposed dosing regimen of SKYSONA administered by intravenous (IV) infusion has demonstrated clinical efficacy with a tolerable safety profile; therefore, the proposed dosing regimen is acceptable. From a clinical pharmacology standpoint, the BLA is acceptable to support approval.

2 INTRODUCTION

SKYSONA (elivaldogene autotemcel, eli-cel, Lenti-D) is an adenosine triphosphate [ATP]-binding cassette, sub-family D, member 1 (*ABCD1*) autologous gene addition therapy. The SKYSONA active drug substance consists of an autologous cluster of differentiation 34 positive (CD34+) cell-enriched population from patients with cerebral adrenoleukodystrophy (CALD) that contains cells transduced with Lenti-D lentiviral vector (LVV) that encodes *ABCD1* complementary deoxyribonucleic acid (cDNA) for human adrenoleukodystrophy protein (ALDP).

Patients with CALD, an X-linked recessive disease, have mutations within the *ABCD1* gene located on the X chromosome at position Xq28. The *ABCD1* gene codes for ALDP, which is a peroxisomal membrane protein involved in the transport and metabolism of very long-chain fatty acids (VLCFA). Mutations in the *ABCD1* gene result in diminished or absent ALDP expression and/or function and subsequent accumulation of VLCFAs in plasma and all tissue types, but most prominently in the adrenal cortex and white matter of the brain and spinal cord. Cerebral ALD is the most severe form of ALD, affecting approximately 40% boys with ALD, typically during childhood. CALD is characterized by rapidly progressive cerebral demyelination leading to progressive, irreversible loss of neurologic function and death. The goal of treatment for CALD is to stabilize neurologic function by delaying or, ideally, preventing the development of major functional disabilities (MFDs) which compromise the ability to function independently.

SKYSONA adds functional copies of the *ABCD1* cDNA into patients' HSCs through ex vivo transduction of autologous CD34+ cells with Lenti-D LVV. After SKYSONA infusion, transduced CD34+ HSCs engraft in the bone marrow and differentiate into various cell types, including monocytes (CD14+ cells) capable of producing functional ALDP. Functional ALDP can then enable the local degradation of VLCFAs, which in turn can prevent or slow further inflammation and demyelination. Expression of ALDP in HSCs and their progeny is controlled by an internal, modified form of the MND promoter (MNDU3).

SKYSONA is a suspension for intravenous infusion, in which cells are suspended in a cryopreservation solution containing 5% dimethyl sulfoxide (DMSO). SKYSONA is intended for a single dose (one-time treatment) intravenous (IV) infusion and the proposed minimum dose of SKYSONA is 5.0×10^6 CD34+ cells/kg.

This clinical pharmacology of eli-cel was evaluated in Studies ALD-102 and ALD-104 in subjects with CALD who were less than 18 years of age. Subjects were followed for 24 months after dosing in these studies. Treated subjects who completed or discontinued from either of these studies were asked to enroll in a long-term follow-up Study LTF-304.

3 SUMMARY OF IMPORTANT CLINICAL PHARMACOLOGY FINDINGS

Key clinical pharmacology findings are summarized below:

General Pharmacodynamics

- One month after infusion of SKYSONA, lentiviral vector copy was detected in peripheral blood leukocytes (PB VCN) and CD14+ cells (CD14+ VCN), demonstrating the early presence of transduced cells. Levels of PB VCN and CD14+ VCN stabilized by Month 6. Subjects had a Month 6 median (min, max) PB VCN levels of 0.38 (0.07, 2.23) c/dg in Study ALD-102 (N=25) and 1.04 (0.03, 3.13) c/dg in Study ALD-104 (N=32). Median CD14+ VCN levels at Month 6 were 0.61 (0.07, 3.96) c/dg (N=29) and 1.41 (0.04, 3.82) c/dg (N=28), for Studies ALD-102 and ALD-104 respectively. VCN levels in peripheral blood and CD14+ cells generally remained stable as of the data cut-off date, although high inter-subject variability of PB VCN and CD14+ VCN kinetic profiles was observed.
- All subjects who received SKYSONA with at least 1 month of follow-up produced ALDP in peripheral blood leukocytes and CD14+ cells, demonstrating early expression of the transgene. The %ALDP+ cell counts stabilized at 6 months after SKYSONA infusion. Subjects had a Month 6 median (min, max) %ALDP+ CD14+ cell count of 16% (2%, 71%) in Study ALD-102 (N=23) and 26% (2%, 86%) in Study ALD-104 (N=25) respectively. The %ALDP+ CD14+ cells generally remained stable through Month 24 with a median (min, max) of 15% (6%, 45%) in Study ALD-102 (N=23) and 28% (2%, 40%) in Study ALD-104 (N=11). As of the data cut-off date of January 07, 2022, ALDP expression in CD14+ cells was detected in 3 of 7 subjects who had the last follow-up through Month 60 in Study ALD-102 (N=7), indicating long-term expression of transgenic ALDP in the progeny of hematopoietic stem cells.
- Subjects with higher PB VCNs generally had higher PB %ALDP+ cells at a given timepoint. There was a positive linear relationship between PB VCN and PB %ALDP+ cells at Month 6.
- ALDP is a peroxisomal membrane protein involved in the transport and metabolism of very long-chain fatty acids (VLCFA). VLCFA levels in fasting serum were highly variable in study subjects treated with SKYSONA. There was a decrease in VLCFAs as observed by median

values of C26:0 LysoPC and C26:0/C22:0 ratios from Baseline to Month 24 post-administration of SKYSONA.

Dosing Characteristics and Responses

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

Pharmacodynamic Responses and Clinical Outcomes

- PD responses and MFD-free survival: Compared to subjects who achieved MFD-free survival at Month 24 after eli-cel infusion, the median levels of the following PD parameters were substantially lower in subjects who developed MFD or underwent allo-HSCT due to disease progression: PB VCN at Month 6, 24-month exposure of PB VCN, and CD14+ %ALDP+ Cells at Month 6.
- PD responses and MDS: among subjects with at least 6 month follow up period, the median levels of PB VCN at Month 6 and maximum PB VCN during observation period were substantially higher in subjects diagnosed with MDS (N=3), compared to subjects who did not have MDS (N=62). All three subjects diagnosed with MDS had maximum PB VCN levels more than 2.0 c/dg (median (range): 3.13 (2.15, 4.82)). The median (min, max) value of maximum PB VCN was 0.96 (0.11, 3.40) c/dg in subjects who did not have MDS. Note that there were 5 subjects with Max PB VCN > 2 c/dg and did not develop MFD. However, the clinical reviewer identified 3 of these subjects with findings potentially concerning for increased risk malignancy. Refer to the clinical review for additional details.

4 LABELING COMMENTS

The clinical pharmacology reviewer has reviewed the package insert for BLA 125755 and finds it acceptable pending the following revisions shown below.

The Applicant proposed following indication in the original labeling: SKYSONA is indicated for the treatment of patients less than 18 years of age with early cerebral adrenoleukodystrophy (CALD) who do not have an available and willing human leukocyte antigen (HLA)-matched sibling hematopoietic stem cell (HSC) donor. The proposed indication in the label was revised based on negotiations with the clinical review team as following: SKYSONA is indicated to slow the progression of neurologic dysfunction in boys 4-17 years of age with early, active cerebral adrenoleukodystrophy (CALD). Early, active CALD refers to asymptomatic or mildly symptomatic (neurologic function score, NFS ≤ 1) boys who have gadolinium enhancement on brain magnetic resonance imaging (MRI) and Loes scores of 0.5-9.

Please see clinical review for details.

12. CLINICAL PHARMACOLOGY

Reviewer's Comments:

1. Certain language in Mechanism of Action section was considered promotional without supportive data and was removed.
2. Data in Pharmacodynamics section were updated to only include subjects treated with commercial lots with data cut-off date of January 07, 2022 to be in line with the clinical evaluation.
3. Data were rounded to whole numbers.

12.1. Mechanism of Action

SKYSONA adds functional copies of the *ABCD1* cDNA into patients' HSCs through transduction of autologous CD34+ cells with Lenti-D LVV. ~~Although the mechanism of action is not fully understood, it is hypothesized that a~~ After SKYSONA infusion, transduced CD34+ HSCs engraft in the bone marrow and differentiate into various cell types, including monocytes (CD14+) **capable of producing functional ALDP.** ~~that migrate to the brain where they are believed to further differentiate into macrophages and cerebral microglia that can produce functional ALDP. The f~~ Functional ALDP can then **participate in** ~~enable~~ the local degradation of very long chain fatty acids (VLCFAs) ~~in the brain, which is believed to slow or possible in turn can stabilize the disease by preventing further inflammation and demyelination. Following successful engraftment with genetically modified cells, the expression of ALDP is expected to be life long.~~

12.2. Pharmacodynamics

All patients who received SKYSONA with at least 1 month of follow-up produced ALDP in CD14+ cells (N=~~2325, ALD-102~~ **Study 1**; N=~~3121, Study 2~~ **ALD-104**), demonstrating early expression of the transgene. ~~The SKYSONA is associated with stable %ALDP+ cell counts~~ **stabilized** at 6 months **after SKYSONA infusion** ~~post transplantation~~. Patients had a Month 6

median (min, max) %ALDP+ CD14⁺ cells of ~~16~~22.2% (2.0%, 71.4%) in Study ~~1 ALD-102~~ (N=~~23~~ 27) and 26% (2%, 86%) in Study 2 (N=25).

In peripheral blood, the %ALDP+ CD14⁺ cells remained generally stable through Month 24 with a median (min, max) of ~~15~~17.0% (~~65.8~~%, 45.0%) in Study ~~1 ALD-102~~ (N=~~23~~29) and 28% (2%, 40%) in Study 2 (N=11). ALDP expression ~~was detected in 43% of the subjects who had continued to be stable at last follow-up through Month 60 in Study 1 (N=7), demonstrating~~ indicating long-term expression of transgenic ALDP in the progeny of hematopoietic stem cells.

12.3. Pharmacokinetics

SKYSONA is an autologous gene therapy ~~consisting of~~ which includes HSCs that have been genetically modified *ex vivo*. The nature of SKYSONA is such that conventional studies on pharmacokinetics, absorption, distribution, metabolism, and elimination are not applicable.

5 RECOMMENDATIONS

The clinical pharmacology information in this BLA is acceptable, provided that satisfactory agreement is reached between the sponsor and the FDA regarding the language in Section 12 of the package insert. Please refer to section 4 for detailed Labeling Recommendations.

6 COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

Reviewer's Comments:

The Applicant submitted data with three data-cut-off dates: March 26, 2021 (original submission), August 18, 2021 (90-day safety updates), and January 07, 2022 (additional updates). To be in line with clinical review, clinical pharmacology evaluation was performed using data with data cut-off date of January 07, 2022. In addition, the analysis excluded 6 subjects in Study ALD-102 due to lack of eli-cel product comparability data.

6.1 Study Design

The clinical pharmacology of SKYSONA (eli-cel)¹ was evaluated in one completed Phase 2/3 study (Study ALD-102), one ongoing Phase 3 study (Study ALD-104), and one ongoing long-term follow-up study (Study LTF-304). Both Studies ALD-102 and ALD-104 are open-label, multicenter, single arm studies evaluating the efficacy and safety of eli-cel at a dose of $\geq 5 \times 10^6$ CD34+ cells/kg in boys (less than 18 years of age) with early, active CALD. Early, active CALD refers to asymptomatic or mildly symptomatic (neurologic function score, NFS ≤ 1) boys who have gadolinium enhancement on brain magnetic resonance imaging (MRI) and Loes scores of 0.5 – 9. Subjects were followed for 24 months after dosing in Studies ALD-102 and ALD-104. All eli-cel treated subjects are followed in the long-term follow-up Study LTF-304, to ensure 15 years of follow-up (Table 1).

Studies ALD-102 and ALD-104 had a similar study design with 4 main phases:

1. screening to determine eligibility,
2. autologous CD34+ cell collection, transduction, disposition of eli-cel, and re-confirmation of eligibility,
3. myeloablative and lymphodepleting conditioning and infusion of eli-cel
4. follow-up, through engraftment and 24 months after eli-cel infusion.

In Study ALD-102, conditioning regimen consisted of busulfan and cyclophosphamide. In Study ALD-104, conditioning regimen consisted of busulfan and fludarabine.

¹ In this review, SKYSONA is also referred as to eli-cel.

Table 1. Overview of Clinical Studies Evaluating Eli-cel and Allo-HSCT in Subjects with CALD

Study Identifier (Status); Location of CSR or Protocol (as applicable)	Study Title	Age, Number of Subjects and Treatment Performed	Conditioning Regimen	Data Cut (LSLV) and Database Lock (for Final CSRs only)
Eli-cel Treatment and Long-term Follow-up				
ALD-102 (complete) Module 5.3.5.2 CSR ALD-102	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 y.o.: 30 planned/ 32 treated with eli-cel	busulfan (IV) and cyclophosphamide (IV)	Data Cut: 26 March 2021 Database Lock: 18 May 2021
ALD-104 (ongoing) Module 5.3.5.2 CSR ALD-104 Second Interim	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 y.o.: 35 planned/ 23 treated with eli-cel	busulfan (IV) and fludarabine (IV)	Data Cut: 05 March 2021
LTF-304 (ongoing) Module 5.3.5.2 CSR LTF-304 Second Interim	Long-term Follow-Up of Subjects with Cerebral Adrenoleukodystrophy Who Were Treated with Lenti-D Drug Product	Long-term follow-up for all subjects with CALD who received eli-cel in parent studies: Approximately 60 planned/ 27 enrolled (27 from Study ALD-102 and 0 from Study ALD-104)	Not applicable (Subjects are not treated with eli-cel in this long-term follow-up study)	Data Cut: 26 March 2021
Allo-HSCT				
ALD-103 (complete) (Sponsor terminated study after 59 subjects were enrolled and analyzed) Module 5.3.5.4 CSR ALD-103 version 2	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 y.o.: 60 planned 59 treated with allo-HSCT	Investigator determined as per institutional guidelines	Data Cut: 06 December 2019 Database Lock: 31 March 2020

ALD-101 (complete) Module 5.3.5.4 CSR ALD-101 version 2	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	Males >3 and <15 y.o. Enrolled: Males >1 and <15 y.o. 137 subjects 72 untreated 65 treated with allo-HSCT	Not applicable (untreated) or Investigator determined as per institutional guidelines	Database Lock: 27 March 2012
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Abbrev.: allo-HSCT, allogeneic hematopoietic stem cell transplantation; CALD, cerebral adrenoleukodystrophy; CSR, clinical study report; IV, intravenous; y.o., years old

Source: Applicant. Module 2, section 2.7.2. Summary of Clinical Pharmacology.

The demographics of subjects in all clinical studies is shown in Table 2.

Table 2. Demographics of Clinical Study Subjects

Study Parameters	TP/ALD-102 (N = 32)	TP/ALD-104 (N = 35)	TPES-103 (N = 27)	TP/ALD-103 (N = 59)
Age at First HSCI (years) ^a Median Min., Max.	6 4, 14	7 5, 13	8 5, 11	8 2, 14
Age at First HSCI Category, n (%)				
≥ 2 to < 6	14 (43.8)	7 (20.0)	3 (11.1)	7 (11.9)
≥ 6 to < 12	17 (53.1)	26 (74.3)	24 (88.9)	49 (83.1)
≥ 12 to < 18	1 (3.1)	2 (5.7)	0	3 (5.1)
Age at CALD diagnosis (years)				
Median	6	6	7	7
Min., Max.	1, 13	2, 13	0, 11	0, 14
Sex, n (%)				
Male	32 (100.0)	35 (100.0)	27 (100.0)	59 (100.0)
Race, n (%)				
White	15 (47)	21 (60)	25 (93)	51 (86)
Black or African American	1 (3)	2 (6)	0	2 (3)
Asian	1 (3)	0	0	1 (2)
Other	5 (16)	2 (6)	2 (7)	3 (5)
Not provided/ unknown/ not reported	10 (31)	10 (29)	0	2 (3)
Ethnicity, n (%)				
Hispanic	12 (38)	5 (14)	7 (26)	12 (20)
Non-Hispanic	17 (53)	24 (69)	11 (41)	32 (54)
Not provided/ unknown/ not reported	3 (9)	6 (17)	9 (33)	15 (25)
Baseline Neurologic Function Score (NFS), n (%)				
0	31 (96.9)	33 (94.3)	26 (96.3)	43 (72.9)
1	1 (3.1)	2 (5.7)	1 (3.7)	7 (11.9)
>1 to ≤4	0	0	0	4 (6.8)
>4	0	0	0	1 (1.7)
Missing	0	0	0	4 (6.8)
Baseline Loes Score				
Median	2.00	2.00	3.00	4.25
Min, Max	1.0, 9.0	1.0, 7.5	1.0, 9.0	0.0, 18.5

Abbrev.: HSCI, hematopoietic stem cell infusion; HSCT, hematopoietic stem cell transplantation; CALD, cerebral adrenoleukodystrophy; TP, transplant population; TPES-103, the population enrolled in Study ALD-103 who meet the strictly eligible criteria for entering Study ALD-102

^a Hematopoietic stem cell infusion refers to either eli-cel infusion in ALD-102 and ALD-104 or allo-HSCT in ALD-103

Source: Applicant. Module 2, section 2.7.2. Summary of Clinical Pharmacology.

6.2 Dosing Characteristics

6.2.1 Busulfan

Prior to infusion of SKYSONA, busulfan was administered to study subjects for myeloablation to deplete endogenous HSCs, to allow repopulation of the subject with HSCs containing the transgene without dilution due to the presence of unablated cells. Busulfan was administered intravenously on Days -10, -9, -8, and -7. Because myeloablation plays a critical role for successful reconstitution with transduced cells, and given the toxicity of busulfan at high doses, busulfan levels were monitored during conditioning to achieve a target cumulative busulfan AUC of 17,000 to 21,000 $\mu\text{mol}\cdot\text{min}/\text{L}$ in Study ALD 102 and 20,706 to 23,180 $\mu\text{mol}\cdot\text{min}/\text{L}$ in Study ALD 104. Table 3 shows the summary of exposure to busulfan during conditioning.

Table 3. Exposure to Busulfan During Conditioning

Parameter	ALD-102 (N = 32)	ALD-104 (N = 35)
Average Daily Dose Busulfan (mg/kg/day) ^a		
n	32	35
Median	3.50	4.20
Min, Max	2.8, 4.2	3.0, 5.3
Estimated Average AUC busulfan per day ($\mu\text{mol}\cdot\text{min}/\text{L}/\text{day}$) ^b		
n	32	35
Median	4717.5	5303.0
Min, Max	4039, 5041	(3478, 5695)

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

^b Estimated average AUC busulfan per day is calculated as the sum of the observed and imputed AUC divided by number of days of conditioning. If a subject had a missing value of AUC, it is imputed to the product of the dose on that day and the mean of the ratios of the observed AUC and the corresponding doses.

Source: Applicant. Module 2, section 2.7.2. Summary of Clinical Pharmacology & IR response.

CALD with gadolinium enhancement detected on MRI is associated with lymphocytic infiltration, inflammation, and demyelination. Study subjects in ALD-102 and ALD-104 also underwent lymphodepletion with either cyclophosphamide (Study ALD-102) or fludarabine (Study ALD-104) to deplete inflammatory lymphocytes. In Study ALD-102, cyclophosphamide IV (50mg/kg/day) was administered for 4 days (Day -5 to Day -2). In Study ALD-104, fludarabine IV was given at a dose of 30 mg/m² for 6 days (Day -8 to Day -3). Because cyclophosphamide has additional myeloablative properties at high doses that fludarabine does not, busulfan dosing in Study ALD-102 (cyclophosphamide for lymphodepletion) targeted a lower PK AUC than in Study ALD-104 (fludarabine for lymphodepletion).

All subjects achieved both neutrophil engraftment (NE) and platelet engraftment (PE) after eli-cel infusion. Neutrophil engraftment was defined as achieving 3 consecutive absolute neutrophil counts (ANC) ≥ 500 cells/ μ L (after initial post-infusion nadir) obtained on different days by Day 43 after SKYSONA infusion. All study subjects (ALD-102 and ALD-104) met neutrophil engraftment criteria on median (min, max) Day 13 (11, 41) after SKYSONA infusion. Platelet engraftment was defined as achieving 3 consecutive platelet counts of $\geq 20 \times 10^9$ cells/L (after initial post-infusion nadir) obtained on different days after SKYSONA infusion, with no platelet transfusions administered for 7 days immediately preceding and during the evaluation period. All evaluable patients treated with SKYSONA met platelet engraftment criteria with a median (min, max) platelet engraftment on Day 29.0 (14, 108) in clinical studies ALD-102 and ALD-104.

6.2.2 SKYSONA (eli-cel)

SKYSONA is a cell suspension for a single dose (one time treatment) intravenous (IV) infusion. The dose of CD34+ cells is based on accepted safe practice to achieve hematopoietic reconstitution with long-term engraftment after autologous transplantation. In the clinical practice of HSCs transplantation, a minimum dose of $\geq 1.5 \times 10^6$ CD34+ cells/kg mobilized peripheral stem cells is associated with favorable engraftment kinetics. Lower cell doses may result in engraftment with delays in neutrophil and platelet recovery relative to higher doses. Literature indicates that optimal neutrophil and platelet engraftment occurs at HSCT doses of CD34+ around 5.0×10^6 cells/kg. Initially, the dose of eli-cel was a single IV infusion of $\geq 3.0 \times 10^6$ CD34+ cells/kg. Data from the first 21 subjects in Study ALD-102 showed that doses of $\geq 6.0 \times 10^6$ CD34+ cells/kg were frequently obtainable and well tolerated. The dose of eli-cel was then increased to $\geq 5.0 \times 10^6$ CD34+ cells/kg. The median (min, max) total cell dose of eli-cel were 11.4 (5.0, 20.1) $\times 10^6$ CD34+ cells/kg (N=32) in Study ALD-102 and 12.8 (5.1, 38.2) $\times 10^6$ CD34+ cells/kg (N=35) in Study ALD-104 (Table 4).

Table 4. Dosing Characteristics of Eli-cel

Parameter	ALD-102 (N = 32)	ALD-104 (N = 35)
Total Cell Dose (CD34+ cells $\times 10^6$ /kg)		
n	32	35
Median	11.4	12.8
Min, Max	5.0, 20.1	5.1, 38.2
DP VCN (c/dg) ^{ab}		
n	32	35
Median	1.2	1.3
Min, Max	0.5, 2.7	0.7, 3.1
DP %LVV+ Cells ^a		
n	31	29
Median	45	53

Min, Max	(b) (4)	(b) (4)
(b) (4) (vector copies per transduced cell) ^a		
n	31	29
Median	(b) (4)	(b) (4)
Min, Max	(b) (4)	(b) (4)

Abbrev: DP %LVV+, percentage of cells transduced in drug product; DP VCN, vector copy number in drug product
TP, transplant population

^a If a subject had multiple lots of drug product, the weighted average using the fractions of cell dose (dose per lot/total dose of all lots) as the weight will be calculated per subject.

^b The DP VCN specification lower limit was increased from 0.5 to 0.7 c/dg on 19 January 2017.

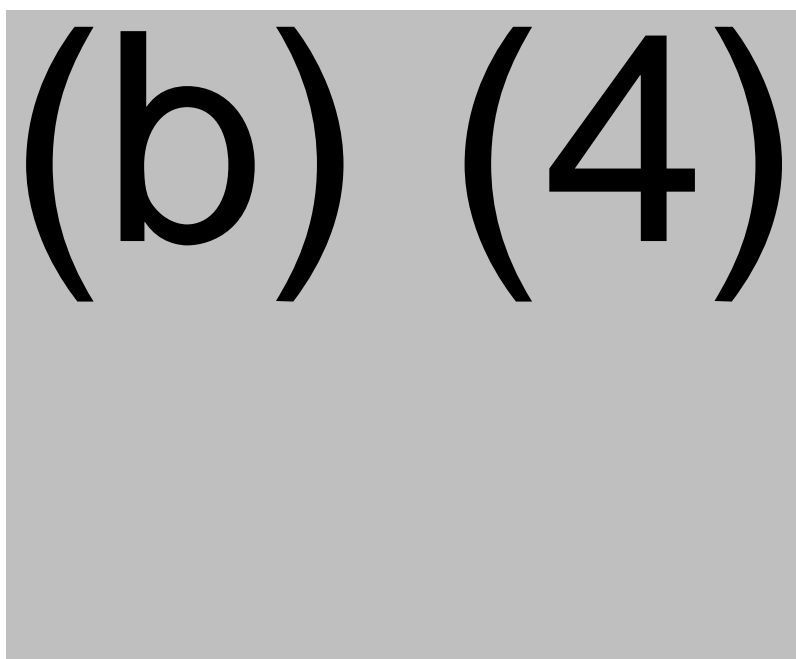
Source: Applicant. Module 2, section 2.7.2. Summary of Clinical Pharmacology & IR response.

During Study ALD-102 and prior to Study ALD-104 initiation, the Applicant made manufacturing changes to (b) (4) transduction efficiency. This may have contributed to the slightly higher median values of DP %LVV+ cells and DP VCN observed in Study ALD-104, compared to Study ALD-102.

DP VCN versus DP %LVV+ Cells

Both DP VCN and DP %LVV+ cells measure drug product characteristics related to transduction efficiency. DP VCN relates to both the number of copies per cell and the percentage of transduced cells in the drug product. DP %LVV+ cells reflect the percentage of transduced cells in drug product. As shown in Figure 1, DP %LVV+ cells increased linearly with the increase of DP VCN up to approximately 60% LVV+ cells, at which point they appear to plateau at higher DP VCNs.

Figure 1. DP VCN Versus DP %LVV+ Cells



Source: Applicant. IR response.

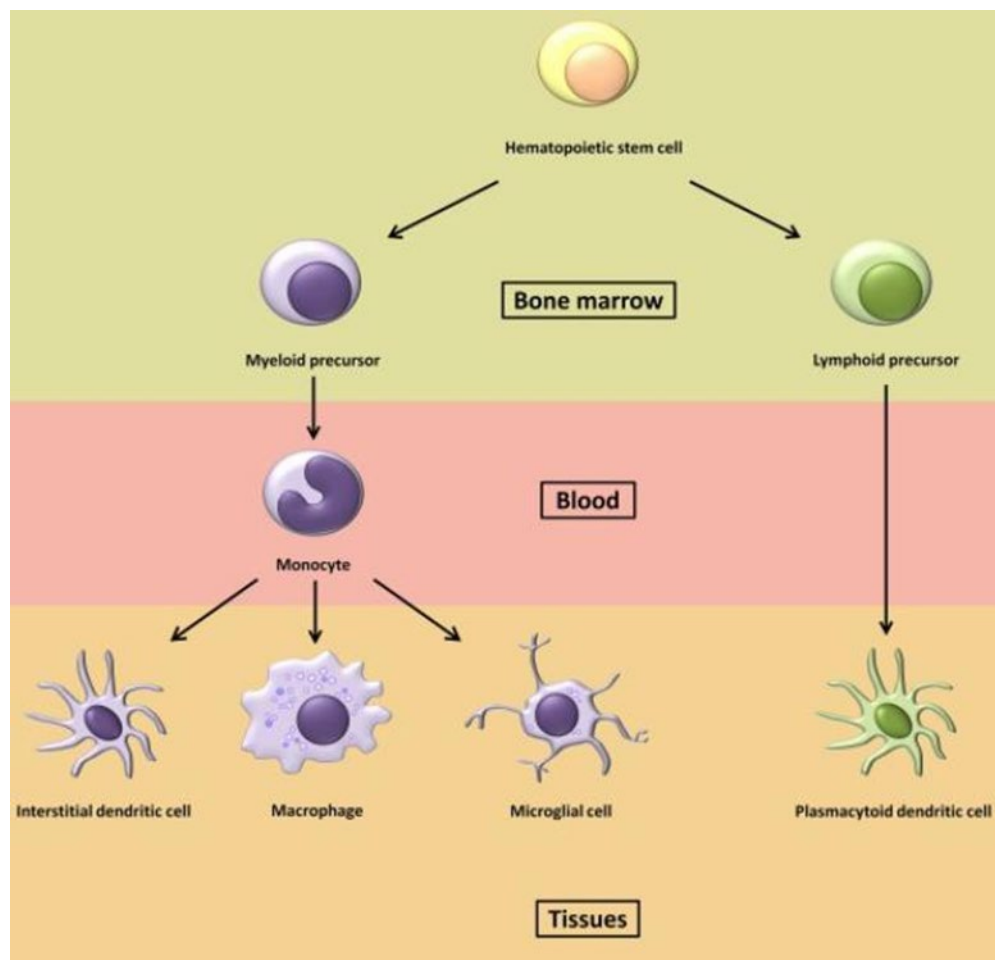
The relationship between eli-cel dosing characteristics and PD responses are analyzed and discussed in Section 6.5.

6.3 General Pharmacology

SKYSONA (eli-cel) is an autologous gene therapy consisting of hematopoietic stem cells (HSCs) transduced with lentiviral vector (LVV) encoding *ABCD1* cDNA for human ALDP. Figure 2 shows differentiation of HSCs into both myeloid and lymphoid cell lineages in peripheral blood, as well as further differentiation of some of these cells into dendritic-like cells in tissues. After SKYSONA infusion, transduced CD34+ HSCs engraft in the bone marrow and differentiate into various cell types, including monocytes (CD14+) capable of producing functional ALDP. Functional ALDP can then participate in the local degradation of very long chain fatty acids (VLCFAs), which is believed to slow or possibly prevent further inflammation and demyelination.

Based on the nature of SKYSONA (eli-cel), conventional studies on pharmacokinetics, absorption, distribution, metabolism, and elimination cannot be used to monitor the presence of the drug product. To evaluate the delivery and persistence of SKYSONA (eli-cel), pharmacodynamic (PD) parameters were measured to detect the presence of integrated proviral sequences and the expression of transgene in differentiated cells in peripheral blood. VCN in peripheral blood and CD14+ cells were measured using quantitative polymerase chain reaction (qPCR). The expression of the *ABCD1* transgene (%ALDP+ Cells) in peripheral blood leukocytes and their CD14+ subpopulation was also measured using flow cytometry method. Other PD parameters, such as VLCFAs in fasting serum were measured for exploratory purpose. The product dosing characteristics were also evaluated for their impacts on PD and clinical outcomes.

Figure 2. Hematopoietic Stem Cell Give Rise to Microglial Cells and Macrophages in Tissues



Source: Applicant. Module 2, section 2.7.2. Summary of Clinical Pharmacology.

6.4 Pharmacodynamics of Eli-cel

Pharmacodynamic evaluation of eli-cel included measurement of the following PD parameters: lentiviral vector copy number (VCN) in peripheral blood leukocytes and CD14⁺ cells, %ALDP⁺ Cells in peripheral blood leukocytes and CD14⁺ cells, and exploratory PD parameters such as VLCFAs in fasting serum. In addition, the relationship between drug product dose characteristics and PD parameters was evaluated for eli-cel.

Due to the nature of the disease, the study subjects were boys (≤ 13 years of age at the time of study entry for Studies ALD-102 and ALD-104). Among subjects in Studies ALD-102 and ALD-104, 56% were white (29% as not reported/other), 27% were Hispanic (13% as not reported). Therefore, impact of factors (such as age, sex, race, ethnicity) on PD responses was not assessed.

6.4.1 Lentiviral Vector Copy Number in Peripheral Blood Leukocytes (PB VCN) and CD14+ Cells (CD14+ VCN)

Successfully transduced patient's HSCs incorporate the *ABCD1* transgene into their genome, and therefore, their progeny contain the *ABCD1* transgene. VCN levels from both peripheral blood leukocytes (PB VCN) and CD14+ cells (CD14+ VCN) were measured using qPCR method. One month after infusion of eli-cel, lentiviral vector copy was detected in peripheral blood (PB VCN) and CD14+ cells (CD14+ VCN). Levels of PB VCN and CD14+ VCN stabilized by Month 6. Subjects had a Month 6 median (min, max) PB VCN level of 0.38 (0.07, 1.77) c/dg in Study ALD-102 (N=25) and 1.04 (0.03, 3.13) c/dg in Study ALD-104 (N=32). Median CD14+ VCN levels at Month 6 were 0.45 (0.07, 2.42) c/dg (N=25) and 1.41 (0.04, 3.82) c/dg (N=28), for Studies ALD-102 and ALD-104, respectively. VCN levels in peripheral blood and CD14+ cells generally remained stable as of the data cut-off date, although high inter-subject variability of PB VCN and CD14+ VCN kinetic profiles was observed (Figure 2 & Table 5).

Figure 3. Vector Copy Number Over Time in Peripheral Blood Leukocytes (PB VCN) and CD14+ Cells (CD14+ VCN)

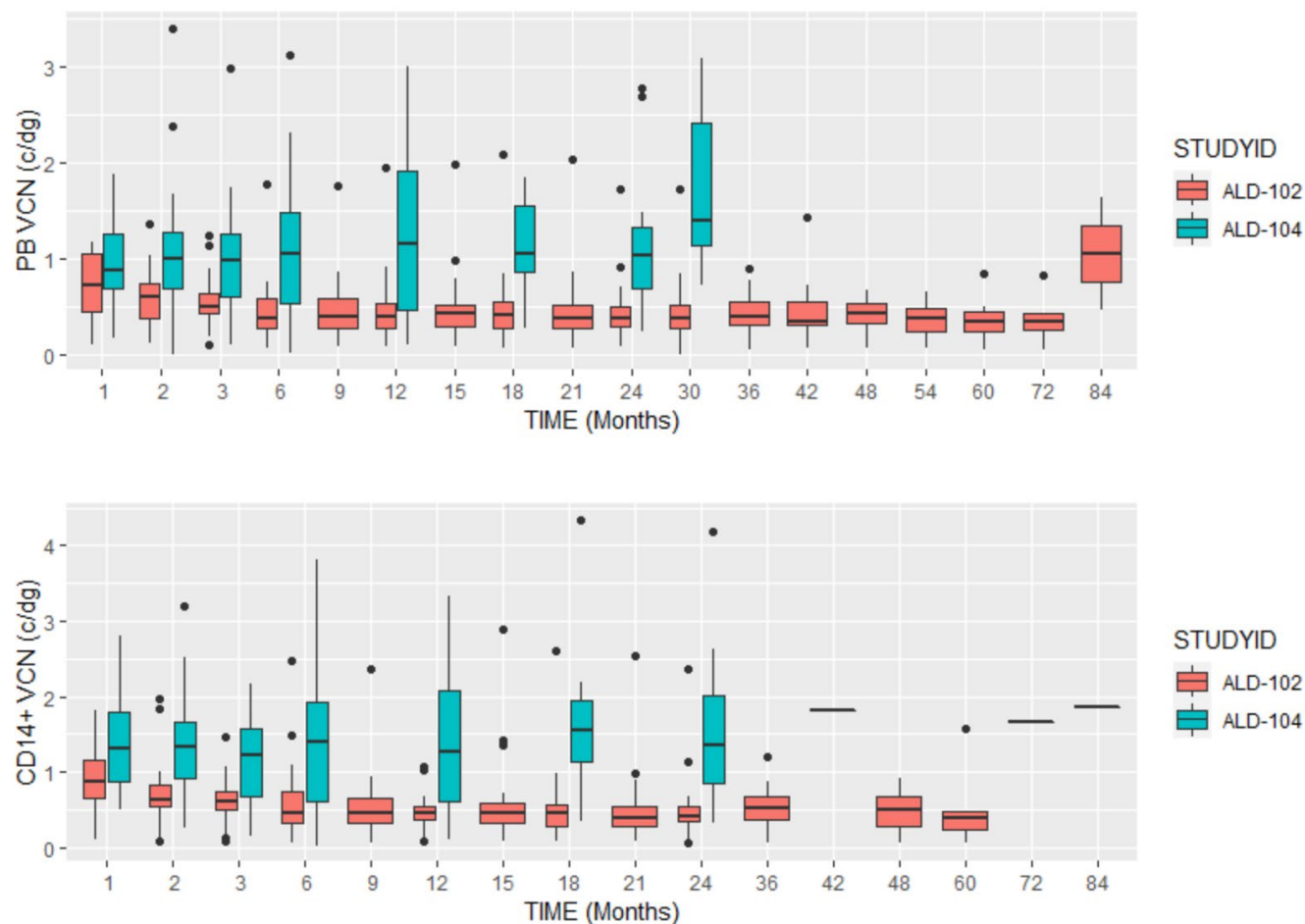


Table 5. Summary of Vector Copy Number in Peripheral Blood Leukocytes (PB VCN) and CD14+ Cells (CD14+ VCN)

Study ALD-102/TP-102

	VCN Over Time (c/dg)								
Cell Type	Month 1	Month 6	Month 12	Month 24	Month 36	Month 48	Month 60	Year 6	Year 7
PBLs									
N	25	25	26	23	22	12	8	6	2
Median	0.71	0.38	0.40	0.37	0.40	0.43	0.35	0.34	1.05
Min, Max	0.10, 1.17	0.07, 1.77	0.07, 1.95	0.08, 1.71	0.05, 0.89	0.06, 0.67	0.05, 0.85	0.05, 0.83	0.47, 1.63
CD14+									
N	24	25	25	23	21	13	8	1	1
Median	0.88	0.45	0.45	0.42	0.51	0.51	0.39	1.96	1.85
Min, Max	0.11, 1.82	0.07, 2.42	0.08, 1.07	0.06, 2.36	0.06, 1.21	0.06, 0.92	0.06, 1.57	1.96, 1.96	1.85, 1.85

Study ALD-104/TP-104

	VCN Over Time (c/dg)				
Cell Type	Month 1	Month 6	Month 12	Month 24	Month 30
PBLs					
N	35	32	18	12	6
Median	0.87	1.04	1.16	1.03	1.39
Min, Max	0.17, 1.88	0.03, 3.13	0.10, 3.00	0.24, 2.77	0.73, 3.09
CD14+					
N	35	28	19	11	-
Median	1.32	1.41	1.26	1.36	-
Min, Max	0.50, 2.81	0.04, 3.82	0.11, 3.33	0.32, 4.26	-

6.4.2 %ALDP+ Cells in Peripheral Blood Leukocytes (PB %ALDP+ Cells) and CD14+ cells (CD14+ %ALDP+ Cells)

Expression of ALDP was evaluated by measuring ALDP+ cells in peripheral blood leukocytes (PB %ALDP+ Cells) and CD14+ cells (CD14+ %ALDP+ Cells). ALDP expression was detected in all subjects who received eli-cel infusion. The %ALDP+ cell counts stabilized at 6 months after SKYSONA infusion. Subjects had a Month 6 median (min, max) %ALDP+ CD14⁺ cell count of 16% (2%, 71%) in Study ALD-102 (N=23) and 26% (2%, 86%) in Study ALD-104 (N=25) respectively. The %ALDP+ CD14⁺ cells generally remained stable through Month 24 with a median (min, max) of 15%

(6%, 45%) in Study ALD-102 (N=23) and 28% (2%, 40%) in Study ALD-104 (N=11). As of the data cut-off date of January 07, 2022, ALDP expression in CD14+ cells was detected in 3 of 7 subjects who had the last follow-up through Month 60 in Study ALD-102 (N=7), indicating long-term expression of transgenic ALDP in the progeny of hematopoietic stem cells (Figure 4 & Table 6).

Figure 4. %ALDP Cells Over Time in Peripheral Blood and CD14+ Cells

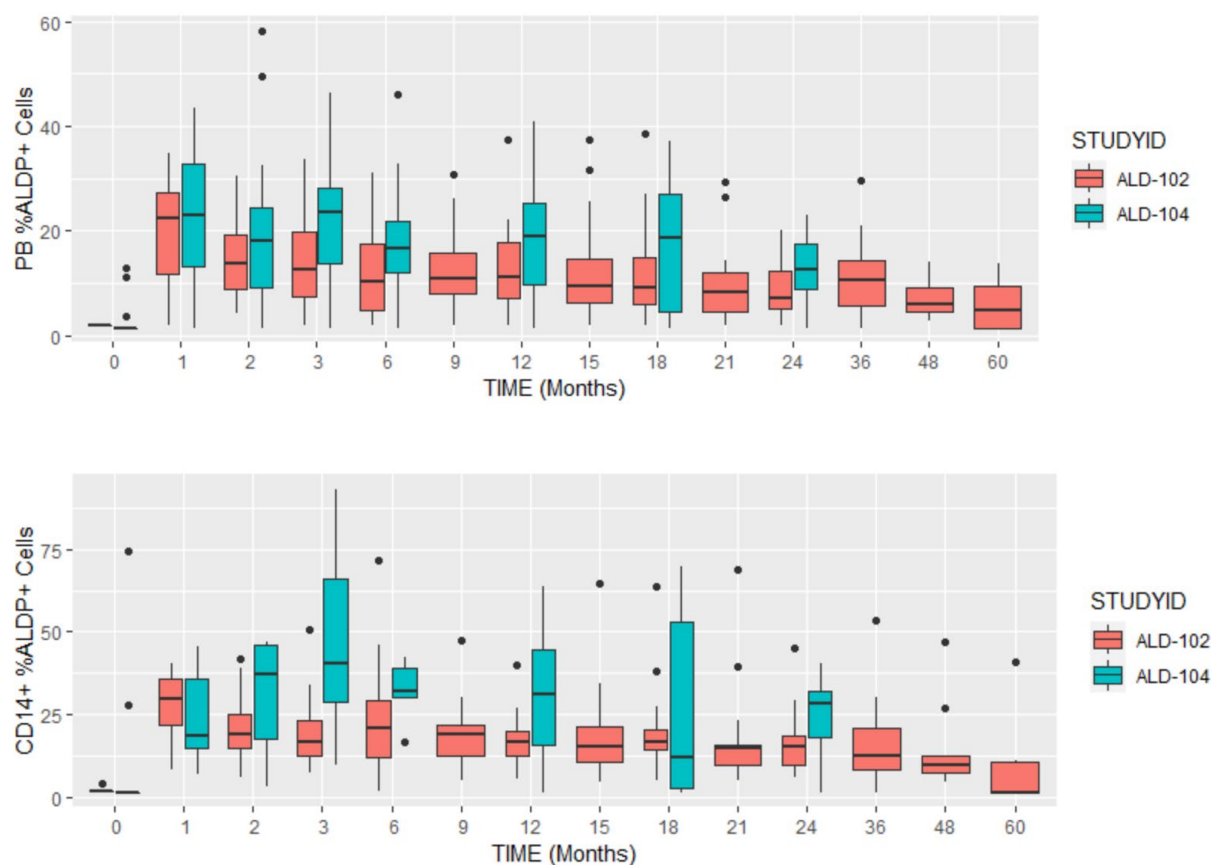


Table 6. %ALDP Cells Over Time

	% ALDP + Cells Study (Study ALD-102)							
Cell Type	Baseline	Month 1	Month 6	Month 12	Month 24	Month 36	Month 48	Month 60
PBLs								
N	25	25	25	26	23	18	12	7
Median	2.00	22.50	10.40	11.20	7.30	10.67	5.99	1.50
Min, Max	2.00, 2.00	2.00, 34.97	2.00, 31.20	2.00, 37.40	2.00, 20.00	1.50, 29.54	2.78, 14.15	1.50, 13.76
Mean	2.00	19.90	12.62	12.66	8.82	11.02	6.85	5.00
SD	(0.000)	(9.724)	(9.670)	(7.728)	(5.096)	(7.080)	(3.453)	(5.093)
CD14+								
N	25	23	23	24	23	18	11	7

Median	2.00	27.40	16.40	15.65	15.40	12.49	9.62	1.50
Min, Max	2.00, 4.20	8.20, 40.40	2.00, 71.40	5.70, 39.80	5.80, 45.00	1.50, 53.40	4.39, 47.08	1.50, 40.92
Mean	2.09	26.01	20.69	16.17	15.73	16.09	13.99	9.73
SD	(0.440)	(10.262)	(15.913)	(7.426)	(8.764)	(12.686)	(12.513)	(14.408)

	% ALDP + Cells Study (Study ALD-104)				
Cell Type	Baseline	Month 1	Month 6	Month 12	Month 24
PBLs					
N	31	31	25	18	11
Median	1.50	22.99	16.60	18.94	12.67
Min, Max	1.50, 12.82	1.50, 43.56	1.50, 46.12	1.50, 40.88	1.50, 23.10
Mean	2.24	22.59	17.04	18.87	13.07
SD	(2.630)	(11.865)	(10.562)	(11.974)	(6.486)
CD14+					
N	31	31	25	18	11
Median	1.50	19.61	25.65	30.99	28.27
Min, Max	1.50, 76.43	1.50, 57.54	1.50, 86.15	1.50, 66.34	1.50, 40.43
Mean	7.10	22.65	27.96	28.56	23.87
SD	(18.802)	(13.501)	(21.745)	(21.808)	(12.207)

Source: IR response.

6.4.3 VLCFAs in Fasting Serum

In patients with CALD, there is accumulation of VLCFAs (fatty acids with > 20 carbon chains) in most tissues of the body. Accumulation of VLCFAs in brain leads to destruction of the protective myelin sheath around nerve cells. Without the myelin sheath, the nerves can no longer quickly and efficiently transmit electrical signals in neurons leading to clinical sequelae.

VLCFAs were measured in fasting serum as exploratory PD biomarkers. C26:LysoPC is typically tested as part of newborn screening for ALD. As shown in Table 7, VLCFA levels were highly variable among the study subjects. After eli-cel infusion, the median levels of VLCFAs generally decreased from Baseline.

Table 7. Summary of Percent Change in VLCFA Over Time in Fasting Serum

Study Visit	Percent Change from Baseline					
	Study ALD-102 (TP)			Study ALD-104 (TP)		
	N	Median	Min, Max	N	Median	Min, Max
C26:0 LysoPC						
Month 6	26	8.6	-64.8, 102.5	-	-	-
Month 12	26	-9.8	-65.5, 165.2	19	-11.4	-55.5, 47.8
Month 24	23	-28.0	-58.5, 37.1	11	-14.6	-59.3, 74.7
Month 36	23	1.7	-57.5, 115.8	-	-	-

Month 48	13	9.76	-43.7, 47.0	-	-	-
Month 60	8	-17.1	-50.6, 78.5	-	-	-
C26:0/C22:0 ratio						
Month 6	26	-5.0	-44.4, 40.0	-	-	-
Month 12	26	-15.5	-60.0, 60.0	19	-24.4	-39.6, 54.3
Month 24	23	-22.2	-50.0, 66.7	11	-29.1	-42.9, -5.4
Month 36	23	-22.2	-60.0, 33.3	-	-	-
Month 48	13	-16.7	-50.0, 0.0	-	-	-
Month 60	8	-7.1	-40.0, 28.6	-	-	-

6.5 Drug Product Dosing Characteristics and Pharmacodynamic Responses, Clinical Outcomes

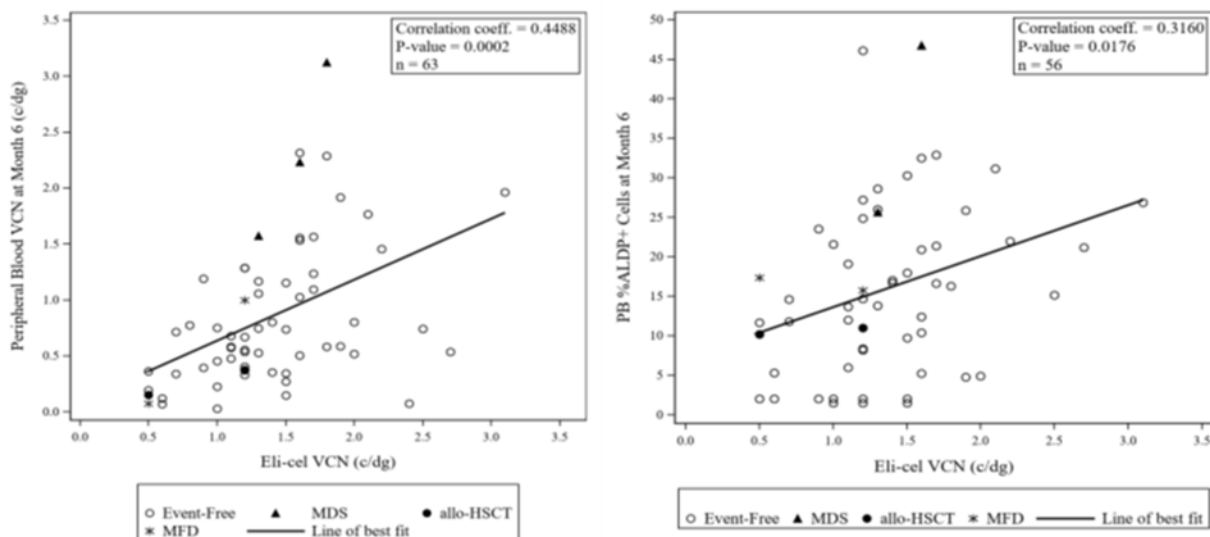
SKYSONA (eli-cel) is an autologous gene therapy consisting of HSCs that have been genetically modified ex vivo and is intended for a one-time treatment with a single dose IV infusion. Considering the heterogeneity of eli-cel product composition and one-time treatment dosing regimen, the product dosing characteristics were also evaluated for their impacts on PD and clinical outcomes.

6.5.1 Drug Product Dosing Characteristics and PD Responses

6.5.1.1 DP VCN versus PB VCN and PB %ALDP+ Cells at Month 6

As shown in Figure 5, positive Pearson correlations were observed between DP VCN and PD parameters in peripheral blood (PB VCN ($p=0.0002$) and PB %ALDP+ cells ($p<0.0001$)) at Month 6.

Figure 5. DP VCN versus PB VCN and PB %ALDP+ Cells at Month 6

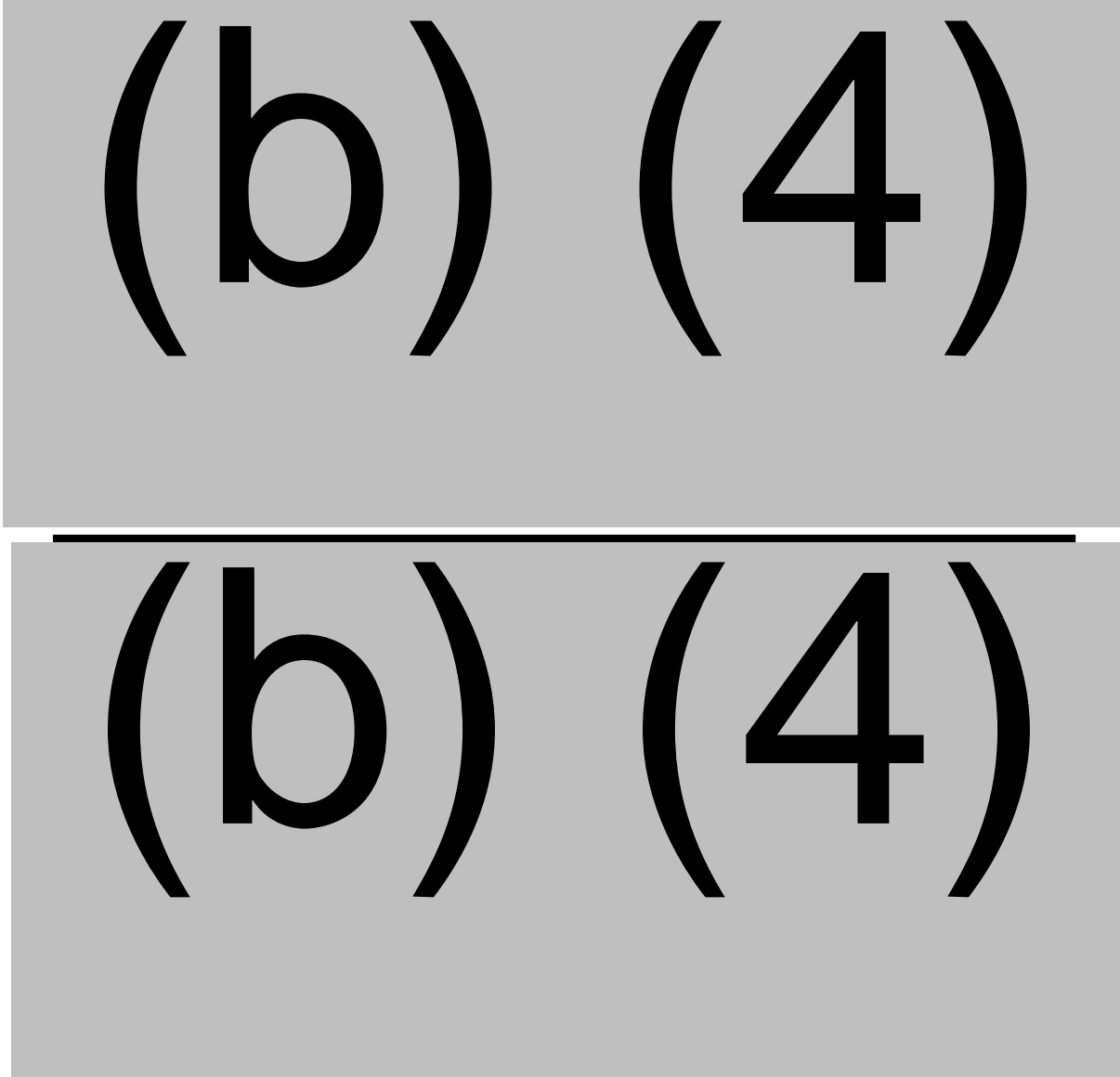


Source: Applicant. IR response.

6.5.1.2 DP %LVV+ Cells versus %ALDP+ Cells in PBLs and CD14+ Cells at Month 6

As shown in Figure 6, positive association was observed between eli-cel DP %LVV+ cells and PB %ALDP+ Cells.

Figure 6. DP %LVV+ Cells versus PB %ALDP+ Cells and CD14+ %ALDP+ Cells at Month 6



Source: Applicant. Module 2, section 2.7.2. Summary of Clinical Pharmacology and IR response.

DP %ALDP+ Cells versus PB %ALDP+ Cells

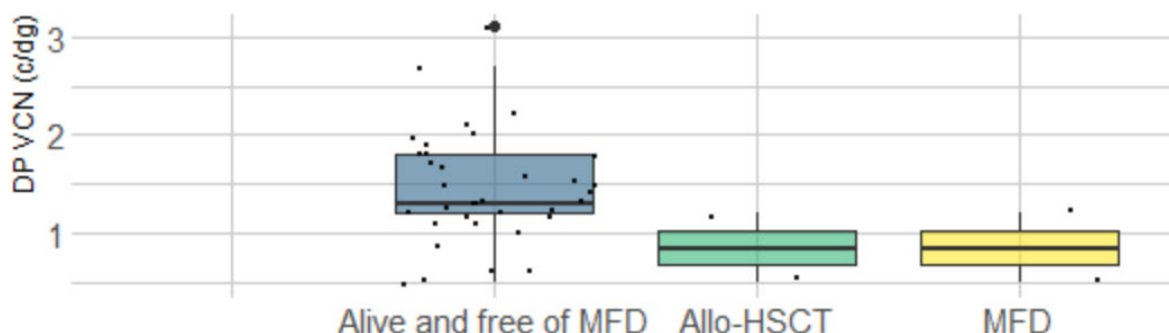
No association was observed between DP %ALDP+ Cells and PB %ALDP+ Cells at Month 6 after eli-cel infusion.

6.5.2 Drug Product Dosing Characteristics and Clinical Outcomes

6.5.2.1 MFD-Free Survival Status at Month 24

Major functional disabilities (MFD) are a subset of neurologic function score (NFS) that are considered largely irreversible clinical neurologic changes in CALD. MFDs were defined as loss of communication, cortical blindness, tube feeding, total incontinence, wheelchair dependence, and complete loss of voluntary movement. The relationship between eli-cel dosing characteristics (DP VCN, DP %LVV+ cells, total cell dose, DP %ALDP+ cells, and VLCFA ratio reduction) and the clinical efficacy outcome, MFD-free survival status at Month 24, was assessed. As shown in Figure 7, DP VCN values were plotted against occurrence of MFDs, or whether a subject would be considered as a failure in the MFD analysis due to treatment with rescue cells or allogeneic hematopoietic stem cell transplantation (allo-HSCT). The DP VCN values of the four subjects who failed to achieve MFD-free survival status were no more than 1.2 c/dg.

Figure 7. SKYSONA DP VCN and MFD-Free Survival Status at Month 24



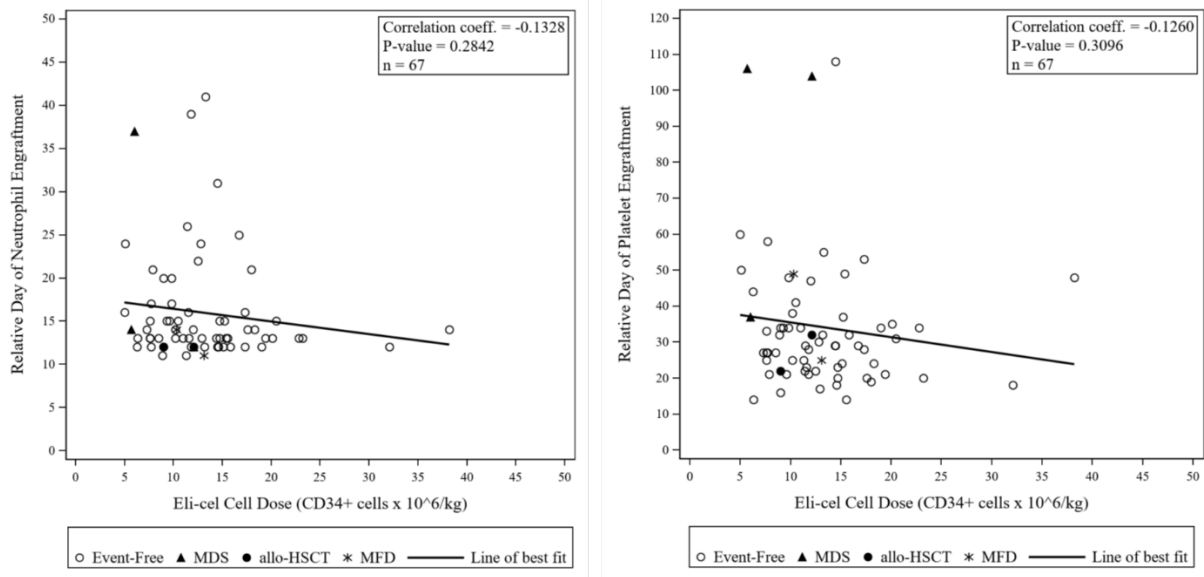
Reviewer's Comments:

Although there is no clear threshold for DP VCN and efficacy (MFD-free survival). The available data suggest subject receiving DP with DP VCN > 1.2 c/dg may be more likely to achieve MFD-free survival.

6.5.2.2 Neutrophil and platelet engraftment

The relationship between total cell dose of eli-cel and engraftment (the day of engraftment for either neutrophil or platelets) was evaluated (Figure 8). No correlation was identified between eli-cel total cell dose and either neutrophil or platelet engraftment. The results suggest the lowest total cell dose of eli-cel investigated in the clinical studies was adequate for effective reconstitution of HSCs in subjects treated with eli-cel.

Figure 8. Cell Dose versus Time to Neutrophil and Platelet Engraftment



Source: Applicant. IR response.

6.6 Pharmacodynamic Responses and Clinical Outcomes

6.6.1 Relationships between Pharmacodynamic Parameters

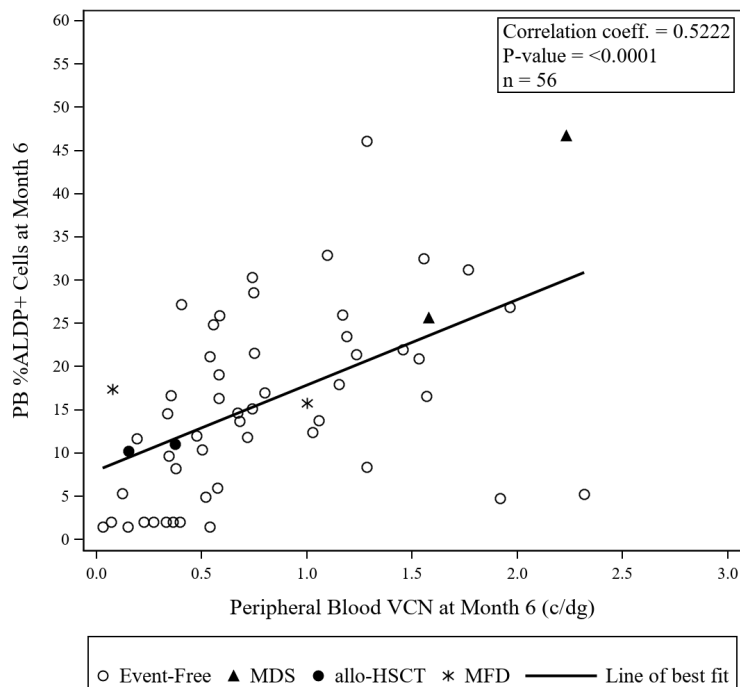
PB VCN versus PB %ALDP+ Cells

Figure 9 shows the relationships between PB VCN and PB %ALDP+ Cells. There was a positive association observed between PB VCN and PB %ALDP+ Cells at Month 6 after eli-cel infusion.

PB VCN at Month 24 versus VLCFAs Percent Change from Baseline at Month 24

There was no association observed between PB VCN and VLCFAs percent change from Baseline at Month 24 after eli-cel infusion.

Figure 9. Relationships between PB VCN and PB %ALDP+ Cells at Month 6



Source: Applicant. IR response.

6.6.2 Pharmacodynamic Responses and Clinical Outcomes

6.6.2.1 MFD-Free Survival Status at Month 24

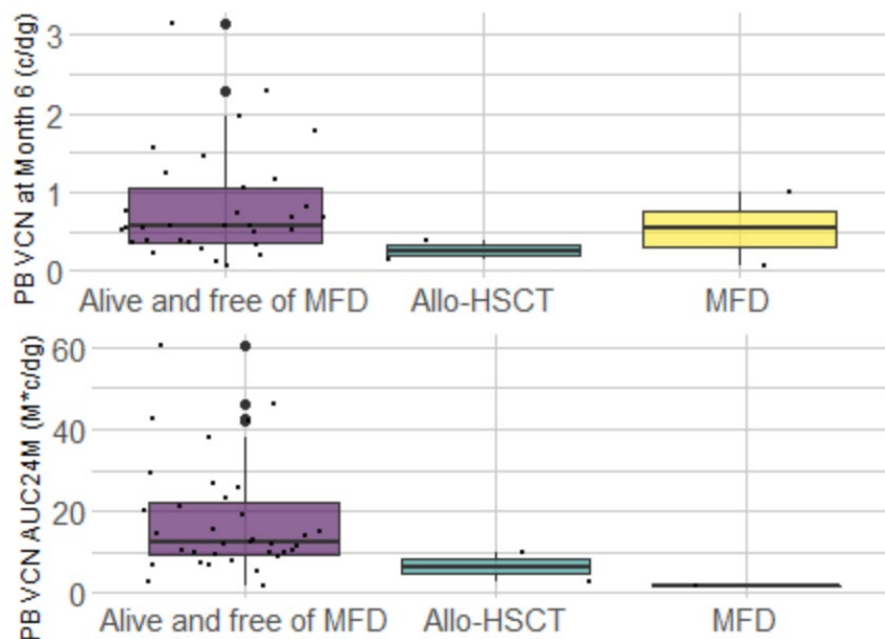
The goal of treatment for CALD is to stabilize neurologic function by delaying or, ideally, preventing the development of major functional disabilities (MFDs). The potential correlations between PD parameters (PB VCN, PB % ALDP+ Cells and CD14+ %ALDP+ Cells) and MFD-free survival status at 24 months post eli-cel infusion were evaluated. As shown in Table 8 and Figure 10, compared to subjects who achieved MFD-free survival at Month 24 post-treatment with eli-cel, the median levels of following PD parameters were substantially lower in subjects who developed MFD or underwent allo-HSCT due to disease progression: PB VCN at Month 6, 24 month exposure of PB VCN, and CD14+ %ALDP+ Cells at Month 6.

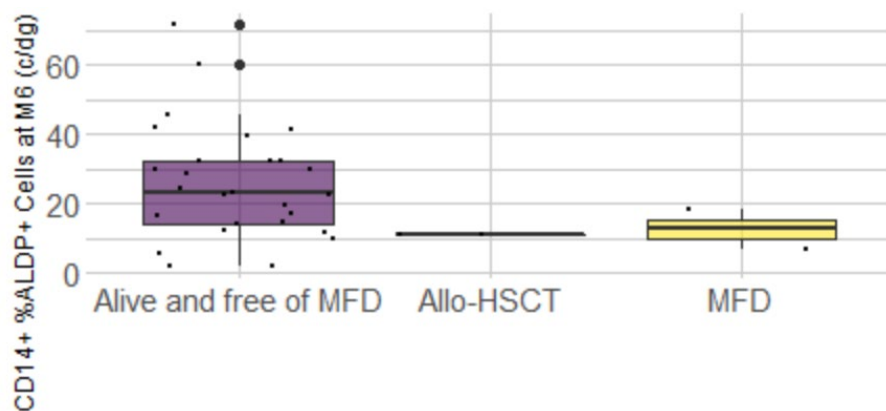
Table 8. Pharmacodynamic Parameters versus MFD-Free/Disease Progression-Free Survival Status at Month 24

	MFD or Allo-HSCT	MFD-Free at Month 24
PB VCN at Month 6 (c/dg)		
N	4	33
Mean (SD)	0.40 (0.42)	0.81 (0.68)
Median (IQR)	0.26 (0.09, 0.84)	0.58 (0.36, 1.11)

Min, Max	0.08, 1.00	0.07, 3.13
PB VCN AUC0-24M (M*c/dg)		
N	3	35
Mean (SD)	4.99 (4.41)	17.73 (13.55)
Median (IQR)	3.00 (1.93, 10.4)	12.28(9.27, 23.20)
Min, Max	1.93, 10.04	1.86, 60.13
PB %ALDP+ Cells at Month 6		
N	4	35
Median (IQR)	13.39 (10.40, 16.99)	15.13 (6.00, 24.90)
Min, Max	10.20, 17.40	2.00, 46.77
CD14+ %ALDP+ Cells at Month 6		
N	4	27
Mean (SD)	11.70 (4.72)	25.95 (16.64)
Median (IQR)	10.85 (7.88, 16.38)	23.30 (14.00, 32.10)
Min, Max	6.9, 18.21	2.00, 71.4

Figure 10. Pharmacodynamic Parameters versus MFD-Free Survival Status at Month 24





Considering the small sample size, non-parametric methods are used to compare the PD parameters between subjects who achieved MFD-free/Disease-free survival at Month 24 and subjects who experienced an MFD or received allo-HSCT due to disease progression after eli-cel treatment.

Wilcoxon rank sum test results show marginal statistical significance between the above two groups of subjects for PB VCN AUC0-24M ($p=0.041$) and CD14+ %ALDP+ Cells at Month 6 ($p=0.055$). The Hodges-Lehmann estimation method is used to calculate the median differences (95% confidence interval) of corresponding PD parameters between above two groups of subjects (Table 9).

Table 9. Wilcoxon Rank Sum Test for Pharmacodynamic Parameters and MFD-Free/Disease Progression Free Survival Status at Month 24

PD Parameter	Wilcoxon Rank Sum Test p Value	Hodges-Lehmann Estimated Median Difference (95% CI)
CD14+ %ALDP+ Cells at Month 6	0.055	12.20 (-1.30, 27.59)
PB VCN AUC0-24M (M*c/dg)	0.041	8.77 (-0.34, 26.12)
PB VCN at Month 6 (c/dg)	0.154	0.29 (-0.20, 0.96)

Reviewer's Comments:

CD14+ %ALDP+ Cells levels reflects the transgene expression in peripheral blood monocytes (CD14+ cells). However, the relationship between CD14+ %ALDP+ Cells and inflammation/demyelination in brain is not very clear.

6.6.2.2 Myelodysplastic Syndrome (MDS)

A major safety signal associated with eli-cel was insertional oncogenesis, and there were 3 cases of MDS, a rare hematologic malignancy in subjects treated with eli-cel. CALD is not associated with an increased risk of hematologic malignancy. As of the data cut-off date on January 07, 2022, MDS has been diagnosed in three subjects treated with eli-cel. In addition, per clinical reviewer's evaluation, nine more subject were identified with potential risk of malignancy. Please see Clinical review for additional details.

The relationship between PB VCN and incidence of MDS and risk of malignancy was assessed. As shown in Table 11 and Figure 11, the median levels of PB VCN at Month 6 and maximum PB VCN during observation period were substantially higher in subjects diagnosed with MDS, compared to subjects who did not have MDS. All three subjects diagnosed with MDS had maximum PB VCN levels more than 2.0 c/dg. Among the 4 subjects who were identified with high risk of malignancy per clinical review, 2 (50%) subjects had maximum PB VCN levels more than 2.0 c/dg. The preliminary analysis results indicate high PB VCN levels (> 2.0 c/dg) may be associated with high risk of development of MDS. The result should be interpreted with caution due to small sample size.

Table 10. PB VCN versus Myelodysplastic Syndrome (MDS)

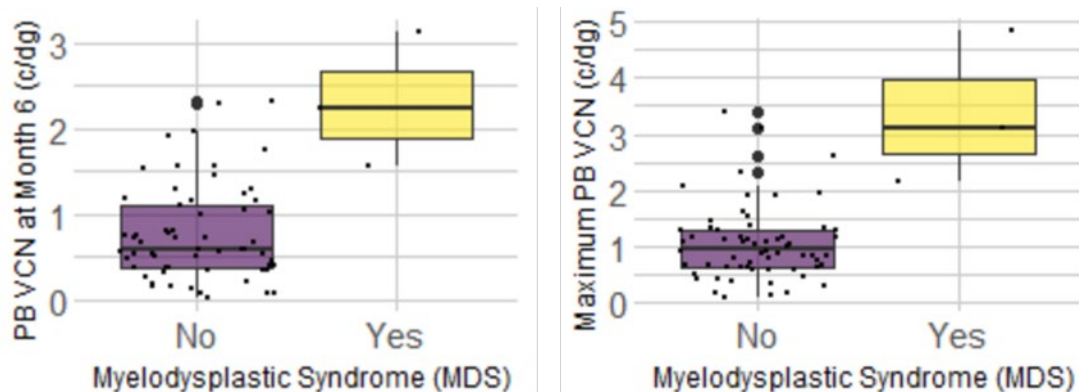
a. PB VCN and MDS

	Myelodysplastic Syndrome (MDS)	
	Yes	No
PB VCN at Month 6 (c/dg)		
N	3	60
Median (IQR)	2.23 (1.58, 3.13)	0.58 (0.37, 1.14)
Min, Max	1.58, 3.13	0.03, 2.32
Maximum PB VCN (c/dg)		
N	3	62
Median (IQR)	3.13 (2.15, 4.82)	0.96 (0.64, 1.29)
Min, Max	2.15, 4.82	0.11, 3.40

b. Maximum PB VCN versus MDS and High Risk of Malignancy

	N	MDS	No MDS	MDS and Risk of Malignancy
Maximum PB VCN > 2.0 c/dg	8	3 (37.5%)	5 (62.5%)	6 (75.0%)
Maximum PB VCN ≤ 2.0 c/dg	57	0 (0.0%)	57 (100.0%)	6 (10.5%)

Figure 11. PB VCN versus Myelodysplastic Syndrome (MDS)



6.7 Clinical Pharmacology Conclusions

Key clinical pharmacology findings are summarized below:

General Pharmacodynamics

- One month after infusion of SKYSONA, lentiviral vector copy was detected in peripheral blood leukocytes (PB VCN) and CD14⁺ cells (CD14⁺ VCN), demonstrating the early presence of transduced cells. Levels of PB VCN and CD14⁺ VCN stabilized by Month 6. Subjects had a Month 6 median (min, max) PB VCN levels of 0.38 (0.07, 2.23) c/dg in Study ALD-102 (N=25) and 1.04 (0.03, 3.13) c/dg in Study ALD-104 (N=32). Median CD14⁺ VCN levels at Month 6 were 0.61 (0.07, 3.96) c/dg (N=29) and 1.41 (0.04, 3.82) c/dg (N=28), for Studies ALD-102 and ALD-104 respectively. VCN levels in peripheral blood and CD14⁺ cells generally remained stable as of the data cut-off date, although high inter-subject variability of PB VCN and CD14⁺ VCN kinetic profiles was observed.
- All subjects who received SKYSONA with at least 1 month of follow-up produced ALDP in peripheral blood leukocytes and CD14⁺ cells, demonstrating early expression of the transgene. The %ALDP⁺ cell counts stabilized at 6 months after SKYSONA infusion. Subjects had a Month 6 median (min, max) %ALDP⁺ CD14⁺ cell count of 16% (2%, 71%) in Study ALD-102 (N=23) and 26% (2%, 86%) in Study ALD-104 (N=25) respectively. The %ALDP⁺ CD14⁺ cells generally remained stable through Month 24 with a median (min, max) of 15% (6%, 45%) in Study ALD-102 (N=23) and 28% (2%, 40%) in Study ALD-104 (N=11). As of the data cut-off date of January 07, 2022, ALDP expression in CD14⁺ cells was detected in 3 of 7 subjects who had the last follow-up through Month 60 in Study ALD-102 (N=7), indicating long-term expression of transgenic ALDP in the progeny of hematopoietic stem cells.
- Subjects with higher PB VCNs generally had higher PB %ALDP⁺ cells at a given timepoint. There was a positive linear relationship between PB VCN and PB %ALDP⁺ cells at Month 6.
- ALDP is a peroxisomal membrane protein involved in the transport and metabolism of very long-chain fatty acids (VLCFA). VLCFA levels in fasting serum were highly variable in study subjects treated with SKYSONA. There was a decrease in VLCFAs as observed by median values of C26:0 LysoPC and C26:0/C22:0 ratios from Baseline to Month 24 post-administration of SKYSONA.

Dosing Characteristics and Responses

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

Pharmacodynamic Responses and Clinical Outcomes

- PD responses and MFD-free survival: Compared to subjects who achieved MFD-free survival at Month 24 after eli-cel infusion, the median levels of the following PD parameters were substantially lower in subjects who developed MFD or underwent allo-HSCT due to disease progression: PB VCN at Month 6, 24-month exposure of PB VCN, and CD14+ %ALDP+ Cells at Month 6.
- PD responses and MDS: among subjects with at least 6 month follow up period, the median levels of PB VCN at Month 6 and maximum PB VCN during observation period were substantially higher in subjects diagnosed with MDS (N=3), compared to subjects who did not have MDS (N=62). All three subjects diagnosed with MDS had maximum PB VCN levels more than 2.0 c/dg (median (range): 3.13 (2.15, 4.82)). The median (min, max) value of maximum PB VCN was 0.96 (0.11, 3.40) c/dg in subjects who did not have MDS. Note that there were 5 subjects with Max PB VCN > 2 c/dg and did not develop MFD. However, the clinical reviewer identified 3 of these subjects with findings potentially concerning for increased risk malignancy. Refer to the clinical review for additional details.

7 APPENDIX - INDIVIDUAL STUDY

7.1 Study #1 – Study ALD-102

Data cut-off date: March 26, 2021.

Title: 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)			
Objectives: To evaluate the safety and efficacy of treatment with Eli-cel in subjects with CALD			
Methodology: Single-arm, open-label, multi-site, single dose study with 4 distinct stages: (1) screening, (2) mobilization with G-CSF with or without plerixafor, and apheresis for autologous cell collection, (3) conditioning with busulfan and cyclophosphamide, followed by drug product infusion on Rel Day 1 (defined as the day of drug product infusion), and (4) follow-up until study completion at Month 24. The "Initial Cohort" includes the first 17 patients treated with eli-cel; the "Overall Cohort" includes all treated subjects. There were no major differences in entry criteria, treatment, or protocol schedule between these cohorts.			
Main Criteria for Inclusion/Exclusion: Subjects were male and < 18 years of age at the time of consent/assent; without a human leukocyte antigen (HLA)-matched sibling donor; diagnosed with CALD as defined by elevated VLCFA levels, and brain MRI demonstrating Loes score between 0.5 and 9 (inclusive); GdE+; an NFS of ≤ 1 .			
Results Relevant to Module 2.7.2 Discussion Disposition			
Table S1: Disposition (ITT)			
	Statistic	Initial Cohort (N = 17)	Overall Cohort (N = 32)
Initiated mobilization (ITT)	n (%)	17 (100.0)	32 (100.0)
Initiated conditioning	n (%)	17 (100.0)	32 (100.0)
Infused with eli-cel (TP)	n (%)	17 (100.0)	32 (100.0)
Successful neutrophil engraftment (NEP)	n (%)	17 (100.0)	32 (100.0)
Completed Study	n (%)	15 (88.2)	29 (90.6)
Discontinued Study	n (%)	2 (11.8)	3 (9.4)
Reasons for study discontinuation			

Death	n (%)	1 (5.9)	1 (3.1)
Subject to receive allo-HSCT	n (%)	1 (5.9)	2 (6.3)
Enrolled in Study LTF-304 ^a	n (%)	15 (88.2)	29 (90.6)
Duration of follow-up (months) in this study(months)	Median	23.9	24.2
	Min., Max.	13.4, 25.3	13.4, 32.3
Subject-years of follow-up (years) ^b		33.0	64.3
Last Visit Completed			
Month 9	n (%)	0	0
Month 12	n (%)	1 (5.9)	1 (3.1)
Month 15	n (%)	0	1 (3.1)
Month 18	n (%)	0	0
Month 21	n (%)	1 (5.9)	1 (3.1)
Month 24	n (%)	15 (88.2)	29 (90.6)

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

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

^b Subject-years were calculated by summing the total of the number of years each subject has been followed after drug product infusion in Study ALD-102; additional follow-up of subjects in LTF-304 is not included.

Results: Pharmacodynamics

Table S2: Summary of Key PD Parameters (TP)

Parameter	Statistic	PB ^a	CD14+ Cell
Month 24 VCN (c/dg)	n	29	29
	Median	0.40	0.48
	Min, max	0.08, 1.71	0.06, 2.36
Month 24 % ALDP+ Cells	n	29	29
	Median	10.40	17.00
	Min, max	2.00, 26.60	5.80, 45.00
Month 24 % Change from Baseline C26:0 LysoPC	n	29	NA
	Median	-22.0	
	Min, max	-58.5, 94.4	
Month 24 % Change from Baseline C26:0/C22:0 Ratio	n	29	NA
	Median	-16.7	
	Min, max	-50.0, 66.7	

Abbrev: ALDP, adrenoleukodystrophy protein; c/dg, vector copies per diploid genome; LysoPC, C26:0 lysophosphatidylcholine; TP, transplant population; VCN, vector copy number

^a PB, peripheral blood cells for VCN and %ALDP+ Cells, fasting serum for VLCFA

Drug Product Characteristics: Cell Dose, DP VCN, and %LVV+ Cells

- Total cell dose administered was a median (min, max) of 11.4 (5.0, 20.1) x 10⁶ CD34+ cells/kg. Median (min, max) DP VCN (N = 32) was 1.2 (0.5, 2.7) c/dg, and median (min, max) %LVV+ cells was 45% (b) (4) resulting in a median (min, max) of (b) (4) vector copies per transduced cell.

All Subjects Maintain VCN in Peripheral Blood Leukocytes (PBLs) and CD14+ Cells

- All subjects with CALD who received eli-cel had vector sequences in peripheral blood cells, with levels stabilizing by 6 months after drug product infusion, with the following values at Month 24 Visit: median (min, max) of 0.40 (0.08, 1.71) c/dg for PB VCN and 0.48 (0.06, 2.36) c/dg for CD14+ VCN (N = 29). These results demonstrate long-term persistence of transduced

repopulating HSCs.

All Subjects Expressed ALDP in PBLs and CD14+ Cells

- All evaluable subjects with CALD who received eli-cel had detectable ALDP+ cells in peripheral blood, with levels stabilizing by 6 months after drug product infusion, with the following values at their Month 24 Visit: median (min, max) %ALDP+ Cells of 10.40% (2.00%, 26.60%) for PBLs and 17.00% (5.80%, 45.00%) for CD14+ cells (N = 29). These results demonstrate expression of the transgene in the progeny of transduced HSCs.

Correlations Between DP VCN versus PB VCN and PB %ALDP+ Cells

- Positive correlations were observed between DP VCN and PB VCN at Month 6 ($r = 0.4091$, $p = 0.0223$), between PB VCN at Month 6 and PB %ALDP at Month 6 ($r = 0.8016$, $p = < 0.0001$), and between DP VCN and %ALDP+ Cells at Month 6 ($r = 0.3817$, $p = 0.0341$), suggesting higher DP VCN values generally result in higher VCN and %ALDP+ Cells in peripheral blood.

Exploratory Biomarkers

- VLCFAs: The majority of subjects showed a decrease in C26:0 LysoPC (23/32, 79.3% of subjects) and C26:0/C22:0 ratios (21/32, 72.4% of subjects) in fasting serum at approximately 24 months after eli-cel infusion, suggesting activity of transgenic ALDP in peripheral blood cells.
- Both MMP and CHIT values were highly variable across subjects.
 - Median values of MMP2, MMP9, and MMP10 in CSF showed a transient increase from Baseline to Month 12, followed by a decrease back towards Baseline at Month 24. The magnitude of the elevation did not appear to be related to clinical outcome. No consistent trend was observed for median values of MMPs in plasma.
 - Median CHIT levels in CSF increased by Month 12, and then leveled off through Month 24. Median CHIT levels in plasma showed a transient increase in values after conditioning, returning to below Baseline values by Month 24. Plasma CHIT levels in Subject (b) (6) (who developed MFDs and died during the study) were strikingly higher than those in the other subjects.

Source: Applicant. Module 2, section 2.7.2. Summary of Clinical Pharmacology.

7.2 Study #2 – Study ALD-104

Interim data cut-off date: March 05, 2021.

Title: 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)

Objectives: To evaluate the efficacy and safety of eli-cel after myeloablative conditioning with busulfan and fludarabine in subjects with CALD.

Methodology:

Study ALD-104 is an international, multi-site, non-randomized, open-label, single-arm, single dose, Phase 3 study in male subjects with CALD (≤ 17 years of age at time of informed consent/assent), with 4 distinct stages after informed consent, as follows:

Stage 1: Screening and Enrollment

Subjects who met eligibility criteria, based on screening assessments, were considered enrolled. Subjects who did not meet eligibility criteria were considered screen failures.

Stage 2: Autologous CD34+ cell collection, transduction, disposition of eli-cel, and re-confirmation of eligibility

Hematopoietic stem cells (HSC) were mobilized using granulocyte colony stimulating factor (G-CSF; filgrastim or lenograstim) and plerixafor, and harvested by apheresis. Harvested cells were enriched for CD34+ cells, transduced with Lenti-D lentiviral vector (LVV), stored frozen in cryopreservation solution in the vapor phase of liquid nitrogen while aliquots were tested to ensure they met product quality specifications, and then shipped to the treatment site.

Stage 3: Myeloablative and lymphodepleting conditioning and infusion of eli-cel

Subjects did not begin conditioning until drug product was released for clinical use and was at the site. Busulfan and fludarabine were administered intravenously for myeloablative conditioning, followed by at least 48 hours for washout before drug product infusion.

On Rel Day 1 (defined as Day of eli-cel infusion), thawed eli-cel was administered via IV infusion as a single dose of $\geq 5.0 \times 10^6$ CD34+ cells/kg.

Stage 4: Follow-up through Month 24

Subjects were to be followed until approximately 24 (± 1) months (Month 24 Visit) after eli-cel infusion in this study. Subsequently, after provision of written informed consent (and assent if applicable), subjects were to be followed for up to an additional 13 years in Study LTF-304, for a total of 15 years after drug product infusion.

Data Monitoring Committee

An independent Data Monitoring Committee (DMC) comprised of members with appropriate scientific and medical expertise monitored the safety of the study.

Main Criteria for Inclusion/Exclusion: Key inclusion criteria included: (Inclusion criterion #2) male and ≤ 17 years of age; (Inclusion criterion #3) active CALD as defined by elevated very long chain fatty acids (VLCFA) levels, brain magnetic resonance imaging (MRI) demonstrating Loes score between 0.5 and 9 (inclusive) on the 34-point scale, and gadolinium enhancement (GdE+) of demyelinating lesions; (Inclusion criterion #4) a Neurologic Function Score (NFS) of ≤ 1 . Key exclusion criteria included: (Exclusion criterion #1) a recipient of an allogeneic hematopoietic stem cell transplant (allo-HSCT) or previous gene therapy.

Results Relevant to Module 2.7.2 Discussion Disposition**Table S2: Disposition**

Parameter	Statistic	ITT (N = 28)
Initiated mobilization (ITT)	n (%)	28 (100)
Initiated conditioning	n (%)	24 (85.7)
Infused with eli-cel (TP)	n (%)	23 (82.1)
Successful neutrophil engraftment (NEP)	n (%)	22 (78.6)
Completed Study	n (%)	0
Discontinued Study	n (%)	0
Duration of follow-up (months)	Median (Min, Max)	12.0 (0.3, 20.8)
Latest Visit Completed		
Month 1	n (%)	1 (4.3)

Month 2	n (%)	1 (4.3)
Month 3	n (%)	5 (21.7)
Month 6	n (%)	3 (13.0)
Month 12	n (%)	6 (26.1)
Month 18	n (%)	6 (26.1)

Abbrev.: ITT, intent-to-treat population; NEP, neutrophil engraftment population; TP, transplant population

Note: TP consists of subjects who underwent an eli-cel infusion while NEP only includes subjects who achieved NE by Rel Day 43.

Results: Pharmacodynamics

Drug product characteristics (median [min, max] on all available data) were as follows:

- DP dose of $12.5 (5.1, 38.2) \times 10^6$ CD34+ cells/kg (N = 23)
- DP VCN: 1.5 (1.2, 3.1) copies per diploid genome (c/dg) (N = 23)

- DP %LVV+ Cells: 60% (b) (4) (N = 13)
- DP %ALDP+ Cells: 55.00% (38.6%, 74.7%) (N = 15)
- Vector copies per transduced cell: (b) (4) (N = 13)

Integrated vector sequences were quantifiable in PBLs and CD14+ cells of all treated subjects

All (21/21) evaluable subjects had quantifiable VCN in PBLs and CD14+ cells, stabilizing by 6 months after drug product infusion, with the longest follow-up for some subjects at Month 18. This result indicates the successful engraftment of transduced HSCs and the stable presence of the transgene in their progeny. At Month 6, median (min, max) VCN was 1.20 c/dg (0.40, 3.13; N = 14) for PBLs and 1.53 c/dg (0.40, 3.82; N = 12) for CD14+ cells.

ALDP+ cells were quantifiable in PBLs and CD14+ cells of all treated subjects

All evaluable subjects had quantifiable ALDP+ PBLs and CD14+ cells after drug product infusion, although results were variable. The presence of ALDP+ cells indicates that the transgene is being expressed in the progeny of transduced HSCs. The %ALDP+ cells generally stabilized by 6 months after drug product infusion, with longest follow-up at Month 18. At Month 6, median (min, max) %ALDP+ cells was 21.68% (13.79%, 27.21%; N = 10) for PBLs and 36.64% (16.87%, 86.15%; N = 10) for CD14+ cells.

Source: Applicant. Module 2, section 2.7.2. Summary of Clinical Pharmacology.

7.3 Study #3 – Study LTF-304

Interim data cut-off date: March 26, 2021.

Title: Long-term Follow-Up of Subjects with Cerebral Adrenoleukodystrophy Who Were Treated with Lenti-D Drug Product
Objectives: Monitor for long-term safety and efficacy of the gene therapy drug product used in a bluebird bio-sponsored clinical studies (i.e., the “parent studies”) in treated subjects with CALD.
Methodology: Single arm, multi-center, noninterventional long-term follow-up study. After monitoring of a subject in the parent study was completed (where the parent study includes approximately 2 years of follow-up after drug product infusion), subjects were asked to enroll in Study LTF-304. During Study LTF-304, subjects were followed every 6 months from 2 years through 5 years post-drug product infusion and then annually from 5 years through 15 years post-drug production infusion.
Main Criteria for Inclusion: Subjects are eligible who have enrolled in a clinical study in which they received eli-cel and who have provided written informed consent/assent for Study LTF-304, as applicable.

Results Relevant to Module 2.7.2 Discussion Disposition

Twenty-seven subjects with CALD treated with eli-cel (all from Study ALD-102) enrolled in Study LTF-304, and the median (min, max) follow-up time was 61.6 (29.0, 83.7) months post-drug product infusion. Twenty-six of these subjects continue to participate in Study LTF-304 as of the CSR interim data cut date for this CSR; 1 subject discontinued after approximately 54 months of follow-up (subject declined further follow-up). No subjects have completed Study LTF-304.

Results: Pharmacodynamics

- VCN values in peripheral blood leukocytes (PB VCN) were stable at the end of parent study ALD-102, and values were stably maintained in all subjects during Study LTF-304. The latest visit for 2 subjects was Year 7 (PB VCNs of 0.52 and 0.28 c/dg). For the 7 subjects who had PB VCN values at Year 6, median (min, max) was 0.70 (0.27, 1.05) c/dg. One subject had CD14+ results at Year 6, with a CD14+ VCN of 1.96 c/dg. These results demonstrate stable, long-term persistence of transduced HSCs in all subjects.
- ALDP+ cells were quantifiable in the peripheral blood of all subjects after treatment with eli-cel (albeit at low levels in subjects with low VCN values), including in several subjects who had completed their Month 60 visit. These results support the long-term expression of transgenic ALDP in hematopoietic cells.
- Fasting serum VLCFA levels for C26:0 LysoPC and C26:0/C22:0 ratio showed a trend to decrease from parent study ALD-102 Baseline levels after drug product infusion, and VLCFA decreases were generally maintained between Month 24 and latest assessment in Study LTF-304.

Source: Applicant. Module 2, section 2.7.2. Summary of Clinical Pharmacology.

7.4 Study #4 – Study ALD-103

Database lock date: March 31, 2020.

Title: 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

Objectives: Evaluate the safety and efficacy of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in subjects with cerebral adrenoleukodystrophy (CALD)

Methodology: Multi-site, global, prospective, and retrospective data collection study for subjects treated with allo-HSCT. This study did not involve the use of an investigational drug or medicinal product.

Main Criteria for Inclusion: Subjects were male and ≤ 17 years of age at the time of treatment for retrospective and partial prospective/retrospective subjects, or at the time of consent/assent for prospective subjects, and had a confirmed diagnosis of CALD as defined by abnormal VLCFA profile and cerebral lesion on brain MRI.

Results Relevant to Module 2.7.2 Discussion Disposition

The population of subjects treated with allo-HSCT (TP-103) was further subdivided into subpopulations, and one of these subpopulations (TPES-103; those enrolled in Study ALD-103 who met the strictly eligible criteria for enrollment in Study ALD-102) satisfied the same baseline eligibility criteria as was used for Study ALD-102. Only key results from VLCFA analyses of TP-103 and TPES-103 are discussed in 2.7.2 and presented in this summary of results.

Table S3: Disposition (TPES, and TP, First Allo-HSCT Period)

	TPES-103 (N = 27)	TP-103 (N = 59)
Initiated conditioning (ITT), n (%)	27 (100.0)	59 (100.0)
Received allo-HSCT (TP), n (%)	27 (100.0)	59 (100.0)
Completed Month 24 Visit, n (%)	13 (48.1)	28 (47.5)
Completed Month 48 Visit, n (%)	4 (14.8)	12 (20.3)
Discontinued from first allo-HSCT Period ^a , n (%)	23 (85.2)	47 (79.7)

Reasons for study discontinuation from first allo-HSCT Period, n (%)		
Decided to receive another allo-HSCT	5 (18.5)	8 (13.6)
Unable to comply with protocol defined visits	1 (3.7)	2 (3.4)
Lost to follow-up	1 (3.7)	2 (3.4)
Death	3 (11.1)	12 (20.3)
Study terminated by Sponsor ^a	13 (48.1)	23 (39.0)
Duration of follow-up in first allo-HSCT Period (months)		
n	27	59
Median	24.31	23.00
Min, Max	0.9, 48.5	0.9, 49.5
^a The Sponsor terminated Study ALD-103 before most subjects completed their Month 48 Visit.		

Results: Pharmacodynamics

VLCFA:

There was a general trend towards decreasing levels of VLCFAs, with the largest median (min, max) decreases appearing to stabilize in TP-103 around Month 12 at -62.62% (-81.9%, 8.7%; N = 11) for C26:0 LysoPC and at -37.50% (-62.5%, 0.0%; N = 11) for the C26:0/C22:0 ratio. Similar results were seen in TPES-103 with VLCFA decreases appearing to stabilize around Month 12 at -62.62% (-81.9%, -38.8%; N = 7) for C26:0 LysoPC and at -37.50% (-62.5%, -16.7%; N = 7) for C26:0/C22:0 ratio. Note that only 11 subjects in TP-103 had C26:0 LysoPC and C26:0/C22:0 values available at both Baseline and Month 12.

Ten of these 11 evaluable subjects showed a decrease for both C26:0 LysoPC and the C26:0/C22:0 ratio at Month 12; however, levels of both VLCFA parameters remained elevated compared to the normal ranges.

Source: Applicant. Module 2, section 2.7.2. Summary of Clinical Pharmacology.