

Clinical Pharmacology BLA Review
Office of Clinical Evaluation (OCE)
Office of Therapeutic Products (OTP)

Submission Number: 125758.00

Product Name: Atidarsagene autotemcel (LENMELDY)

Proposed Indication: Treatment of pediatric patients with pre-symptomatic late infantile (PSLI), pre-symptomatic early juvenile (PSEJ) or early symptomatic early juvenile (ESEJ) metachromatic leukodystrophy (MLD)

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Table of Contents

List of Tables	3
List of Figures	3
1. Executive Summary	4
2. Recommendations	5
3. Background	6
4. Summary of Clinical Pharmacology Findings.....	7
5. Clinical Pharmacology Labeling Comments.....	13
6. Comprehensive Clinical Pharmacology Review	14
6.1. General Pharmacology and Pharmacokinetics	14
6.2. Pharmacodynamic Assessments	16
6.2.1. Transduced Cell Engraftment.....	16
6.2.2. Arylsulfatase A Activity	20
6.2.3. Dose-Response and Correlative Assessments	26
6.2.3.1. Drug Product (DP) Characteristics vs. Pharmacodynamics	27
6.2.3.2. Pharmacodynamics vs. Clinical Outcomes	30
6.3. Immunogenicity Assessments.....	33
6.4. Replication Competent Lentivirus	34
7. Appendix	35
7.1. Study#1- Clinical Study 201222.....	35
7.2. Study#2- Clinical Study 205756	40
7.3. Study #3: Expanded Access Program.....	42

List of Tables

Table 1: Summary of ARSA Activity (nmol/mg/h) in Total Peripheral Blood Mononuclear Cells by Visit in the Patients Treated with LENMELDY	9
Table 2: Minimum and Maximum Recommended Dose of LENMELDY	12
Table 3: Summary of Studies Contributing Evidence of Efficacy for LENMELDY.....	15
Table 4: Summary of Busulfan Conditioning Regimen and Total Area Under the Curve (Integrated Safety Set).....	16
Table 5: Summary of Mean Vector Copy Number (/Cell) in Total Peripheral Blood Mononuclear Cells by Visit (Pre-Symptomatic Late Infantile Subjects, N=20)	18
Table 6: Summary of Mean Vector Copy Number (/Cell) in Total Peripheral Blood Mononuclear Cells by Visit (Pre-Symptomatic Early Juvenile Subjects, N=7).....	18
Table 7: Summary of Mean Vector Copy Number (/Cell) in Total Peripheral Blood Mononuclear Cells by Visit, Early Symptomatic Early Juvenile Subjects (N=10)	19
Table 8: Summary of Administered Dose in Responding vs Non-responding Patients.	32
Table 9: ARSA in PBMC in Responding vs Non-responding Patients.	32

List of Figures

Figure 1: Summary of Vector Copy Number in peripheral blood mononuclear cells (PBMC) 12 Months Following LENMELDY Treatment	19
Figure 2: Plot of Percent Lentiviral Vector Transduced Cells in Bone Marrow.....	20
Figure 3: Longitudinal Plot of Arylsulfatase A Activity in Total Peripheral Blood Mononuclear Cells (PBMC) Following LENMELDY Treatment in PSLI Subjects (N=20).....	22
Figure 4: Longitudinal Plot of Arylsulfatase A Activity in Total Peripheral Blood Mononuclear Cells (PBMC) Following LENMELDY Treatment in PSEJ Subjects (N=7).....	23
Figure 5: Longitudinal Plot of Arylsulfatase A Activity in Total Peripheral Blood Mononuclear Cells (PBMC) Following LENMELDY Treatment in ESEJ Subjects (N=10).....	24
Figure 6: Summary of Arylsulfatase A Activity in Total Peripheral Blood Mononuclear Cells (PBMC) at 12 Months Following LENMELDY Treatment.....	25
Figure 7: Arylsulfatase A Activity in Total Cerebrospinal Fluid (CSF) at 12 Months Following LENMELDY Treatment.....	26

Figure 8: Summary of Dose-response analysis for ARSA activity in PBMC for MLD subtypes 28
Figure 9: Summary of Age at LENMELDY Treatment versus ARSA activity in PBMC for PSLI, PSEJ and ESEJ..... 29
Figure 10: Summary of Total Administered Dose versus ARSA activity in PBMC for PSLI, PSEJ and ESEJ..... 30

1. Executive Summary

LENMELDY (atidarsagene autotemcel) is a gene therapy consisting of autologous CD34+ cells, containing hematopoietic stem cells (HSCs), transduced with a lentiviral vector (LVV) encoding the human the Arylsulfatase A (ARSA) gene. In this BLA submission, the applicant proposes, LENMELDY for the treatment of pediatric patients with pre-symptomatic late infantile (PSLI), pre-symptomatic early juvenile (PSEJ) or early symptomatic early juvenile (ESEJ) metachromatic leukodystrophy (MLD).

The data supporting clinical pharmacology of LENMELDY is based two clinical studies (#201222 and #205756) and Expanded Access Program (EAP) with a total of 37 subjects included in efficacy, pharmacodynamic (PD) and dose-response analysis. The average number of integrated ARSA transgenes vector copy number in peripheral blood mononuclear cells (VCN in PBMC), the proportion of lentiviral vector positive (% LVV+) in the bone marrow (BM) and ARSA in PBMC were evaluated as part of PD assessments. Following administration of LENMELDY, the VCN in PBMC was first detected at Month 1, continue to increase to relatively stable average levels at Month 12 and remained stable at the end the follow-up period for PSLI, PSEJ and ESEJ. The median % LVV+ exceeded the applicant defined 4% threshold at Month 1 and it remained stable throughout the follow-up period for PSLI, PSEJ, and ESEJ. Following administration of LENMELDY, median ARSA activity in PBMCs was at supranormal levels by Month 3 in the PSLI and PSEJ (normal range for ARSA activity is 30.6-198 nmol/mg/h) and supranormal median levels were sustained throughout the duration of follow-up. In ESEJ median ARSA level was within normal range from Month 3 to Year 2 and supranormal level was achieved from Year 3 to 5. The time to reach supranormal ARSA activity is delayed in ESEJ and the actual peak ARSA activity is substantially lower in ESEJ as compared to PSLI. The median [min, max]

ARSA activity in PBMC (nmol/mg/h) was 1239 [46, 6467] for PSLI (n=20), 883 [272, 1976] for PSEJ (n=7) and 130 [55, 688] for ESEJ (n=8). The available data don't allow to establish quantitative relationship between ARSA activity and efficacy outcome hence supranormal ARSA activity should be interpreted carefully.

Dose-response analysis showed a trend for increasing ARSA in PBMC with increasing dose for PSLI and ESEJ. Also, factors such as age at treatment appear to contribute to the observed variability in ARSA activity. Age is negatively correlated with ARSA activity for PSLI. Anti-ARSA antibodies (AAA) were detected in 6 out 39 subjects (15%). There is no indication of decreased ARSA activity in five PSLI patients that developed AAA but there was a trend of decreasing ARSA activity in one PSEJ patient with AAA.

2. Recommendations

This BLA is acceptable for approval from the clinical pharmacology perspective. Labeling recommendations are provided in section 5 of the memo.

3. Background

Metachromatic leukodystrophy (MLD) is a rare autosomal recessive inherited lysosomal storage disorder caused by mutations in the Arylsulfatase A (ARSA) gene that results in deficiency of the enzyme ARSA. The lysosomal enzyme ARSA is essential for the metabolism of sulfatides, a major component of myelin of the nervous system. Deficiency of ARSA results in accumulation of the undegraded sulfatides in the nervous system, and progressive demyelination of the central and peripheral nervous system. MLD primarily manifests clinically as loss of motor, neuro-cognitive functions and ultimately death.

The disease spectrum of MLD can widely vary and the following are subtypes of MLD based on the age of onset of symptoms. About 300 pathogenic variants (mutations) of the *ARSA* gene have been described and these can be functionally divided into two broad groups: null (0) or “severe” alleles associated with little or no enzyme activity, and R alleles encoding for ARSA with some residual enzyme activity. Patients who are clinically classified with the late infantile (LI) MLD usually carry 2 null alleles (0/0 genotype) and barely express any residual ARSA activity, resulting in symptoms manifestation before 30 months of age. LI MLD is the most prevalent MLD variant and the most aggressive form of the disease showing a predictable and severe disease course, characterized by progressive decline in motor and cognitive function and an early death^{1,2}. Patients who are affected by the early juvenile (EJ) subtype typically carries one null allele and one residual allele (0/R genotype) and have symptom onset between the age of 30 months and 7 years. There are currently no therapies approved by the US FDA for the treatment of MLD.

In this BLA submission the applicant developed atidarsagene autotemcel (LENMELDY) for the treatment of pediatric patients with pre-symptomatic late infantile (PSLI), pre-symptomatic early juvenile (PSEJ) or early symptomatic early juvenile (ESEJ) metachromatic leukodystrophy (MLD). LENMELDY (formerly known as OTL-200) is a gene therapy consisting of autologous

¹Gieselmann V , Krägeloh-Mann I. (2010). Metachromatic Leukodystrophy – An Update. *Neuropediatrics*; 41(1): 1-6.

² van Rappard DF, Boelens JJ, Wolf NI (2015). Metachromatic leukodystrophy: Disease spectrum and approaches for treatment. *Best Pract Res Clin Endocrinol Metab.*;29:261-73.

CD34+ cells, containing hematopoietic stem cells (HSCs), transduced with a lentiviral vector (LVV) encoding the human ARSA gene.

The clinical development program (CDP) for OTL-200 includes two clinical studies (# 201222 [N = 20], #205756 [N = 10] and an Expanded Access Program (EAP):

- Compassionate Use Program (CUP) 207394 (N = 1)
- Hospital Exemption (HE) Program 205029 (N = 3)
- CUP 206258 (N = 5)

In total, data from 39 subjects with MLD treated in the OTL-200 clinical studies and expanded access program (EAP) were included as part of the BLA with 37 treated subjects included in the pharmacodynamic and dose-response analysis.

4. Summary of Clinical Pharmacology Findings

Pharmacokinetics (PK)

- LENMELDY is an autologous cellular therapy which includes HSCs that have been genetically modified ex vivo. The nature of LENMELDY is such that conventional pharmacokinetic studies on absorption, distribution, metabolism, and excretion are not applicable.
- Myeloablative conditioning is required prior to administering LENMEDLY; in the clinical trial, busulfan was given. Blood samples were collected to determine the PK/exposure of busulfan.
- The total busulfan area under the curve (AUC) was within the predefined target range and total AUC was comparable among PSLI, PSEJ and ESEJ. No difference in total AUC was noted across the evaluated age and bodyweight range.

Pharmacodynamics (PD)

I. Engraftment of transduced cells

The average number of integrated ARSA transgenes vector copy number in peripheral blood mononuclear cells (VCN in PBMC) and the proportion of lentiviral vector positive (LVV+) in the bone marrow (BM) were monitored as part of PD assessment of LENMELDY.

- Following administration of LENMELDY, the VCN in PBMC was first detected at Month 1, continued to increase to relatively stable levels at Month 12 and remained stable until at the end the follow-up period for PSLI, PSEJ and ESEJ. The median VCN in PBMC is about 5-fold higher in PSLI /PSEJ versus ESEJ at Month 12 following treatment with LENMELDY. The median [min, max] VCN in PBMC at Month 12 is 1.6 [0.1, 4.1], 1.7 [0.9, 2.4] and 0.3 [0.1, 0.8] in PSLI, PSEJ and ESEJ, respectively.
- The median VCN in PBMC at Month 12 is about 5-fold higher in PSLI/PSEJ versus ESEJ. The median [min, max] VCN in PBMC is 1.6 [0.1, 4.1], 1.7 [0.9, 2.4] and 0.3 [0.1, 0.8] in PSLI (n=20), PSEJ (n=7) and ESEJ (n=8), respectively.
- The median % LVV+ exceeded the applicant defined 4% threshold at the at Month 1 assessment and it remained stable throughout the follow-up period for PSLI, PSEJ, and ESEJ.
- The median [min, max] %LVV+ in bone marrow is 77 % [20, 100], 67 % [40, 91] and 32 % [18, 61] in PSLI (n=17), PSEJ (n=7) and ESEJ (n=8), respectively.
- Overall, the median engraftment parameters (i.e., VCN in PBMC and LVV+) are lower for ESEJ as compared to PSLI/PSEJ, but it is important to note that engraftment parameters for ESEJ is still within the range of the PSLI/PSEJ.

II. ARSA Activity

ARSA activity in PBMC was monitored to provide pharmacodynamic activity of LENMELDY. Assessment of ARSA activity in cerebrospinal fluid (ARSA in CSF) was also performed to provide supportive evidence of pharmacodynamic activity of LENMELDY.

- Prior to start of conditioning, the ARSA activity in PBMC was at or near the limit of quantification (25.79 nmol/mg/h). It should be noted that most baseline values were below the limit of quantification and it is difficult to fully characterize the baseline values for different MLD subtypes based on age or other characteristics (e.g. genotypes).
- Following administration of LENMELDY, median ARSA activity in PBMCs was at supranormal levels by Month 3 in the PSLI and PSEJ (normal range for ARSA activity is 30.56-198.02 nmol/mg/h) and supranormal median levels were sustained throughout the duration of follow-up (Table 1). In ESEJ median ARSA level was within normal range from Month 3 to Year 2 and supranormal level was achieved from Year 3 to 5 (Table 1).

- The ARSA activity for ESEJ was lower than PSLI/PSEJ but it was within the normal or supranormal range. It should be noted that the available data do not allow the establishment of a quantitative relationship between ARSA activity and efficacy outcome; supranormal ARSA activity should be interpreted carefully.
- For efficacy evaluable population at Month 12, the median [min, max] ARSA activity in PBMC (nmol/mg/h) was 1239 [46, 6467] for PSLI (n=20), 883 [272, 1976] for PSEJ (n=7) and 130 [55, 688] for ESEJ (n=8). The median ARSA activity (Month 12) in PBMC is 9.5-fold higher in PSLI vs ESEJ and it was 1.4 higher in PSLI vs PSEJ. However, it is important to note the high variability, and the ARSA activity in PBMC for PSEJ and ESEJ is still within the range of PSLI.
- Following treatment with LENMELDY, reconstitution of ARSA activity in CSF was observed in PSLI, PSEJ, and ESEJ. The median ARSA activity in CSF increased to within the reference range (0.3 to 2.8 nmol/mg/h) by 3 to 6 Month and were maintained within the reference range throughout the follow-up period for PSLI, PSEJ and ESEJ.

Dose-Response Analysis

Despite the median supranormal ARSA activity is achieved, the available data are insufficient to establish a quantitative relationship between ARSA activity and clinical outcomes.

- The median [min, max) administered dose ($\times 10^6$ CD34+cells/kg) was 14.2 [4.2, 30] for PSLI, 17.6 [9, 30] for PSEJ and 9.5 [6,30] for ESEJ.
- The median dose was 1.5-fold higher for PSLI vs ESEJ and 1.8-fold higher for PSEJ vs ESEJ.
- There was a trend for increasing ARSA activity in PBMC with increasing dose for PSLI ($R^2=0.2$, $p=0.05$) and ESEJ ($R^2=0.7$, $p=0.01$).
- It should be noted that confounding factors such as age at treatment may contribute to the observed variability for ARSA activity for MLD subtypes. For example, this reviewer noted a negative correlation between age at treatment and ARSA activity PSLI ($R^2=0.3$, $p=0.01$) suggesting that early treatment with LENMELDY may result in a higher ARSA activity in PBMC.

- In PSLI, all patients with adequate follow-up were responding with overall higher ARSA level across the wide dose range evaluated and the minimum effective dose was 4.2×10^6 CD34+ cells/kg.
- For PSEJ, the ARSA level in one non-responding patient was higher than the average level in responding PSEJ patient. The administered dose for this non-responding patient (i.e., 17.6×10^6 CD34+ cells/kg) was higher than the minimum dose for responding PSEJ patient (i.e., 9×10^6 CD34+ cells/kg).
- For ESEJ, the ARSA level in non-responding patients were numerically lower than the value for responding patients. The minimum effective dose for ESEJ was 6.6×10^6 CD34+ cells/kg.
- Dose-response relationship was also explored using total administered dose (i.e., CD34 cell/kg multiplied by bodyweight). Again, a trend for increasing ARSA activity with increasing total administered dose was noted for PSLI and ESEJ.
- These dose-response relationship for ARSA suggests a more robust ARSA activity with higher administered total dose. Clinical efficacy assessment also identifies some qualitative relationship between ARSA activity and clinical outcomes. Given these dose-response relationship, this reviewer believes that there is insufficient information to extrapolate dose and efficacy outside of the current minimum age (8.2 months) and bodyweight (7.2 kg).

Overall, clinical, and clinical pharmacology teams discussed the minimum dose proposed by the applicant ($(b)(4) \times 10^6$ CD34+ cells/kg for PSLI, PSEJ and ESEJ). Based on the results of the dose-response relationship for ARSA in PBMC and clinical outcome data, the minimum FDA recommended dose should reflect the minimum dose that result in therapeutic effect for each MLD subtypes. The applicant did not define maximum dose in the USPI. Considering potential for thrombosis risk the FDA recommended defining maximum dose based on the maximum dose of 30×10^6 CD34+ cells/kg that was administered in the clinical studies.

Accordingly, the FDA recommended minimum and maximum dose is summarized in Table 2.

Table 1: Summary of ARSA Activity (nmol/mg/h) in Total Peripheral Blood Mononuclear Cells by Visit in the Patients Treated with LENMELDY

Visit	Variable	PSLI (N=20)	PSEJ (N=7)	ESEJ (N=10)
Month 3	Patient Samples, n	18	6	10
--	Median (Min, Max)	682 (61, 3398)	1140 (314, 1300)	210 (50, 426)
Month 6	Patient Samples, n	16	6	7*
	Median (Min, Max)	1095 (37, 2716)	983 (150, 1804)	107 (26, 444)
Year 1	Patient Samples, n	20	7	8
	Median (Min, Max)	1239 (46, 6467)	883 (272, 1976)	130 (55, 688)
Year 2	Patient Samples, n	18*	6	7
	Median (Min, Max)	935 (26, 5935)	1063 (328, 2205)	82 (70, 219)
Year 3	Patient Samples, n	18*	4	6
	Median (Min, Max)	1558 (26, 7091)	1156 (537, 2173)	234 (30, 1271)
Year 5	Patient Samples, n	9	0	3
	Median (Min, Max)	756 (28, 3474)	-	363 (282, 793)

* One value was below the lower limit of quantification (LLQ) or not detected or not quantifiable, and has been imputed as the LLQ (26 nmol/mg/h).

Source: Applicant's Table X43.24; Response clinical IR#8

Table 2: Minimum and Maximum Recommended Dose of LENMELDY

MLD Subtype	Minimum Recommended Dose (CD34⁺ cells/kg)	Maximum Recommended Dose (CD34⁺ cells/kg)
Pre-symptomatic late infantile	4.2 x 10 ⁶	30 x 10 ⁶
Pre-symptomatic early juvenile	9 x 10 ⁶	30 x 10 ⁶
Early symptomatic early juvenile	6.6 x 10 ⁶	30 x 10 ⁶

Source: FDA reviewer analysis

Immunogenicity Assessments

- No subjects tested positive for anti-ARSA antibodies (AAA) at baseline.
- At the time of the data cut-off for the BLA (01 Nov 2022), AAA had been detected in 6 subjects out of the 39 subjects (15%) treated with LENMELDY in the clinical development program.
- Five occurrences of AAA formation occurred in PSLI patients with a 0/0 ARSA genotype, and one occurrence was in an PSEJ patient with a 0/UNK genotype.
- The proportion of subjects with the 0/0 variant in the Integrated Safety Set who developed AAA was 26%.
- There is no indication of decreased ARSA activity in PBMC in PSLI subjects that developed AAA following administration of LENMELDY. There was a trend of decreasing ARSA activity in one PSEJ patient with AAA.
- Overall, there is insufficient data to fully evaluate the impact of AAA on ARSA activity in PBMC, and we recommend (b) (5)

[REDACTED]

5. Clinical Pharmacology Labeling Comments

Section 2.1. Dose

- The applicant proposed a minimum dose of $(b) (4) \times 10^6$ CD34+ cells/kg for PSLI, PSEJ and ESEJ. Considering dose-response and clinical outcome data, we requested to separately define the following dose for each MLD subtype: 4.2×10^6 CD34+ cells/kg for PSLI, 9×10^6 CD34+ cells/kg for PSEJ, 6.6×10^6 CD34+ cells/kg for ESEJ.
- The applicant did not define maximum dose. We requested to define maximum dose (30×10^6 CD34+ cells/kg) for all indications. This is the maximum number of cells received in the clinical trial and given the risk of thrombosis, we do not believe there is data to support a higher dose.

Section 12.1. Mechanism of Action

- Requested to include data supported statement to describe the mechanism of action and avoid speculative claims of untested mechanism of action.

Section 12. 2. Pharmacodynamics

- Requested to remove descriptive and speculative statements about engraftment parameters.
- The applicant initial presented pooled analysis ARSA in PBMC. We requested to present longitudinal ARSA in PBMC for efficacy evaluable population for each MLD subtypes separately.

Section 12.3. Pharmacokinetics

- Requested to remove a nonclinical biodistribution information as this was not relevant or reviewed as part of the clinical pharmacology data.

Section 12.6. Immunogenicity

- Requested to provide details about MLD subtypes and time of resolution of anti-ARSA antibodies.

- Included about the lack of sufficient information to characterize the impact of anti-ARSA antibodies on ARSA activity in PBMC.

6. Comprehensive Clinical Pharmacology Review

6.1. General Pharmacology and Pharmacokinetics

LENMELDY is prepared from the patient's own HSCs, which are collected via apheresis procedure(s). The autologous cells are enriched for CD34+ cells, then transduced *ex vivo* with a recombinant replication-incompetent self-inactivating (SIN) human immunodeficiency virus-1 (HIV-1) based LVV that has been modified to carry the ARSA deoxyribonucleic acid (cDNA) sequence under the human phosphoglycerate kinase (PGK) promoter. The transduced CD34+ cells are washed, formulated into a suspension, and then cryopreserved. LENMELDY is intended for one-time administration to add functional copies of the human ARSA gene into the patient's own HSCs. LENMELDY inserts one or more functional copies of the human ARSA complementary cDNA into the patients' HSCs, through transduction of autologous CD34+ cells with ARSA LVV. After LENMELDY infusion, transduced CD34+ HSCs engraft in bone marrow, repopulate the hematopoietic compartment and produce ARSA enzyme. Functional ARSA enzyme can play role in breakdown of sulfatides.

The clinical studies contributing to clinical pharmacology assessments are summarized in Table 3. The nature of LENMELDY is such that conventional pharmacokinetic studies on absorption, distribution, metabolism, and excretion are not applicable. PK analysis is performed to adjust dosing of the conditioning regimen (i.e., busulfan). In the LENMELDY clinical development program two busulfan regimens were used:

- Sub-myeloablative conditioning (SMAC) regimen is defined as a target cumulative AUC of 67,200 $\mu\text{g}\cdot\text{h}/\text{L}$ (range 58,800 to 78,400 $\mu\text{g}\cdot\text{h}/\text{L}$).
- Myeloablative conditioning (MAC) regimen is defined as a target cumulative AUC of 85,000 $\mu\text{g}\cdot\text{h}/\text{L}$ (range 76,500 to 93,500 $\mu\text{g}\cdot\text{h}/\text{L}$). The MAC regimen was designed to

produce an approximately 10% higher cumulative busulfan AUC with the objective of reducing the variability in transduced cell engraftment.

The busulfan regimen administered to each subject depended upon the specific study protocols (see the appendix). In the integrated safety populations, a total of 13 subjects (33%) were treated with a SMAC regimen and 26 subjects (67%) were treated with a MAC regimen. As expected, subjects who received a SMAC regimen received a lower total dose (mg) and lower total dose per body weight (mg/kg) than subjects who received a MAC regimen (Table 4). The geometric mean cumulative AUC in subjects who received a SMAC and MAC regimen were consistent with the target AUC. There was low variability (based on % CV) in busulfan AUC for both SMAC and MAC regimens (Table 4).

Reviewer comments: The total busulfan AUC was comparable for the three MLD subtypes and no difference in total AUC was noted for different age groups.

Table 3: Summary of Studies Contributing Evidence of Efficacy for LENMELDY

	Clinical Studies		Expanded Access Program		
Study IDs	201222	205756	CUP 207394	HE 205029	CUP 206258
Number of subjects	20	10	1	3	
Study Design	Non-randomized, open-label, prospective, single-center	Non-randomized, open-label, prospective, single center	Single patient CUP	Hospital exemption	Compassionate use program
Study Population	Pre-symptomatic LI MLD; Pre- or early symptomatic EJ MLD	Pre-symptomatic, early-onset MLD	Early symptomatic, EJ MLD	Pre-symptomatic, LI MLD	Pre-symptomatic, early-onset MLD
Dose Range Per Protocol (CD34+Cells/kg)	2-20 x 10 ⁶	3-30 x 10 ⁶	2-25 x 10 ⁶	2-20 x 10 ⁶	2-30 x 10 ⁶

Source: Table 1; Module 2.7.3

Table 4: Summary of Busulfan Conditioning Regimen and Total Area Under the Curve (Integrated Safety Set)

Parameter, Summary Statistic	SMAC Regimen (N = 13)	MAC Regimen (N = 26)
Total Dose (mg)		
Geometric Mean	146.7	179.5
Median (Min, Max)	155.3 (72, 268)	158.5 (79.30, 408)
%CV	43.3	44.9
Total Dose/kg (mg/kg)		
Geometric Mean	12.7	14.5
Median (Min, Max)	13.4 (9, 16.2)	14.5 (10.1, 31.8)
% CV	17.2	26.9
AUC (µg*h/L)		
Geometric Mean	71596	82453
Median (Min, Max)	70843 (63420, 79447)	80036 (78000, 88310)
% CV	6.5	3.8

Source: Table 6 &7; Module 2.7.4

6.2. Pharmacodynamic Assessments

6.2.1. Transduced Cell Engraftment

The engraftment parameters for LENMELDY were measured by the average number of integrated ARSA transgenes vector copy number in peripheral blood mononuclear cells (VCN in

PBMC) and the proportion of lentiviral vector positive (LVV+) hematopoietic stem and progenitor cells that stably engraft in the bone marrow (BM).

The VCN in PBMC was detected beginning at 1-month, continue to increase to relatively stable level at Year 1 post-treatment and throughout the course of follow-up for PSLI (Table 5), PSEJ (Table 6) and ESEJ (Table 7). For PSLI, PSEJ, and ESEJ % LVV+ cells in BM mononuclear cells exceeding the 4% threshold identified in the initial clinical study (Study# 201222). The median % LVV+ exceeded the 4% threshold at the first assessment at 1-month post-treatment and engraftment remained stable throughout the follow-up period in the PSLI, PSEJ, and ESEJ.

Reviewer comment: During the BLA review, the FDA updated the MLD subtypes data for three subjects, and we accordingly requested to update the engraftment parameters focusing on efficacy evaluable population (N=37). For the updated data, we preformed comparative analysis of engraftment parameters at 12 Month following treatment with LENMELDY. We selected Month 12 since it represents a period where a stable engraftment was achieved, and data were available from most subjects at 12 Month (n=35 for VCN in PBMC) following treatment with LENMELDY. The FDA analysis for efficacy evaluable population is summarized as follows:

- **The median VCN in PBMC is about 5-fold higher in PSLI /PSEJ versus ESEJ. The median [min, max] VCN in PBMC is 1.6 [0.1, 4.1], 1.7 [0.9, 2.4] and 0.3 [0.1, 0.8] in PSLI, PSEJ and ESEJ, respectively (Figure 1).**
- **The median [min, max] % LVV+ in bone marrow is 77 % [20, 100], 67 % [40, 91] and 32 % [18, 61] in PSLI, PSEJ and ESEJ, respectively (Figure 2).**
- **Overall, the median engraftment parameters (i.e., VCN in PBMC and LVV+) are lower for ESEJ as compared to PSLI/PSEJ, but it is important to note that the engraftment parameters for ESEJ is still within the range of the PSLI/PSEJ (Figure 1& Figure 2).**

Table 5: Summary of Mean Vector Copy Number (/Cell) in Total Peripheral Blood Mononuclear Cells by Visit (Pre-Symptomatic Late Infantile Subjects, N=20)

Visit	n	Median	Min	Max
Day 28	20	0.315	0.030	1.03
Day 60	19	0.600	0.080	2.26
Month 3	19	0.710	0.070	3.95
Month 6	18	1.185	0.060	4.69
Year 1	20	1.615	0.100	4.08
Year 2	18	1.750	0.150	5.96
Year 3	18	1.875	0.220	7.34
Year 4	14	1.470	0.140	5.49
Year 5	9	1.150	0.270	3.69
Year 8	5	1.190	0.200	1.97

Source: Applicant's Table X43.1; Response clinical IR#8

Table 6: Summary of Mean Vector Copy Number (/Cell) in Total Peripheral Blood Mononuclear Cells by Visit (Pre-Symptomatic Early Juvenile Subjects, N=7)

Visit	n	Median	Min	Max
Day 28	7	0.480	0.050	0.790
Day 60	7	0.690	0.300	1.310
Month 3	6	0.855	0.480	1.320
Month 6	7	0.890	0.470	2.520
Year 1	7	1.680	0.870	2.380
Year 2	6	1.220	0.790	2.670
Year 3	5	1.180	1.160	2.550
Year 4	3	1.500	1.200	1.560
Year 5	1	1.19	1.19	1.19
Year 8	1	1.33	1.33	1.33

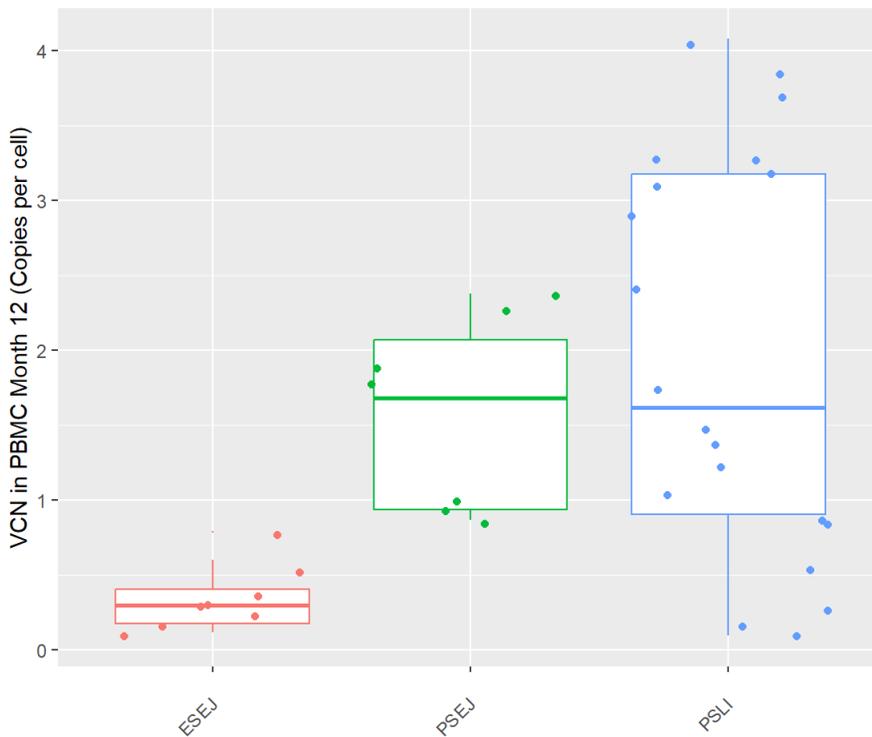
Source: Applicant's Table X43.2; Response clinical IR#8

Table 7: Summary of Mean Vector Copy Number (/Cell) in Total Peripheral Blood Mononuclear Cells by Visit, Early Symptomatic Early Juvenile Subjects (N=10)

Visit	n	Median	Min	Max
Day 28	10	0.16000	0.0300	0.6500
Day 60	10	0.28000	0.1200	0.7700
Month 3	10	0.32000	0.0700	0.5900
Month 6	7	0.25000	0.1200	0.6300
Year 1	8	0.30000	0.1200	0.7900
Year 2	8	0.23500	0.0800	0.7700
Year 3	6	0.35000	0.1200	0.8600
Year 4	4	0.33500	0.1200	0.6800
Year 5	3	0.280	0.130	0.390
Year 8	1	0.310	0.310	0.310

Source: Applicant's Table X43.3; Response clinical IR#8

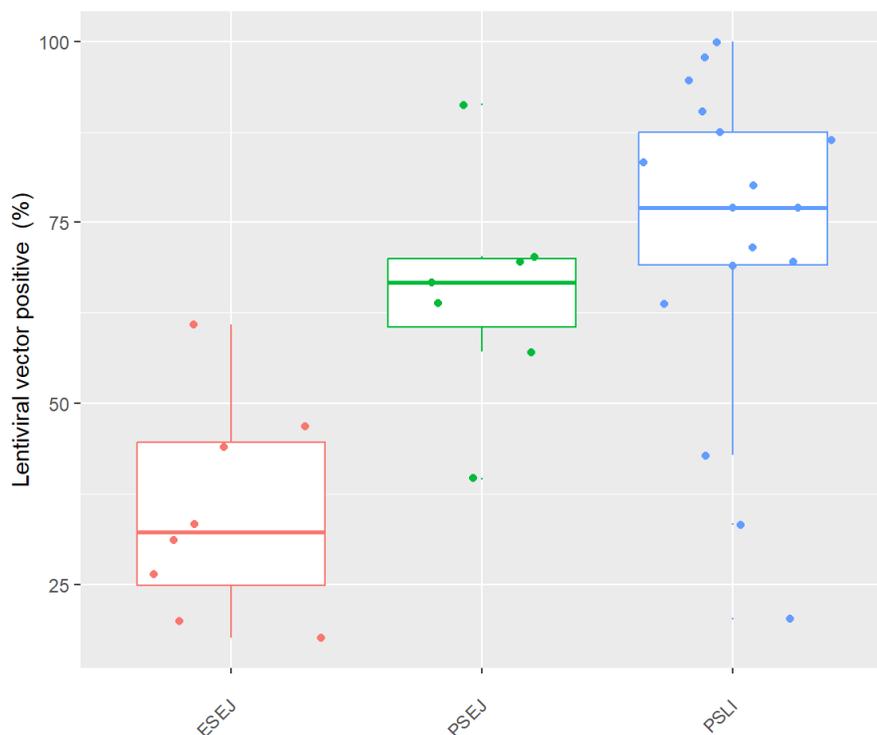
Figure 1: Summary of Vector Copy Number in peripheral blood mononuclear cells (PBMC) 12 Months Following LENMELDY Treatment



Source: FDA reviewer analysis

Figure 2: Plot of Percent Lentiviral Vector Transduced Cells in Bone Marrow

Mononuclear 12 Months Following LENMELDY Treatment



Source: FDA reviewer analysis

6.2.2. Arylsulfatase A Activity

Deficiency of ARSA is known to be the cause of MLD, so demonstrating reconstitution of ARSA activity in cells of the hematopoietic lineage would provide pharmacodynamic evidence of successful engraftment in ARSA-expressing cells. Assessment of ARSA activity in CSF was also performed to provide supportive evidence of pharmacodynamic activity.

Arylsulfatase A Activity (ARSA) in PBMC

Prior to start of conditioning, the ARSA activity in PBMC was at or near the limit of quantification (LLQ; 25.79 nmol/mg/h) for all three MLD subtypes. A high intra- and inter-individual variability ARSA activity in PBMC was observed for PSLI (Figure 3), PSEJ (

Figure 4) and ESEJ (

Figure 5). After treatment with LENMELDY, the ARSA activity levels in PBMC increased to within the normal reference range (30.6 to 198 nmol/mg/h) within Day 28-90 post-treatment for most PSLI, PSEJ, and ESEJ subjects. The ARSA activity reached supranormal level for most PSLI (18 out of 20, 90%) and PSEJ (6 out of 7, 86%) by Month 6 post-treatment with LENMELDY (Figure 3 and

Figure 4). In two PSLI subjects ARSA activity was below the normal reference range occasionally up to Month 36 but at later time points it exceeded the normal reference range.

As shown in Figure 5, ARSA activity was within the normal range for most ESEJ over 3 years following treatment with LENMELDY. In one ESEJ subject ARSA level was below LLQ until Month 6 and remain below normal range until Month 24. After Month 24, the ARSA level for this subject was within normal to supranormal level up to last follow-up. In some ESEJ values supranormal level were observed after 3 years (

Figure 5).

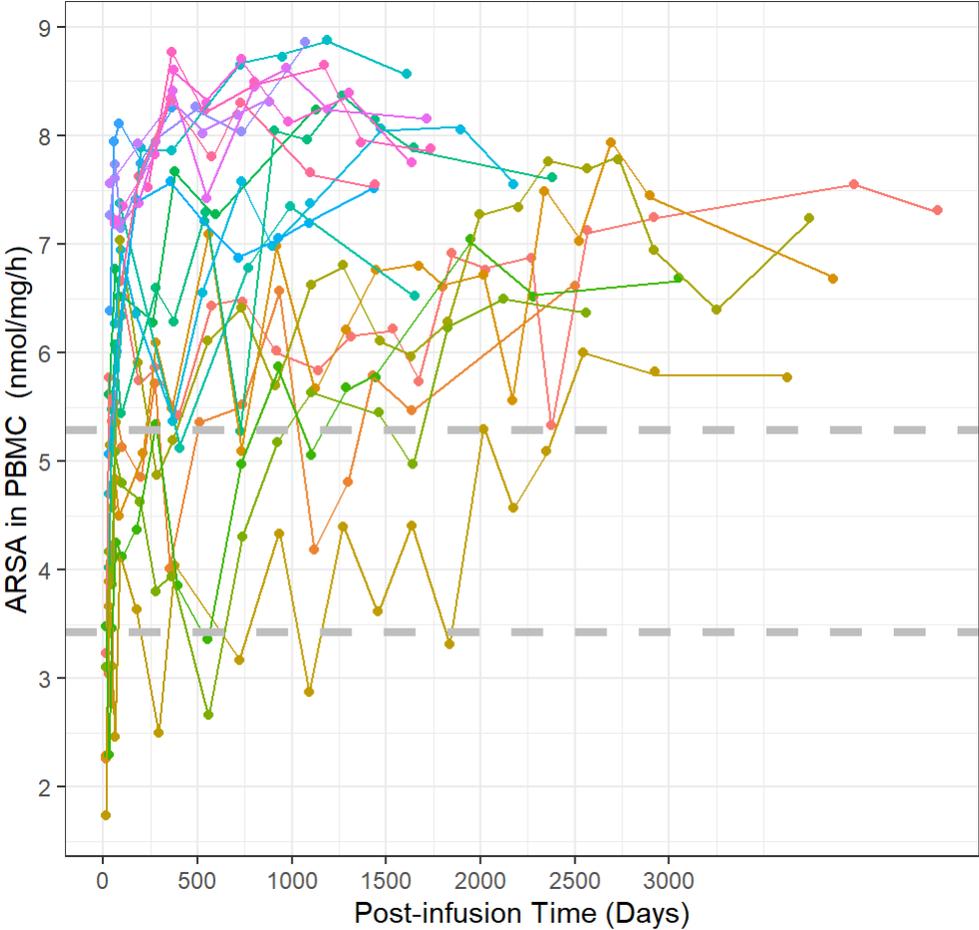
ARSA in Cerebrospinal Fluid (CSF)

At Baseline, ARSA activity levels in CSF were at the LLQ (0.0032 nmol/mg/h) in all subjects. Reconstitution of ARSA activity in CSF was observed in each of the PSLI, PSEJ, and ESEJ. The geometric mean values for ARSA activity in CSF increased to within the reference range (0.3 nmol/mg/h to 2.8 nmol/mg/h established based on CSF samples from children without MLD) by 3 to 6 months posttreatment and were maintained within the reference range throughout follow-up period.

Reviewer comments: We preformed comparative assessment of ARSA in PBMC and CSF for the three MLD subtypes using Month 12 data for efficacy evaluable population.

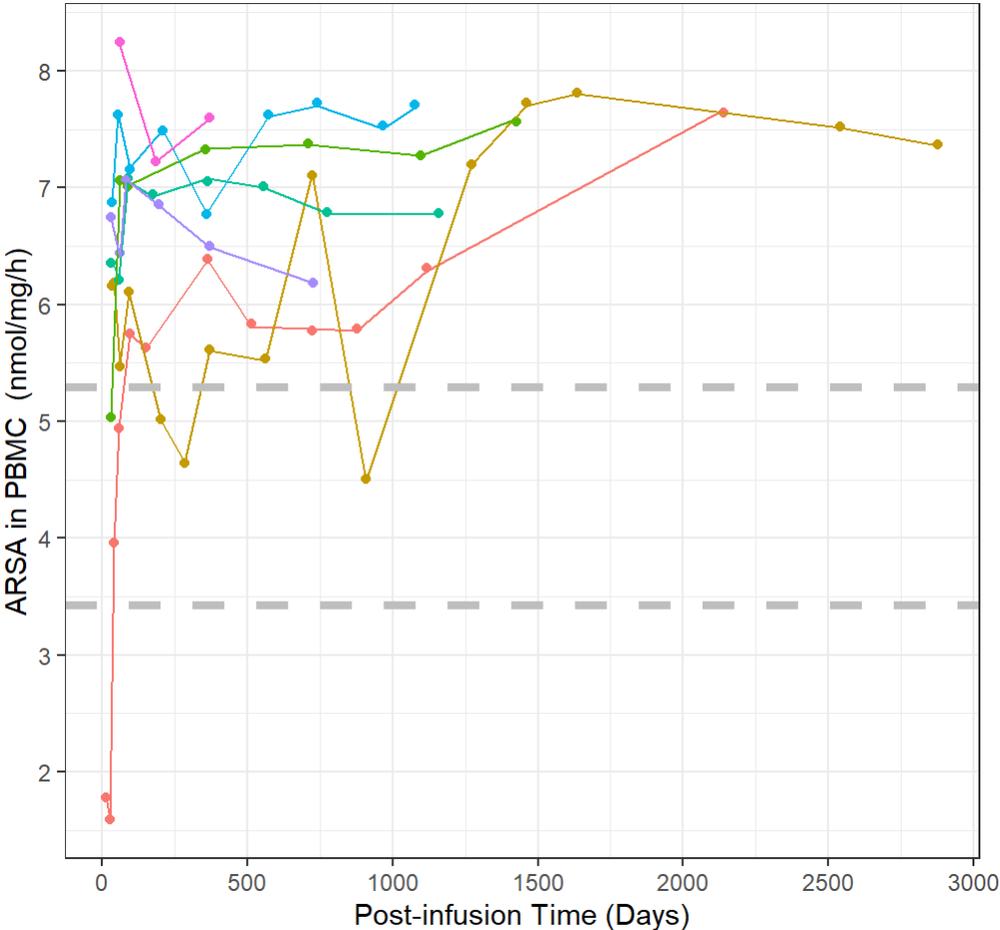
- **The median ARSA activity in PBMC is 9.5-fold higher in PSLI vs ESEJ and it was 1.4 higher in PSLI vs PSEJ. However, it is important to note the high variability and the ARSA activity in PBMC for PSEJ and ESEJ is still within the range of PSLI (Figure 6). The median [min, max] ARSA activity in PBMC (nmol/mg/h) was 1239 [46, 6467], 883 [272, 1976] and 130 [55, 688] in PSLI, PSEJ and ESEJ, respectively (Figure 6).**
- **The median ARSA activity in CSF is comparable among the three MLD subtypes (Figure 7).**

Figure 3: Longitudinal Plot of Arylsulfatase A Activity in Total Peripheral Blood Mononuclear Cells (PBMC) Following LENMELDY Treatment in PSLI Subjects (N=20)



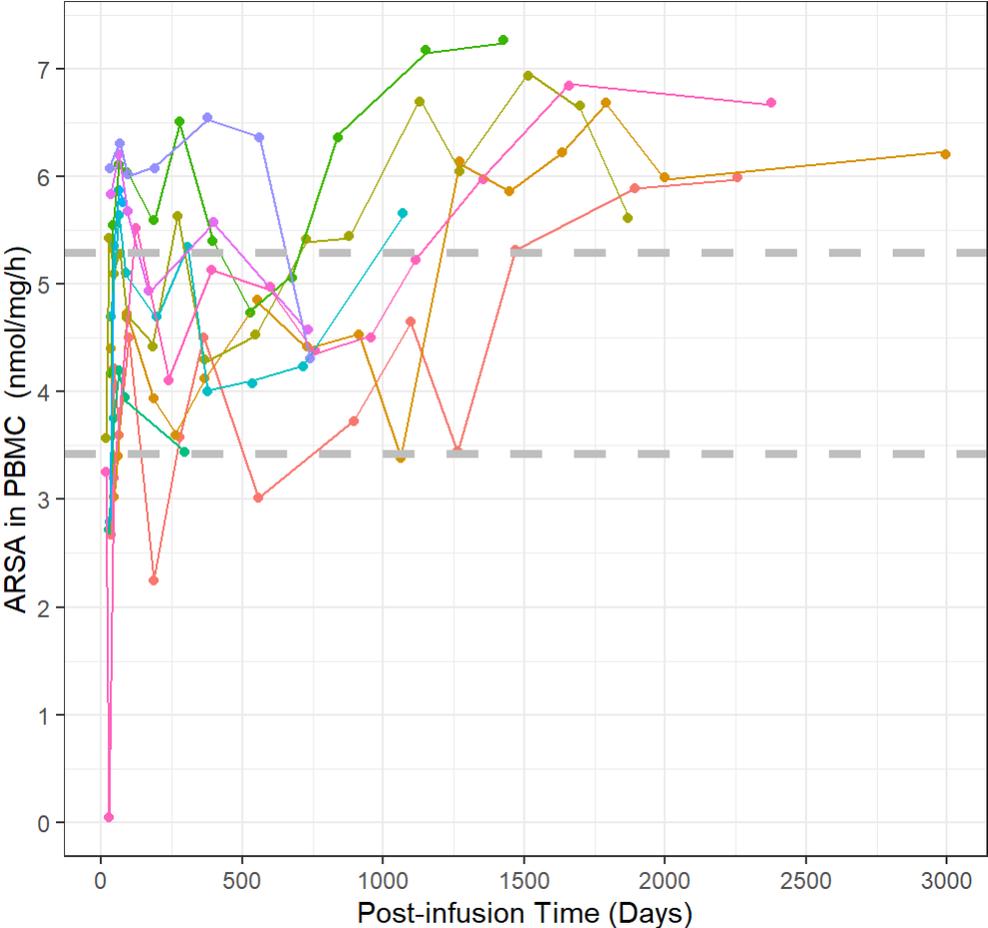
Note: The gray broken lines denote normal ARSA levels in PBMC samples obtained from subjects without MLD. ARSA data were log-transformed. Source: Reviewer analysis

Figure 4: Longitudinal Plot of Arylsulfatase A Activity in Total Peripheral Blood Mononuclear Cells (PBMC) Following LENMELDY Treatment in PSEJ Subjects (N=7)



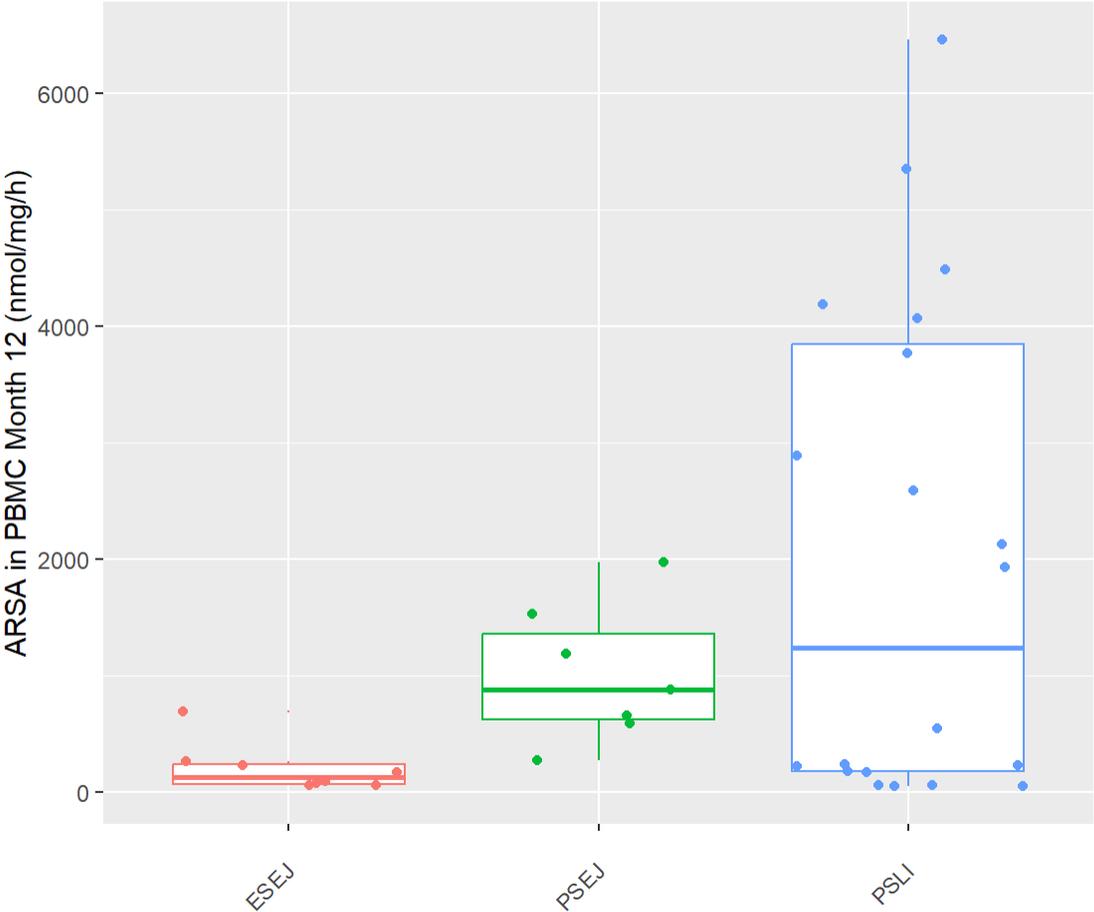
Note: The gray broken lines denote normal ARSA levels in PBMC samples obtained from subjects without MLD. ARSA data were log-transformed. Source: Reviewer analysis

Figure 5: Longitudinal Plot of Arylsulfatase A Activity in Total Peripheral Blood Mononuclear Cells (PBMC) Following LENMELDY Treatment in ESEJ Subjects (N=10)



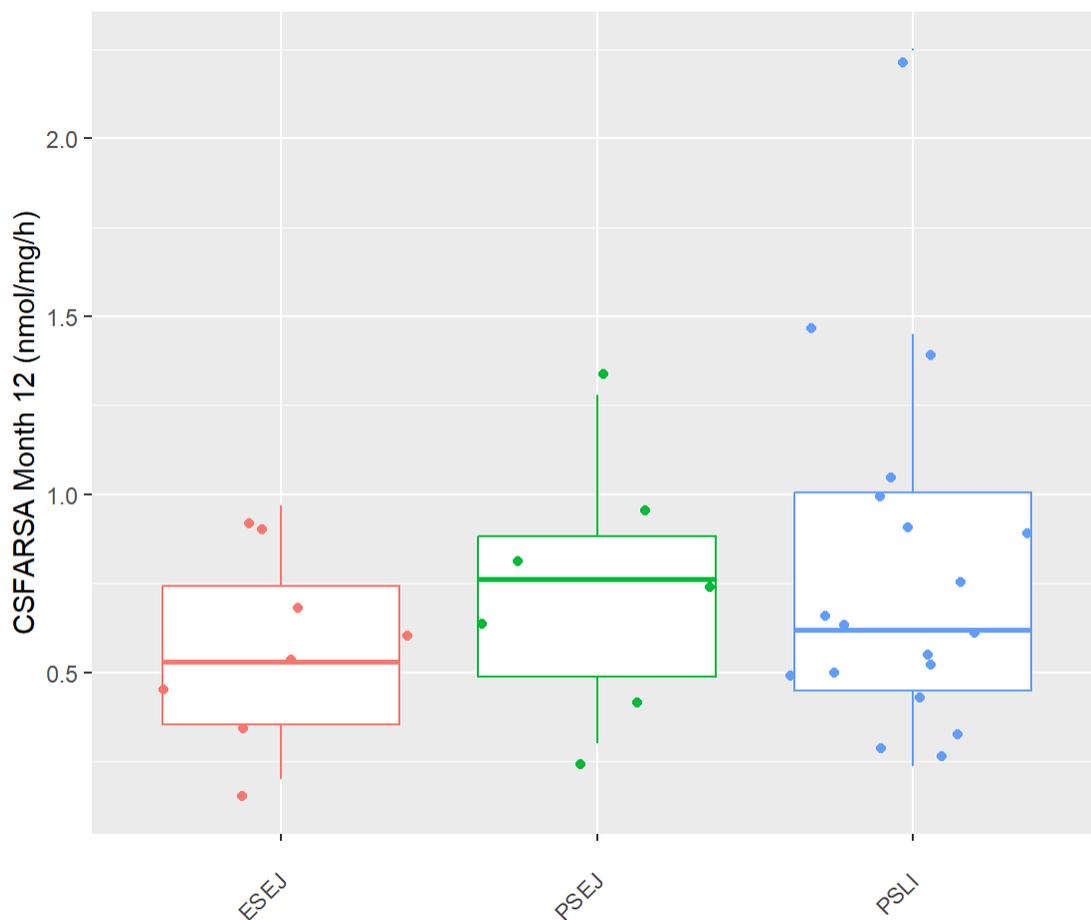
Note: The gray broken lines denote normal ARSA levels in PBMC samples obtained from subjects without MLD. ARSA data were log-transformed. Source: Reviewer analysis

Figure 6: Summary of Arylsulfatase A Activity in Total Peripheral Blood Mononuclear Cells (PBMC) at 12 Months Following LENMELDY Treatment



Source: FDA reviewer analysis

Figure 7: Arylsulfatase A Activity in Total Cerebrospinal Fluid (CSF) at 12 Months Following LENMELDY Treatment



Source: Reviewer analysis

6.2.3. Dose-Response and Correlative Assessments

The relationships between product characteristics, engraftment parameters, enzyme levels, and clinical outcomes were explored using scatter plots and correlation analysis.

6.2.3.1. Drug Product (DP) Characteristics vs. Pharmacodynamics

The following drug product characteristics were explored for correlative assessment:

- (b) (4) (CD34+ [%] and $\times 10^6/\text{kg}$), (b) (4)
- (b) (4)
- Transduction efficiency (TE [%]), VCN (/cell), ARSA (transduced).

No consistent correlations were observed between drug product characteristics and engraftment parameters (%LVV+ and VCN in total PBMC) or ARSA levels (in total PBMC or CSF) using pooled data from all treated subjects when evaluated at Month 6, Year 1, 2, and 5.

Reviewer comments: Considering several drug product characteristics, patient related factors and limited sample size for each MLD subtypes it is difficult to draw definitive conclusion from the correlative assessments between drug product vs pharmacodynamic parameters. Also, we don't agree with the pooled correlative analysis of the three MLD subtypes as the disease phenotype and age at treatment were different for the MLD subtypes. We conducted dose-response analysis focusing on ARSA activity in PBMC at Month 12 separately for the three MLD subtypes using Log transformed ARSA data for efficacy evaluable population. The results of the FDA dose-response analysis are summarized as follows:

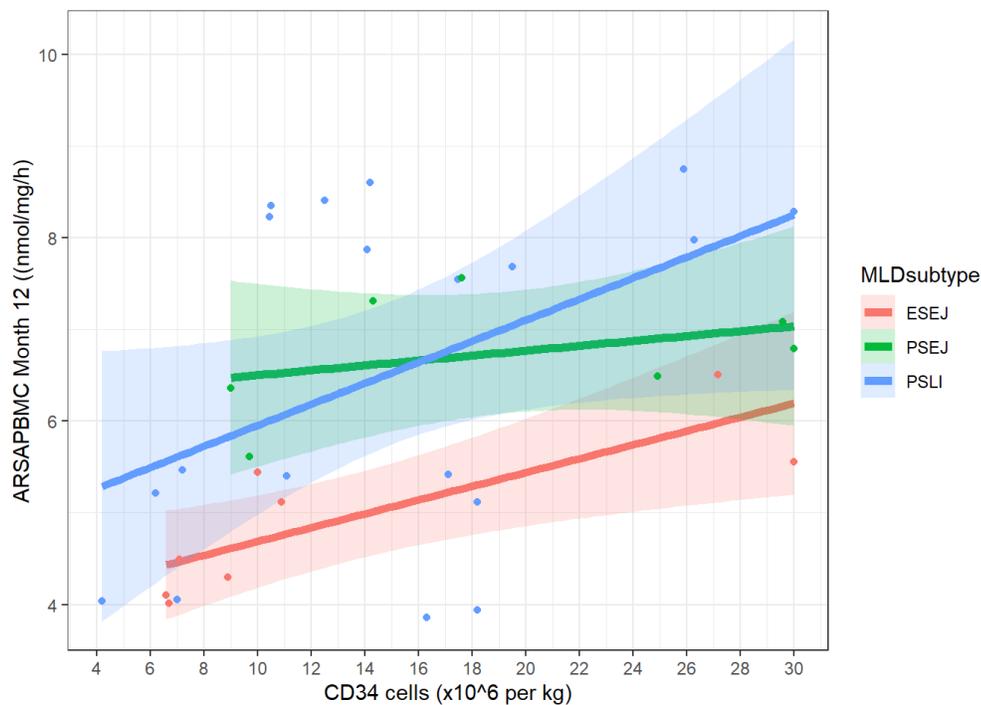
- The median [min, max) administered dose ($\times 10^6$ CD34+cells/kg) was 14.2 [4.2, 30] for PSLI (n=20), 17.6 [9, 30] for PSEJ (n=7) and 9.5 [6,30] for ESEJ (n=10).
- The median dose was 1.5-fold higher for PSLI vs ESEJ and 1.8-fold higher for PSEJ vs ESEJ.
- There was a trend for increasing ARSA activity in PBMC with increasing dose for PSLI ($R^2=0.2$, $p=0.05$) and ESEJ ($R^2=0.7$, $p=0.01$;
- Figure 8).
- It should be noted that confounding factors such as age at treatment may contribute to the observed variability of ARSA activity. As example, we evaluated the relationship between age and ARSA activity for the three MLD subtypes. As shown (Figure 9) age is negatively correlated with ARSA level for PSLI ($R^2=0.3$,

p=0.01) suggesting that treatment at early age with OTL-200 may result in a higher ARSA activity in PBMC. Although not significant a similar trend is seen for PSEJ and ESEJ.

Dose-response relationship was also explored using total administered dose (i.e. CD34 cell/kg multiplied by bodyweight). A trend for increasing ARSA activity with increasing total administered dose was noted for PSLI and ESEJ (Figure 10).

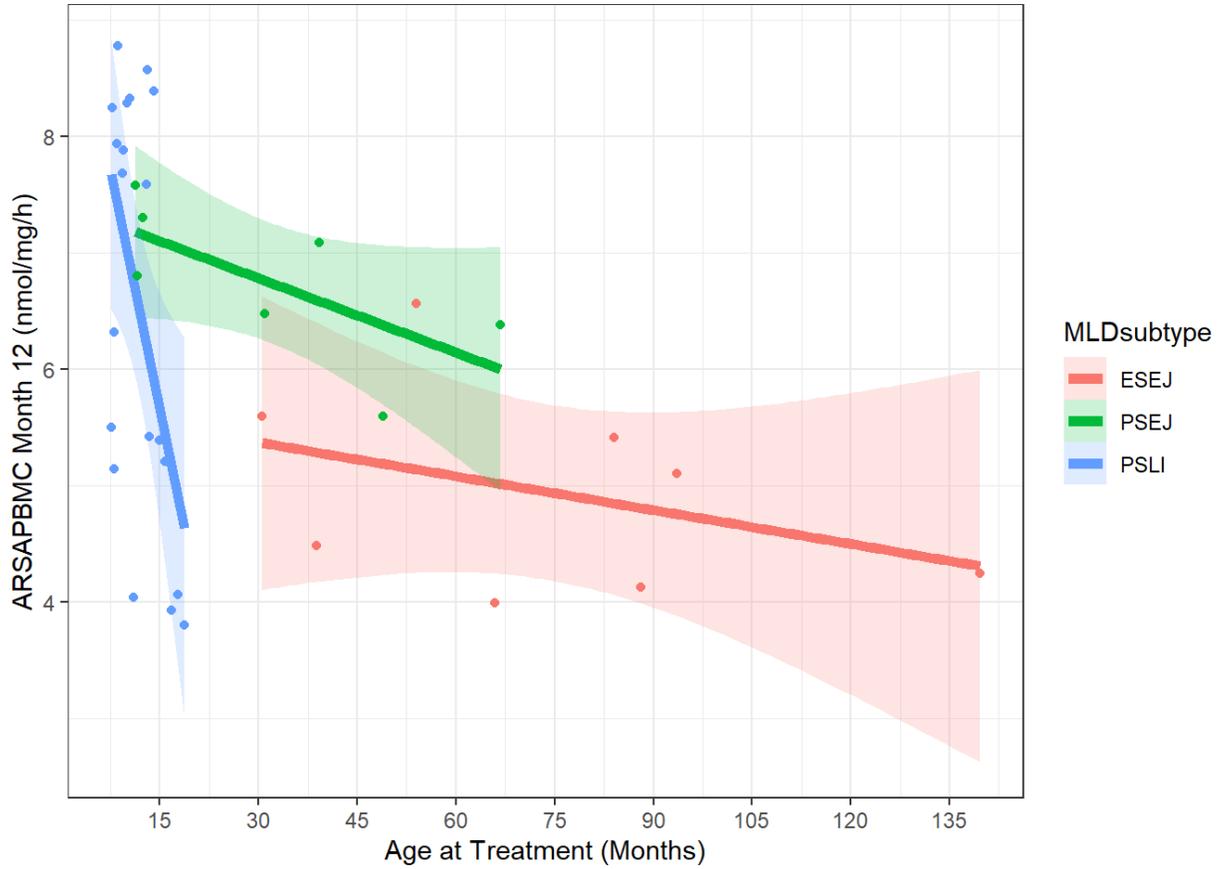
Overall, the dose-response relationship for ARSA suggests a more robust ARSA activity with higher administered total dose. Clinical efficacy assessment also identifies some qualitative relationship between ARSA activity and clinical outcomes. Given these dose-response relationship, this reviewer believes that there is insufficient information to extrapolate dose and efficacy outside of the current minimum age (8.2 months) and bodyweight (7.2 kg).

Figure 8: Summary of Dose-response analysis for ARSA activity in PBMC for MLD subtypes



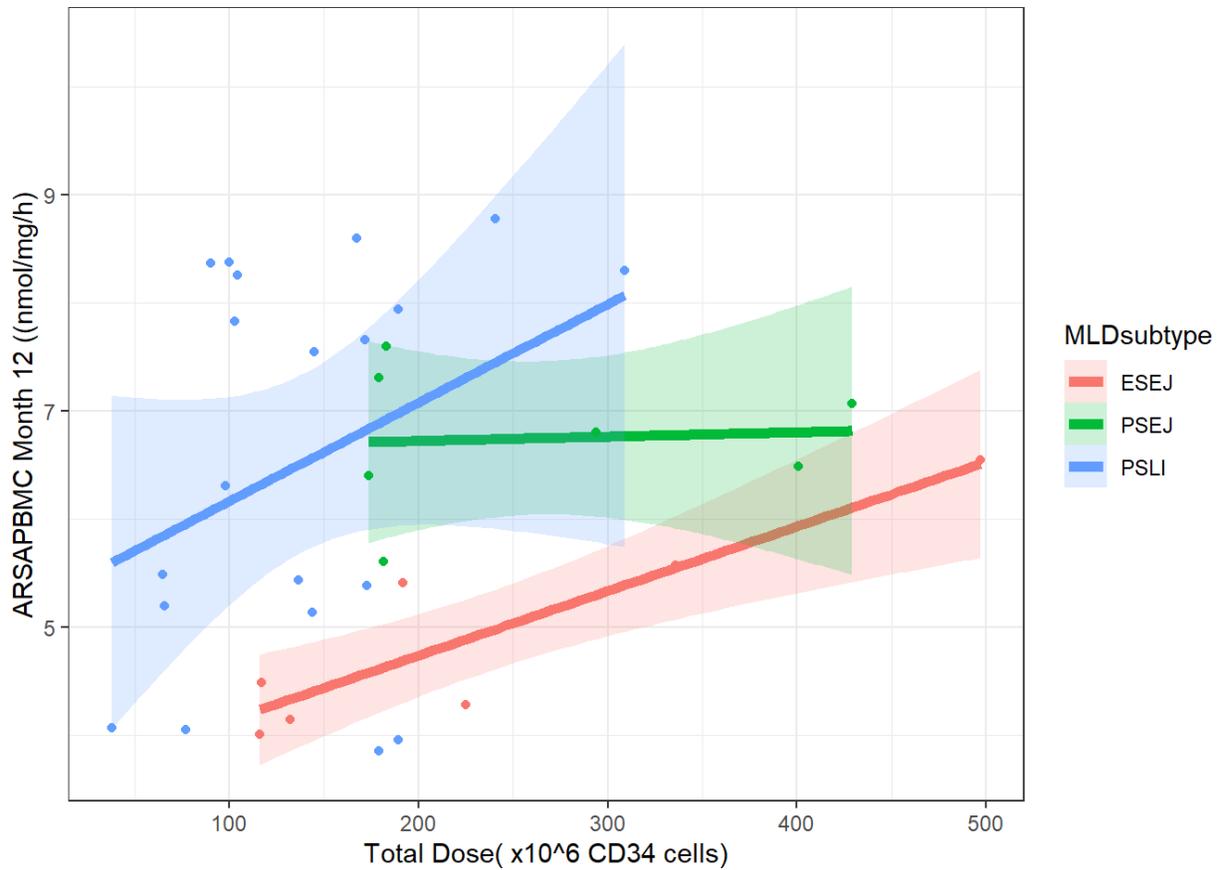
Note: ARSA data were log-transformed. Source: FDA reviewer analysis

Figure 9: Summary of Age at LENMELDY Treatment versus ARSA activity in PBMC for PSLI, PSEJ and ESEJ



Note: ARSA data were log-transformed. Source: FDA reviewer analysis

Figure 10: Summary of Total Administered Dose versus ARSA activity in PBMC for PSLI, PSEJ and ESEJ



Note: ARSA data were log-transformed. Source: FDA reviewer analysis

6.2.3.2. Pharmacodynamics vs. Clinical Outcomes

A correlation was observed between VCN in PBMC vs ARSA in PBMC a for PSLI ($R^2=0.7$, $p<0.001$) and ESEJ ($R^2=0.7$, $p=0.01$). A correlation was also observed between percent LVV and ARSA in total PBMC at Month 12 for PSLI ($R^2 =0.6$, $p<0.001$). No correlations were observed between engraftment parameters (%LVV+ and VCN in total PBMC) and ARSA level in CSF.

The following clinical outcomes were explored as part of correlative assessments:

- Gross Motor Function Classification for Metachromatic Leukodystrophy (GMFC-MLD) level,
- Gross Motor Function Measure (GMFM) total score,
- Brain MRI total score,
- Cognitive performance standard score.

No relevant correlations were observed between engraftment parameters (%LVV+ and VCN in total PBMC) and clinical outcomes (GMFC-MLD level, GMFM total score, brain MRI total score, performance standard score). Also, no relevant correlations were observed between ARSA levels (in PBMC and CSF) and clinical outcomes (GMFC-MLD level, GMFM total score, brain MRI total score, cognitive performance standard score).

Reviewer comments: It is difficult to establish a quantitative relationship between pharmacodynamic parameters and clinical outcomes due to the small sample size and a higher variability of the engraftment parameters and ARSA levels. For dose determination we looked at the relationship of clinical outcome (responding vs non-responding based on the FDA clinical assessment) vs dose or ARSA in PBMC at Month 6 and 12 (Table 8 & Table 9).

- In PSLI, all patients with adequate follow-up were responding with overall higher ARSA level at Month 6 & 12 across the wide dose range evaluated and the minimum effective dose was 4.2×10^6 CD34+ cells/kg (Table 8 & Table 9).
- For PSEJ, the ARSA level in one non-responding patient was higher than the average level in responding PSEJ patient (Table 7). The administered dose for this non-responding patient (i.e., 17.6×10^6 CD34+ cells/kg) was also higher than the minimum dose for responding PSEJ patient (i.e., 9×10^6 CD34+ cells/kg).
- For ESEJ, the ARSA level in non-responding patients were numerically lower than the value for responding patients (Table 9). The minimum effective dose for ESEJ was 6.6×10^6 CD34+ cells/kg.

Overall, based on the results of the dose-response relationship for ARSA in PBMC, and clinical outcome data, the clinical and clinical pharmacology team recommend

defining the minimum recommended dose based on the minimum dose that result in therapeutic effect for each MLD subtype. Accordingly, the recommended minimum dose for each MLD subtype is as follows:

- 4.2 x10⁶ CD34+ cells/kg for PSLI,
- 9 x10⁶ CD34+ cells/kg for PSEJ,
- 6.6 x10⁶ CD34+ cells/kg for ESEJ.

Table 8: Summary of Administered Dose in Responding vs Non-responding Patients.

	PSLI	PSEJ		ESEJ	
	Responder*	Responder	Non-Responder	Responder	Non-Responder
n	20	3	1	3	4
Mean	15.0 (6.9)	16.1 (11.7)	17.6 (NA)	9.2 (2.3)	7.7 (2.3)
Median	14.2	9.7	17.6	10	6.9
Min, Max	4.2, 30	9.0, 29.6	NA	6.6, 10.9	6.0, 11.1

*All PSLI patients with adequate follow-up were responding. NA-not applicable

Source: Reviewer analysis

Table 9: ARSA in PBMC in Responding vs Non-responding Patients.

	PSLI	PSEJ		ESEJ	
	Responder*	Responder	Non-Responder	Responder	Non-Responder
Month 6					
n	16	3	1	2	2
Mean	1196 (1060)	482 (470)	1377 (NA)	156 (149)	58 (69)
Median	1095	276	1377	51	58
Min, Max	37, 2716	150, 1020	NA	51, 261	9, 107
Month 12					
n	20	3	1	3	2
Mean	1983 (2103)	682 (463)	1976 (NA)	152 (84)	73 (25)
Median	1239	590	1976	169	73
Min, Max	46, 6467	272, 1184	NA	62, 227	55, 91

*All PSLI patients with adequate follow-up were responding. NA-not applicable

Source: Reviewer analysis

6.3. Immunogenicity Assessments

Treatment with LENMELDY can stimulate adaptive immune response to ARSA and produce anti-ARSA antibodies. An anti-ARSA (b) (4) antibody (b) (4) immunoassay was initially developed for screening subjects in # Study 201222. A second method was developed and validated for the determination of anti-ARSA antibodies in human plasma using an (b) (4) (b) (4) that followed a tiered testing scheme where positive screening is followed by confirmation of positivity and titration with defined cut-off points. At the time of the data cut-off for the BLA (01 Nov 2022), anti-ARSA antibodies had been detected in 6 subjects out of the 39 subjects (15%) treated with LENMELDY in the clinical development program. Five occurrences of anti-ARSA antibody formation occurred in PSLI patients with a 0/0 ARSA genotype, and one occurrence was in an PSEJ patient with a 0/UNK genotype. The proportion of subjects with the 0/0 variant in the Integrated Safety Set who developed anti-ARSA antibodies was 26%. No subjects tested positive for anti-ARSA antibodies at baseline. The six subjects who tested positive for anti-ARSA antibodies were:

- In the EAP, four PSLI patients tested positive for anti-ARSA antibodies when tested using the (b) (4) assay. Antibody titers in all 4 subjects were generally low and resolved after a dose of rituximab (n = 3) or spontaneously (n = 1). In all subjects with positive anti-ARSA antibody tests, there was no evidence of reduced ARSA activity in PBMC or CSF.
- In Study 205756, two subjects tested positive for anti-ARSA antibodies using the (b) (4) assay. Titers were reported at 1:400 in both subjects. One event resolved spontaneously (PSLI), and 1 event was ongoing (PSEJ) as of the data cut-off date; however, no specific treatment was recommended due to lack of clinical concerns by the investigator. In both subjects with positive anti-ARSA antibody tests, there was no evidence of reduced ARSA activity.

Reviewer comments: There is no indication of decreased ARSA activity in PBMC in PSLI subjects that developed anti-ARSA antibodies following administration of LENMELDY. The median [min, max] ARSA activity in PBMC (nmol/mg/h) at Month

12 was 2889 [171, 5348] and 239 [46, 6467] in PSLI with (n=5) and without (n=15) anti-ARSA antibodies, respectively. The median [min, max] ARSA activity in PBMC (nmol/mg/h) at Month 12 was 660 and 1034 [272, 1976] in PSEJ with (n=1) and without (n=6) anti-ARSA antibodies, respectively. The median ARSA level is higher in PSLI subjects that developed anti-ARSA antibodies, and the level falls within the range of subjects who did not develop anti-ARSA antibodies. One PSEJ subject was tested positive for AAA at Day 371 and 726 and the ARSA level was above the normal range but with a decreasing trend noted after Month 6.

6.4. Replication Competent Lentivirus

Replication competent lentivirus (RCL) was assessed via (b) (4) for (b) (4). No samples were tested positive for RCL based on the available clinical results at the time of BLA submission.

Reviewer comments: We recommend yearly monitoring for RCL for up to 15 years per FDA guidance for industry³.

³ Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up, Guidance for Industry: www.fda.gov/media/113790/download

7. Appendix

7.1. Study#1- Clinical Study 201222

Title: A Phase I/II Clinical Trial of Hematopoietic Stem Cell Gene Therapy for the Treatment of Metachromatic Leukodystrophy

Objectives:

The co-primary efficacy endpoints were:

- **Gross Motor Function Measure-88 (GMFM-88):** An improvement of > 10% of the GMFM-88 total score in treated subjects, when compared to the GMFM-88 total scores in the historical control MLD population, evaluated 2 years after treatment.
- **ARSA activity:** Change from Baseline in residual ARSA activity as compared to pre-treatment values, measured in total peripheral blood mononuclear cells (PBMCs) at 2 years after treatment.

Clinical pharmacology relevant secondary endpoints were:

- **Engraftment of transduced cells:** Transduced cell engraftment above 4% in BM-derived clonogenic progenitor cells at Year 1 after transplant. Vector copy number (VCN) per cell in total PBMC, total BM, and PB and BM cell subpopulations was also evaluated.
- **Correlations between transduced cell engraftment parameters and busulfan exposure:** Evaluations of correlations occurring between transduced cell engraftment parameters (ie, percentage of lentiviral vector-positive [%LVV+], VCN in total PBMC, and VCN in total BM) at Year 1 and busulfan exposure (ie, total AUC) during the conditioning phase.
- **ARSA activity in hematopoietic cells and other cell types:** Change from Baseline in residual ARSA activity as compared to pretreatment values, measured in total BM mononuclear cells (MNCs), and PB and BM subpopulations at 2 years after treatment. ARSA activity was also measured in cerebrospinal fluid (CSF) at multiple visits.

Methodology: Phase 1/2 study, a non-randomized, open-label, prospective, single-center study in children with LI MLD or EJ MLD treated with OTL-200 and followed for safety and efficacy endpoint measures post-treatment (NCT01560182). Initially, Study 201222 planned to enroll and treat 8 subjects (pre-symptomatic LI and pre- or early symptomatic EJ). The sample size and proportions of pre-symptomatic LI and pre-symptomatic or early symptomatic EJ subjects were revised multiple times during the course of the study in order to treat a total of 20 subjects. The Study 201222 comprises the following protocol phases:

1. Screening phase: The conditions required by the clinical protocol for subject enrollment were assessment and the study inclusion/exclusion criteria were evaluated.
2. Baseline phase (end of the Screening phase to the day before busulfan administration [ie, Day ^{(b) (4)}]): Clinical and instrumental evaluations were conducted to establish a subject's disease status and general clinical condition at the latest possible timepoint prior to treatment.
3. Treatment phase (Day -35 to Day 0): The Treatment phase started with the purification of a subject's stem cells from a BM harvest or from the mobilization of peripheral blood stem cell (PBSC) for the investigational DP manufacture, as well as unmanipulated back-up to be infused in the event of engraftment failure, technical issues during DP manufacture, or for additional DP manufacture. The Treatment and Baseline phases overlap, as the Treatment phase is inclusive of purification and mobilization procedures for back-up, and baseline assessments should be performed as close to administration of the investigational DP as possible. **Note:** all subjects in this study had back-up cell harvest on Day -35 and cell harvest for DP manufacture on Day ^{(b) (4)}. The Treatment phase also included busulfan conditioning (Day -4 to Day -1).
4. Follow-up phase: This comprises an initial follow-up period of 3 years after infusion of OTL-200 to evaluate study endpoints. During this phase, subjects are intended to reach the interim 2- and 3-year timepoints for assessment of efficacy and safety endpoints. Subjects continue to be followed under this protocol for 15 years post-treatment, with the option to roll-over into a long-term follow-up study under a separate protocol once available; this study will also follow patients for up to 15 years post-treatment.

Drug treatment:

OTL-200: The dose of OTL-200 was significantly influenced by the number of CD34+ HSPCs that the subject was able to donate as a starting material and, hence, the dose of OTL-200 was not fixed. Harvesting of $> 5 \times 10^6$ CD34+ cells/kg was recommended to achieve the minimum dose of 2×10^6 transduced CD34+ cells/kg (maximum 20×10^6 CD34+ cells/kg) for intravenous (IV) administration.

Busulfan:

1. Sub-myeloablative busulfan conditioning (SMAC) regimen with therapeutic drug monitoring was initially employed. The following busulfan dosage was used based on subject bodyweight:
 - <9 kg: 1 mg/kg/dose
 - 9 to <16 kg: 1.2 mg/kg/dose
 - 16 to 23 kg: 1.1 mg/kg/dose
 - >23 to 34 kg: 0.95 mg/kg/dose
 - >34 kg: 0.8 mg /kg/dose

Subjects received a total of 14 doses, as a 2-hour IV infusion administered every 6 hours from Day -4 to Day -1. Busulfan plasma levels were monitored by serial sampling following the first and the fifth or sixth (depending on pharmacokinetic [PK]-based dose adjustment requirements) administrations. The busulfan exposure, based on the individual concentration versus time curves, was calculated. The fifth and subsequent doses of busulfan were adjusted using the AUC, derived after the first dose. The dose adjustment was performed using a target AUC of $4800 \mu\text{g}\cdot\text{h}/\text{L}$ (range 4200 to $5600 \mu\text{g}\cdot\text{h}/\text{L}$), which corresponds to an expected total cumulative AUC of $67,200 \mu\text{g}\cdot\text{h}/\text{L}$ (range 58,800 to $78,400 \mu\text{g}\cdot\text{h}/\text{L}$). The additional adjustment at the ninth or tenth dose could be made, depending on the AUC value derived after the fifth or sixth dose.

2. Myeloablative conditioning (MAC)

In 2013, a protocol amendment (protocol version 6.0 [10 Dec 2013]) included a modification of the busulfan conditioning regimen to one that was myeloablative (MAC), designed to produce an approximately 10% higher cumulative busulfan area under the curve (AUC) with the objective of reducing the variability in transduced cell engraftment

observed in the first 9 treated subjects, who received the SMAC regimen. The MAC regimen according to subject's age is given below:

≤ 1 year of age: 80 mg/m²/dose

> 1 year of age: 120 mg/m²/dose

Under the modified MAC regimen, subjects received a total of 4 doses, administered as a 3-hour IV infusion every 20 to 24 hours from Day -4 to Day -1. Busulfan plasma levels were monitored by serial sampling following the first and the second administrations. The exposure to busulfan, based on the individual concentration versus time curves, was calculated. The second and subsequent doses were adjusted based on the AUC derived after the first dose. The adjustment was performed using a target total cumulative AUC of 85,000 $\mu\text{g}\cdot\text{h}/\text{L}$ (range 76,500 to 93,500 $\mu\text{g}\cdot\text{h}/\text{L}$). A further adjustment could be made at the third dose based on the AUC values derived from the second dose. Like the SMAC, administration of OTL-200 occurred at least 24 hours after the end of the last IV busulfan dose to allow for the safe administration of stem cells. Busulfan plasma levels were monitored just before the administration of OTL-200

Study Disposition: Among the 20 subjects treated with OTL-200, 9 subjects met the protocol-defined classification of LI MLD, and 10 subjects met the protocol-defined classification for EJ MLD. One subject was originally classified as PSLI, but subsequent information suggested that the subject and the subject's older sibling are affected by a clinical subtype of intermediate severity between the classical LI and EJ forms of MLD. For the applicant data analysis this subject has been included in the EJ subgroup. Among the LI subjects, the median age at the time of OTL-200 administration was 14.95 months (range 7.6 to 23.3 months).

Among the EJ subjects, the median age at the time of OTL-200 administration was 66.66 months (range 18.8 to 139.7 months) was 55.03 months (range 20.2 to 74.1 months). Two symptomatic EJ subjects discontinued study participation due to death at approximately 15 months and 8 months after treatment. Both deaths were attributed to rapid disease progression. The median duration of post-treatment follow-up was 9.52 years (range 6.52 to 11.03 years) in the treated LI subjects and 7.46 years (range 0.64 to 10.61 years) in treated EJ subjects. The median age at last contact or death was

10.8 years (range 7.19 to 12.15 years) in the LI subjects and 12.5 years (range 6.56 to 19.09) in the EJ subjects.

7.2. Study#2- Clinical Study 205756

Title: A Single Arm, Open-Label Clinical Study of Cryopreserved Autologous CD34+ Cells Transduced with Lentiviral Vector Containing Human ARSA cDNA (OTL-200), for the Treatment of Early-Onset Metachromatic Leukodystrophy

Objectives: The primary efficacy endpoint is GMFM-88 total score at 24 months post-treatment with OTL-200. Clinical pharmacology relevant secondary endpoints were:

Engraftment/Pharmacodynamics of the cryopreserved formulation of OTL-200, as measured by:

- Percent LVV+ clonogenic progenitors in BM at Day 30 post-treatment and at multiple visits over time
- VCN (in BM MNC) at Day 30 post-treatment and at multiple visits over time
- VCN (in PB MNC) at Day 60 post-treatment and at multiple visits over time
- ARSA activity in PBMC
- ARSA activity in PB CD15+ cells
- ARSA activity in PB CD14+ cells
- ARSA activity in CSF at Day 90 post-treatment and at multiple visits over time

Methodology: Preliminary data from the ongoing Study 201222 informed the design of Study 205756 in terms of subject population and efficacy and safety endpoints. Study 205756 is an open-label, single-arm study conducted in pre-symptomatic subjects with early-onset MLD (ie, either LI, EJ, or an intermediate subtype between LI/EJ) and early symptomatic subjects with the EJ MLD subtype. The stem cell harvest for DP manufacture occurred during the Baseline phase of the study on a date tailored to each subject's condition and clinical needs. Typically, the harvest occurred several weeks prior to reinfusion into the subject to support the cryopreservation step and to enable the commencement of product-specific release testing. All subjects treated with OTL-200 will be followed up for a minimum period of 8 years post-treatment within the study. Beyond 8 years, subjects will continue to be followed for 15 years post-treatment in a long-term follow up study, in-line with prevailing regulatory guidelines.

Study Disposition: This study enrolled pediatric subjects with:

- Pre-symptomatic MLD with the LI subtype
- Pre- or early symptomatic MLD with the EJ subtype

To date, 10 subjects have received treatment with OTL-200 in this study and are included in the Enrolled, Intent-to-Treat, and Safety populations. Four subjects met the protocol-defined classification for LI MLD; 6 subjects met the protocol-defined classification for EJ MLD. The median age at MLD diagnosis was 5.77 months (range: 0.4 to 10.7 months) for the LI

subjects and 26.10 months (range: 3.8 to 51.7 months) for the EJ subjects. The median predicted age at onset was 15.0 months (range: 13 to 19 months) for LI subjects and 53.0 months (range: 42 to 60 months) for EJ subjects. The median age at OTL-200 administration was 9.28 months (range: 7.8 to 13.0 months) and 30.65 months (range: 11.6 to 54.1 months) for LI and EJ subjects, respectively. Nine of the subjects (90%) had completed a visit at the primary endpoint (defined as completion of 24 months after treatment with OTL-200) as of the data-cut (Table 22), and all subjects have been followed up for at least 2 years with follow up continuing. Eight of 10 subjects (80%) treated with OTL-200 in this study have been followed up beyond their predicted age of symptom onset based on the index case in the family. The median follow-up among all 10 subjects was 2.6 years (range: 2.0 to 3.9 years).

Study Treatments:

OTL-200: The recommended dose range for cryopreserved OTL-200 was between 3 and 30 x 10⁶ CD34+ cells/kg. The actual dose given depended on the yield of cells available after transduction. Cryopreserved OTL-200 was administered via intravenous (IV) infusion as a single dose.

Busulfan: A MAC regimen using busulfan was employed for 4 consecutive days immediately prior to treatment as previously described in Study 201222.

Pharmacokinetic/Pharmacodynamic Analysis: As previously described in Study 201222.

7.3. Study #3: Expanded Access Program

The expanded access (EAP) programs were based on the study design and preliminary results obtained from Study 201222. The following studies were included as EAP:

1. Single Subject Compassionate Use Program (CUP 207394)

Title: Gene Therapy Protocol Using Autologous Hematopoietic Stem Cells for MLD-C02, A Patient with Metachromatic Leukodystrophy

The overall objective of the program was to provide a mechanism to supply OTL-200 on a compassionate use basis for the treatment of a single subject. The individualized program was designed to allow collection of efficacy and safety data under a comparable schedule of assessments and clinical procedures that had been employed in Study 201222.

2. Hospital Exemption (205029) and Compassionate Use Program (206258)

Title: Early Access Program for Hematopoietic Stem Cell Gene Therapy OTL-200 in Subjects with Early-Onset Metachromatic Leukodystrophy: (HE Protocol 205029 and CUP 206258).

The HE and CUP designs were based on the design of Study 201222, including safety and efficacy assessments. Three subjects were treated under an HE framework and 5 subjects were treated under CUP. Both the HE and CUP protocols specify subjects are to be followed for 8 years after treatment with OTL-200 to evaluate safety and efficacy. Each subject was to undergo regular follow-up for a period of 3 years after treatment with OTL-200 to assess the efficacy and safety of the treatment and then enter a long-term follow-up period until 8 years after OTL-200. After 8 years, subjects will continue to be followed for 15 years in the long-term follow up study, in line with prevailing regulatory guidelines.