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*A registered 501(c)(3) non-profit serving families worldwide*

October 4th, 2025

WA State NBS Technical Advisory Committee

vie email: wsboh@sboh.wa.gov

Re: Consideration of adding Metachromatic Leukodystrophy to the WA State NBS Screening Panel

Dear TAC members,

The MLD Foundation is a 25-year-old organization that supports families, researchers, public health professionals, clinicians, and industry to optimize the quality of life and care for individuals with metachromatic leukodystrophy.

The MLD Foundation was the nominating organization in August 2024 for MLD to be reviewed by the ACHDNC and considered for addition to the RUSP by the HHS Secretary. Even though the federal advisory committee was disbanded in April, the ACDNC's previously authorized independent Expert Evidence Review was completed with an excellent positive response. A subsequent Federal Register Notice With Request for Comment: Consideration of Adding Metachromatic Leukodystrophy to the Recommended Uniform Screening Panel closed mid-September, and we are awaiting a response from the HHS Secretary Kennedy.

We strongly suggest the WA NDS TEC consider a review of MLD as a candidate for the WA newborn screening panel.

MLD has the perfect trifecta of …

1) A screen that is straightforward to implement on existing multiplexed equipment (throughput and cost efficient), is highly repeatable with no false positives, and to date, no false negatives. New York State launched population-wide MLD screening on September 12th, 2025. Illinois, Minnesota, and Pennsylvania are actively working to implement population-wide screening, with North Carolina hosting an opt-in program, Utah reviewing the addition of MLD to their panel, and Tennessee working on a MLD pilot. The screen had previously been piloted on over half a million babies, with five babies identified, four of whom have had therapy, and one is being monitored.

2) A proven"one and done" ex-vivo lentiviral curative gene therapy approved by the EMA in 2000, and by the FDA in 2024 (it was developed in Italy, hence it was approved in the EU first), with 13+ years of patient outcome data, and

3) Superb independent value assessments by ICER (US) and NICE (UK).

**The "catch" is that the therapy and value depend on identifying patients pre-clinically symptomatic** … and newborn screening is the most thorough, equitable, cost-effective, and accurate means to identify the expected one baby born per week in the US.

RUSP-alignment plus the individual state efforts reported above, equate to the expedited implementation of screening for over 64% of the babies born in the US, saving at least one baby's life, and empowering them to live normal, healthy, disease-free lives.

The time is now for Washington State to join its peers in implementing MLD newborn screening.

**History**
In August of 2024, on behalf of the MLD community, MLD Foundation submitted a nomination to the Federal HHS ACHDNC. The ACHDNC referred the nomination to their independent External Expert Evidence Review workgroup (Kemper, et al.), which resulted in a recent favorable publication. Despite the recent dissolution of the ACHDNC, we have completed the processes defined by the Committee and are now actively working to obtain the HHS Secretary's signature to put MLD on the RUSP.

**Newborn Screening Assay**
We've been collaborating on MLD newborn screening since 2008, notably with Professor Gelb to define and then develop, pilot, optimize, and refine the MLD NBS assay. Teryn Suhr recommended sulfatide as the first-tier in the MLD flow to eliminate false-positives due to pseudo-deficiency. With over 500k babies in MLD identified-baby pilot studies in the EU, there have been five babies detected and zero reports of false negatives. In 2017, MLD Foundation established an MLD NBS Expert Working Group, which led to a federal RUSP Nomination submission in 2024.

**Genotype-Phenotype Predictability**
More recently, we've worked with Dr. Gelb and Dr. Adang on an MLD genotype-phenotype algorithm and prediction matrix so that proper therapeutic recommendations can be made based on a screen-positive NBS result. 72% predictability has been increased to over 95% using Gelb-inspired algorithms and research. MLD Foundation has an ongoing research project underway to continue to refine this predictability.

**FDA Approved Gene Therapy**
In 2005, we began collaborating with the San Rafaelle (Italy) researchers, who developed and conducted the clinical trials for MLD's first ex vivo gene therapy. That therapy was approved by the EMA in 2020 (branded Libmeldy®) and by the FDA in early 2024 (branded Lenmeldy™). We've seen the gene therapy papers and the

science for decades. More importantly, we've met dozens of gene therapy patients and can say the therapy is curative when patients access it pre-symptomatically, which is what MLD newborn screening will do.

**Supporting Policy**
MLD Foundation began its federal policy work in 2007, with a focus on newborn screening, access, and reimbursement for rare diseases. We inspired the language of the county's first state RUSP alignment legislation in California (SB 1095 in 2016), and are proud to have continued to help advance state RUSP alignment legislation, as well as additional MLD-specific lab efforts and legislation to bump the 53% of babies born in RUSP-aligned states to over 64% for MLD screening – assuming all RUSP-aligned states proceed to implementation of MLD NBS.

**Expert Independent Value Assessments**
Value The MLD Foundation served as an expert reviewer for the ICER value assessment of Libmeldy, providing detailed technical contributions to the UK's NICE assessment, both of which were highly favorable. Both independent expert conclusions are that not only is the quality of life for the baby optimized, but also that the cost of the therapy is cheaper than the costs of medical care in the absence of treatment. UCSF is a Lenmeldy Qualified Treatment Center (QTC), and the Leukodystrophy Center at Primary Children's Hospital/University of Utah is actively undergoing certification to be the nation's 6th US QTC.

**Reference Documents**
Attached are two reference documents with extensive references and details that we are happy to elaborate on. With over 25 years of experience in MLD advocacy, academia, clinical research, clinical trials, newborn screening, clinical care, and patient/family perspectives, we are happy to respond to any questions, issues, or concerns HRSA or the Secretary might have.

MLD Foundation strongly suggests that the WA TEC actively consider the addition of MLD to the Washington State NBS panel..

Sincerely,

Dean Suhr, President
Teryn Suhr RN (retired), Executive Director
MLD Foundation

# MLD Newborn Screening Justification and Resources

## Source

Dean Suhr, President, MLD Foundation
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## Metachromatic Leukodystrophy

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 a rare terminal genetic (autosomal recessive) neurometabolic leukodystrophy and lysosomal disease that most often affects infants. Late infantile MLD represents approximately 60% of MLD cases with clinical symptoms evident at 12-18 months. These children will typically die within 5-6 years without therapy. Early juvenile MLD (symptom onset at 30 months to 7 years) represents another 15-20% of cases, and while progression is a bit slower, these children die within a decade or a decade and a half. Late juvenile and adult forms of the disease also exist, but are not the initial target of this application.

## Newborn Screening for MLD

MLD can be detected with a demonstrably high reliability, high accuracy, and no false positive MS/MS assay. Babies identified and treated within the first 6-9 months of life with the FDA-approved (2024) autologous MLD gene therapy are essentially cured.

Recommended Three-Tier Screening Flow

1. NBS Lab … DBS
   Tier 1 - MS/MS Sulfatide level … DBS
   Tier 2 - MS/MS ARSA level … DBS
   Tier 3 - Sequencing … DBS … tool to determine the form of disease for therapeutic referral/timing

   *Screening notes:*
   - To date, states are sending samples for the Tier 2 ARSA screen to the Mayo Clinic in Rochester, MN (Matern).
   - To optimize the timing of the return of results, Tier 2 and Tier 3 are often performed simultaneously.

2. Notify pediatrician/family
3. Diagnostic confirmation… whole blood and urine
    - ARSA activity in blood
    - Sulfatide level in urine
4. Therapeutic recommendation and referral

The MLD NBS suggested flow is high volume standard flow MS/MS for elevated levels of C16:0 and C16:1-OH sulfatides in DBS utilizing an HPLC-MS/MS assay that can be multiplexed with typical MS/MS processes already performed in NBS labs. This sulfatide first tier eliminates the high-incidence 1:12 pseudo-deficiency false positives that an ARSA enzyme first tier would be challenged with. A second-tier MS/MS assay looks for decreased ARSA levels, and a third-tier uses sequencing to help determine the form of MLD and guide treatment and timing decisions. Sequencing is estimated to show a 76% (and improving) predictive ability for the form of disease.

This three-tier screen flow avoids pseudo-deficiency false positives and eliminates PSAP and multiple sulfatase disease reflexing that cannot be treated with the current gene therapy. (An MSD therapy is close to a clinical trial.)

In August of 2024, a RUSP Nomination was submitted to the Federal HHS ACHDNC. The ACHDNC referred the nomination to their independent External Expert Evidence Review workgroup, which resulted in a recent favorable publication (Lam, et al. 2025). Despite the recent dissolution of the ACHDNC, we have completed the processes defined by the Committee, and are now actively working to obtain the HHS Secretary's signature to put MLD on the RUSP. The federal ACHDNC called for an independent

ref:

https://MLDnewbornScreening.org/MLDruspNomination
https://MLDnewbornScreening.org/MLDgenotypephenotype2023
https://MLDnewbornScreening.org/achdncMLDexpertReview202507 (Lam)
https://MLDnewbornScreening.org/MLDnbs1601

## Treatments for MLD

Atidarsagene autotemcel (arsa-cel), "LENMELDY™ " is an autologous ex vivo lentiviral gene therapy that was approved by the FDA in March of 2024 (and the EMA in December 2020). The one-time treatment should be provided before 6-9 months of age to balance optimizing the mass of the baby while minimizing disease progression. The first patient was treated over 13 years ago in 2012, with published data from approximately 40 children (median of nearly 8 years post-therapy as of 2025) published.

When treated well before symptoms can be identified in the clinic, these children will live normal cognitive and motorskill lives. No ongoing major post-therapy MLD-induced medical expenses should be incurred. They will grow to be healthy, physically and mentally active, employed, taxpaying citizens, rather than being beneficiaries of costly medical services until they pass.

All states refer to qualified Lenmeldy gene therapy centers for treatment. There are currently five treatment centers:

- Benioff Children's Hospital at University of California at San Francisco (UCSF)
- University of Minnesota Fairview (UMN), Minneapolis, MN
- Children's Hospital of Philadelphia (CHOP), Philadelphia, PA
- Children's Healthcare of Atlanta (CHOA), Atlanta, GA
- Texas Children's Hospital, Baylor University, Houston, TX
- Note: the Leukodystrophy Center at Primary Children's Hospital/University of Utah is actively seeking certification as the nation's 6th therapeutic site.

Both ICER (US) and NICE (UK) completed very positive external expert Value Assessments of the therapy. The therapy is very economically viable for public and private payors.

ref:

https://MLDnewbornScreening.org/MLDruspNomination
https://MLDnewbornScreening.org/ICERMLDataglance
https://MLDnewbornScreening.org/ICER2022final
https://MLDnewbornScreening.org/NICEMLDfinal2022
https://MLDnewbornScreening.org/LenmeldyUStreatmentCenters

## Incidence

MLD has an estimated incidence of 1 in 40,000 to 1 in 100,000 births. Nationally, with 3.6M births, it's approximately 36-90 babies per year … a bit over 1 per week.

ref:

https://mldnewbornscreening.org/mldruspnomination

## Other State Population Screening and Pilots for MLD

Illinois, Minnesota, New York, and Pennsylvania are actively working to implement population-wide MLD NBS over the next 6-12 months. Tennessee is working on a pilot for MLD. North Carolina has an opt-in MLD screen.

## Recommended Uniform Screening Panel (RUSP) Status

MLD was submitted to the ACHDNC in June of 2024 and referred to expert review in August of 2024. The ACHDNC has been dissolved (for now), but the independent External Expert Evidence Review was completed with very positive results.

MLD Foundation is currently spearheading an effort to obtain the HHS' Secretary's approval onto the RUSP.

ref:

https://MLDnewbornScreening.org/MLDruspNomination

## MLD NBS Pilot History

Over 500k babies have been screened in and identified in pilot studies in Germany and Austria. Five babies have been identified. Four have received therapy, and one is being monitored for later onset disease.

## Consensus Care Guidelines

Consensus Care Guidelines have been published for the treatment of MLD.

ref:

https://MLDnewbornScreening.org/MLDnbsNEJM2024
https://MLDnewbornScreening.org/consensusguidelinesUSA
https://MLDnewbornScreening.org/MLDnbsNEJM2024supplement

The clinicians, the NBS biochemists, the state & foreign pilots, the payors, and the patient are all reporting superb outcomes when gene therapy is received pre-symptomatically, usually approximately six months after birth.

Prepublication Release

PEDIATRICS

OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

# Evidence Regarding Metachromatic Leukodystrophy Newborn Screening

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# Evidence Regarding Metachromatic Leukodystrophy Newborn Screening

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**Abbreviations:** ACHDNC (Advisory Committee on Heritable Disorders in Newborns and Children), FDA (Food and Drug Administration), MLD (metachromatic leukodystrophy), RUSP (Recommended Uniform Screening Panel)

**Article Summary:** This article describes the benefits and harms of adding metachromatic leukodystrophy to state newborn screening panels in the United States.

**Contributors' Statement**

Dr. Lam, Dr. Ream, Dr. Grosse, Mr. Ojodu, Ms. Jones, Dr. Smith, Dr. Comeau, Dr. Tanksley, and Dr. Kemper conceptualized and participated in the evidence review. Dr. Kemper drafted the initial manuscript. All authors critically reviewed and revised the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

**Abstract**

Metachromatic leukodystrophy (MLD) is a lysosomal disorder affecting about 1 per 100,000 newborns. It is caused by biallelic variations in the arylsulfatase A (*ARSA*) gene, leading to deficiency of ARSA enzyme activity leading to elevation of sulfatides. Most affected individuals have the late-infantile or early-juvenile phenotype, associated with significant and progressive neurologic degeneration and death. For these phenotypes, treatment with a lentiviral gene therapy in infancy can improve survival and motor function. However, in the absence of screening or an affected older sibling, most cases are diagnosed much later. A two-tiered newborn screen, based on the presence of elevated sulfatides in dried-blood spots followed by finding low ARSA enzyme activity, can accurately identify newborns with the early-onset phenotypes of MLD for timely gene therapy. An MLD screening study with consent is ongoing in 8 hospitals in New York City and population-based newborn screening has been implemented in several regions in Europe. Although the false-positive rate is low, only one of these MLD newborn screening activities, in Hannover, Germany, has reported identifying cases. At least four newborn screening programs in the United States are in the process of implementing MLD screening.

## Introduction

The Recommended Uniform Screening Panel (RUSP) is a list of conditions maintained by the United States (US) Secretary of Health and Human Services that can feasibly be detected through newborn screening and for which screening leads to improved health outcomes. Individual states use the RUSP to inform which conditions are included in their newborn screening panels. Twelve states, which together represent about half the births in the US, have legislative requirements to screen for the core conditions included on the RUSP. The Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC), established in 2003, provided recommendations to the Secretary of Health and Human Services about which conditions should be included on the RUSP based on the expected impact and feasibility of screening.

In August 2024, the ACHDNC voted to consider metachromatic leukodystrophy (MLD) newborn screening for addition to the RUSP by requesting an evidence-based review from the ACHDNC Evidence Review Group. This report summarizes the key evidence related to MLD newborn screening, including an overview of the condition, the feasibility and accuracy of newborn screening for MLD, and the expected impact of presymptomatic detection compared with usual case detection, to help inform policy makers about whether MLD should be included in state newborn screening panels.

## Overview of Metachromatic Leukodystrophy

MLD is a lysosomal disorder caused by variations in the arylsulfatase A (*ARSA*) gene, leading to deficiency of ARSA enzyme activity and elevation of sulfatides (OMIM #250100). Untreated, exposure to excess sulfatides in microglia leads to progressive demyelination in the central and peripheral nervous system that causes significant and often rapidly progressive neurologic impairment, leading to death in childhood for those with the early-onset phenotypes. These individuals often develop seizures, cortical blindness, motor impairment, dysphagia, and gallbladder abnormalities (e.g., gallstones, wall thickening, polyps, carcinoma). [1,2]

MLD has a range of phenotypes based on the timing of presentation. The focus of newborn screening is to identify the more severe and rapidly progressing early-onset phenotypes, including those referred to as late-infantile (i.e., symptom onset before 30 months of age) and early-juvenile (i.e., symptom onset from 30 months to 7 years of age) MLD, as children with these phenotypes are potentially eligible for gene therapy. The later-onset phenotypes, late-juvenile (i.e., 7 to 16 years of age) and adult-onset MLD, are not currently eligible for gene therapy and not a target of newborn screening.

Age of symptom onset is also strongly associated with the rate of progression, with earlier-onset disease leading to more rapid decline.[3] Cases that present at younger ages tend to have a motor-predominant presentation compared with later-onset cases, which are associated with neuropsychological symptoms.

A systematic review of studies published through March 2022 found that the overall birth prevalence of clinically diagnosed cases of MLD in 14 studies from 13 countries ranged from 0.16 per 100,000 in Japan to 1.85 per 100,000 in Portugal.[4] The review also identified 17 studies of the phenotypic distribution. The late-infantile phenotype was the most common in 10 of these studies (39.2% to 80.5% of the MLD cases) and the early-juvenile phenotype was the second most common overall. These epidemiological findings should be interpreted with caution because of the different approaches to case finding, the degree to which phenotypes were reported, and varying approaches to determining the underlying population denominator.

Two studies in Germany described the natural history of MLD among 21 and 23 subjects with late-infantile MLD and 36 and 38 subjects with early- and late-juvenile-onset MLD.[5,6] Common initial symptoms of late-infantile MLD were motor impairment, including gait disturbance and abnormal movement, and developmental delay, with median age of onset of 17

months (range 9-27 months) and a median time to diagnosis of 12 months (range 9-27 months) later. Juvenile-onset MLD showed similar symptoms and was more likely to have other co-occurring findings, including fine motor delay and behavior problems. The median age of onset was 76 months (range 32-162 months) and a median time to diagnosis was 21 months (range 1-282 months). These studies also found that those with late-infantile MLD and early-juvenile MLD had rapid progression of motor impairment.

A natural history study from Italy included 22 subjects with late-infantile MLD and 14 subjects with early-juvenile MLD.[7] The five-year survival after symptom onset was 36% for late-infantile MLD and 64% for early-juvenile MLD.

**Metachromatic Leukodystrophy Newborn Screening**

The current approach to newborn screening for late-infantile and early-juvenile MLD begins with sulfatide measurement in dried-blood spots as a first-tier screen. First-tier screening with liquid chromatography-tandem mass spectrometry can measure different sulfatides. Screening for the combination of elevated C16:0-sulfatide (i.e., 16:0 saturated fatty acyl group attached to the sphingosine amino group) and C16:1-OH-sulfatide is highly sensitive and specific, with fewer false-positive results than for first-tier C16:0-sulfatide screening alone.[8] This first-tier testing can be multiplexed with newborn screening for other lysosomal storage disorders and X-linked adrenoleukodystrophy. If sulfatides are elevated, ARSA enzyme activity is measured as a second-tier screening test, which can be done in the same dried-blood spot as the first-tier screen. Screening for other lysosomal disorders included in the RUSP is based on measuring specific enzyme activity levels. For MLD, first-tier screening for elevated sulfatides avoids the detection of ARSA enzyme pseudodeficiency. To expedite diagnosis and to help with phenotypic prediction, the *ARSA* gene can also be sequenced from the same dried-blood spot as an optional third-tier test.

There are two conditions biochemically similar to MLD that can be detected by MLD newborn screening: multiple sulfatase deficiency and prosaposin deficiency. Multiple sulfatase deficiency (OMIM #272200) is an ultra-rare disorder (less than 1 case per million individuals) caused by reduced activity of an enzyme that modifies other sulfatase enzymes.[9] Prosaposin deficiency (OMIM *176801) is another ultra-rare disorder causing deficiency of saposin B. Because saposin B is an ARSA enzyme activator, affected individuals also have elevated sulfatide levels. No targeted therapy is available for either multiple sulfatase deficiency or prosaposin deficiency, and therefore neither is a proposed target of newborn screening.

Laboratory studies with de-identified dried-blood spots, sometimes referred to as pre-pilot studies, have been used to develop the thresholds for a positive first-tier screen. The largest of these was based on more than 27,000 dried-blood spots including 40 from newborns with MLD from 4 separate centers (University of Washington, Manchester University, Rouen University Hospital, and ARCHIMEDlife in France).[8] This study found with a specific cut-off for C16:1-OH-sulfatide testing (2.70 multiple-of-the-median) that the first-tier positive rate is <0.05% (0/592 from the University of Washington, 0/3687 from Manchester University, 2/5000 from Rouen University Hospital, and 39/126,545 from ARCHIMEDlife) with no missed cases.[8]

From October 2021 through July 2023, a prospective MLD newborn screening pilot study was conducted in Hannover, Germany.[10] This is the largest published population-based study of MLD newborn screening. This study used a different first-tier screening test (C:16:0-sulfatide ≥0.17 μmol/L or C16:1-OH-sulfatide ≥0.05 μmol/L) than the more recent laboratory report,[8] leading to a greater number of first-tier positive results. Of the 109,259 newborns screened, 3881 (0.35%) had a positive first-tier screen (elevated C:16:0-sulfatide and C:16:1-OH-sulfatide), of whom 3 (0.8%) had a positive second-tier screening (low ARSA enzyme activity) and each carried two pathogenic *ARSA* variants. Two of these infants received gene therapy for having a treatment-eligible early-onset MLD phenotype and the other

infant, who has a later-onset MLD phenotype, is being monitored for the development of signs and symptoms.

From March 2023 through February 2025, a prospective population-based pilot MLD newborn screening with consent was conducted in the Tuscany region of Italy.[11] Dried-blood spot first-tier screening involved measuring four sulfatides (C:16:0-sulfatide, C16:1-OH-sulfatide, C:16:0-OH-sulfatide, C16:1-sulfatide). If elevated on repeat testing in the same dried-blood spot, ARSA enzyme activity was measured as a second-tier test on the same dried-blood spot. If the ARSA enzyme activity was <20% of daily controls, a new dried-blood spot was collected for additional testing. Of the 42,262 newborns screened for MLD, >99% of those approached for consent, 90 (0.21%) had a positive first-tier test. Of these, 6 infants had a positive second-tier screen and 4 infants had insufficient dried-blood spot material for second-tier screening, leading to the request for an additional dried-blood spot for 10 infants (0.02% of the total screened or 0.11% with a positive second-tier test). On follow-up testing, the 10 infants had normal ARSA enzyme activity and therefore no cases of MLD were identified.

## Predicting the Phenotype of Metachromatic Leukodystrophy

More than 300 *ARSA* gene variants have been described.[12] Genomic analyses of affected individuals suggest that genotype is predictive of loss of function associated with the early-onset phenotypes of MLD.[13] For example, in a study of 489 subjects with MLD, phenotype could be predicted with 76% accuracy. The most common genotype (c.465 + 1G > A/c.456 + 1G > A) was present in 6.8% of the subjects, of whom 32 were reported to have infantile/late-infantile MLD and 1 had an unknown phenotype.[14] Phenotypic prediction is also possible based on the amount of residual ARSA enzyme activity: <2% of expected enzyme activity is associated with early-onset phenotypes of MLD, 2-4% of expected enzyme activity is associated with moderate phenotypes of MLD, and 4%-13% of expected enzyme activity is associated with mild phenotypes of MLD.[14] In contrast, low ARSA enzyme activity with non-elevated sulfatides

suggests ARSA pseudodeficiency. Use of ARSA enzyme activity for phenotype prediction is particularly useful for previously undescribed variants or when the significance is unclear. [13]

Age of symptom onset is also strongly associated with the rate of progression, with earlier-onset disease leading to more rapid decline. [3] Cases that present at younger ages tend to have a motor-predominant presentation compared with later-onset cases, which are associated with neuropsychological symptoms.

**Metachromatic Leukodystrophy Treatment**

A gene therapy for MLD, atidarsagene autotemcel was approved in 2020 by the European Medicines Agency (Libmeldy; Orchard Therapeutics) and in 2024 by the US Food and Drug Administration (Lenmeldy; Orchard Therapeutics) with an indication for "the treatment of children with pre-symptomatic late infantile (PSLI), pre-symptomatic early juvenile (PSEJ) or early symptomatic early juvenile (PSEJ) metachromatic leukodystrophy (MLD)." [15] This gene therapy, also called arsa-cel, is not recommended for those with later-onset disease, for whom allogenic hematopoietic stem cell transplantation is recommended after becoming minimally symptomatic, with the goal of stopping further MLD disease progression.. [3,16]

Use of the arsa-cel gene therapy begins with harvesting CD34+ cells from the affected individual, which are then transfected with a lentivirus to integrate functional *ARSA* into the genome. The individual then undergoes myeloablative conditioning, typically with busulfan, followed by infusion of the modified stem cells. For individuals with the late-infantile or early-juvenile phenotype, gene therapy should be initiated in infancy. According to international expert panel consensus guidelines, the goal of timing for gene therapy is within 6 months of age for late-infantile MLD and within 12 months of age for early-juvenile MLD. [3]

Most MLD gene treatment outcome data come from patients who were not identified by newborn screening, but instead because they had an older affected sibling. A report published in 2025 summarized the efficacy of gene therapy among 37 subjects (18 presymptomatic late-infantile, 8 presymptomatic early-juvenile, 11 early symptomatic early-juvenile) compared with 43 untreated subjects (26 late-infantile; 17 early-juvenile), with a primary end point of survival without severe motor impairment.[17] Among patients with presymptomatically treated late-infantile MLD, none died during follow-up, with age at last contact from 3.2 to 13.4 years, and only one subject developed severe motor impairment, at age 7.2 years. Follow-up of untreated patients was more variable, with age at last contact ranging from 2.7 to 20.4 years. Survival curve probabilities to age 6 years, which take into account differences in follow-up time, for untreated patients with late-infantile MLD were estimated as 59.0% (95% CI: 37.2%-75.5%) overall survival and 8.8% (95% CI: 1.5%-24.3%) for survival without severe motor impairment. Among subjects with early-juvenile MLD, an estimated 87.5% of those treated with presymptomatic MLD and 80% of those with treated early-symptomatic MLD survived without severe motor impairment to 10 years of age. In contrast, only an estimated 11.2% (95% CI, 0.9 to 36.4) of subjects with untreated early-juvenile MLD survived without severe motor impairment to 10 years of age. One subject with treated presymptomatic early-juvenile MLD died from an ischemic cerebral infarction at 2.1 years and two with treated early-symptomatic early-juvenile MLD died due to disease progression. None of these deaths were deemed associated with gene therapy.

The previously described report describing MLD newborn screening in Hannover, Germany, led to two cases referred for gene therapy.[10] One underwent cell harvesting at 10 months of age followed by gene therapy at 12 months of age. The patient developed mucositis

during conditioning that resolved. At 18 months of age the patient had above normal ARSA enzyme activity and reached all developmental milestones age appropriately. The patient had patterns of demyelination based on nerve conduction studies at 11 months of age that had improved. The patient also had a normal brain MRI. The other patient underwent cell harvesting at 11 months of age and gene therapy at 12 months of age. This patient developed catheter-associated thrombosis during apheresis requiring treatment with heparin and enoxaparin treatment and also developed mucositis during the conditioning that resolved. At 18 months of age, the last available follow-up, this patient also had achieved developmental milestones age appropriately and had a normal brain MRI. This patient also had gall bladder thickening before gene therapy, which had improved after treatment.

**Metachromatic Leukodystrophy Newborn Screening in the United States**

A pilot study requiring parental consent in 8 hospitals in New York City, ScreenPlus, offers MLD newborn screening (first-tier sulfatide screening, second-tier ARSA enzyme activity measurement).[18] From 2020 through May 2025, there were 61,285 infants born in these hospitals, of which 80% (N=49,197) were approached for screening and nearly 30,000 were screened for MLD, with no positive results (Melissa Wasserstein, MD, The Children's Hospital at Montefiore, Bronx, NY, email communication, May 8, 2025).

At least four states are working to add MLD to their state newborn screening panels. New York is planning for statewide pilot screening for MLD the Summer of 2025. In January 2024, Illinois passed legislation to add MLD screening and is planning for implementation. In April 2025, the Pennsylvania Division of Newborn Screening and Genetics has proposed adding MLD to its newborn screening panel by January 2026. Also in April 2025, Minnesota's Advisory Committee on Heritable and Congenital Disorders recommended that MLD be added to its newborn screening panel to the Minnesota Commissioner of Health, who approved it in May 2025.

**Summary**

MLD affects about 1 per 100,000 newborns, most of whom will have the late-infantile or early-juvenile phenotypes, which if untreated leads to profound neurologic impairment and death in childhood. Gene therapy delivered in the first year after birth, prior to when cases can be clinically detected, can lead to significant improvement in health outcomes for most infants. Laboratory studies, a pilot study in New York, and two large-scale population-based screening studies in Europe have found that two-tiered screening, with sulfatides followed by ARSA enzyme activity, has a low rate of false-positives. The prospective screening program in Hannover, Germany, has reported identifying cases of MLD leading to treatment with gene therapy. In the US, at least four states are now working to implement MLD newborn screening.

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# MLD NBS Nomination Evidence Dossier

1. Metachromatic Leukodystrophy Description

**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 (Asbreuk, 2025). This includes the late infantile (LI) and early juvenile (EJ) subtypes.

Metachromatic leukodystrophy is typically classified based on the age of symptom onset into four subtypes (Gomez-Ospina, 2024; Asbreuk, 2025):

| | | | |
|---|---|---|---|
| Early-Onset | Late infantile (LI) | ≤ 30 months | 50-60% |
| | Early Juvenile (EJ) | 30 months to <7 years | 20-40%[a] |
| Late-Onset | Late Juvenile (LJ) | 7 years to <17 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 350 pathogenic variants of the *ARSA* gene have been described (Cesani, 2016; ClinVar, 2025). 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 (Asbreuk, 2025; 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, 2024a).

**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, 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; Zambon, 2025).

**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 <17 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 (Asbreuk, 2025; 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; Asbreuk, 2025).

2. Incidence

The incidence (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. MLD is pan-ethnic, with affected patients described in multiple populations: birth prevalence is estimated as 1 in 8,000 among Arab groups in Israel, 1 in 75 in the Jewish Habbanite community, 1 in 2,500 in the Yup'ik ancestry of southern Alaska and 1 in 2,520 live births in western Navajo Nation (Zlotogra, 1980; Bonkowsky, 2018; Söderholm, 2020; Heim, 1997; Poorthuis, 1999; Pinto, 2004; Holve, 2001; Pastor-Soler, 1995; Lugowska, 2011; Hult, 2014; Stellitano, 2016; Chang, 2024;Asbreuk, 2025). The total prevalent population of MLD has not been published in the literature.

3. Clinical Diagnosis of MLD

Most children with MLD are diagnosed when they are overtly symptomatic and beyond the window for intervention with a disease-modifying therapy (Adang, 2024a; Mohajer, 2025). 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; Mohajer, 2025). 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 (Fumagalli, 2025).

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

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, 2017; Ammann-Schnell, 2021; Pang, 2021; Sevin, 2022; Kehrer, 2025; MLD PFDD, 2023).

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).

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. In the juvenile and adult forms, the type of presenting symptoms (motor vs cognitive) rather than age of onset is predictive of disease progression (Asbreuk, 2025). Patients with motor symptoms at onset have significantly faster disease progression than those with cognitive symptoms alone.

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, 2024).

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, the absence of brain MRI or ENG abnormalities does not rule out MLD, in contrast to other leukodystrophies, such as X-ALD. (Beerepoot, 2019; Schoenmakers, 2022a; Laugwitz, 2024a, Asbreuk, 2025).

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.

4. Benefit of Newborn Screening

Both currently recommended treatment options for MLD, arsa-cel for early-onset subtypes and allogeneic HSCT for late onset subtypes, have limited or no effectiveness in MLD patients who are symptomatic or who have entered the rapidly progressive phase of their disease (Fumagalli, 2025; Boucher, 2015; Groeschel, 2016; Schoenmakers, 2022b; Adang, 2024a). 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 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, 2024a; Adang 2024a).

MLD and other leukodystrophies are less likely to be diagnosed in indigenous, ethnic, and minority populations (Bonkowsky, 2018). The implementation of universal NBS for MLD will help address the issues of inequities in diagnosis related to demographic or socioeconomic factors and ensure that all patients are eligible to receive disease-modifying treatment when they are in a presymptomatic state. 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 older sibling.

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.

5. Treatment of MLD

Consensus treatment guidelines for children diagnosed with MLD pre-symptomatically and/or through NBS have been published and represent both international and US-based expertise (Laugwitz, 2024a; Adang, 2024a). These expert consensus guidelines recommend arsa-cel treatment as the standard of care for this group of early-onset MLD patients (Laugwitz, 2024a; Adang, 2024a). Arsa-cel is not FDA or EMA approved for treatment of late-onset MLD (Laugwitz, 2024a; Adang, 2024a, https://clinicaltrials.gov/study/NCT04283227).

Arsa-cel is an autologous hematopoietic stem cell (HSC) gene therapy (HSC-GT) consisting of CD34+ hematopoietic stem and progenitor cells (HSPCs) transduced ex vivo with a lentiviral vector (LVV) encoding the human ARSA cDNA, with constitutive ARSA expression driven by a human phosphoglycerate kinase promoter (Fumagalli, 2025). It is a one-time treatment.

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, 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, 2025; Lenmeldy USPI).

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, 2025). Arsa-cel treatment was associated with a significantly lower risk of death or severe motor impairment (time from birth to first occurrence of loss of locomotion and sitting without support or death from any cause) than no treatment in patients with PSLI ($p < 0.001$), PSEJ ($p = 0.04$) and ESEJ ($p < 0.001$) MLD. Motor function, cognitive function, and language skills were better maintained in patients with PSLI MLD who were treated with arsa-cel than in untreated LI patients (Fumagalli, 2025). Additionally, peripheral neuropathy assessed by NCV was significantly ameliorated in PSLI patients treated with arsa-cel compared to untreated patients of similar age, and younger age at treatment was associated with increased NCV in the ulnar and median nerves (Zambon, 2025). No evidence of insertional oncogenesis has been found with arsa-cel (Fumagalli, 2025). The vector used for arsa-cel does not contain the viral promoter that has been implicated in hematological cancers associated with a different lentiviral gene therapy used to treat cerebral adrenoleukodystrophy (Duncan, 2024).

The benefit of presymptomatic treatment is particularly apparent in comparing the difference in outcomes between PSEJ and ESEJ patients. All surviving patients with PSEJ who were treated with arsa-cel had age-appropriate motor, cognitive, and language skills at last follow-up, with five of seven having surpassed the age at which the onset of symptoms had occurred in their untreated sibling and four of seven having surpassed the median age at which untreated EJ patients have onset of severe motor impairment, unable to move or sit without assistance (Fumagalli, 2025). Outcomes in patients with ESEJ were much more variable. Most surviving patients with ESEJ were free of severe motor impairment and continued to acquire verbal skills as expected for age, while the untreated ESEJ patients all experienced severe motor and cognitive impairment. However, two of the treated patients with ESEJ died due to disease progression, which were not considered by the investigators to be related to treatment with arsa-cel (Fumagalli, 2025).

The results of a recent analysis of the large differences in motor outcomes in age-matched treated vs. untreated sibling pairs reinforces the importance of early diagnosis through NBS to enable all early-onset patients to have the opportunity for presymptomatic treatment, not just the younger siblings of symptomatic patients (Calbi, 2025).

Newborns diagnosed with MLD via NBS and treated pre-symptomatically with arsa-cel have limited follow up to demonstrate long term benefit, but long-term data from the clinical development program predict that the treatment will be very effective in

preventing the motor and cognitive deterioration always seen in untreated children with MLD.

Allogeneic hematopoietic stem cell transplantation (HSCT) has also been used as a one-time treatment for MLD. There are multiple publications on this topic (Bredius, 2007; Martin, 2013; Bley, 2013; Boucher, 2015; van Rappard, 2016; Groeschel, 2016; Tan, 2019; Beschle, 2020), which were 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. The data for patients with LJ MLD has been mixed, and longer-term follow-up suggested that any improvement or stabilization in cognitive and gross motor function was not likely to be maintained. Outcomes of HSCT in patients with adult MLD are relatively sparse.

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, 2024).

6. 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.

7. Newborn Screening Methodology

A two-tiered approach for NBS starting with LC-MS/MS analysis with a 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).

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.

**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). An

international collaboration of scientists experienced in MLD NBS recently published protocols with full details so that the methods can be readily adopted by NBS laboratories (Shaff, 2025).

Internal standards to measure C16:0 sulfatide and C16:1-OH sulfatide are commercially available from Revvity, Inc. and Enfanos, LLC. A multiplex kit for sulfatide analysis and the reagents to measure ARSA activity using LC-MS/MS are available through Enfanos, LLC. Revvity, Inc. and Enfanos, LLC currently provides Quality Control (QC) DBS for sulfatide measurement, and Enfanos, LLC provides QC DBS for ARSA enzymatic activity. The CDC has also started to make QC materials for MLD NBS.

The ARSA enzyme assay in DBS is currently available at Mayo Medical Laboratories and is being validated by RevvityOmics, and Greenwood Genetics Center. Additionally, *ARSA* gene sequencing has been shown to be feasible in DBS and is widely available.

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, measurements of C16:0 and C16:1-OH sulfatides can be carried out via a multiplexed assay with the same LC-MS/MS methodology that is used for all lysosomal storage disorders on the RUSP (Hong, 2020b).

**Prospective Pilot Study Data:**

A manuscript describing the NBS and treatment experience from the world's first prospective NBS pilot study for MLD has recently been published (Laugwitz, 2024b). The study was initiated in the Hannover 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.

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, 2024).

**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 (Hong, 2020a). Prosaposin 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 much rarer than MLD.

**Other MLD NBS Research and Adoption**

**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). Dried blood spot samples from 27,355 newborns were tested. The cutoff value for C16:0 was established based on archived DBS samples from 15 MLD patients (10 LI and 5 juvenile onset) and 2000 random newborns. Of the 27,355 newborns tested, 195 were identified with elevated C16:0 levels (0.71%). The second-tier screening identified two specimens with 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 was possible.

**More recent retrospective analysis.** A prepilot study in Manchester, UK assessed the feasibility of the same two-tier screening approach established by Hong et al., 2021. Evaluation of 3,687 samples and assay validation studies resulted in the refinement of cutoff values proposed for C16:0 screening. This study also resulted in the incidental identification of a late infantile MLD patient leading to an urgent ethics review. The child was subsequently evaluated, diagnosed with pre-symptomatic LI-MLD, and treated with arsa-cel at 11 months of age. Preliminary data from this study suggested C16:1-OH sulfatide is more specific for MLD than C16:0 alone. Subsequently, the addition of C16:1-OH was assessed in a multicenter analysis of pilot program data (Bekri, 2024). This study used cutoff values based on 40 DBS samples from confirmed MLD patients that were expressed as MoM values to enable comparison across centers. After screening 135,824

DBS samples of newborns the combination of C16:0 and C16-OH was found to be superior to C16:0 alone as a first-tier biomarker for MLD, with a false positive rate estimated at 0.031% compared to 1.8% for C16:0 alone and 0.048% for C16:1-OH alone.

As of June 2024, DBS from approximately 158,000 newborns from the Hannover region of Germany and approximately 35,000 newborns from Vienna, Austria have been tested (Olivia, 2024). Additional pilot studies are occurring in Normandy, Italy, and Saudi Arabia (Shaff, 2025).

The results of the prospective pilot MLD NBS study in Tuscany that analyzed DBS from approximately 42,000 newborns over two years support the validity and feasibility of a multi-tier approach (Malvagia, 2025). Although no screen-positive cases of MLD were identified, the testing algorithm was accurate in retrospectively identifying patients with symptomatic, genetically confirmed MLD.

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. Following early results from ScreenPlus, the New York State Department of Health is implementing a state-wide NBS pilot study for MLD, which will use quantification of C16:0 and C16:1-OH in the first-tier assay to reduce the number of first-tier screen positives (NY State Department of Health, 2024).

In early 2025, MLD was added to the newborn screening panels in Minnesota and Pennsylvania. In 2024, New York State was awarded a NICHD grant to conduct an IDIQ pilot for MLD. In 2023, Illinois passed legislation (SB67) to mandate screening for MLD. Implementation is currently underway in Minnesota, Pennsylvania, and Illinois. All three states include MLD as mandated conditions on their state NBS panel. New York is currently scaling screening for MLD and statewide NBS for MLD in New York is slated to begin in July of 2025.

In 2024, Norway became the first country to implement national NBS for MLD (HSPM Network 2024).

8. Confirmatory Diagnosis and Care Pathway

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 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, 2023) 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.

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).

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 2024a; Laugwitz 2024b).

Recent expert consensus guidelines (Laugwitz, 2024a; Adang 2024a) 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.

Recent expert consensus guidelines (Laugwitz, 2024a; 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 2024a).

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 2024a).

9. Other conditions identified via NBS

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 2024a; Laugwitz 2024b). Likewise, diagnosis and management of patients with MSD and prosaposin B deficiency have also been described (Ahrens-Nicklas, 2018, Schlowata, 2019, Kolnikova, 2019)

10. Potential Harms of MLD NBS

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.

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. Published management guidelines are available to assist in the monitoring of patients where subtype remains uncertain (Laugwitz, 2024a; 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.

11. Availability of Treatment

Arsa-cel is offered at a network of Qualified Treatment Centers (QTCs) (https://lenmeldy.com/treatment-centers/; https://www.libmeldy.eu/treatment-process-2/# ). QTCs in the United States include Benioff Children's Hospital at UCSF, University of Minnesota Fairview, Children's Hospital of Philadelphia, Children's

Healthcare of Atlanta, and Texas Children's Hospital, Baylor University. QTCs in Europe include Skåne University Hospital in Norway, Royal Manchester Children's Hospital in the UK, UMC Utrecht in the Netherlands, Universitätsklinikum Tübingen in Germany, Hôpital Robert-Debré in France, and San Raffaele Telethon Institute for Gene Therapy in Italy.

Thus, for those states in the US currently implementing MLD NBS the treatment will be available in-state in Minnesota and Pennsylvania. In New York and Illinois patients with confirmed diagnoses will be referred to one of the centers in the QTC network and care delivered at the QTC. It is the sole discretion of the patients and their healthcare professionals to determine which QTC is the best fit for them.

12. Review of MLD by the RUSP

A nomination to add MLD to the U.S. Recommended Uniform Screening Panel (RUSP) has been submitted. On August 9th, the ACHDNC unanimously voted to move the RUSP nomination for metachromatic leukodystrophy to the evidence review phase. The ACHDNC was expected to recommend MLD for the RUSP at its May 2025 meeting which was cancelled due to the dissolution of the ACHDNC.

**13.** Cost Effectiveness

A cost-effectiveness study to evaluate adding a three-tier algorithm for MLD to the routine NBS program in the UK calculated the total annual cost of screening 704,328 newborns to be £122,625 (£0.17 per newborn) (Bean, 2024b). In this analysis, based on a decision-tree framework for each MLD subtype using long-term outcomes derived from an established survival and Markov economic model, NBS remained cost-effective under a wide range of factors, including fluctuations in MLD incidence, discount rates, screening test costs, and response to treatment. Most importantly, model results showed that only ~2.6 of the 7 expected annual MLD cases would be identified early enough to receive arsa-cel treatment without NBS. With NBS, however, all cases are expected to be identified in time for pre-symptomatic treatment, improving survival and quality of life for MLD-affected newborns. Overall, the study supports that NBS for MLD is a cost-effective use of National Health Services resources and should be included in the official NBS program in the UK (Bean, 2024b). Limited information from two congress abstracts indicated similar results for cost-effectiveness in the USA (Bean, 2024a; Bean, 2025). A model based on the 2021 annual birth cohort in California (420,608 infants) showed that screening for MLD in California would result in a positive net economic benefit >$70 million and the avoidance of 5.09 premature deaths (Bean, 2025). Similarly, a cost-benefit analysis demonstrated that NBS for MLD by LC-MS/MS is a cost-effective use of resources in the USA, due to the positive long-term health outcomes associated with pre-symptomatic treatment of MLD patients (Bean, 2024a). Overall, these results support the inclusion of MLD into routine NBS programs.

14. Additional Info

**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.

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