

NEWBORN SCREENING IN

METACHROMATIC LEUKODYSTROPHY

European consensus-based recommendations on clinical management

EUROPEAN REFERENCE NETWORKS
FOR RARE, LOW PREVALENCE AND COMPLEX DISEASES

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ERN-RND unites 64 of Europe's leading expert centers as well as 4 affiliated partners in 24 member states and includes highly active patient organizations. Centers are located in Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Slovenia, Spain and Sweden.

The following disease groups are covered by ERN-RND:

- Ataxias and Hereditary Spastic Paraplegias
- Atypical Parkinsonism and Genetic Parkinson's Disease
- Dystonia, Paroxysmal Disorder and Neurodegeneration with Brain Iron Accumulation
- Frontotemporal Dementia
- Huntington's Disease and other Chorea
- Leukodystrophies

Specific information about the network, the expert centers and the covered diseases can be found on the network's website www.ern-rnd.eu.

Recommendation for clinical use:

The European Reference Network for Rare Neurological Diseases strongly recommends the use of the following recommendations as best clinical practice for patients with metachromatic leukodystrophies.

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METHODOLOGY

The recommendations for Newborn Screening in MLD were endorsed as recommendation for clinical use by the Disease Group for Leukodystrophies of ERN-RND upon unanimous consent.

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Endorsement process:

- Proposal to endorse guidelines to Disease Group in terms of annual meeting group discussion – 27 October 2025
- Consent to endorse guidelines by whole Group: 24 June 2026
- Publication of endorsed guidelines: 28 July 2026

REFERENCES

Laugwitz L, Schoenmakers DH, Adang LA, Beck-Woedl S, Bergner C, Bernard G, Bley A, Boyer A, Calbi V, Dekker H, Eichler F, Eklund E, Fumagalli F, Gavazzi F, Grønborg SW, van Hasselt P, Langeveld M, Lindemans C, Mochel F, Oberg A, Ram D, Saunier-Vivar E, Schöls L, Scholz M, Sevin C, Zerem A, Wolf NI, Groeschel S. Newborn screening in metachromatic leukodystrophy - European consensus-based recommendations on clinical management. Eur J Paediatr Neurol. 2024 Mar;49:141-154. doi: 10.1016/j.ejpn.2024.03.003. Epub 2024 Mar 9. PMID: 38554683.

RECOMMENDATIONS FOR NBS IN MLD (AS OF NEXT PAGE)

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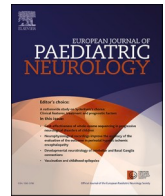
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Original article

Newborn screening in metachromatic leukodystrophy – European consensus-based recommendations on clinical management

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ABSTRACT

Introduction: Metachromatic leukodystrophy (MLD) is a rare autosomal recessive lysosomal storage disorder resulting from arylsulfatase A enzyme deficiency, leading to toxic sulfatide accumulation. As a result affected individuals exhibit progressive neurodegeneration. Treatments such as hematopoietic stem cell transplantation (HSCT) and gene therapy are effective when administered pre-symptomatically. Newborn screening (NBS) for MLD has recently been shown to be technically feasible and is indicated because of available treatment options. However, there is a lack of guidance on how to monitor and manage identified cases. This study aims to establish consensus among international experts in MLD and patient advocates on clinical management for NBS-identified MLD cases.

Methods: A real-time Delphi procedure using eDELPHI software with 22 experts in MLD was performed. Questions, based on a literature review and workshops, were answered during a seven-week period. Three levels of consensus were defined: A) 100%, B) 75–99%, and C) 50–74% or >75% but >25% neutral votes. Recommendations were categorized by agreement level, from strongly recommended to suggested. Patient advocates participated in discussions and were involved in the final consensus.

Results: The study presents 57 statements guiding clinical management of NBS-identified MLD patients. Key recommendations include timely communication by MLD experts with identified families, treating early-onset MLD with gene therapy and late-onset MLD with HSCT, as well as pre-treatment monitoring schemes. Specific knowledge gaps were identified, urging prioritized research for future evidence-based guidelines.

Discussion: Consensus-based recommendations for NBS in MLD will enhance harmonized management and facilitate integration in national screening programs. Structured data collection and monitoring of screening programs are crucial for evidence generation and future guideline development. Involving patient representatives in the development of recommendations seems essential for NBS programs.

Abbreviations		HPLC	High-Performance Liquid Chromatography
		HSCT	Hematopoietic Stem Cell Transplantation
ACMG	American College of Medical Genetics and Genomics	MLD	Metachromatic Leukodystrophy
ARSA	Arylsulfatase A	MLDi	Metachromatic Leukodystrophy Initiative
arsa-cel	atidarsagene autotemcel	MSD	Multiple Sulfatase Deficiency
BAER	Brainstem Auditory Evoked Responses	MS	Mass Spectrometry
DBS	Dried Blood Spots	NBS	Newborn Screening
EDTA	Ethylenediaminetetraacetic acid	NfL	Neurofilament Light Chain
EMA	European Medicines Agency	OMIM	Online Mendelian Inheritance in Man
ERN-RND	European Reference Network on Rare Neurological Diseases	PSAP	Prosaposin
GMFC-MLD	Gross Motor Function Classification for Metachromatic Leukodystrophy	TLC	Thin Layer Chromatography
GMFM-88	Gross Motor Function Measure-88	VKS	Dutch Association for Inherited Metabolic Diseases
		WHO	World Health Organization

1. Introduction and background

Metachromatic leukodystrophy (MLD, OMIM #250100) is a rare, autosomal recessive lysosomal storage disorder with an estimated birth prevalence of 1 in 40.000–100.000 in Europe [1,2]. The disease is caused by biallelic disease-causing variants in the ARSA gene, which encodes the lysosomal enzyme arylsulfatase A (ARSA). ARSA deficiency results in accumulation of toxic sulfatides throughout the body. This impacts primarily myelinating cells of the central and peripheral nervous system, leading to progressive neurodegeneration [1–3].

1.1. Clinical subtypes of metachromatic leukodystrophy

Four **clinical subtypes of MLD** have been delineated based on the age at which symptoms arise: late-infantile (<30 months), early-juvenile (2.5–6 years), late-juvenile (>6–16 years), and adult (>16 years). The onset of symptoms at a younger age is typically associated with a quicker progression of the disease and a shorter life expectancy [4,5]. Compared to the early-onset forms (including late-infantile and early-juvenile), the disease course is slower in adolescents and adults. These late-onset forms are characterized by early cognitive, behavioural, and psychiatric symptoms. Motor regression also often occurs later in the disease course,

with a slower course of decline [4,5].

1.2. Diagnosis of metachromatic leukodystrophy

The **diagnosis** of MLD relies on the measurement of **ARSA enzyme activity in blood** [6,7], the detection of **elevated sulfatides in urine** [8–12] and the **sequencing of the ARSA gene** (OMIM *607 574).

Strong correlations between residual enzyme activity and phenotype are subject to debate and have so far been difficult to establish. Although improved assays in single center studies have shown promising results that indicate a correlation between residual ARSA activity and MLD subtypes, and a high positive predictive value for early-onset MLD, these results await confirmation [7]. For urinary sulfatides different quantification methods are available and well established, including thin layer chromatography (TLC), high performance liquid chromatography (HPLC), mass spectrometry (MS), and liquid chromatography tandem mass spectrometry (LC/MS/MS) [13]. However, their use in differentiating between disease subtypes is hazardous. Sulfatide level in urine and/or blood are age dependent and increase along the disease course [14,15].

Currently, over 280 disease-causing variants have been identified in the ARSA gene. Genotype-phenotype studies showed a reliable associations with loss-of-function (LoF) variants, resulting in minimal or no residual enzyme activity and a late-infantile course [7,16,17]. However,

due to the abundance of rare or private variants, anticipating the clinical subtype is still a challenge, especially for missense variants, with some exceptions [4,17–19].

Moreover, a condition called ARSA pseudo-deficiency occurs in individuals possessing pseudo-deficiency alleles (c.1055A>G and/or c.*96A>G) in the ARSA gene. In these individuals, residual ARSA activity can be in the range of MLD patients', but the carriers remain healthy throughout their life and exhibit no or negligible sulfatide accumulation in urine [19,20].

In rare cases, sulfatide degradation is impaired due to disease-causing variants in the PSAP gene, encoding an ARSA activator protein leading to Saposin B-dependent MLD (OMIM #249900) or due to variants in the SUMF1 gene, that encodes an enzyme responsible for the synthesis of different degrading enzymes causing multiple sulfatase deficiency (OMIM #272200). Saposin B-dependent MLD resembles MLD on a biochemical level, resulting in raised sulfatides with little to no decrease of ARSA enzyme activity. MSD leads to both elevated sulfatide levels and reduced ARSA activity. Unlike MLD, neither the PSAP-mediated disorder nor MSD is currently treatable.

1.3. Characteristic disease features

Characteristic brain MRI abnormalities in MLD include diffuse T2-hyperintense signal in the corpus callosum and the central and periventricular white matter [21]. Specific MLD MRI scores have been applied to visually quantify the severity of MRI abnormalities in MLD patients [22,23]. The severity of MRI abnormalities is correlated to clinical severity and clinical symptoms of MLD typically align with abnormal MRI findings [24–28]. However, in cases of early-onset MLD, signs and symptoms may precede MRI abnormalities. In these very young patients, clear central nervous system signs and peripheral neuropathy may be present even in the absence of distinct abnormalities on brain MRI [29]. As **peripheral neuropathy** occurs in MLD the measurement of nerve conduction velocities (NCV) is one of the diagnostic pillars of MLD as a decrease is typically found early in the disease course [5,30–37]. A possible correlation of reduced NCV with certain genotypes, disease severity or progression is still under discussion [3]. Other tools in evaluating demyelination in MLD can be visual evoked potentials (VEP) [37–41] and brainstem auditory evoked responses (BAER) [5,38,41], but these tests have not yet been evaluated systematically in larger cohorts.

Secondary to sulfatide accumulation gall bladder abnormalities may occur before MLD becomes neurologically manifest. Characteristic sonographic abnormalities include sludge, wall thickening, collapsed gall bladder, and polyps [42].

Neurofilament light chain (NfL) has been evaluated as a non-MLD-specific marker in MLD. Increased levels in blood and cerebrospinal-fluid (CSF) in symptomatic MLD patients were linked to a more severe phenotype with rapid decline [43]. Nonetheless, the usefulness of NfL in pre-symptomatic individuals is still unresolved, and the question whether an elevation in NfL precedes clinical symptoms of MLD is being debated.

1.4. Treatment options

To date, several treatment strategies are available:

Hematopoietic stem cell transplantation (HSCT) has been used in the treatment of MLD for the past three decades [44]. Long-term results show that individuals with late-juvenile and adult onset disease benefit from HSCT if transplanted during the pre-symptomatic or early symptomatic stages of disease [45–49]. They reveal an improved survival and a stabilization of cognitive and motor functions compared to the untreated MLD patients [45–49]. However, uncertainties on the long-term outcomes of HSCT in late-juvenile MLD exist and data on adult MLD is sparse [46,50,51].

In 2020 the European Medicines Agency (EMA) granted approval for

the **autologous hematopoietic stem and progenitor cell gene therapy, atidarsagene autotemcel (arsa-cel)**, in late-infantile MLD subtype during the presymptomatic and in early-juvenile MLD during the pre- and early-symptomatic disease stage [23,52,53]. Arsa-cel has demonstrated notable efficacy and safety in individuals with pre-symptomatic early-onset MLD as the majority of infants exhibit an almost age appropriate cognitive and motor development [23,52,53]. The ARSA gene is expressed at supranormal levels, which may yield a faster and more significant clinical benefit than allogeneic HSCT [23, 52–55]. This therapy is currently available and reimbursed in several European countries.

1.5. Newborn screening in MLD

In the absence of NBS, pre-symptomatic patients are typically identified after an older affected sibling has been diagnosed [23]. To enable early treatment different approaches to NBS are under investigation. Concurrently, successful establishment of biochemical and genetic testing for MLD in dried blood spots have been achieved [56,57], presently undergoing large-scale testing in pilot projects.

1.5.1. Biochemical screening

To identify newborns with MLD, a two-tier biochemical screening was established, followed by a third-tier genetic test. The biochemical screening quantifies C16:0-sulfatides using liquid chromatography tandem mass spectrometry (LC-MS/MS) in the first-tier [56,58]. A cut-off value for sulfatides to achieve 100% sensitivity, was sought in a retrospective study of 27 000 neonates. As the second-tier analysis to reduce false positives, the ARSA activity is measured. The third-tier analysis consisted of sequencing of the ARSA gene to confirm the positive biochemical screening result as well as the PSAP and SUMF1 gene to exclude biochemically similar disorders. Only DBS samples that displayed elevated C16:0-sulfatide levels, a reduction in ARSA activity and disease-causing variants in the ARSA gene were considered screen positives. This study showed that neonatal screening for MLD is feasible with a near 100% assay specificity, however, the screening could not differentiate between MLD subtypes [58]. The first prospective pilot study in Hannover, applies a two-tier method using sulfatide screening as the first-tier and genetic testing (including the ARSA, PSAP and SUMF1 gene) as the second-tier. As of December 2023, within a period of 21 months, 109 259 samples were screened among which three screen positives were identified. In all these screen positives, MLD was confirmed afterwards. There have been no false positive cases reported during the first two years of screening [59].

1.5.2. Genetic screening

Genetic-based screening methods, such as next-generation sequencing and multiplex sequencing, are also analysed. In this approach, individuals carrying disease causing variants in the ARSA gene are identified as screen positive. They then require further biochemical and genetic evaluation, including parental carrier testing, to confirm the diagnosis of MLD. Genetic screening studies typically include likely pathogenic or pathogenic ARSA variants. In a subset of these studies, only the most common disease-causing ARSA variants were reported. The sensitivity and specificity of such studies are still under investigation. However, due to the genetic heterogeneity inherent in MLD and the presence of numerous rare and/or private variants especially in non-Caucasian groups, genetic screening has the risk of being less sensitive than biochemical screening methods [60].

Considering that early treatment is crucial in all MLD subtypes [47, 48,52], NBS programmes are highly advocated. However, the identification of pre-symptomatic neonates with MLD via NBS entails clinical challenges concerning adequate phenotype prediction, monitoring, and treatment of NBS-identified MLD cases.

Scientific uncertainties about predicting phenotype and treatment eligibility persist, hindering evidence-based decision-making.

Regulatory and reimbursement challenges surrounding innovative therapies delay access and cause disparities in MLD care across countries, even within Europe. National or local introduction of different types of newborn screening programs for MLD driven by several academic and commercial parties make these differences even larger. A uniform and evidence-based approach is urgently needed.

The goal of this project was to attain an unbiased agreement among experts in MLD on the clinical management and prognosis of MLD cases discovered through NBS. By doing so, we intend to establish a standardized clinical management approach which will benefit cross-border harmonization of care and appropriate use of treatments in MLD.

2. Methods

2.1. Expert panel and patient advocates

The MLD initiative (MLDi), in collaboration with the MLD Guideline Working Group from the European Reference Network on Rare Neurological Diseases (ERN-RND), established a list of potential panelists from MLD expert centers. A multidisciplinary panel of MLD experts was identified based on expertise in MLD diagnosis, treatment, and published research. All candidates were sent an email and given the opportunity to express their interest by completing a survey on Microsoft Forms and all but one accepted. The multidisciplinary expert panel ($n = 22$) was composed of nine pediatric neurologists, three adult neurologists, three physicians with expertise in pediatric inherited metabolic diseases, two physicians with expertise in adult inherited metabolic diseases, two pediatric hematologists, one adult hematologist, two pediatricians, and one geneticist. Experts were located across Canada ($n = 1$), Denmark ($n = 1$), France ($n = 2$), Germany ($n = 6$), Israel ($n = 1$), Italy ($n = 2$), the Netherlands ($n = 4$), Norway ($n = 1$), Sweden ($n = 1$), the United Kingdom ($n = 1$), and the United States of America ($n = 2$). In addition, patient advocates from the European Leukodystrophy Association (ELA) and the Dutch Association for inherited metabolic diseases (VKS) participated in writing and reviewing the manuscript.

2.2. Real-time delphi procedure

To establish consensus statements, we performed a real-time Delphi procedure, utilising the web-based software tool eDELPHI (<https://www.edelphi.org/>). The topics and questions provided in the real-time Delphi approach were determined through a review of the available literature, existing clinical decision algorithms (University of Tübingen and San Raffaele Hospital in Milan), and consultation with clinical MLD experts ($n = 15$) and biochemical/screening professionals ($n = 18$), during two digital workshop meetings held on April 26, 2023, and June 27, 2023. Additionally, clinical decision algorithms developed by the University of Tübingen, San Raffaele Hospital in Milan and Meyer Hospital in Florence were taken into consideration. The expert panel identified seven predetermined themes for the topic clustering: clinical management of NBS positive cases, confirmatory diagnostics, phenotypic prediction, monitoring, treatment, and specificity and sensitivity of screening. A background document providing a literature overview addressing all relevant topics for this procedure, as well as a recommended reading list were shared with the panel.

During a seven-week period from October 11, 2023 to November 29, 2023, the platform was open to ongoing discussion with the ability to review the anonymous justifications from other panelists, respond to queries, debate differences in opinions and revise answers. The moderator (DHS) monitored responses to ensure all panelists actively participated. Responses required panelists to indicate agreement or disagreement using a 3-point Likert scale, select preferences in single/multiple choice questions, or provide open-ended answers. Three levels of consensus were defined based on the percentage of agreement: A) strongly recommended (100% agreement), B) recommended (75–99% agreement), and C) suggested (50–74% agreement or more than 75%

agreement, but more than 25% of neutral votes). The moderator evaluated automatically generated visual reports from the eDELPHI system to monitor the procedure progress. Those reports were then distributed to all panellists. The comments and arguments were subject to qualitative analysis. Whenever new queries were suggested, or recommendations were to be reworded, the moderator updated the queries accordingly. Upon reaching consensus on all items, the outcomes were descriptively analysed, and the level of agreement was calculated for all queries. A final meeting was held on November 29th, 2023, to address any remaining topics of discussion. All items with >50% neutral votes were explicitly addressed in this meeting and a decision was made to either keep the recommendation as a level C recommendation or to remove it as a recommendation and instead mention it as a research gap. The patient advocates reviewed and refined the output from the Delphi process.

3. Results

3.1. Clinical management of NBS positive cases

The expert panel developed 57 consensus recommendations through a Delphi consensus procedure (Table 1).

3.1.1. Initial counselling and family involvement

Question 1 to 13 addressed the counselling of families of an individual with a positive screening result (Table 1): It is strongly recommended that the family is informed about the contact at an MLD expert center when a positive screening result is communicated (level A). MLD expert centers for initial counselling were defined as either nationally recognized expertise centers or centers with significant expertise in diagnosing, treating, and managing MLD patients, staffed by a multidisciplinary team of experts in pediatric neurology, neurology, genetics, hematology and various therapy fields and engaged in networks aimed at advancing MLD care. Whenever feasible, it is recommended that the initial contact with the family should be handled by experts in MLD (level B). Families should be informed that a positive screening result requires confirmatory diagnostics before being considered an established diagnosis (level A). The importance of timely interaction with the family was stressed by the panel and patient advocates, but no recommendations on number of days not to be exceeded were made as this is subject to national regulatory standards. In addition to counselling by an MLD expert and a geneticist (level A), a multidisciplinary team should be available to assist the family following diagnosis. Moreover, the family should be informed about patient support groups and offered support by psychosocial support (level A). Additionally, discussing the option of participating in international registries and/or research projects with the caregivers should be considered (level A).

It is recommended to base treatment decisions on the consensus of an international expert round (level A). Therefore, families should be asked for permission to discuss their case and informed about this process (level B). MLDi and ERN-RND facilitate international panel discussions to provide case-based treatment advice [62,63]. The expert panel should be composed, as outlined by MLDi (<https://www.mldinitiative.com/or-professionals/treatment-eligibility/>).

In case of uncertainty of the phenotype prediction a geneticist should attend the treatment eligibility panel (level B). The panel intends to discuss all NBS-identified patients in Europe and offers recommendations on therapeutic options, predicted disease onset, timing of therapy, additional diagnostics, and monitoring before treatment. The time from positive screening result to confirming the diagnosis and reaching a treatment decision by the treatment eligibility panel should not exceed 3 months (level B).

Key considerations of patient representatives substantiating these recommendations were that maintaining a transparent decision-making process is crucial in counselling affected families. Especially neonatal screening for disorders with variable onset throughout life requires a

Table 1

Expert-consensus recommendations.

Number	Level	Recommendations	Agreement	Neutral votes
		COMMUNICATION AND COUNSELLING		
1	A	It is strongly recommended that the family is informed about the contact at an expert center when a positive screening result is communicated.	100%	0%
2	B	It is recommended that an expert for metachromatic leukodystrophy (MLD) from a national referral center contacts the family after a positive screening result.	95%	5%
3	A	It is strongly recommended to inform the family that the positive screening results need to be confirmed by diagnostic tests.	100%	5%
4	A	After confirming the diagnosis, it is strongly recommended to offer genetic counselling to the family.	100%	5%
5	A	After confirming the diagnosis, it is strongly recommended to inform the family about patient support groups .	100%	18%
6	A	After confirming the diagnosis, it is strongly recommended to offer the family support by psychosocial services of the referral center.	100%	14%
7		It is recommended to counsel medically by a multidisciplinary MLD team including medical experts:		
		paediatric neurologists	86%	
		metabolic paediatricians	55%	
		geneticist	55%	
		<i>optional:</i> hematologist/transplanter and paramedical experts	45%	
		social workers	64%	
8	A	After confirming the diagnosis, it is strongly recommended to inform the family about research programs and registries , including the MLD initiative registry.	100%	18%
9	B	After confirming the diagnosis, it is recommended to ask the family's permission to discuss the case with international experts .	95%	14%
10	A	It is strongly recommended to arrange a treatment eligibility panel discussion according to the procedure from the ERN-RND and the MLD initiative to discuss the treatment eligibility.	100%	0%
11	B	It is recommended that in case of uncertainty about the phenotype a geneticist attends the treatment eligibility panel discussion.	88%	16%
12		It is recommended that the treatment eligibility panel formulates a consensus-based advice on:		
		• therapeutic options	90%	
		• predicted disease onset	86%	
		• timing of therapy	86%	
		• additional confirmatory diagnostics when applicable	73%	
		• monitoring scheme before treatment	64%	
13	B	It is recommended that the entire timeline after a positive screening result is established to treatment	<i>Likert scale</i> 95%	0%

Table 1 (continued)

Number	Level	Recommendations	Agreement	Neutral votes
		decision should not exceed 3 months.		
		CONFIRMATORY DIAGNOSTICS		
		Genetic assessment		
14	A	It is strongly recommended to perform confirmatory genetic testing of the index patient and its parents in all newborn screening positive cases.	100%	18%
15	A	It is strongly recommended to include the ARSA gene in the genetic test.	100%	5%
16	B	It is recommended to include testing of the pseudodeficiency alleles in the genetic test.	89%	18%
17	A	It is strongly recommended to classify genetic variants according to American College of Medical Genetics (ACMG)	100%	18%
18	A	It is strongly recommended to perform functional studies including ARSA activity in blood and urinary sulfatides for diagnostic confirmation in all variants of unknown significance (class 3) in the ARSA gene.	100%	14%
19	C	It is suggested to discuss the reclassification of class 3 variants in ARSA based on functional and statistical evidence as available.	100%	27%
20	A	It is strongly recommended to offer comprehensive genetic counselling to identify potentially affected relatives.	100%	0%
21	B	In case the screening does not include genetic testing, it is recommended to actively exclude mimicking disorders. This means that Prosaposin deficiency caused by pathogenic variants in the PSAP gene and multiple sulfatase deficiency caused by pathogenic variants in the SUMF1 gene should be excluded.	89%	18%
		Biochemical assessment		
22	A	It is strongly recommended to perform a confirmatory ARSA enzyme activity test in all newborn screening positive cases.	100%	9%
23	A	It is strongly recommended to assess the ARSA enzyme activity in EDTA blood .	100%	9%
24	C	It is suggested to measure and evaluate ARSA enzyme activity according to local standards and reference values.	100%	33%
25	B	It is recommended to measure urinary sulfatides in all newborn screening positive cases.	88%	23%
26	C	It is suggested to measure and evaluate sulfatide excretion in urine according to local standards and reference values.	100%	29%
27	C	It is suggested to conduct additional testing, including fibroblast studies or other <i>in vitro</i> systems, if all previous genetic and biochemical tests are inconclusive.	86%	36%
28	A	It is strongly recommended to standardize laboratory procedures for assessing measuring ARSA enzyme activity and urinary sulfatides across laboratories to enable cross-lab comparison.	100%	11%

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Table 1 (continued)

Number	Level	Recommendations	Agreement	Neutral votes
29	A	PERSERVATION OF BIOMATERIAL	100%	0%
		It is strongly recommended to archive biosamples collected in newborn screening identified cases to enable future studies according to local ethics votes. • Dried blood spots • Blood-EDTA plasma • Urine • DNA Optional: • Blood heparin plasma • Blood serum • Blood – Paxgene tube • Fibroblasts when collected in context of routine diagnostics/care • Cerebro spinal fluid when collected in context of routine diagnostics/care		
30	A	PREDICTION OF SYMPTOM ONSET	100%	5%
		It is strongly recommended to predict the age of symptom onset based on the following aspects ordered by priority: • family history, i.e. age of symptom onset in relative with identical genotype • genotype and reported onset literature • ARSA enzyme activity in blood		
31	C	It is suggested to anticipate late-infantile onset in case biallelic loss of function variants in the ARSA gene are detected.	100%	27%
32	C	It is suggested to anticipate late-infantile onset in case a loss of function variant is detected in compound heterozygous state with a class 4/5 variant with reported late-infantile onset in literature.	100%	36%
33	C	It is suggested to anticipate late-infantile onset in case biallelic class 4/5 variants with reported late-infantile onset in literature are detected.	100%	36%
34	C	It is suggested to anticipate early juvenile onset in case of individuals harbouring a known genotype with mostly earlyjuvenile onset.	92%	36%
35	C	It is suggested to anticipate early juvenile onset in case of individuals harbouring a loss of function variant in compound heterozygous state with the c.1283C>T variant.	89%	55%
36	B	It is recommended to anticipate earlyjuvenile onset instead of late-juvenile onset, even if the reported onset in literature for the respective genotype is late-juvenile in ≥80% of the reported cases.	78%	14%
37	C	It is suggested to anticipate late onset in case of individuals harbouring the c.542T>G variant in compound heterozygous state with another class 4/5 variant.	90%	50%
38	C	It is suggested to anticipate late onset (late-juvenile or adult) in case of individuals harbouring the c.1283C>T variant in homozygous state.	86%	32%
39		Late onset can be predicted for individuals harboring a known	100%	0%

Table 1 (continued)

Number	Level	Recommendations	Agreement	Neutral votes
40	C	genotype with late-juvenile or adult onset well reported in literature.	83%	37%
		DEFINITION OF DISEASE ONSET It is suggested to define the symptom onset as the onset of MLD-related symptoms. MLD-related symptoms are defined as clinical manifestations of the neurodegeneration (incl. developmental stagnation) that can be experienced by the patient and/or its caretakers or noted as changed function.		
41	A	It is strongly recommended to interpret the following measurable abnormalities as (sub)clinical evidence for the disease onset when associated with a MLD related genotype. - Brain MRI abnormalities (e.g. T2-hyperintensity in corpus callosum) - Presence of electro-neurophysiological abnormalities (e.g. decreased nerve conduction velocities in peripheral nerves, BAER) - Sonographic gall bladder abnormalities (e.g. thickened gall bladder wall, sludge, or polyps) - Abnormalities at neurological examination	100%	11%
		TREATMENT It is strongly recommended to treat MLD patients before they exhibit MLD-related symptoms. Late-infantile MLD		
42	A	It is strongly recommended that late-infantile patients are treated with autologous hematopoietic stem cell gene therapy (arsa-cel).	100%	0%
43	A	It is strongly recommended to treat late-infantile patients with allogeneic hematopoietic stem cell transplantation.	100%	0%
44	C	It is <u>not</u> recommended to treat late-infantile patients with allogeneic hematopoietic stem cell transplantation.	93%	41%
45	C	It is suggested to schedule late-infantile patients for apheresis between 5 and 9 months depending on weight (>5 kg body weight) and feasibility at the treatment center.	80%	59%
46	A	It is strongly recommended <u>not</u> to wait with apheresis until there is observable (sub-)clinical evidence for disease in late-infantile patients.	100%	45%
47	C	Early-juvenile MLD It is suggested to treat early-juvenile patients with autologous hematopoietic stem cell gene therapy (arsa-cel).	100%	32%
48	A	It is recommended to schedule earlyjuvenile patients for apheresis between 9 and 12 months (>8 kg body weight).	100%	0%
49	A	It is recommended to treat pre-symptomatic early-juvenile patients with allogeneic hematopoietic stem cell transplantation only in case arsa-cel is not available.	100%	0%
50	C	Late-juvenile MLD It is suggested to treat late-juvenile patients with allogeneic hematopoietic stem cell transplantation.	100%	45%

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Table 1 (continued)

Number	Level	Recommendations	Agreement	Neutral votes
51	C	It is suggested to schedule late-juvenile patients for allogeneic hematopoietic stem cell transplantation as soon as there is subclinical evidence for the disease.	77%	32%
52	C	Adult MLD It is suggested to treat adult patients with allogeneic hematopoietic stem cell transplantation.	75%	50%
53	A	It is <u>not</u> recommended to schedule adult patients for allogeneic hematopoietic stem cell transplantation at a predefined age, but to be guided by a case-to-case decision of the treatment eligibility panel.	100%	0%
54	C	It is suggested to schedule adult patients for allogeneic hematopoietic stem cell transplantation as soon as there is subclinical evidence for the disease MLD.	77%	32%
55	A	Unpredicted MLD subtype In case of patients with an unpredictable MLD subtype it is strongly recommended to choose the best treatment option and schedule treatment as soon as there is subclinical evidence for the disease MLD.	100%	11%
56	A	MONITORING Regular post-treatment follow-up in an expert center is recommended in all newborn screening identified patients.	100%	0%
57	A	NEWBORN SCREENING FOR MLD Newborn screening for MLD is recommended and aligns with the established criteria [61].	100%	0%

thoughtful and sensitive communication with identified families.

3.2. Confirmatory diagnostics

Next, the Delphi approach addressed the recommended procedures for the confirmation of the diagnosis of MLD in a newborn who has been identified by the NBS (questions 14 to 29). A positive screening result for MLD needs to be validated by confirmatory diagnostics which includes a combination of biochemical and genetic testing (level A).

3.2.1. Genetic assessment

To establish the molecular diagnosis of MLD in individuals with positive screening results, it is necessary to confirm the molecular diagnosis by sequencing of the ARSA gene from the blood of the index case and its biological parents to determine biallelic localization of the detected variants (level A). In order to identify potentially affected relatives, comprehensive genetic counselling is strongly recommended (level A). The variants identified in the ARSA gene must be classified based on the American College of Medical Genetics (ACMG) criteria (level A). If variants of unknown significance are detected in the ARSA gene, functional and statistical evidence is necessary to establish the molecular diagnosis of MLD including elevated urinary sulfatides and reduced ARSA activity in blood (level A). To prove the pathogenicity of variants of uncertain significance an ARSA assay in fibroblasts and other *in vitro* models can be of use (level C). Based on the functional and statistical evidence available variants of unknown significance (class 3) can be reclassified (level C).

To avoid false positive screening results, the genetic sequencing should include the reporting of pseudo-deficiency alleles in the ARSA gene (level B).

Individuals with biochemically similar disorders such as MSD or Prosaposin deficiency should be excluded preferably during the screening process, but at the latest during the confirmatory diagnostic process by genetic sequencing of the PSAP and SUMF1 genes (level B).

3.2.2. Biochemical assessment

The biochemical assessment to confirm the diagnosis of MLD in individuals identified by NBS comprises the measurement of ARSA enzyme activity and urinary sulfatides. It is indispensable to perform the ARSA enzyme assay in leukocytes as confirmatory diagnostics using EDTA tubes of the index case (level A). Only if standard biochemical and genetic tests are inconclusive, ARSA activity in fibroblast and other *in vitro* models may be useful (level C). Urinary sulfatides should be measured in all newborns identified by NBS (level B). Due to the absence of an internationally standardized assay for ARSA activity in blood and sulfatides in urine, it is suggested to perform the measurements in centers with expertise in MLD to ensure the accuracy of reference values (level C).

As use of different laboratory techniques and units hampers cross-center comparisons, it is strongly recommended to standardize laboratory procedures for assessing measuring ARSA enzyme activity and urinary sulfatides (level A).

3.2.3. Perservation of biomaterial for future research

To endorse future research, it is strongly recommended to collect and archive biosamples of MLD patients (level A). Dried blood spots, plasma from EDTA tubes, urine and DNA are considered important by the expert panel (level A) whereas other samples are considered as optional (as listed in Table 1 recommendation 29).

3.3. Prediction of MLD subtypes

The next cluster of questions (30–39) developed recommendations regarding the prediction of MLD subtypes in pre-symptomatic neonates. It is strongly recommended to predict the age of symptom onset based on family history (level A), genotype (level A), and ARSA enzyme activity (level A). The panel underlined the predictive importance of family history to predict the onset, yet the number of index cases without helpful family history will increase due to NBS programs. Moreover, the intrafamilial variability regarding disease onset can be considerable, in particular across the juvenile cohort, less so in late-infantile MLD [18]. In neonates without affected relatives the prediction relies primarily on genotype-phenotype correlations as published in literature and public databases. Despite remaining uncertainty, the panel acknowledged the predictive value of the following genetic predispositions for disease onset with the prerequisite that future genotype data will be published: Late-infantile onset is linked to biallelic LoF variants in the ARSA gene including homozygosity for the most common ARSA variant c.465+1G>A. This association is supported by a level C consensus, with unanimous agreement (100%), except for 27% of neutral votes; It is suggested to anticipate late-infantile disease onset, if biallelic disease-causing variants (class 4 and 5) in the ARSA gene are detected that have been reported consistently in individuals with late-infantile disease onset (level C; 100% agreement, except 36% neutral votes). It is suggested to anticipate early-juvenile onset in case of individuals harbouring a known genotype with mostly early-juvenile onset (level C; 92% agreements, except 36% neutral votes). It is recommended to anticipate early-juvenile onset instead of late-juvenile onset, even if the reported onset in literature for the respective genotype is late-juvenile in ≥80% of the reported cases (level B; 78% agreement, except 14% neutral votes). In line, it is suggested to anticipate an early-juvenile disease onset if the second most frequent variants c.1283C > T, p. (Pro428Leu) is detected in compound-heterozygous state with a LoF

variant (level C; 89%, except 55% neutral votes). The panel deliberated on the predictive likelihood of late-onset subtypes associated with specific ARSA variants that result in relatively high residual ARSA activity. This discussion included consideration of the divided expert opinion on the matter, given that such variants are reported in late-onset phenotypes: homozygosity for the c.1283C>T, p.(Pro428Leu) variant is in all probability associated with late-juvenile or adult onset (level C;

agreements 86%, except 32% neutral votes); compound-heterozygosity for the c.542T>G, p.(Ile181Ser) variant is mostly associated with late-juvenile and adult onset of MLD (level C; 90% agreement, 50% neutral votes). Several experts underlined that despite these firm genotype-phenotype correlations, biological outliers have been observed and respective individuals need to be monitored clinically to detect subclinical evidence of disease progression.

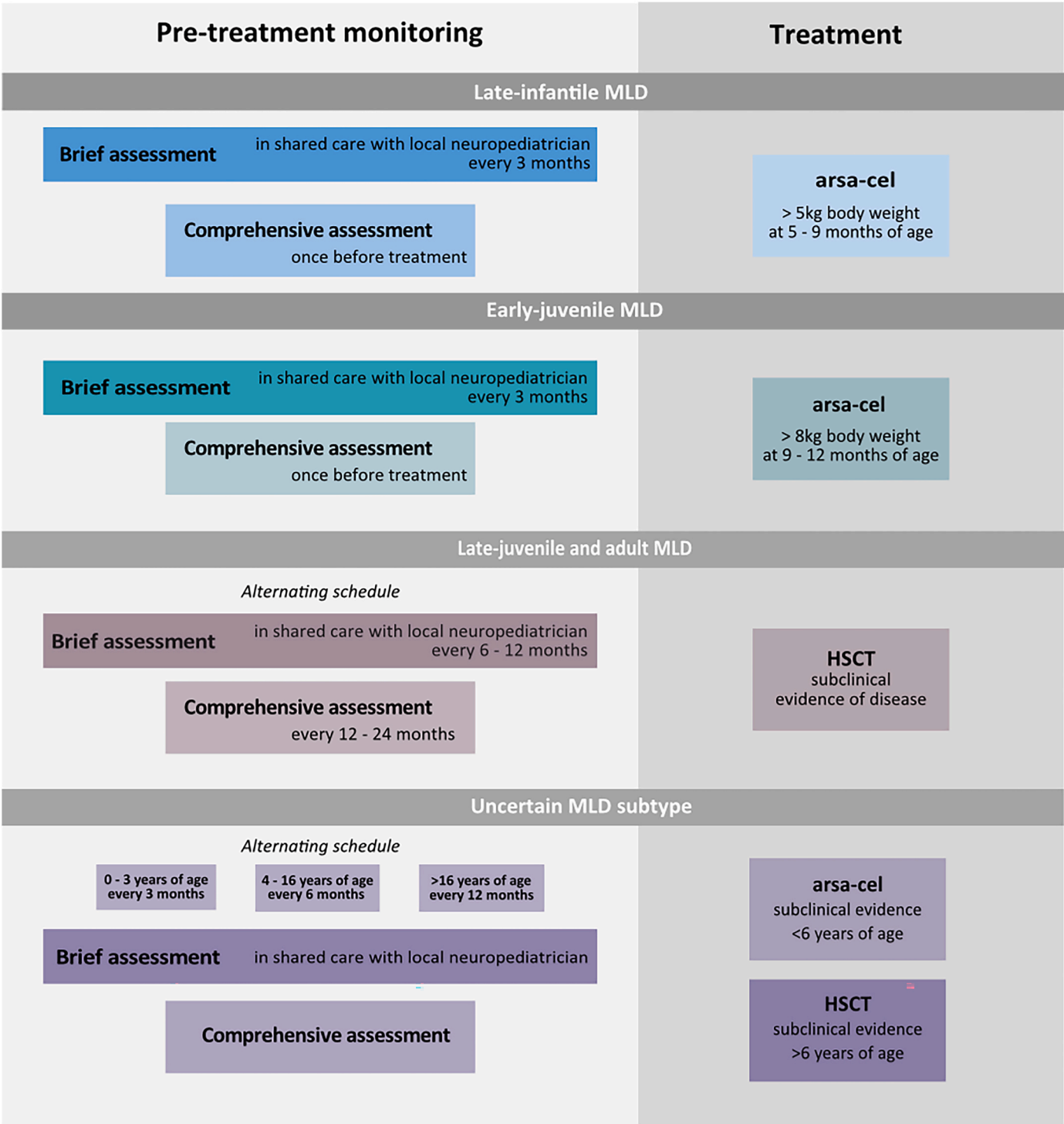


Fig. 1. Monitoring and management algorithm for individuals identified by newborn screening for metachromatic leukodystrophy. The monitoring and management recommendations are designed for four scenarios: 1) predicted late-infantile onset 2) predicted early-juvenile onset 3) predicted late onset (including late-juvenile and adult) and 4) predicted uncertain onset. Monitoring includes brief assessments and comprehensive assessments in alternating schedules (Table 2). This allows for collaborative care with local neuropsychiatricians and neurologists. Periodic post-treatment assessments are required according to the local standards of each MLD treatment center.

3.4. Definition of MLD symptom onset

The expert panel suggested defining the symptom onset of MLD as the onset of MLD-related symptoms due to neurodegeneration, that can be experienced by the patient and/or its caretakers or noticed as changed neurological function (Table 1). The panel emphasized to maintain the widely adopted definition of MLD subtypes based on the age of symptom onset in natural, i.e., untreated, disease course, and acknowledged the difficulties in classifying pre-symptomatic NBS-identified patients according to this definition. Instead, clear descriptions of the certainty of the MLD diagnosis including the available evidence for clinically relevant signs of MLD were advocated by the expert panel. In this description it is strongly recommended to interpret measurable abnormalities on brain MRI, electro-neurophysiological tests and gall bladder ultrasound or even subtle abnormalities in the neurological examination (such as mildly reduced or increased reflexes) as subclinical evidence for the disease (level A).

3.5. Treatment

The workgroup examined considerations of treatment strategies for identified individuals (questions 42–55). The panel decided to establish a clinical decision algorithm based on four scenarios: 1) late-infantile onset with great certainty; 2) early-juvenile onset with great certainty; 3) late-juvenile/adult onset with great certainty (this was combined as to date late-juvenile and adult onset cannot be distinguished based on genotype data in pre-symptomatic individuals); 4) uncertain onset (cannot be predicted with reasonable probability). Great certainty means highest possible likelihood based on currently available evidence.

Recommendations are made for each scenario in terms of the type of treatment, timing of treatment and monitoring (Fig. 1).

It is strongly recommended to initiate treatment in identified individuals with MLD before they exhibit MLD-related symptoms (level A).

3.5.1. Arsa-cel for early-onset MLD subtypes

It is strongly recommended to treat pre-symptomatic late-infantile (level A) and pre-/early-symptomatic early-juvenile (level B; agreement 100%, except 32% neutral votes) MLD patients with arsa-cel (Fig. 1). HSCT is not recommended as treatment for MLD patients with predicted late-infantile onset (level C; agreement 93%, except 41% neutral votes). If arsa-cel is available, it is not recommended to treat early-juvenile with HSCT (level A).

These recommendations encompass the notable effectiveness and safety demonstrated by arsa-cel in individuals with pre-symptomatic late-infantile MLD, as well as those with early- or pre-symptomatic early-juvenile MLD [23,52,53]. Despite the absence of treatment-related serious adverse events or fatalities associated with arsa-cel [23,52,53], the panel stressed that uncertainties persist regarding its long-term (>10 years) effectiveness and safety, necessitating post-authorization monitoring.

3.5.2. Timing of arsa-cel treatment for early-onset MLD

It is strongly advised to treat any individual with MLD who has been identified by NBS with predicted late-infantile or early-juvenile disease onset before the onset of symptoms (level A). Therefore, apheresis should be initiated for individuals with late-infantile, around 5–9 months of age (currently the lowest feasible body weight for apheresis is 5 kg, but most centers prefer a higher apheresis weight (level C). Apheresis appointments for individuals with predicted early-juvenile onset should be arranged between 9 and 12 months of age, when body weight is at least 8 kg (level C).

3.5.3. Treatment for early-onset MLD in low-resource countries

Only if arsa-cel is not available, e.g., due to regional availability and when a referral to a treatment center abroad is not possible, HSCT may

be considered for presymptomatic individuals with predicted onset of early-juvenile disease (level A). Treatment should then be scheduled between 9 and 12 months. Nevertheless, the panel highlighted that individuals with predicted late-infantile onset do not benefit from HSCT, that might even accelerate the natural disease course [46].

3.5.4. Hematopoietic stem cell transplantation for late-onset MLD

It is suggested to treat individuals with a predicted late-juvenile onset with HSCT (level C, 100% agreement, except 45% neutral votes). It is also suggested that individuals with a predicted adult onset should undergo HSCT (level C, 75% agreement, except 50% neutral votes). The panel stressed, however, that data on long-term outcomes in adult patients following HSCT are sparse and have not been systematically evaluated in larger cohorts.

3.5.5. Timing of hematopoietic stem cell transplantation for late-onset MLD

The panel suggested scheduling treatment in predicted late-onset cases as soon as there is subclinical evidence for disease onset (level C). It is not recommended to schedule late-onset MLD patients for HSCT at a predefined age, but to be guided by a case-to-case decision of the treatment eligibility panel (level A).

This question of timing the treatment resulted in divided expert opinions: Therefore, several experts and the patient advocacy groups were in favour of treating suspected late-onset MLD patients as early as possible considering the following arguments: 1) Late-juvenile MLD individuals show best outcomes when treated with HSCT in the pre-symptomatic stage [46–48]. 2) The impact of HSCT takes 12–18 months post-transplantation before the disease stabilizes, and 3) the HSCT-associated morbidity and mortality are lower at younger ages. Conversely, the majority of the panel support a deferred approach until subclinical evidence of disease onset is present because of the following considerations. 1) There is uncertainty about the long-term outcomes of HSCT in late-juvenile MLD exist and data on adult MLD is sparse [46,50, 51]. 2) It is currently challenging to distinguish between late juvenile and adult onset in newborns based on genotype or ARSA activity, unless symptomatic relatives with an identical genotype are present [7]. 3) Better therapies for late-onset MLD might be available in the near future as an ongoing trial on arsa-cel for late-juvenile MLD patients has completed enrollment (<https://clinicaltrials.gov/study/NCT04283227>) 4) HSCT-treated patients may become ineligible for these forthcoming treatments, potentially depriving them of optimal care.

The assessment of risks and benefits was emphasized as a crucial aspect of individual counselling. Considering the predicted age of onset and implementing a rigorous pre-treatment monitoring scheme were deemed essential in making well-informed decisions about the initiation of treatment in each case.

3.5.6. Treatment decision for uncertain subtypes

The prediction of disease onset poses a significant challenge for NBS programmes for MLD in cases of rare and/or unique genotypes. For those with unclear disease onset, a systematic monitoring scheme is crucial to enable early treatment during pre- and early-symptomatic disease stages (Fig. 1).

If disease onset cannot be predicted, those affected should undergo frequent monitoring and receive treatment promptly upon displaying subclinical evidence of disease (level A). The appropriate treatment option should be selected based on the age at which subclinical disease features appear.

3.6. Monitoring

Monitoring is defined as the surveillance of patients before they are eligible for treatment. The panel recommended to closely monitor NBS-identified MLD patients to identify subclinical evidence of the disease enabling timely treatment in pre-symptomatic disease stages. The panel agreed that, based on the phenotype prediction, different intensities of

monitoring schemes should be applied with age-tailored sets of assessments as shown in Fig. 1. The monitoring scheme comprises *comprehensive assessments* to be done at the MLD expert center and *brief assessments* that can be offered in shared care with the local neuro-pediatrician or neurologist (Fig. 1, Table 2). The frequency of assessments during the pre-symptomatic phase of the disease should be tailored within the proposed schedule to the needs of the family to balance the burden of the disease.

3.6.1. Clinical examination and motor function assessment

It is strongly recommended to perform a neurological examination including an evaluation of developmental milestones at every visit of the monitoring regardless of age (level A). The suggested frequency of clinical evaluation depends on the predicted subtype (Fig. 1) and can be done in collaboration with the local paediatrician or neurologist. In particular, patients with uncertain onset should be followed closely depending on the age (Fig. 1). It is strongly recommended that the GMFC-MLD level (level A) and optionally the GMFM-88 are determined during each *comprehensive assessment*.

3.6.2. Brain MRI

Depending on the age and MLD subtype of the patient, it is strongly recommended to perform an MRI every 12–24 months during the surveillance phase (level A). In patients with uncertain onset, MRI should be performed more frequently according to age as suggested in Fig. 1. Additionally, it is strongly recommended to perform an MRI shortly, defined as 0–3 months, before starting treatment (level A). The panel furthermore acknowledged the logistic challenges, such as MRI requiring sedation or general anaesthesia in young children, that may affect feasible timing and frequencies of the MRIs.

3.6.3. Neurophysiology

The panel strongly recommended to conduct the NCV measurement as part of the *comprehensive assessment* every 12–24 months (level A). In patients with uncertain onset, NCV should be performed more frequently according to age as suggested in Fig. 1. The panel acknowledged the need for further standardization and research [10], see also below.

Due to the lack of data in pre-symptomatic MLD patients, VEP and BAER remain optional and can be done in a research context to evaluate their predictive impact.

3.6.4. Gall bladder ultrasound

The panel strongly recommended performing a gall bladder ultrasound every 12–24 months in MLD patients (level A). However, several experts pointed out that there is no evidence that gall bladder abnormalities correlate with central nervous disease onset. Hence, this would only justify organ specific therapeutic interventions such as a cholecystectomy.

Table 2
Monitoring assessments.

Brief assessment	Evaluation of developmental milestones Neurological examination
Comprehensive assessment	• Neurological examination • Gross motor function classification for MLD (GMFC-MLD) • MRI • Nerve conduction velocities • Gall bladder ultrasound • Neuropsychological assessment Optional: Gross motor function measurement 88 (GMFM-88) Optional: preservation of biomaterial in a research context

3.7. Research agenda

The panel faced challenges in achieving consensus on specific items due to insufficient evidence. These particular areas are marked by significant scientific uncertainty, and there is an urgent need for recommendations. The panel collectively acknowledged that these research gaps should be given priority in future research, as outlined in Table 3, underscoring the significance of systematic data collection and continuous evidence generation, critical research aspects encompass.

3.7.1. Genotype-phenotype correlations

Further understanding of genotype-phenotype correlations is essential to unravel the uncertainties regarding disease prediction in pre-symptomatic neonates.

3.7.2. Predictive biomarkers

Identification and validation of potential biomarkers for predicting disease onset and monitor progression is highly warranted. This includes:

Accurate measurements of **ARSA enzyme activity** in blood as a correlation between residual ARSA activity *in vivo* and the age of onset is suggested [7,64]. In particular for the early-onset subtype, its positive predictive value has been reported as 100% when residual ARSA activity is below 1% [7]. However, the lack of standardization of ARSA assays in large cohorts obstructs cross-comparison between centers, and its predictive value depends on local standards. To establish residual enzyme activity as an additional parameter in the prediction of disease onset, internationally harmonized laboratory procedures are essential.

Future studies evaluating the predictive value of **sulfatides levels** in blood, dried blood spots (DBS), and/or urine are warranted.

The evaluation of **NfL** in large cohorts of pre-symptomatic MLD patients and over the course of the disease is necessary to evaluate its use as a predictive and/or monitoring parameter.

3.7.3. Instrumental tests

Further studies on clinically and biologically meaningful **MRI biomarkers** especially in pre-symptomatic individuals are needed [65–67].

A systematic evaluation of **NCV** during the pre-symptomatic disease stage for understanding when abnormalities can first be detected and whether this correlates with disease severity, disease progression and/or distinct genotypes.

Further tools to measure the degree of central demyelination such as **VEP** and **BAER** need to be studied in larger cohorts of MLD patients. To date there is a lack of evidence when abnormalities in VEP and BAER tests become apparent in the disease course and whether these predict disease progression.

Determining when **gall bladder abnormalities** become apparent in the natural history of the disease and whether they correlate with disease progression requires further investigations.

3.7.4. Comparative efficacy and safety of HSCT and arsa-cel

Further insight into the comparative efficacy and safety of HSCT and arsa-cel in late-onset MLD is essential for guiding treatment decisions.

3.7.5. Timing for HSCT in predicted late-onset MLD

Understanding when to schedule HSCT for late-onset MLD patients is a critical knowledge gap that requires attention for optimizing treatment outcomes.

3.7.6. Psychological and psychosocial burden on families

Addressing the psychological and psychosocial burden on patients and their families when late or unclear onset is predicted is essential for providing holistic care and support.

Despite the need to generate more evidence-based data, particularly for the cohort of pre-symptomatic MLD patients, all panel members agreed that newborn screening for MLD is recommended and aligns with

Table 3

Knowledge gaps.

1. Further understanding of genotype-phenotype correlations
2. Identification and validation of potential biomarkers to predict disease onset including
 - a. ARSA enzyme activity
 - b. Sulfatides
 - c. NFL
3. Improved understanding of instrumental tests as potential markers of disease activity in pre-symptomatic MLD, including
 - a. MRI
 - b. NCV
 - c. VEP and BAER
 - d. Gall bladder ultrasound
4. Insights into the comparative efficacy and safety of HSCT and arsa-cel in late-onset MLD.
5. Insights into when to schedule HSCT for late-onset MLD patients
6. The psychological and psychosocial burden on families when late or unclear onset is predicted

the established criteria (level A) [61].

4. Discussion

Since its discovery a century ago, MLD has presented a significant challenge as an incurable disease. HSCT has been the only available treatment for MLD; yet outcomes have exhibited variability, with compelling evidence limited to pre- and early-symptomatic cases of late-onset subtypes. The recent integration of the highly effective arsa-cel into standard of care for early-onset subtypes represents a pivotal moment, shifting the perspective from early-onset MLD as an irreversible neurodegenerative disorder to a condition that can be treated. With these treatment options available, **delayed diagnosis has become the major hurdle**: as two-thirds of patients are diagnosed too late to be eligible to benefit from treatment [68]. Also, for late-onset MLD, pre-symptomatic diagnosis by NBS offers time during which clinical decisions can be tailored to the best interest of the patient at affordable costs. Hence, the key to timely detection and successful treatment lies in the inclusion of MLD in national neonatal screening programs.

Pilot screening programs for MLD, led by various commercial and academic parties, are rapidly emerging. This brings unanswered questions regarding management of identified cases already in daily clinical practices of MLD clinicians. The premature introduction of screening without the relevant infrastructure to support families and divergent management approaches in different countries may impact successful outcomes. Recognizing the differences in healthcare practices across countries, there is a pressing need for uniform care pathways for individuals with MLD identified by NBS to ensure a consistent management and treatment approach.

Our recommendations are designed to navigate this new era of MLD treatment, by supporting physicians and acknowledging existing knowledge gaps. These recommendations, endorsed by patient advocates and various MLD experts, encompass paediatric and adult specialties and reflect a comprehensive approach.

Monitoring of screening programs is considered important as regular evaluations and adjustments based on emerging data are necessary. From other disease fields it is known that the disease spectrum changes after introduction of neonatal screening [69,70]. With neonatal identification of patients, the known phenotypic variation of the disease becomes larger and typically milder phenotypes become more prevalent.

The ethical implications surrounding early diagnosis by NBS in late-onset and uncertain onset MLD cases are substantial and warrant careful consideration. While early diagnosis facilitates close monitoring and timely treatment, it also poses the risk of inducing emotional and psychological distress for both individuals and their families. This includes practical hurdles like restricted life choices and limited access to certain services. Ensuring a delicate balance between the benefits of early diagnosis and the potential negative effects on quality of life necessitates the implementation of comprehensive psychosocial follow-up programs for affected individuals and their families, in particular when late or uncertain onset is predicted. Unlike other disorders discussed as

candidates for NBS programs, such as Krabbe disease [71], the exclusion of late-onset subtypes in MLD is not applicable. The primary reason for this is that predicting purely adult onset is currently not feasible based on genotype or other biomarkers. Late-onset genotypes may manifest in childhood, adolescence, or later stages of life and consequently some may require an early treatment intervention during childhood. A similar situation concerns adrenoleukodystrophy (ALD) where NBS is now part of several national screening programs and where onset of the cerebral form cannot be predicted. A survey of Dutch ALD patients resulted in a strong consensus of screening boys (100%) and all newborns regardless of sex (80%) [72].

Addressing the limitation of our consensus-based recommendation, we have to outline the lack of evidence and the limited number of experts in the field as inherent challenge in the context of rare diseases. We acknowledge that the recommendations are influenced by the initial questions posed. To mitigate this bias, we designed the questionnaire based on collaborative workshops involving MLD experts. Despite conducting the query among MLD experts, there is a considerable percentage of neutral votes for certain questions regarding subtype prediction or treatment. As we outlined, this reflects the lack of evidence regarding subtype prediction and the need for ongoing research (Table 3). It is also due to the multidisciplinary composition of the expert panel, for example an MLD geneticist can comment on the prediction of MLD subtype but not on the timing of treatment.

Emphasizing the significance of structured data collection and ongoing evidence generation, future studies should focus on key research aspects. These include assessing the predictive impact of measurable markers to anticipate disease onset during the presymptomatic stage. To achieve this, there is a need for additional data collection and analysis of MRI and NCV data in pre-symptomatic MLD patients, along with the evaluation of additional prognostic biomarkers in larger cohorts. Predicting MLD subtypes in neonates necessitates extended phenotype-genotype correlation studies. Additionally, harmonizing ARSA enzyme activity in leukocytes is crucial for more accurate predictions in this context. Therefore, initiatives like MLDi can serve as a tool for consolidating information and fostering collaboration between expert centers, patient advocacy groups, and regulatory authorities.

Despite uncertainties and challenges mentioned in this manuscript, all experts in MLD unanimously supported the implementation of NBS programs for MLD. This endorsement is driven by the recognized effectiveness of treatment when administered during the pre-symptomatic stage of the disease and the technical feasibility of NBS.

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Declaration of competing interest

L. A. Adang is a consultant to Takeda Pharmaceuticals, Orchard Therapeutics, and is a site sub-investigator for the Takeda trial. G. Bernard is/was a consultant for Calico (2023-present), Orchard Therapeutics (2023-present), Passage Bio Inc (2020–2022) and Ionis (2019). She is/was a site investigator for the Alexander's disease trial of Ionis (2021-present), Metachromatic leukodystrophy of Shire/Takeda (2020–2021), Krabbe (2021–2023) and GM1 gene therapy trials of Passage Bio (2021-present), GM1 natural history study from the University of Pennsylvania sponsored by Passage Bio (2021-present) and Adrenoleukodystrophy/Hematopoietic stem cell transplantation natural history study of Bluebird Bio (2019), a site sub-investigator for the MPS II gene therapy trial of Regenxbio (2021-present) and the MPS II clinical trial of Denali (2022-present). She has received an unrestricted educational grant from Takeda (2021–2022). Orchard Therapeutics: V. Calbi works as consultant for Orchard Therapeutics. F. Fumagalli is investigator of gene therapy clinical trials for MLD sponsored by Orchard Therapeutics, the licence holder of investigational medicinal product arsa-cel. Dr. Fumagalli has acted as ad-hoc consultants for Orchard Therapeutics advisory boards. The MLD gene therapy was licensed to GlaxoSmithKline in 2014, and then to Orchard Therapeutics in 2018. Telethon and Ospedale San Raffaele are entitled to receive milestone payments and royalties for such a therapy. M. Langeveld is involved in a premarketing studies with Sanofi-Genzyme, Protalix/Chiesi and Idorsia. Financial arrangements were made through AMC Research BV. No fees, travel support or grants were obtained from Pharmaceutical Industry. F. Mochel has participated in advisory boards arranged by Minoryx Therapeutics and Vigil Neuroscience. Her research work is funded by the French Ministry of Health (PHRC), the French Ministry of Research (ANR), the Paris Brain Institute and Minoryx Therapeutics. L. Schöls is a consultant to Vico Therapeutics and a site principal investigator for trials of Vigil Neuroscience, Stealth Biotherapeutics and PTC Therapeutics. C. Sevin is PI of the Takeda clinical trial and consultant for Orchard Therapeutics. A. Zerem is a site sub-investigator for the Takeda clinical trial and local PI for the Ionis clinical trial. Institutional research support from Shire plc and Orchard. Advisor and coinvestigator for trials in MLD (Shire/Takeda, Orchard) without personal payments. N. I. Wolf is researcher at the MLD initiative, which is a publicly funded academic registry and collaborative platform for metachromatic leukodystrophy and local PI for several multicenter trials for leukodystrophies (Takeda, Ionis, VigilNeuro). She does consultancies for Takeda, Ionis, VigilNeuro, Eli Lilly, PassageBio, Sana Biotech, Sanofi, without personal payments.

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References

- [1] J. Berger, B. Loschl, H. Bernheimer, et al., Occurrence, distribution, and phenotype of arylsulfatase A mutations in patients with metachromatic leukodystrophy, *Am. J. Med. Genet.* 69 (3) (Mar 31 1997) 335–340.
- [2] V. Gieselmann, I. Krageloh-Mann, Metachromatic leukodystrophy—an update, *Neuropediatrics* 41 (1) (Feb 2010) 1–6, <https://doi.org/10.1055/s-0030-1253412>.
- [3] M.H. Farah, C.I. Dali, S. Groeschel, et al., Effects of sulfatide on peripheral nerves in metachromatic leukodystrophy, *Ann. Clin. Transl. Neurol.* (Dec 26 2023), <https://doi.org/10.1002/acn3.51954>.
- [4] C. Kehler, S. Elgun, C. Raabe, et al., Association of age at onset and first symptoms with disease progression in patients with metachromatic leukodystrophy, *Neurology* 96 (2) (Jan 12 2021) e255–e266, <https://doi.org/10.1212/WNL.00000000000011047>.
- [5] F. Fumagalli, A.A. Zambon, P.M.V. Rancoita, et al., Metachromatic leukodystrophy: a single-center longitudinal study of 45 patients, *J. Inher. Metab. Dis.* (Apr 15 2021), <https://doi.org/10.1002/jimd.12388>.
- [6] S. Strobel, N. Hesse, V. Santhanakumaran, et al., Optimization of enzyme assays to enhance reliability of activity measurements in leukocyte lysates for the diagnosis of metachromatic leukodystrophy and gangliosidoses, *Cells* 9 (12) (2020) 2553, <https://doi.org/10.3390/cells9122553>.
- [7] V. Santhanakumaran, S. Groeschel, K. Harzer, et al., Predicting clinical phenotypes of metachromatic leukodystrophy based on the arylsulfatase A activity and the ARSA genotype? - chances and challenges, *Mol. Genet. Metabol.* 137 (3) (Sep 30 2022) 273–282, <https://doi.org/10.1016/j.ymgme.2022.09.009>.
- [8] S. Parikh, G. Bernard, R.J. Leventer, et al., A clinical approach to the diagnosis of patients with leukodystrophies and genetic leukoencephalopathies, *Mol. Genet. Metabol.* 114 (4) (Apr 2015) 501–515, <https://doi.org/10.1016/j.ymgme.2014.12.434>.
- [9] D.F. van Rappard, J.J. Boelens, N.I. Wolf, Metachromatic leukodystrophy: disease spectrum and approaches for treatment, *Best Pract. Res. Clin. Endocrinol. Metabol.* 29 (2) (Mar 2015) 261–273, <https://doi.org/10.1016/j.beem.2014.10.001>.
- [10] S. Beerepoot, S. Nierkens, J.J. Boelens, C. Lindemans, M. Bugiani, N.I. Wolf, Peripheral neuropathy in metachromatic leukodystrophy: current status and future perspective, *Orphanet J. Rare Dis.* 14 (1) (Nov 4 2019) 240, <https://doi.org/10.1186/s13023-019-1220-4>.
- [11] A. Lugowska, A. Tylki-Szymanska, J. Berger, B. Molzer, Elevated sulfatide excretion in compound heterozygotes of metachromatic leukodystrophy and ASA-pseudodeficiency allele, *Clin. Biochem.* 30 (4) (Jun 1997) 325–331, [https://doi.org/10.1016/s0009-9120\(97\)00033-7](https://doi.org/10.1016/s0009-9120(97)00033-7).
- [12] M.A. Tan, M. Fuller, Z.A. Zabidi-Hussain, J.J. Hopwood, P.J. Meikle, Biochemical profiling to predict disease severity in metachromatic leukodystrophy, *Mol. Genet. Metabol.* 99 (2) (Feb 2010) 142–148, <https://doi.org/10.1016/j.ymgme.2009.09.006>.
- [13] M. Mirzaian, G. Kramer, B.J. Poorthuis, Quantification of sulfatides and lysosulfatides in tissues and body fluids by liquid chromatography-tandem mass spectrometry, *J. Lipid Res.* 56 (4) (Apr 2015) 936–943, <https://doi.org/10.1194/jlr.M057232>.
- [14] M. Barcenas, T.R. Suhr, C.R. Scott, F. Turecek, M.H. Gelb, Quantification of sulfatides in dried blood and urine spots from metachromatic leukodystrophy patients by liquid chromatography/electrospray tandem mass spectrometry, *Clin. Chim. Acta* 433 (Jun 10 2014) 39–43, <https://doi.org/10.1016/j.cca.2013.12.016>.
- [15] Z. Spacil, A.B. Kumar, H.C. Liao, et al., Sulfatide analysis by mass spectrometry for screening of metachromatic leukodystrophy in dried blood and urine samples, *Clin. Chem.* 62 (1) (Jan 2016) 279–286, <https://doi.org/10.1373/clinchem.2015.245159>.
- [16] A. Polten, A.L. Fluharty, C.B. Fluharty, J. Kappler, K. von Figura, V. Gieselmann, Molecular basis of different forms of metachromatic leukodystrophy, *N. Engl. J. Med.* 324 (1) (1991/01/03 1991) 18–22, <https://doi.org/10.1056/NEJM199101033240104>.
- [17] M. Cesani, L. Lorioli, S. Grossi, et al., Mutation update of ARSA and PSAP genes causing metachromatic leukodystrophy, *Hum. Mutat.* 37 (1) (Jan 2016) 16–27, <https://doi.org/10.1002/humu.22919>.
- [18] S. Elgün, J. Waibel, C. Kehler, et al., Phenotypic variation between siblings with metachromatic leukodystrophy, *Orphanet J. Rare Dis.* 14 (1) (2019), <https://doi.org/10.1186/s13023-019-1113-6>, 136–136.
- [19] S. Beerepoot, S.J.M. van Dooren, G.S. Salomons, et al., Metachromatic leukodystrophy genotypes in The Netherlands reveal novel pathogenic ARSA variants in non-Caucasian patients, *Neurogenetics* 21 (4) (Oct 2020) 289–299, <https://doi.org/10.1007/s10048-020-00621-6>.

- [20] L. Laugwitz, V. Santhanakumaran, M. Spieker, et al., Extremely low arylsulfatase A enzyme activity does not necessarily cause symptoms: a long-term follow-up and review of the literature, *JIMD Rep.* 63 (4) (Jul 2022) 292–302, <https://doi.org/10.1002/jimd2.12293>.
- [21] M.S. van der Knaap, R. Schiffmann, F. Mochel, N.I. Wolf, Diagnosis, prognosis, and treatment of leukodystrophies, *Lancet Neurol.* 18 (10) (Oct 2019) 962–972, [https://doi.org/10.1016/S1474-4422\(19\)30143-7](https://doi.org/10.1016/S1474-4422(19)30143-7).
- [22] F. Eichler, W. Grodd, E. Grant, et al., Metachromatic leukodystrophy: a scoring system for brain MR imaging observations, *AJNR Am. J. Neuroradiol.* 30 (10) (Nov 2009) 1893–1897, <https://doi.org/10.3174/ajnr.A1739>.
- [23] M. Sessa, L. Lorioli, F. Fumagalli, et al., Lentiviral haemopoietic stem-cell gene therapy in early-onset metachromatic leukodystrophy: an ad-hoc analysis of a non-randomised, open-label, phase 1/2 trial, *Lancet* 388 (10043) (Jul 30 2016) 476–487, [https://doi.org/10.1016/S0140-6736\(16\)30374-9](https://doi.org/10.1016/S0140-6736(16)30374-9).
- [24] S. Groeschel, C. Kehrner, C. Engel, et al., Metachromatic leukodystrophy: natural course of cerebral MRI changes in relation to clinical course, *J. Inher. Metab. Dis.* 34 (5) (Oct 2011) 1095–1102, <https://doi.org/10.1007/s10545-011-9361-1>.
- [25] S. Groeschel, C. i Dali, P. Clas, et al., Cerebral gray and white matter changes and clinical course in metachromatic leukodystrophy, *Neurology* 79 (16) (Oct 16 2012) 1662–1670, <https://doi.org/10.1212/WNL.0b013e31826e9ad2>.
- [26] J. Feldmann, P. Martin, B. Bender, et al., MR-spectroscopy in metachromatic leukodystrophy: a model free approach and clinical correlation, *Neuroimage Clin.* 37 (2023) 103296, <https://doi.org/10.1016/j.nicl.2022.103296>.
- [27] L.B. Amedick, P. Martin, J. Beschle, et al., Clinical significance of diffusion tensor imaging in metachromatic leukodystrophy, *Neuropediatrics* (May 10 2023), <https://doi.org/10.1055/a-2073-4178>.
- [28] M. Strolin, I. Krageloh-Mann, C. Kehrner, M. Wilke, S. Groeschel, Demyelination load as predictor for disease progression in juvenile metachromatic leukodystrophy, *Ann. Clin. Transl. Neurol.* 4 (6) (Jun 2017) 403–410, <https://doi.org/10.1002/actn3.420>.
- [29] D.H. Schoenmakers, S. Beerepoot, I. Krageloh-Mann, et al., Recognizing early MRI signs (or their absence) is crucial in diagnosing metachromatic leukodystrophy, *Ann. Clin. Transl. Neurol.* 9 (12) (Dec 2022) 1999–2009, <https://doi.org/10.1002/actn3.51692>.
- [30] A. Biffi, M. Cesani, F. Fumagalli, et al., Metachromatic leukodystrophy - mutation analysis provides further evidence of genotype-phenotype correlation, *Clin. Genet.* 74 (4) (Oct 2008) 349–357, <https://doi.org/10.1111/j.1399-0004.2008.01058.x>.
- [31] P.S. Bindu, A. Mahadevan, A.B. Taly, R. Christopher, N. Gayathri, S.K. Shankar, Peripheral neuropathy in metachromatic leukodystrophy. A study of 40 cases from south India, *J. Neurol. Neurosurg. Psychiatr.* 76 (12) (Dec 2005) 1698–1701, <https://doi.org/10.1136/jnnp.2005.063776>.
- [32] C.L. Cameron, P.B. Kang, T.M. Burns, B.T. Darras, H.R. Jones Jr., Multifocal slowing of nerve conduction in metachromatic leukodystrophy. Article, *Muscle Nerve* 29 (4) (2004) 531–536, <https://doi.org/10.1002/mus.10569>.
- [33] X. Chen, D. Gill, P. Shaw, R. Ouvrier, C. Troedson, Outcome of early juvenile onset metachromatic leukodystrophy after unrelated cord blood transplantation. Review, *J. Child Neurol.* 31 (3) (2016) 338–344, <https://doi.org/10.1177/0883073815595078>.
- [34] C. Dali, N.W. Barton, M.H. Farah, et al., Sulfatide levels correlate with severity of neuropathy in metachromatic leukodystrophy, *Ann. Clin. Transl. Neurol.* 2 (5) (May 2015) 518–533, <https://doi.org/10.1002/actn3.193>.
- [35] O.N. Koc, J. Day, M. Nieder, S.L. Gerson, H.M. Lazarus, W. Krivit, Allogeneic mesenchymal stem cell infusion for treatment of metachromatic leukodystrophy (MLD) and Hurler syndrome (MPS-IH), *Bone Marrow Transplant.* 30 (4) (Aug 2002) 215–222, <https://doi.org/10.1038/sj.bmt.1703650>.
- [36] A. Raina, S. Nair, C. Nagesh, B. Thomas, M. Nair, S. Sundaram, Electroneurography and advanced neuroimaging profile in pediatric-onset metachromatic leukodystrophy. Article, *J. Pediatr. Neurosci.* 14 (2) (2019) 70–75, <https://doi.org/10.4103/jpn.JPN.155.18>.
- [37] M. Solders, D.A. Martin, C. Andersson, et al., Hematopoietic SCT: a useful treatment for late metachromatic leukodystrophy, *Bone Marrow Transplant.* 49 (8) (Aug 2014) 1046–1051, <https://doi.org/10.1038/bmt.2014.93>.
- [38] O. Artigalás, V.L. Lagranha, M.L. Saraiva-Pereira, et al., Clinical and biochemical study of 29 Brazilian patients with metachromatic leukodystrophy, *J. Inher. Metab. Dis.* 33 (Suppl 3) (Dec 2010) S257–S262, <https://doi.org/10.1007/s10545-010-9140-4>.
- [39] L.D. De Hosson, B.P.C. Van De Warrenburg, F.W.M.B. Preijers, et al., Adult metachromatic leukodystrophy treated by allo-SCT and a review of the literature, Review, *Bone Marrow Transplantation* 46 (8) (2011) 1071–1076, <https://doi.org/10.1038/bmt.2010.252>.
- [40] S. Jabbehdari, E. Rahimian, N. Jafari, S. Sanii, S. Khayatadehkhaki, H. Nejad Biglari, The clinical features and diagnosis of Metachromatic leukodystrophy: a case series of Iranian Pediatric Patients, *Iran J. Child Neurol. Summer* 9 (3) (2015) 57–61.
- [41] H.R. Martin, M.D. Poe, J.M. Provenzale, J. Kurtzberg, A. Mendizabal, M.L. Escolar, Neurodevelopmental outcomes of umbilical cord blood transplantation in metachromatic leukodystrophy, *Biol. Blood Marrow Transplant.* 19 (4) (Apr 2013) 616–624, <https://doi.org/10.1016/j.bbmt.2013.01.010>.
- [42] D.F. van Rappard, M. Bugiani, J.J. Boelens, et al., Gallbladder and the risk of polyps and carcinoma in metachromatic leukodystrophy, *Neurology* 87 (1) (Jul 5 2016) 103–111, <https://doi.org/10.1212/WNL.0000000000002811>.
- [43] S. Beerepoot, H. Heijst, B. Roos, et al., Neurofilament light chain and glial fibrillary acidic protein levels in metachromatic leukodystrophy, *Brain* (Aug 16 2021), <https://doi.org/10.1093/brain/awab304>.
- [44] P.L. Musolino, T.C. Lund, J. Pan, et al., Hematopoietic stem cell transplantation in the leukodystrophies: a systematic review of the literature, *Neuropediatrics* 45 (3) (Jun 2014) 169–174, <https://doi.org/10.1055/s-0033-1364179>.
- [45] D.F. van Rappard, A. Klausner, M.E. Steenweg, et al., Quantitative MR spectroscopic imaging in metachromatic leukodystrophy: value for prognosis and treatment, *J. Neurol. Neurosurg. Psychiatry* 89 (1) (Jan 2018) 105–111, <https://doi.org/10.1136/jnnp-2017-316364>.
- [46] A.A. Boucher, W. Miller, R. Shanley, et al., Long-term outcomes after allogeneic hematopoietic stem cell transplantation for metachromatic leukodystrophy: the largest single-institution cohort report, *Orphanet J. Rare Dis.* 10 (Aug 7 2015) 94, <https://doi.org/10.1186/s13023-015-0313-y>.
- [47] S. Groeschel, J.S. Kuhl, A.E. Bley, et al., Long-term outcome of allogeneic hematopoietic stem cell transplantation in patients with juvenile metachromatic leukodystrophy compared with nontransplanted control patients, *JAMA Neurol.* 73 (9) (Sep 1 2016) 1133–1140, <https://doi.org/10.1001/jamaneurol.2016.2067>.
- [48] D.F. van Rappard, J.J. Boelens, M.E. van Egmond, et al., Efficacy of hematopoietic cell transplantation in metachromatic leukodystrophy: the Dutch experience, *Blood* 127 (24) (Jun 16 2016) 3098–3101, <https://doi.org/10.1182/blood-2016-03-708479>.
- [49] B.T.A. van den Broek, K. Page, A. Paviglianiti, et al., Early and late outcomes after cord blood transplantation for pediatric patients with inherited leukodystrophies, *Blood Adv.* 2 (1) (Jan 9 2018) 49–60, <https://doi.org/10.1182/bloodadvances.2017010645>.
- [50] M. Al-Saady, S. Beerepoot, B.C. Plug, et al., Neurodegenerative disease after hematopoietic stem cell transplantation in metachromatic leukodystrophy, *Ann. Clin. Transl. Neurol.* (May 22 2023), <https://doi.org/10.1002/actn3.51796>.
- [51] C. Videbaek, J. Stokholm, H. Sengelov, et al., Allogeneic hematopoietic stem cell transplantation in two siblings with adult metachromatic leukodystrophy and a systematic literature review, *JIMD Rep.* 60 (1) (Jul 2021) 96–104, <https://doi.org/10.1002/jimd2.12221>.
- [52] F. Fumagalli, V. Calbi, M.G. Natali Sora, et al., Lentiviral haematopoietic stem-cell gene therapy for early-onset metachromatic leukodystrophy: long-term results from a non-randomised, open-label, phase 1/2 trial and expanded access, *Lancet* 399 (10322) (Jan 22 2022) 372–383, [https://doi.org/10.1016/S0140-6736\(21\)02017-1](https://doi.org/10.1016/S0140-6736(21)02017-1).
- [53] A. Biffi, E. Montini, L. Lorioli, et al., Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy, *Science* 341 (6148) (Aug 23 2013) 1233158, <https://doi.org/10.1126/science.1233158>.
- [54] C.O. Miranda, P. Brites, M. Mendes Sousa, C.A. Teixeira, Advances and pitfalls of cell therapy in metabolic leukodystrophies, *Cell Transplant.* 22 (2) (2013) 189–204, <https://doi.org/10.3727/096368912X656117>.
- [55] R. Penati, F. Fumagalli, V. Calbi, M.E. Bernardo, A. Aiuti, Gene therapy for lysosomal storage disorders: recent advances for metachromatic leukodystrophy and mucopolysaccharidosis I, *J. Inher. Metab. Dis.* 40 (4) (Jul 2017) 543–554, <https://doi.org/10.1007/s10545-017-0052-4>.
- [56] X. Hong, A.B. Kumar, J. Daiker, et al., Leukocyte and dried blood spot arylsulfatase A assay by tandem mass spectrometry, *Anal. Chem.* 92 (9) (May 5 2020) 6341–6348, <https://doi.org/10.1021/acs.analchem.9b05274>.
- [57] X. Hong, J. Daiker, M. Sadilek, et al., Toward newborn screening of metachromatic leukodystrophy: results from analysis of over 27,000 newborn dried blood spots, *Genet. Med.* (Nov 20 2020), <https://doi.org/10.1038/s41436-020-01017-5>.
- [58] X. Hong, J. Daiker, M. Sadilek, et al., Toward newborn screening of metachromatic leukodystrophy: results from analysis of over 27,000 newborn dried blood spots, *Genet. Med.* 23 (3) (Mar 2021) 555–561, <https://doi.org/10.1038/s41436-020-01017-5>.
- [59] M.A.J. Samuel Groeschel, Laimdota Zizmare, Stefanie Beck-Wödl, Claire Cannet, Hartmut Schäfer, Jürgen Okun, Christoph Trautwein, Nils Janzen, David Kaspar, Lucia Laugwitz, Predicting Phenotypes in Metachromatic leukodystrophy: Implications for Newborn Screening, presented at, SSIEM, 2023, <https://onlineibrary.wiley.com/doi/abs/10.1002/jimd.12668>.
- [60] M. Trinidad, X. Hong, S. Froelich, et al., Predicting disease severity in metachromatic leukodystrophy using protein activity and a patient phenotype matrix, *Genome Biol.* 24 (1) (Jul 21 2023) 172, <https://doi.org/10.1186/s13059-023-03001-z>.
- [61] J.M.G. Wilson, G. Jungner, W.H. Organization, Principles and Practice of Screening for Disease, 1968.
- [62] MLDi, Treatment Eligibility Panel, 2023. <https://www.mldinitiative.com/f-or-professionals/treatment-eligibility/>.
- [63] D.H. Schoenmakers, S. Beerepoot, S. van den Berg, et al., Modified Delphi procedure-based expert consensus on endpoints for an international disease registry for Metachromatic Leukodystrophy: the European Metachromatic Leukodystrophy initiative (MLDi), *Orphanet J. Rare Dis.* 17 (1) (Feb 14 2022) 48, <https://doi.org/10.1186/s13023-022-02189-w>.
- [64] M. Cesani, A. Capotondo, T. Plati, et al., Characterization of new arylsulfatase A gene mutations reinforces genotype-phenotype correlation in metachromatic leukodystrophy, *Hum. Mutat.* 30 (10) (Oct 2009) E936–E945, <https://doi.org/10.1002/humu.21093>.
- [65] M.D. Stellingwerf, P.J.W. Pouwels, S.D. Roosendaal, F. Barkhof, M.S. van der Knaap, Quantitative MRI in leukodystrophies, *Neuroimage Clin.* 38 (May 3 2023) 103427, <https://doi.org/10.1016/j.nicl.2023.103427>.
- [66] P. Martin, G.E. Hagberg, T. Schultz, et al., T2-Pseudonormalization and microstructural characterization in advanced stages of late-infantile metachromatic leukodystrophy, *Clin. Neuroradiol.* (Nov 23 2020), <https://doi.org/10.1007/s00062-020-00975-2>.

- [67] S. Groeschel, G.E. Hagberg, T. Schultz, et al., Assessing white matter microstructure in brain regions with different myelin architecture using MRI, *PLoS One* 11 (11) (2016) e0167274, <https://doi.org/10.1371/journal.pone.0167274>.
- [68] C. Horgan, K. Watts, D. Ram, et al., A retrospective cohort study of Libmeldy (atidarsagene autotemcel) for MLD: what we have accomplished and what opportunities lie ahead, *JIMD Rep.* 64 (5) (Sep 2023) 346–352, <https://doi.org/10.1002/jmd2.12378>.
- [69] E.M. Maier, U. Mutze, N. Janzen, et al., Collaborative evaluation study on 18 candidate diseases for newborn screening in 1.77 million samples, *J. Inherit. Metab. Dis.* 46 (6) (Nov 2023) 1043–1062, <https://doi.org/10.1002/jimd.12671>.
- [70] S. Timmermans, M. Buchbinder, Patients-in-waiting: living between sickness and health in the genomics era, *J. Health Soc. Behav.* 51 (4) (Dec 2010) 408–423, <https://doi.org/10.1177/0022146510386794>.
- [71] D. Matern, K. Basheeruddin, T.L. Klug, et al., Newborn screening for Krabbe disease: status quo and recommendations for improvements, *Int. J. Neonatal Screen* (1) (Jan 28 2024) 10, <https://doi.org/10.3390/ijns10010010>.
- [72] H.A.F. Yska, L. Henneman, R.W. Barendsen, M. Engelen, S. Kemp, Attitudes of patients with adrenoleukodystrophy towards sex-specific newborn screening, *Int. J. Neonatal Screen* (3) (Sep 2 2023) 9, <https://doi.org/10.3390/ijns9030051>.



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