MLD NEWBORN SCREENING RUSP APPLICATION

Name of the Targeted Condition: Metachromatic Leukodystrophy

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The Condition

1. What is the clinical case definition for the condition being proposed for newborn screening (i.e., the targeted condition)? Be specific about whether screening is for a particular phenotype and for a specific biochemical profile or genotype?

The primary target of the proposed newborn screening (NBS) is the severe, early-onset phenotype of metachromatic leukodystrophy (MLD), a rare autosomal recessive lysosomal storage disorder caused by biallelic pathogenic variants in the arylsulfatase A (*ARSA*) gene that result in deficiency of the encoded lysosomal ARSA enzyme (Gomez-Ospina, 2006). This includes the late infantile (LI) and early juvenile (EJ) subtypes.

Metachromatic leukodystrophy is classified based on the age of symptom onset into four subtypes (Gomez-Ospina, 2006; Eicher, 2022):

Overall Phenotype	Subtype	Symptom Onset	Percentage of Cases
Early-Onset	Late infantile (LI)	≤ 30 months	50-60%
	Early Juvenile (EJ)	30 months and 7 years	20-40%[a]
Late-Onset	Late Juvenile (LJ)	7 years and 16 years	
	Adult	≥ 17 years	10-20%

[a] *Percentage applies to both EJ and LJ cases combined*

The early-onset MLD phenotype includes the LI and EJ subtypes, and the late-onset MLD phenotype includes the LJ and adult subtypes. The majority of MLD patients have an early-onset phenotype.

In addition to age and type of symptom onset in the patient or an affected sibling, MLD phenotypes can often be characterized based on their *ARSA* genotype and the level of residual ARSA enzyme activity in leukocytes, especially if an assay with increased sensitivity is employed (Kehrer, 2021; Santhanakumaran, 2022).

Historically, the EJ and LJ subtypes of MLD have often been collectively referred to as "juvenile" MLD, but recent data describing the relationship between type of symptoms at onset and disease course strongly support the age-of-onset-based classification and the existence of distinct, clinically meaningful EJ and LJ subtypes (MacFaul, 1982; Kehrer, 2021; Fumagalli, 2021).

Approximately 300 pathogenic variants of the *ARSA* gene have been described (Cesani, 2016; ClinVar, 2024). Pathogenic variants of *ARSA* can be functionally divided into 2 broad groups differing in predicted severity: null (0) or "severe" variants associated with little or no enzyme activity, and R variants encoding for ARSA with higher levels of residual enzyme activity (Gomez-Ospina, 2006; Cesani, 2016; Santhanakumaran, 2022; Trinidad, 2023). The

most common null variant (0) is c.465+1G>A, affecting a splice donor site, and 2 of the most common residual variants (R) are c.1283C>T (p. Pro428Leu) and c.542T>G (p.Ile181Ser). The classification of less common variants in the *ARSA* gene as "0" or "R" can be determined from a combination of factors, including evidence from published literature and public databases on the severity of the phenotype caused by the variant in a homozygous or compound heterozygous state, in vitro expression studies, and whether the variant is a nonsense or frameshift mutation (which typically results in a null variant). Certain R variants may result in more severe phenotypes than other R variants, likely due to the relative level of residual ARSA activity produced by each variant in vivo (Cesani, 2016; Santhanakumaran, 2022; Trinidad, 2023). Consensus-based guidelines for using *ARSA* genotype to help predict MLD subtype have been published (Laugwitz, 2024).

Late Infantile MLD

Patients with LI MLD invariably carry 2 null *ARSA* variants (0/0 genotype) (Cesani, 2016; Santhanakumaran, 2022; Trinidad, 2023). LI MLD patients manifest first symptoms at or before 30 months of age and, within months, suffer from predictable and homogenous rapid disease progression to severe disability and eventual early death. Patients with LI MLD may show a relative delay or stagnation in motor milestone achievement, especially at the age of independent walking (Zlotogora, 1980; MacFaul, 1982; Bindu, 2005; Harrington, 2019; Adang, 2023; Adang, 2024b). Once symptoms appear, often as an abnormality in gait, there is invariably rapid psychomotor regression and loss of the motor, language, and cognitive skills previously acquired (van Rappard, 2015; Gieselmann, 2010; Kehrer, 2014). Severe peripheral neuropathy is an early and characteristic finding in LI MLD (MacFaul, 1982; van Rappard, 2015; Bindu, 2005; Beerepoot, 2019).

Early Juvenile MLD

EJ MLD is the second subtype of early-onset MLD. Patients who are affected by EJ MLD typically carry one null variant and one residual variant of *ARSA* (0/R genotype) and have symptom onset between the age of 30 months and 7 years. Rarely, patients with EJ MLD may have an R/R genotype (e.g., homozygous for the missense variant c.931G>A (p.Gly311Ser); Cesani, 2016; Pekgül, 2020; Mahdieh, 2021). Patients with EJ MLD may develop behavioral and cognitive deterioration at the same time or even slightly earlier than the invariable deterioration of motor function that comprises their initial symptoms (Gordon 1978; MacFaul, 1982; Kehrer, 2021).

Late-Onset MLD

Late-onset MLD includes the LJ subtype, with symptom onset occurring between 7 and 16 years of age, and the adult subtype, where first symptoms present after 17 years of age. Patients who are affected by late-onset MLD typically have an 0/R or R/R genotype and at least one such common genotype (homozygous c.1283C>T; p.Pro428Leu) is detected exclusively in late-onset MLD patients in association with relatively high residual ARSA values (Santhanakumaran, 2022; Trinidad, 2023).

First symptoms in patients with the LJ subtype are typically behavioral or cognitive issues and patients with adult MLD frequently present with cognitive decline, behavioral and psychiatric disturbances, ataxia, polyneuropathy, and epileptic seizures (Gomez-Ospina, 2006; Kehrer, 2021). Age of disease onset and disease progression are more variable in the late-onset subtype of MLD than in the early-onset LI and EJ subtypes (Elgün, 2019; Gomez-Ospina, 2006).

2. What is the estimated prevalence of the condition? This could be in the United States or outside of the United States. If information is not available about the prevalence in newborns, describe the prevalence at other ages.

MLD is pan-ethnic, with affected patients described in multiple populations. The birth prevalence of MLD (all subtypes) is estimated at approximately 1 per 100,000 live births (range 1 per 40,000 to 160,000) worldwide; birth prevalence is estimated as 1 in 8,000 among Arab groups in Israel, 1 in 2,500 in the western portion of the Navajo Nation, and may be even higher in the Native American population of southern Alaska (Bonkowsky, 2018; Söderholm, 2020; Heim, 1997; Poorthuis, 1999; Pinto, 2004; Holve, 2021; Pastor-Soler, 1995; Lugowska, 2011; Hult, 2014; Stellitano, 2016; Chang, 2024).

3. Without newborn screening, what is the typical age of diagnosis?

The majority of children with MLD are diagnosed when they are overtly symptomatic and beyond the window for intervention with a disease-modifying therapy. The initial signs and symptoms of MLD can be subtle and non-specific and can go unrecognized or misdiagnosed for months or years, (Harrington, 2019; Eichler, 2022). By the time a patient receives a diagnosis of MLD they may already be experiencing rapid disease progression or be on the brink of rapid decline. In one study of LI MLD patients (Harrington, 2019), the mean ages at symptom onset and at diagnosis were 1.5 ± 0.4 years and 2.6 ± 1.7 years, respectively, values that are similar to others reported for LI MLD patients in the literature (Artigalás, 2010; Mahmood 2010; Kehrer, 2011; Morton, 2022) and in untreated LI patient group analyzed as part of the atidarsagene autotemcel (arsa-cel) clinical development program (Orchard Therapeutics BLA data on file).

For juvenile MLD, the gap between age at initial onset of symptoms and age at MLD diagnosis can be even longer (Harrington, 2019; Morton, 2022). For the untreated EJ MLD patient group analyzed as part of the arsa-cel clinical development program, the median age at diagnosis was 4.4 years (range 2.6 to 7.6 years; Orchard Therapeutics BLA data on file).

With very rare exceptions, the only way that early-onset MLD patients are diagnosed while still in the presymptomatic state is by having an older, symptomatic sibling.

4. Without newborn screening, what is the process for establishing the condition after the development of clinical symptoms?

Guidelines for the definitive diagnosis of MLD are consistent in the United States and around the world and are based on a combination of medical history, examination, and laboratory findings, including biochemical and genetic testing (Wang, 2011; Gomez-Ospina, 2006).

A patient with clinical symptoms of progressive neurological dysfunction suggestive of MLD typically receives the following diagnostic tests to confirm the diagnosis:

- Leukocyte ARSA activity
- Urine sulfatides
- Molecular genetic testing to identify biallelic pathogenic *ARSA* variants

In addition, a brain MRI to document changes consistent with leukodystrophy and electroneurography (ENG) to document peripheral polyneuropathy may be performed. However, absence of brain MRI or ENG abnormalities does not rule out MLD (Beerepoot, 2019; Schoenmakers, 2022a; Laugwitz, 2024).

Decreased ARSA activity alone is not sufficient for the diagnosis of MLD because it may reflect the presence of ARSA pseudodeficiency in a healthy individual; though true MLD usually shows decreased activity as compared to pseudodeficiency. The pseudodeficiency alleles of the *ARSA* gene are variants that result in lower-than-average ARSA enzyme activity, but do not cause MLD either in a homozygous state or in a compound heterozygous state with a pathogenic *ARSA* variant (Laugwitz, 2022).

The presence of abnormally high levels of urine sulfatides unequivocally distinguishes patients with any subtype of MLD from those who carry a single pathogenic *ARSA* variant (carriers) or who have low ARSA activity due to pseudodeficiency (Wang, 2011; Laugwitz, 2022).

Other early signs or subclinical symptoms (strabismus, cranial nerve enhancement, gallbladder abnormalities) may also provide supportive evidence of MLD.

5. What treatment guidelines are available for individuals with the targeted condition diagnosed clinically?

Standard of Care for Early-Onset MLD

Arsa-cel is indicated for the treatment of children with pre-symptomatic late infantile (PSLI), presymptomatic early juvenile (PSEJ) or early symptomatic early juvenile (ESEJ) MLD. Arsa-cel is the only approved treatment for early-onset MLD. It was approved in the United

States in March 2024 under the trade name Lenmeldy™ and was previously approved in the European Union, the United Kingdom, Iceland, Liechtenstein, Switzerland, and Norway under the trade name Libmeldy®. The most favorable gross motor and cognitive outcomes with arsa-cel are observed in patients treated in the presymptomatic period (Fumagalli 2022; Lenmeldy USPI). Expert consensus guidelines strongly recommend treatment with arsa-cel as the standard of care for this group of early-onset MLD patients (Laugwitz, 2024; Adang, 2024a). Arsa-cel is not currently FDA approved for treatment of late-onset MLD.

Standard of Care for Late-Onset MLD

Allogeneic hematopoietic stem cell transplantation (HSCT) has been used for the treatment of MLD for the past three decades. There are multiple publications on this topic (Bredius, 2007; Martin, 2012; Bley, 2013; Boucher, 2015; van Rappard, 2016; Groeschel, 2016; Tan, 2019; Beschle, 2020), which has recently been reviewed (Armstrong, 2023).

Long-term results show that individuals with late juvenile and adult MLD benefit from HSCT if transplanted during the presymptomatic or early symptomatic stages of disease, with improved survival and a stabilization of cognitive and motor functions compared to untreated MLD patients. Uncertainties on the long-term outcomes of HSCT in LJ MLD still exist and data on adult MLD is relatively sparse.

Symptomatic management of MLD

For MLD patients who are diagnosed too late to be considered eligible for disease-modifying treatments, supportive and palliative care includes physical therapy and anti-spasticity medications to maintain mobility and manage complications of being bedridden, respiratory physiotherapy and management of pulmonary infections, dietary support and enteral nutrition in the advanced disease stage, anti-epileptic drugs to control seizures, pain management treatments, and family and psychological and psychiatric counseling.

Expert guidelines for the medical care of symptomatic leukodystrophy patients have been developed and published (Bonkowsky, 2021; Keller, 2021; Adang, 2017) and general considerations for the care of MLD patients have recently been summarized (Adang, 2024a; Gomez-Ospina, 2006).

6. Briefly describe the current outcomes and ages associated with clinical diagnosis (e.g., premature death, need for mechanical ventilation, neurologic impairment).

Please refer to our response to Question 1. As the disease progresses in patients with the LI subtype, they develop spasticity, seizures, and respiratory and feeding problems. Untreated patients with the LI subtype experience devastatingly severe motor and cognitive impairment between 2 and 4 years of age (Kehrer, 2011; Kehrer, 2014). Beyond this stage, some patients with the LI subtype may survive for many more years; however, their severely impaired functional state requires intensive supportive care by both healthcare providers and caregivers, including physical transfers and positioning, pain management, control of

seizures and spasticity, and responsibility for all their personal care, including toileting, washing, and feeding, before their ultimate death during childhood (Mahmood, 2010; Bonkowsky, 2021; Keller, 2021; Adang, 2017; Van Haren, 2015; Eichler, 2016; Harrington, 2019; Brown, 2018; Ammann-Schnell, 2021; Pang, 2021; Sevin, 2022; Kehrer, 2022; MLD PFDD, 2023).

The mean time between symptom onset and loss of independent walking in EJ patients (20 months) is about four times longer than that in LI patients (5 months) but once the ability to walk is lost, patients with the LI and EJ subtypes have a similarly steep decline in motor function (Kehrer, 2011; Kehrer, 2021). Initial disease progression in children with EJ MLD is slower and occurs at an older age than in patients with the LI subtype, however, subsequent disease progression and loss of skills in EJ MLD follows the same rapid, characteristic, predictable and devastating course and requires the same type of intensive supportive care and burden on the caregivers as described above for LI MLD patients.

7. Please describe any patient registries

A post-marketing, prospective, observational study to assess and characterize the risk of secondary malignancies and long-term safety following treatment with arsa-cel (Study OTL-200-12) is planned in the US. This study will enroll a minimum of 17 subjects. The enrolled patients will be followed for 15 years after product administration.

The major leukodystrophy centers in the United States have an established registry and natural history study for MLD as part of the Global Leukodystrophy Initiative Clinical Trial Network (GLIA-CTN, https://theglia.org/gliactn/about). There are currently over 10 academic sites across the United States contributing to this work and the study is open to all interested participants. GLIA-CTN has regular engagement with patient associations and industry partners. The US MLD sites are currently aligning to leverage existing infrastructure to capture the post-NBS population. The approach will be based on similar models which have been implemented for other NBS monitoring programs, including those with uncertain phenotypes or age of onset such as the one for X-ALD (https://aldnr.umn.edu/). Additionally, the MLD community is actively aware of other ongoing efforts for longitudinal follow-up after NBS by HRSA and CDC and are prepared to actively engage in these efforts.

The MLD initiative (MLDi) is an international MLD registry that utilizes multi-stakeholder collaboration to initiate and coordinate research projects on MLD with the objective of improving disease management of MLD (Schoenmakers, 2022b). Currently, experts from 15 centers are involved, including several in the US. The MLDi closely collaborates with patient associations, regulatory authorities and drug developers.

Screening

8. What approach is recommended for newborn screening? Please be specific regarding the type of sample and screening algorithm leads to diagnostic referral.

A two-tiered approach for NBS starting with LC-MS/MS analysis with an optional third molecular sequencing tier is recommended as follows:

1st tier = C16:0 sulfatide and C16:1-OH sulfatide in dried blood spots (DBS) using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

2nd tier = ARSA activity in DBS using LC-MS/MS.

3rd tier = *ARSA* gene sequencing (feasible in DBS).

Rationale for Testing Strategy:

ARSA is essential for the metabolism of sulfatides, and its deficiency in MLD results in accumulation of the undegraded substrate in the lysosomes of oligodendrocytes, microglia, certain neurons of the central nervous system (CNS), Schwann cells, and macrophages of the peripheral nervous system. The abnormal accumulation of sulfatides (seen in both DBS and urine) and resultant pathophysiology commence long before clinical symptoms become apparent.

Because of ARSA stability considerations and the difficulty of using DBS to distinguish patients with commonly occurring pseudodeficiency alleles from those with MLD based on measurement of ARSA activity alone, a two-tier algorithm was developed in DBS that assessed C16:0 sulfatide by LC-MS/MS as the primary, first-tier test. Assessment of ARSA enzyme activity was performed only when abnormally high C16:0 sulfatide levels were detected. The feasibility of this algorithm was demonstrated through the first population study performed on 27,335 de-identified newborn DBS (Hong, 2020a; Hong, 2021; also see Question 9).

The screening algorithm has subsequently been improved by adding measurement of 16:1-OH sulfatide to the first-tier test (Wu, 2024; Bekri, 2024), and it has been incorporated in the prospective, population based MLD NBS study in Germany described below in the response to Question 9.

Additional Information regarding Testing Methodology:

Methods to quantify C16:0 sulfatide and C16:1-OH sulfatides in DBS using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and to measure ARSA activity in DBS using LC-MS/MS were developed in the Gelb lab (Hong, 2020a; Hong, 2021). Revvity, Inc. and GelbChem, LLC have recently made the internal standards (now commercially available) to measure C16:0 sulfatide and C16:1-OH sulfatide. The reagents to measure

ARSA activity using LC-MS/MS are available from GelbChem. Revvity, Inc. currently provides Quality Control (QC) DBS for sulfatide measurement, and GelbChem provides QC DBS for ARSA enzymatic activity. The CDC has started to make QC materials for MLD NBS.

The ARSA enzyme assay is currently being validated by Mayo Medical Laboratories. Additionally, *ARSA* gene sequencing has been shown to be feasible in DBS (see Question 9).

These methods can be applied to high-throughput, tiered NBS for MLD that is compatible with methods and equipment used for other lysosomal storage diseases in NBS programs worldwide. Importantly, measurement of C16:0 and C16:1-OH sulfatides can be carried out via a multiplexed assay with the same LC-MS/MS assay as used for all lysosomal storage disorders on the RUSP (Hong, 2020b).

9. What information is available from studies of screening for the targeted condition? Outcomes could be one or more screening test characteristics (e.g., sensitivity, specificity, positive predictive value, negative predictive value), birth prevalence of the targeted condition, or rates of detection of non-targeted phenotypes of conditions. If possible, focus on prospective studies of screening. Do not include studies of only anonymized dried blood spots without follow-up of human subjects.

A manuscript describing the NBS and treatment experience from the world's first prospective NBS pilot study for MLD has been submitted for publication (Laugwitz, 2024 manuscript in review). The study was initiated in the Hanover region of Germany and used the treatment center in Tübingen.

The aim of the study was to evaluate the technical feasibility of MLD NBS and to implement a comprehensive care pathway providing prompt confirmatory diagnostics, clinical follow-up, and treatment. DBS samples from 109,259 newborns were analyzed in a three-tiered screening program, including sulfatide levels, ARSA enzyme activity, and genetic sequencing (see Appendix 1).

Three newborns were identified as screen positives, and a diagnosis of pre-symptomatic MLD was confirmed in all three newborns. Early treatment with arsa-cel was initiated in two of the three newborns based on the prediction of early-onset MLD (early juvenile subtype). One patient was predicted to have late-onset disease and is being monitored regularly ahead of planned allogeneic hematopoietic stem cell therapy. Since the manuscript was written, one additional newborn was confirmed to have early-onset MLD (late infantile subtype) (Oliva, 2024), and arsa-cel treatment has been initiated. No false-positive cases were identified in this pilot program using the three-tier algorithm.

Sulfatide levels were quantified in the first-tier analysis. Samples with elevated sulfatide levels underwent ARSA enzyme testing as second tier when it was available, and all underwent DNA sequencing as third tier. A positive screening result was defined by elevated sulfatides in combination with low ARSA activity and two homozygous or heterozygous clinically relevant variants in the *ARSA* gene.

First tier: Sulfatide screening in DBS

First-tier sulfatide screening used the MS/MS high throughput screening method described previously (Hong, 2020a; Hong, 2021; Bekri, 2024). Three hundred eighty-one of the 109,259 DBS samples had elevated C16:0 and/or C16:1-OH using defined cut-off values.

Second tier: ARSA enzyme activity testing in DBS

For the second tier, ARSA enzyme activity was measured in the DBS using the LC-MS/MS method described previously (Hong, 2020a; Wu, 2024) using a cut-off of ≤0.015 μmol/L/h. Due to early technical challenges that were subsequently resolved, 230 of the 381 samples were analyzed for ARSA activity. Twenty of the 230 samples had enzyme activity below the cut-off. The three screen positive cases were in this group of 20.

Third tier: DNA sequencing in DBS

Genomic DNA was isolated from the 381 DBS samples that were positive in the first tier sulfatide analysis. Next generation sequencing (NGS) was performed for *ARSA*, *SUMF1*, and *PSAP*. Biallelic pathogenic variants in *SUMF1* and *PSAP* cause two biochemically similar disorders, multiple sulfatase deficiency (MSD, OMIM #272200) and prosaposin B deficiency (OMIM #249900). *SUMF1* and *PSAP* were included to examine potential differential diagnoses in patients with elevated sulfatides (Gomez-Ospina, 2006).

Overall performance of the NBS tests.

1. First-Tier Sulfatides Followed by Second-Tier DNA sequencing. Of the 109,259 newborns tested, 381 had 16:0 sulfatide or 16:1-OH sulfatide levels above the cutoff. DNA sequence analysis of all 381 confirmed the 3 cases of MLD. The remaining 378 showed no indication of MLD, including a lack of inconclusive genotypes due to the presence of one or more variants of uncertain significance (VUS). Six of 381 had one pathogenic *ARSA* variant, which suggests an MLD carrier status and were not deemed screen positive results. Thus, the false positive rate was zero using this two-tier algorithm in the NBS pilot study.

Four samples with one clinically relevant variant in *PSAP* and three samples with one clinically relevant variant in *SUMF1* were also detected.

2. First-Tier Sulfatides Followed by Second-Tier ARSA enzyme activity then Third-Tier DNA sequencing. Two hundred and seventy of the 381 high sulfatide containing DBS were submitted to ARSA enzyme activity testing (second-tier). Twenty specimens had ARSA enzyme activity below the cutoff. After DNA sequence analysis (third tier), the three MLD cases were identified. The false positive rate was zero in this three-tier analysis. Second-tier ARSA enzyme activity testing is cheaper and easier to do compared to DNA sequencing; thus, it is recommended to use the 2-tier strategy of sulfatides followed by ARSA enzyme analysis with an optional third molecular tier.

The investigators who carried out this prospective pilot study have not been made aware of any reported cases of MLD that were missed by the pilot screening. Given the timeline of

the pilot, missed cases may still be possible, but no missed diagnoses have been suspected in the screened population to date.

Note: Programs utilizing a second tier ARSA enzyme test will not detect prosaposin B deficiency because ARSA enzyme activity should be normal in these cases. However, it is thought that propsaposin B-deficient MLD is extremely rare relative to ARSA-deficient MLD (Cesani, 2016). Individuals with multiple sulfatase deficiency should have reduced ARSA enzyme activity and reduced iduronate 2-sulfatase enzyme activity (measured in NBS for MPS II). Low values for both enzymes would trigger a suspicion of multiple sulfatase deficiency rather than MLD. Multiple sulfatase deficiency is thought to be much rarer than MLD.

Birth Prevalence Results of the pilot MLD NBS program

The observed birth prevalence of MLD in this study (3/109,259 = 1/36,420) is consistent with the birth prevalences reported previously for MLD in Europe (Chang, 2024).

Updated data and other MLD NBS studies

As of June 2024, DBS from approximately 158,000 newborns from the Hanover region of Germany and approximately 35,000 newborns from Vienna, Austria have been tested (Olivia, 2024). Other prospective pilots in Europe using the same algorithm include the LysoNeo study in Rouen, France and a study in Florence, Italy. These studies have not been published, but principal investigators are available for further information.

In the United States, MLD is among the 14 disorders included in the ScreenPlus prospective pilot NBS study in the New York State NBS program (Kelly, 2024). This study is designed to evaluate the feasibility, efficacy, and accuracy of screening assays, as well as the birth prevalence of these treatable diseases. As of June 2024, there have been approximately 20,000 infants screened. No screen-positive cases of MLD have been detected; but feasibility of screening for MLD in a US-based high-throughput NBS program has been demonstrated.

10. What other conditions could be identified by screening for the targeted condition as nominated? This includes phenotypes of the condition that are not nominated for newborn screening (e.g., late-onset disease, mild variants). Will screening for the targeted condition identify carriers? What is the recommended or expected follow-up for these non-targeted conditions?

As described above, the established MLD NBS approach may identify patients with late-onset MLD, unaffected carriers of MLD, and has the potential to identify individuals with MSD or prosaposin B deficiency, depending upon the use of 2nd-tier ARSA activity and/or third-tier genetic sequencing. However, MSD and prosaposin B deficiency are extremely rare, so detection of these cases would be infrequent.

NBS programs may choose to only consider a result as screen-positive if the combination of sulfatide level (elevated), ARSA activity (low), and molecular analysis (two pathogenic, likely pathogenic, or VUS *ARSA* variants) are suggestive of MLD. Programs may choose to also provide molecular analysis for *SUMF1* and/or *PSAP* as desired by the program and/or their clinical specialists.

Recommended evaluation and subsequent treatment or follow-up of all MLD screen positive patients and potential differential diagnoses has been described (Laugwitz 2024; Laugwitz manuscript in review; also see replies to Questions 11 and 12 below). Likewise, diagnosis and management of patients with MSD and prosaposin B deficiency have also been described (Ahrens-Nicklas, 2018, Schlowata, 2019, Kolnikova, 2019)

11. What is the process for confirming the condition after newborn screening? Please also describe if there are non-targeted phenotypes or non-targeted conditions including later-onset conditions that would be identified during the diagnostic evaluation.

Biochemical and genetic testing to confirm the diagnosis of MLD (ARSA activity in leukocytes, urine sulfatides, and *ARSA* genotyping (of the proband and biologic parents) is well-established. ARSA activity in leukocytes and *ARSA* genotype are expected to predict the age of overt disease onset and disease progression for most patients with a positive screen for MLD, including those with the early-onset subtypes of MLD (Santhanakumaran, 2022; Trinidad, 2023). This will guide appropriate counseling and prompt treatment decisions.

A panel of MLD experts has developed disease-specific guidelines based on healthcare resources in the United States with best-practice recommendations in NBS, diagnosis, early treatment, and clinical management of all subtypes (including situations where subtype is uncertain) of MLD and potential differentials (MSD and prosaposin B deficiency) from screening (Adang, 2024a; see also answers to Questions 10 and 12).

A detailed description of the methodology used to predict the MLD subtypes of the three screen positive patients in the prospective Hannover NBS study is available (Laugwitz 2024; Laugwitz manuscript in review).

12. What treatment guidelines are available for individuals who are diagnosed through newborn screening or who are known to have the condition for other reasons (e.g., prenatal diagnosis, affected sibling) but are presymptomatic?

Consensus treatment guidelines for children diagnosed with MLD presymptomatically and/or through NBS have been published and represent both international and US-based expertise (Laugwitz, 2024; Adang, 2024a).

Timing of arsa-cel treatment for early-onset MLD

Arsa-cel consists of autologous CD34+ cells transduced ex vivo with a lentiviral vector encoding the human *ARSA* cDNA with constitutive expression driven by a human PGK promoter, infused intravenously after busulfan conditioning.

Recent expert consensus guidelines (Laugwitz, 2024; Adang 2024a) strongly recommend arsa-cel as the treatment for any presymptomatic individual with a predicted LI or EJ subtype. Therefore, apheresis for collection of CD34+ hematopoietic stem cells for manufacturing of arsa-cel should be initiated for individuals with LI onset around 5 to 9 months of age. Currently the lowest feasible body weight for apheresis is 5 kg, but most centers prefer a higher apheresis weight. Apheresis appointments for individuals with predicted EJ onset should be arranged between 9 to 12 months of age, when body weight is at least 8 kg.

Timing of allogeneic HSCT for late-onset MLD

Recent expert consensus guidelines (Laugwitz, 2024; Adang 2024a) suggest that any individual with predicted late-onset MLD who has been identified by NBS be carefully monitored, with HSCT scheduled as soon as there is subclinical evidence for disease onset. The panel discussion and rationale for the timing of LJ and adult treatment to be decided on a case-by-case basis rather than at a predefined age has been summarized (Laugwitz 2024).

Patients with predicted LI MLD who have been identified by NBS should not be treated with allogeneic HSCT and, assuming arsa-cel is available, neither should those with predicted EJ onset (Laugwitz 2024)

Treatment guidelines for children diagnosed with presymptomatic MLD are also covered in the response to Question 5 above.

Impact of Screening

13. What is the expected benefit to infants from detection through newborn screening compared with clinical identification?

Both arsa-cel and allogeneic HSCT have limited or no effectiveness in MLD patients who are symptomatic or who have entered the rapidly progressive phase of their disease (Fumagalli, 2022; Boucher 2015; Groeschel 2016). This is because the mechanism of action of both treatments requires sufficient time for hematopoietic cells to engraft and for their progeny to migrate and produce ARSA in the nervous system, a process that is insufficient to counteract the speed at which irreversible neurophysiological deterioration occurs in such patients.

Disease progression in untreated patients with the LI and EJ forms of MLD is highly predictable, with fast deterioration in motor and cognitive function occurring within

months after patients enter the rapidly progressive phase of the disease (Kehrer, 2011; Kehrer, 2021). As with other pediatric neurodegenerative diseases, treatment of early-onset MLD patients with arsa-cel as early as possible is expected to provide the greatest clinical benefit, given the narrow window to intervene before serious progression occurs. The importance of early diagnosis and urgent treatment of MLD was repeatedly noted by experts at the MLD Scientific Workshop in 2022 (MLD Scientific Workshop Summary, 2023) and the vital role of NBS in this paradigm is stressed in recent expert consensus guidelines (Laugwitz, 2024; Adang 2024a).

Results from the arsa-cel clinical program demonstrate the benefit of early treatment of LI and EJ MLD, with the best outcomes observed in subjects treated prior to symptom onset (Fumagalli, 2022; Fumagalli, 2024; Lenmedly USPI). The benefit of pre-symptomatic treatment is particularly apparent in comparing the difference in outcomes between EJ subjects treated with arsa-cel prior to symptom onset (PSEJ) and EJ subjects treated in the early symptomatic stages of the disease (ESEJ). The data indicate the benefit of treating subjects with arsa-cel as soon as possible before the onset of symptoms. While arsa-cel-treated ESEJ subjects did benefit from treatment and experienced stabilization or slower decline in motor function compared to untreated natural history subjects, every surviving PSEJ subject had normal motor function with GMFC-MLD Level 0 at last follow up. In addition, 4 of those 7 subjects have surpassed the median age (6.4 years) at which untreated EJ subjects enter GMFC-MLD Level 5, unable to move or sit without assistance. In pre-symptomatic subjects, 7 of 8 LI subjects and 3 of 4 EJ subjects retained the ability to walk (GMFC-MLD Level ≤ 2), and among these subjects, 4 PSLI subjects and 3 PSEJ subjects were able to walk without support (GMFC-MLD Level 0 or 1) at the time of the last recorded score before data cut-off. This is in contrast to outcomes in untreated subjects who followed a trajectory of rapid deterioration in gross motor function that was in-line with that described by Kehrer and colleagues (Kehrer, 2011; Kehrer, 2021). These findings demonstrate the ability of arsa-cel to stabilize or prevent the loss of gross motor function in subjects with early-onset MLD who are treated before the onset of symptoms.

As described in Question 2, MLD is more common in certain populations, but leukodystrophies, in general, are less likely to be diagnosed in these and other minority populations (Bonkowsky, 2018). As a result, the implementation of NBS for MLD in areas where these populations are found will serve to address the issues of inequities in diagnosis and subsequent eligibility for treatment.

14. What is the expected benefit to families from detection through newborn screening compared with clinical identification?

Detection of MLD through NBS will ensure that all patients can have disease-modifying treatment initiated when they are in a presymptomatic state, regardless of their phenotype. As summarized above, this is the optimal time to receive such treatments. Currently the majority of MLD patients who are diagnosed when they are presymptomatic

are only recognized as a result of an affected sibling. With NBS, parents will have the opportunity to consider family planning.

Furthermore, diagnosis of MLD in its presymptomatic stage through NBS will prevent families from having to endure a prolonged diagnostic odyssey, the long and difficult process of getting the correct diagnosis for a rare disease, which can take months or years, involve multiple doctors and specialists, and cause significant physical, emotional, and financial hardship. In the case of progressively symptomatic MLD patients, the correct diagnosis will typically be accompanied by the families being told that their child is too severely affected to be eligible for disease-modifying therapy.

A recent survey of 20 MLD families in the UK and Republic of Ireland, most of whom had children with early-onset MLD, revealed a high degree of support for NBS, with 95% of caregivers describing it as very or extremely important and 86% believing that detection of MLD at birth would have changed their child's future (Morton, 2022).

The urgent need for MLD NBS to ensure that patients can be diagnosed and treated before symptom onset is further highlighted in a recent retrospective cohort study describing the real-world experience of the Royal Manchester Children's Hospital, the only specialized arsa-cel treatment center in the United Kingdom, (Horgan, 2023). The study describes 17 MLD patients who were referred for treatment with arsa-cel after it was approved. The vast majority of referred children were deemed ineligible due to advanced disease. Four patients met eligibility criteria and have been treated. Three out of four treated patients were diagnosed after MLD was diagnosed in a symptomatic older sibling. Eleven patients were not eligible for treatment, of whom 10 were symptomatic patients with LI MLD and one was a symptomatic EJ MLD patient with cognitive decline.

15. Are there known harms to infants or to families from detection through newborn screening compared with clinical identification?

There have been no published studies illustrating harms to families as a result of NBS for MLD. Per Goldenberg 2016, a harm in NBS is defined as "any adverse impact (i.e. event, risk, or burden) resulting from screening or related follow-up with respect to the well-being of a newborn or the psychosocial health of the family and can occur at any point within the stages of screening." This group goes on to highlight several potential harms within the context of NBS and these are addressed further below as they directly pertain to MLD NBS:

False positive or false negatives of screening

At this time, little research is available to show lasting psychological impacts of false positive results after NBS. Indeed, studies published to date suggest that psychological impacts are transient, and these have less to do with the result itself and more to do with the context in which the result is communicated and whether appropriate supports are available to the family at the time of notification (Hayeems, 2016; Chudleigh, 2020;

Chakraborty, 2021). This suggests that effects of false positive results can be mitigated by improved notification and communication strategies from NBS programs.

As presented above in Questions 8 and 9, both retrospective and prospective analysis of the MLD screening algorithm has resulted in high screening performance and extremely low false positive findings, surpassing performance metrics seen in many current NBS conditions today. These studies have already provided insights into enhanced screening algorithms (i.e., utilization of C16:1-OH species and the second-tier ARSA assay) which US programs can employ from the outset. Thus, it is not anticipated that significant harms in terms of false positive and/or negative results will be an outcome of screening for MLD.

The rate of false negatives for NBS for MLD cannot be stated with certainty, but the following results argue that the false negative rate is expected to be extremely low. Using the first-tier NBS method where both 16:0 and 16:1-OH sulfatide are above the cutoff, 40 out of 40 newborn DBS from patients that received a clinical diagnosis if MLD would test positive after this first-tier analysis (Bekri, 2024). These newborn samples were obtained from NBS labs that store residual DBS.

Uncertain diagnoses

Uncertainty in healthcare, and especially in rare genetic diseases, is a well-documented experience even in the absence of NBS (Biesecker, 2008). Uncertainty as a specific result from NBS and how this differs from the uncertainty stemming in general from complex diseases is difficult and less studied.

Like the potential harms generated from false positive results, there is little work assessing lasting harms from prognostic uncertainty coming from NBS. However, studies in this arena have invariably come to similar conclusions - that more supportive and accurate approaches to communication along with the provision of well-crafted resources can help lessen negative impacts of uncertainty and move patients and families to adaptation (Biesecker, 2008; Johnson, 2019; Raspa, 2024).

The study conducted by Azzopardi, et al in 2020 suggested that healthcare providers need a consistent approach and guidelines to case management where uncertainty exists and strengthened networks between clinical centers in order to best meet patient and caregiver needs and help clinicians navigate uncertainty.

To address the potential for uncertainty, a considerable amount of work has been done in MLD to delineate genotype-phenotype relationships and to provide guidance in the prediction of phenotypic subtype (see Question 1 and 11). Published management guidelines are available to assist in the monitoring of patients where subtype remains uncertain (Laugwitz, 2024; Adang, 2024a).

Taking into account these studies, the MLD Community is also working with experts on the development of information targeted to families with screen-positive results and the providers managing these patients in order to help ensure that families are provided

appropriate and supportive information throughout their diagnostic and therapeutic journeys.

Disparities in access to diagnostic testing and treatment

The community recognizes the financial impact of Lenmeldy™ and that new economic financing models are needed in the US to address new one-time therapies. However, current costs of treatments for NBS conditions over the lifetime of patients easily reach similar financial impacts.

Lenmeldy™ will be available to eligible patients through a network of Qualified Treatment Centers (QTCs) in key regions throughout the United States in an effort to reduce travel required by families. Five treatment centers with specialized expertise in HSCT and the treatment of neurometabolic diseases such as MLD are being activated. These centers include the following sites: M Health Fairview Masonic Children's Hospital in Minnesota, Children's Healthcare of Atlanta, Children's Hospital of Philadelphia, Texas Children's Hospital, and UCSF Benioff Children's Hospital San Francisco.

Follow-up of patients who receive this treatment will be conducted by specialists at the QTCs and by the patients' local health care providers.

Other Considerations

16. Please share any information that is not captured above but important for the ACHDNC to understand. This may be left blank.

Use of LC-MS/MS: While, historically, NBS laboratories in the US have used flow-injection MS/MS, the addition of several recent diseases (X-ALD, MPS II) to the RUSP has necessitated a move to LC-MS/MS (Gelb, 2022).

Additional studies of *ARSA* Genotype/Phenotype Relationship. A challenge that comes with the interpretation of genotypes is the presence of variants of uncertain significance (VUS). Recently, ~250 VUS found in the gnomAD human genome database (~240,000 genomes) were biochemically annotated by expression of *ARSA* in human cells to determine residual enzymatic activity. This study (Trinidad, 2024) shows that ~20% of the VUS in the *ARSA* gene are predicted to be 0 type and will likely contribute to an MLD phenotype. This work is expected to help predict the phenotypic subtype of screen positive infants with novel *ARSA* genotypes.

Initial retrospective analysis. The prospective NBS pilot studies were preceded by a large scale population MLD NBS study at the University of Washington in collaboration with the Washington NBS laboratory that utilized de-identified DBS (Hong, 2021). Approximately 29,000 randomly selected DBS were tested. Approximately 200 had elevated C16:0-sulfatide levels. The cutoff was based on sulfatide levels in newborn DBS from 17 patients who received a clinical diagnosis of MLD after birth. Two hundred DBS were submitted for

second-tier ARSA activity analysis and two specimens had low ARSA activity (8% and 0% of normal activity). Third-tier sequencing of the *ARSA* gene showed that the 8% case was a heterozygote (carrier) and would have been a false positive. The 0% case had two known pathogenic *ARSA* variants. This study indicated that high precision NBS for MLD is possible.

More recent retrospective analysis. Retrospective analysis of data from four MLD NBS pilots [Hanover (Germany), University of Washington (USA), Manchester Centre for Genomic Medicine (UK), and Rouen University Hospital (France)] indicated that the number of second-tier ARSA enzyme tests could be reduced about 10-fold if the first-tier algorithm was changed so that only those DBS with 16:0 sulfatide and 16:1-OH sulfatide above their respective cutoffs are reflexed to the second-tier test (Bekri, 2024). With the new sulfatide first-tier algorithm, 40 out of 40 true MLD cases would be detected suggesting that the false negative rate should be extremely low.

Using the new first-tier algorithm the following rate of first-tier positives would have been observed (Bekri, 2024): University of Washington (0 out of 592); Manchester (0 out of 3,687); Rouen University Hospital (2 out of 4,000); Hanover (39 out of 126,545). This very low rate of ARSA second-tier tests needed should reduce the workload in NBS programs.

Appendix

Appendix 1: Screening Algorithm Used in the German Prospective Pilot

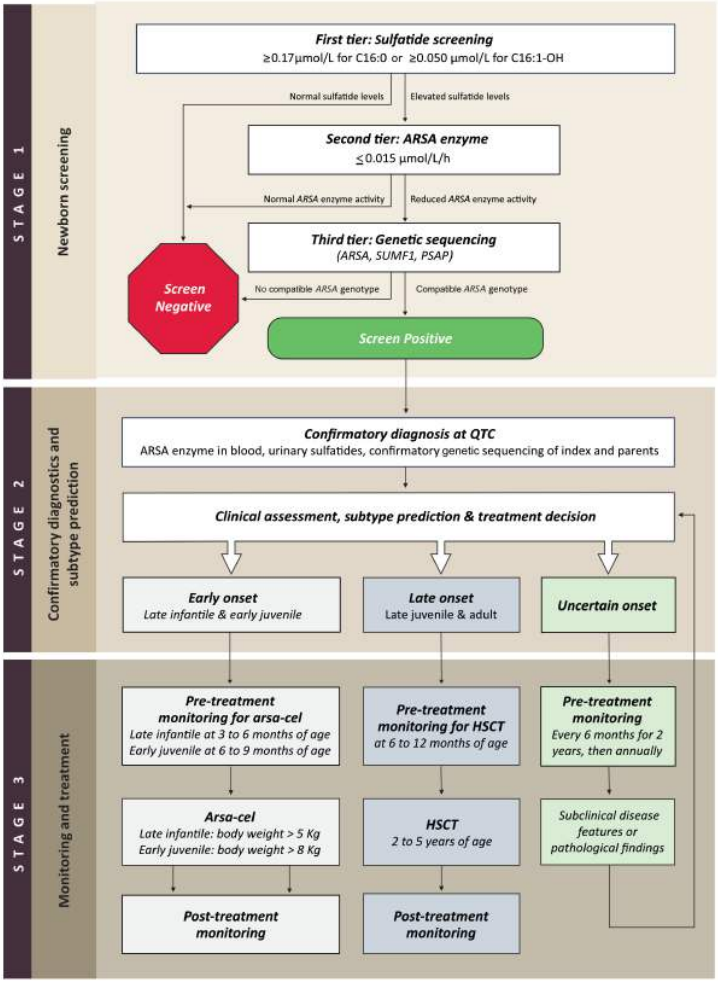

Figure 1. Proposed screening algorithm and care pathway

Stage 1, Newborn screening: A three-tiered screening is proposed. A screen positive is defined as elevated sulfatides, reduced ARSA activity in DBS samples as well as the detection of one homozygous or two (presumably compound) heterozygous, clinically relevant variants in *ARSA*.

Stage 2, Confirmatory diagnostics, clinical assessment and subtype prediction: Confirmatory diagnostics include urinary sulfatides, ARSA activity in leukocytes, and genetic sequencing of index cases and their parents. All confirmed MLD cases are clinically assessed at the QTC. Disease onset prediction and treatment decisions are based on genotype data from literature and public databases.

Stage 3, Monitoring and treatment: All individuals with confirmed MLD undergo standardized pre-treatment monitoring at the QTC, including biochemical assessments, neuropediatric exams, brain MRI, gallbladder ultrasound, and neurophysiological tests. Those with predicted early-onset MLD receive arsa-cel therapy, while those with predicted late-onset MLD are scheduled for allogeneic HSCT. Individuals with uncertain disease onset, based on genotype or residual ARSA activity, undergo monitoring, with treatment options based on the age at subclinical symptom onset.

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