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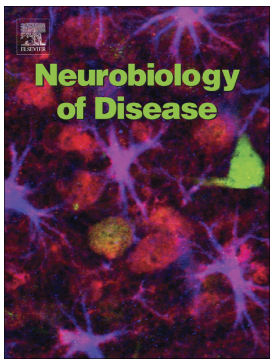

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Glial cells in the driver seat of leukodystrophy pathogenesis

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Abbreviations:

PMD	Pelizaeus-Merzbacher disease
PLP	proteolipid protein
ER	endoplasmic reticulum
UPR	unfolded protein response
OL	Oligodendrocyte
ADLD	Autosomal Dominant Leukodystrophy
AHDS	Allan-Herndon-Dudley syndrome
T3	triiodothyronine
T4	thyroxine
BBB	blood brain barrier
CNS	central nervous system
H-ABC	Hypomyelination and Atrophy of Basal ganglia and Cerebellum
MT	microtubule
OPC	Oligodendrocyte progenitor cells
GALC	galactosylceramidase
IL	Interleukins,
TNF	Tumor necrosis factor
ALSP	Adult-onset Leukoencephalopathy with Axonal Spheroids and Pigmented Glia
PLOSL	Polycystic Lipomembranous Osteodysplasia with Sclerosing Leukoencephalopathy
X-ALD	X-linked adrenoleukodystrophy
VCLFA	very long chain fatty acid
MLD	Metachromatic leukodystrophy
ARSA	arylsulfatase

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AxD Alexander's disease

VWM *Vanishing White* Matter *Disease*

AGS Aicardi-Goutières syndrome

IFN- α interferon- α

ISG interferon-stimulated gene

CXCL10 C-X-C motif chemokine 10

MLC Megalencephalic Leukoencephalopathy

MLC1 Megalencephalic Leukoencephalopathy with Subcortical Cysts 1

GlialCAM Glial cell adhesion molecule

UCBT Umbilical cord blood transplantation

HSCT Hematopoietic stem cell transplantation

NPC neural progenitor cell

ASO Anti-sense oligonucleotide

DITPA, Diiodo-thyropropionic Acid

PTU, propylthiouracil

LT4, L-thyroxine

BMT, Bone marrow transplantation

ERT, enzyme replacement therapy

AAV, Adeno-associated virus

HCT, Hematopoietic cell transplantation

ISR, Integrated Stress Response

RTI, Reverse Transcriptase Inhibitor

ABSTRACT

Glia cells are often viewed as support cells in the central nervous system, but recent discoveries highlight their importance in physiological functions and in neurological diseases. Central to this are leukodystrophies, a group of progressive, neurogenetic disease affecting white matter pathology. In this review, we take a closer look at multiple leukodystrophies, classified based on the primary glial cell type that is affected. While white matter diseases involve oligodendrocyte and myelin loss, we discuss how astrocytes and microglia are affected and impinge on oligodendrocyte, myelin and axonal pathology. We provide an overview of the leukodystrophies covering their hallmark features, clinical phenotypes, diverse molecular pathways, and potential therapeutics for clinical trials. Glial cells are gaining momentum as cellular therapeutic targets for treatment of demyelinating diseases such as leukodystrophies, with no current treatment options. Here, we bring the much needed attention to role

of glia in leukodystrophies, an integral step to furthering disease comprehension, understanding mechanisms and developing future therapeutics.

Key words: Leukodystrophy, glia, astrocyte, oligodendrocyte, microglia, white matter

INTRODUCTION

Leukodystrophies are a group of genetic neurodegenerative disorders, originally described as primarily affecting myelin in the central nervous system (CNS). The term “leukodystrophy” by itself refers to wasting (*dystrophy*) of the white (*leuko*) matter (van der Knaap and Bugiani, 2017; Vanderver et al., 2015). However, the definition has now evolved to encompass ‘heritable disorders that affect the white matter, with abnormalities in myelin sheath, and neuropathology involving glial cells along with significant axonal pathology’ (Vanderver et al., 2015). In addition to the CNS pathology, many leukodystrophies also affect myelination in the peripheral nervous system (PNS) (Ashrafi and Tavasoli, 2017; Vanderver et al., 2015). Leukodystrophies are rare, with a reported incidence ranging from 1 in 7,500 to 2 in 100,000 live births (Bonkowsky et al., 2010). Within this broad class of disorders, progressive motor and cognitive dysfunction are common (Adang et al., 2017). Most individuals affected by leukodystrophy begin to show clinical symptoms in childhood and demonstrate abnormalities in white matter signal on neuroimaging. Magnetic resonance imaging (MRI) has long been the standard diagnostic tool for leukodystrophies, but molecular techniques such as whole-exome sequencing (WES) and whole-genome sequencing (WGS) have resulted in rapid diagnostics and this combination is now used for clinical diagnosis (van der Knaap et al., 2019). Unfortunately, there are few targeted therapies available for the leukodystrophies, and clinical options are often limited to supportive care. The challenges to develop the treatment are multifactorial: small populations, diagnostic difficulties, lack of clear clinical outcomes, limited epidemiological data, and unknown disease mechanisms (Bonkowsky et al., 2010). In this review, we discuss the leukodystrophies classified based on dysfunction of specific glial cell types, provide insight into their disease models, pathological mechanisms, and lastly the potential therapeutic treatments options (**Table 1**).

Glial cells

‘Glia’ originates from the Greek word “glue”, referred to as the ‘nerve glue’ of the CNS, holding together the neurons. The primary glial cells of the CNS are astrocytes, oligodendrocytes, and microglial cells with emerging role of the oligodendrocyte precursor cells as the fourth glial cell. The new functions associated with glia signify that their role in CNS has evolved, as specialized cells that are more than just glue.

Astrocytes

Historically, astrocytes are broadly classified as protoplasmic astrocytes in the gray matter and fibrous astrocytes in the white matter. An emerging view is that astrocytes are a fairly heterogenous population based on their morphological, regional, molecular, functional and physiological properties driven by their surrounding microenvironment (Ben Haim and Rowitch, 2017; Chen and Swanson, 2003; Farmer et al., 2016; Lanciotti et al., 2013). Astrocytes perform crucial homeostatic functions in the CNS including buffering of potassium and calcium ions, regulating synaptic events via glutamate transporters, providing metabolic support to neurons, and modulating synaptic inputs through release of gliotransmitters (Kelley et al., 2018; Lanciotti et al., 2013; Poskanzer and Molofsky, 2018; Ridet, 2000; Vainchtein et al., 2018). Astrocytes also play an integral role in shaping oligodendrocytes throughout development (Baumann and Pham-Dinh, 2001). They secrete soluble factors (PDGF, FGF-2, LIF, CNTF) important for survival, proliferation and differentiation of oligodendrocyte precursor cells into mature oligodendrocytes and directly regulate oligodendrocyte development and myelination through connexin (Cx) mediated gap junctions (Domingues et al., 2016; Orthmann-Murphy et al., 2008). Astrocyte end-feet help establish the blood-brain barrier (BBB) in juxtaposition with endothelial cells and pericytes, making CNS an immune privileged site.

In response to stress or an insult to the CNS, astrocytes become activated leading to ‘reactive astrogliosis’, which involves morphological and transcriptional changes with upregulation of the intermediate filament protein glial fibrillary acidic protein (GFAP) along with cytokines, chemokines, growth factors, and inflammatory mediators (Norris et al., 2005; Pekny and Nilsson, 2005; Pekny et al., 2014). Reactive astrogliosis can exert both beneficial and detrimental effects such as limiting tissue damage and also modulating the immune response in the context of the injury or insult (Chen and Swanson, 2003; Hol and Pekny, 2015; Lanciotti et al., 2013; Ridet, 2000). Astrocytes and microglia influence each other, as microglia conduct the sentinel-like functions in the CNS. Recent work classifies astrocytes based on their activation state, as A1 with pro-inflammatory and neurotoxic features or A2 astrocytes with anti-inflammatory and neuroprotective properties (Liddelow et al., 2017). It is unsurprising these myriad roles of astrocytes profoundly affects white matter pathology and CNS health in leukodystrophies and here we discuss how understanding them has helped shed light on disease to develop potential therapies (Lanciotti et al., 2013).

Oligodendrocytes

Oligodendrocytes (OL) are the myelinating cells of the CNS and arise from progenitors called oligodendrocyte precursor cells (OPC). OL generation and myelination is a tightly regulated process

comprised of proliferation, migration, and differentiation of OPCs into a mature OL that conducts myelination of axon bundles (Bradl and Lassmann, 2010; Genoud et al., 2002). Myelin sheaths are immensely compact structures composed of lipids (70%) that provide axonal insulation and myelin specific proteins (30%), that stabilize the myelin structure. Major myelin proteins in the CNS include myelin associated glycoprotein (MAG), myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP) (Morell P, 1999). Functionally, myelin sheaths are essential for nerve conduction and establishment of axonal domains such as nodes of Ranvier, paranodes, and juxtaparanodes, necessary for saltatory conduction and providing metabolic support for axons (Barres, 2008). Both acquired demyelinating disorders (Multiple Sclerosis and Neuromyelitis Optic Spectrum Disorder) and inherited demyelinating disorders (Pelizaeus-Merzbacher disease, Krabbe disease, and Hypomyelination with Atrophy of Basal Ganglia and Cerebellum) result in injury to OLs and the myelin sheath. Here we discuss some of the inherited leukodystrophies resulting in damage or loss of the OL lineage cells.

Microglia

Microglia are cells that vastly differ from their glial counterparts in the CNS, as they are derived from yolk sac erythro-myeloid progenitor cells of the hematopoietic lineage, and conduct sentinel immune functions in the CNS (Bennett et al., 2018; Oosterhof et al., 2018). Similar to astrocytes, in the context of disease or injury, microglia become activated and undergo morphological and functional transformation such as release of proinflammatory cytokines and phagocytosis (Nicaise et al., 2016; Oosterhof et al., 2017; Oosterhof et al., 2018). In a context dependent manner, activated microglia exhibits differential states from a pro-inflammatory or cytotoxic (referred as M1) to an anti-inflammatory state (referred as M2) (Nicaise et al., 2016). While this classification of microglia into M1 and M2 phenotypes is important for understanding their response to pathogens and/or cytokines, it does little to describe the unique disease specific microglial activation or phenotype. Additionally, recent transcriptome-based analyses of microglia have identified a wide spectrum of macrophage activation states beyond that of the M1 and M2 model; further highlighting its' limitations (Chiu et al., 2013; Nicaise et al., 2016; Xue et al., 2014). While most leukodystrophies have microglia involvement only as a secondary effect, they are directly implicated in certain leukodystrophies as described in this review (Bergner et al., 2019).

Astrocyte-associated leukodystrophies

The leukodystrophies classified under this section are associated with mutations in astrocyte specific gene and/or pathomechanisms indicating astrocyte driven pathology, sometimes preceding the disease

symptoms. Hence, we discuss them here as astrocyte-associated leukodystrophies albeit obvious pathology in OLs, axons and other glial cells (Figure 1).

Alexander's Disease (AxD)

Alexander's Disease (AxD) is an autosomal dominant leukodystrophy typically arising due to *de novo* mutations in the gene encoding GFAP (Lanciotti et al., 2013; Messing, 2019; Olabarria and Goldman, 2017). GFAP is an astrocyte-specific intermediate filament with at least 10 different isoforms and typically used as a marker of reactive astrogliosis that is upregulated in response to injury, such as trauma, ischemia, or neurodegeneration (Hol and Pekny, 2015; Olabarria and Goldman, 2017). Under physiological conditions, GFAP provides cytoskeletal support to the cell, however, mutations in *GFAP* result in cytotoxic accumulation of GFAP protein in astrocyte cell body, processes, and endfeet (Hagemann et al., 2005; Olabarria and Goldman, 2017). The common clinical features of infantile AxD are megalocephaly, seizures, bulbar dysfunction, psychomotor regression, developmental delay, and low life expectancy (Lanciotti et al., 2013; Prust et al., 2011). The distinction between the Type I and Type II forms of AxD, based on clinical, radiologic, and genetic profile provides a better understanding between age of onset, genotype of GFAP and clinical presentation (Prust et al., 2011). Characteristic MRI imaging of AxD patient shows demyelination of cerebellum and middle cerebellar peduncles, frontal predominance, brainstem involvement, and spinal cord atrophy (Kohler et al., 2018). MRI imaging also shows distinctive ventricular garlands, which are believed to consist of blood vessels surrounded by the hall mark perivascular Rosenthal fibers (van der Knaap et al., 2006). Rosenthal fibers are eosinophilic ubiquinated protein aggregates consisting of GFAP, vimentin, small heat shock proteins αβ-crystallin and Hsp27, and plectin present within astrocytes of post-mortem brain tissue of AxD patients (Lanciotti et al., 2013). Currently, there are no accepted mechanisms on how GFAP mutations result in the accumulation of Rosenthal fibers (Brenner et al., 2001).

Several transgenic mouse models have been developed to understand mechanism of pathologic *GFAP* variants in AxD. *Gfap*-null mice are normal and viable, however, mice overexpressing wild-type (WT) human *GFAP (GFAP*^{Tg}) and mutant *Gfap*$^{R236H/+}$ and *Gfap*$^{R76H/+}$ (both orthologues to the human R79 and R239 mutations respectively) exhibit characteristic inclusions of Rosenthal fibers in astrocytes (Brenner et al., 2001; Hagemann et al., 2005; Sosunov et al., 2013). This indicates that *GFAP* mutations result in a toxic gain-of-function in AxD (Hagemann et al., 2005). *Gfap*$^{R236H/+}$ mice have a normal life span but have elevated GFAP levels in the brain and cerebrospinal fluid (CSF) (Jany et al., 2013). However, the double transgenic mice *GFAP*^{Tg}::*Gfap*$^{R236H+}$ exhibit seizures and die at ~P30 expressing a seven-fold increase in GFAP protein compared to *Gfap*$^{R236H/+}$ and *GFAP*^{Tg} mice (Jany et al., 2013; Olabarria and Goldman, 2017). The morphology of astrocytes in these double transgenic

mice appear abnormal with bushy, thickened processes and high expression of Cluster of Differentiation 44 (CD44), indicative of reactive astrocytes (Sosunov et al., 2013). The astrocytes are also highly dysmorphic and multinucleated suggesting GFAP accumulation inhibits cell division (Olabarria and Goldman, 2017; Sosunov et al., 2013).

While murine models share some features, they do not recapitulate the demyelination phenotype observed in AxD. The development of human induced pluripotent stem cell (iPSC) derived from AxD affected individuals has been especially helpful to study these phenotypes. iPSC derived astrocytes from AxD individuals display the characteristic Rosenthal fibers. Importantly, when AxD astrocytes are co-cultured with normal OPCs, they cause a decrease in OPC proliferation, OL maturation and myelination. Thus, iPSC models have helped elucidate that AxD astrocytes impede OL development and contribute to loss of OLs and demyelination (Lanciotti et al., 2013).

AxD astrocytes show a decrease in gap junctional coupling and glutamate buffering due to diminished glutamate transporter-1 (GLT-1) activity. This loss of buffering potentially contributes to hyperexcitability, seizures and cell death in adjacent neurons in AxD (Olabarria and Goldman, 2017). An increase in levels of the inflammatory mediators CXCL10, CCL2 and lipocalin2 occurs in AxD astrocytes, which further activates microglia; potentially contributes to seizures and neuronal dysfunction (Olabarria and Goldman, 2017; Olabarria et al., 2015). The ongoing accumulation of GFAP in astrocyte leads to activation of a number of cellular pathways including mechanistic target of rapamycin (mTOR), mitogen-activates protein kinase 3 (MAPK3) and c-Jun N-terminal kinase (JNK); perhaps to inhibit the proteasome activity (Olabarria and Goldman, 2017; Sosunov et al., 2013). Post-mortem samples from AxD patients and iPSC astrocytes exhibit an increase in the expression of the chitinase-3-like protein 1 (CHI3L1) (Li et al., 2018). CHI3L1 is a cytokine expressed in reactive astrocytes during chronic inflammation in Multiple Sclerosis, Amyotrophic Lateral Sclerosis, Alzheimer's disease and also in AxD, where it inhibits proliferation of OPCs (Ansari et al., 2020; Bonneh-Barkay et al., 2010; Canto et al., 2015; Kyrgios et al., 2012; Li et al., 2018). While currently elevated GFAP levels in the CSF serves as a biomarker for AxD, CHI3L1 is emerging as a CSF biomarker in other neurological diseases and could be explored in the future for AxD (Gaur et al., 2020; Jany et al., 2013).

Vanishing White Matter (VWM)

VWM occurs due to biallelic mutations in genes encoding the five subunits of eukaryotic translation initiation factor 2B (eIF2B). The eIF2B protein complex regulates protein synthesis under both normal and cellular stress conditions (Atzmon et al., 2018; Raini et al., 2017). Classically, VWM disease presents with episodic and progressive neurological deterioration, often induced by stress or trauma (Abbink et al., 2019; Zhou et al., 2019). The early onset form of the disease is characterized by

symptoms of progressive spastic ataxia, optic atrophy, and premature death (Atzmon et al., 2018; Clayton and Popko, 2016; Raini et al., 2017). In the late onset forms, affected individuals often display slow progressive ataxia and females may also develop ovarian failure. VWM post-mortem samples show an increase in the number of proliferating OPCs and OLs with 'foamy cytoplasm' appearance resulting in loss of myelin and axonal damage in the brain and spinal cord (Abbink et al., 2019; Atzmon et al., 2018; Bugiani et al., 2011; Clayton and Popko, 2016; Dooves et al., 2016; Leferink et al., 2018). Astrocytes derived from post-mortem samples and iPSCs of individuals with VWM appear dysmorphic with multiple nuclei and blunt short processes compared to control individuals. This provides support that astrocyte dysfunction is central to the pathology of this disease (Dietrich et al., 2005; Zhou et al., 2019). An additional characteristic pathology examined is the translocation of Bergmann glia (specialized astrocytes), from the Purkinje layer into the molecular layer of cerebellum in patient tissues (Dooves et al., 2018).

The development of cellular and animal models have been helpful in recapitulating VWM disease pathology and exploring the underlying mechanisms (Zhou et al., 2019). The mutations in *eIF2B5* and *eIF2B4* subunits in humans result in severe forms of VWM. The heterozygous and homozygous crosses of *eif2b5*$^{R191H/R191H}$ and *eif2b4*$^{I484W/D384W}$ mice reproduce a spectrum of clinically observed VWM phenotypes (Dooves et al., 2016; Wisse et al., 2018). These mice present with symptoms of low body weight, progressive gait ataxia and sporadic epileptic seizures. Tissue histopathology shows a decrease in myelination with extensive white matter vacuolization in cerebellar white matter cross sections, which correlates with the disease severity and serve as an indicator of disease progression (Dooves et al., 2016). The astrocytes in the white matter are immature and have atypical morphology in all VWM mice regardless of the severity and these astrocytic deficits occur well before disease onset and myelination deficits (Dooves et al., 2016). Albeit ongoing proliferation of OPCs, there are fewer mature OLs in the white matter, as VWM OPCs fail to differentiate and mature. Co-culture studies in mice reveals that VWM astrocytes secrete hyaluronan, an inhibitory molecule that prevents OL maturation, also elevated in brain samples from VWM patients (Back et al., 2005; Bugiani et al., 2013; Dooves et al., 2016).

eI2FB is critical for protein translation and regulates the integrated stress response (ISR), which is activated due to stressors such as oxidative damage, amino acid starvation, and endoplasmic reticulum (ER) stress (Carter, 2007). *eif2b* missense mutations cause a decrease in its gene expression leading to nuclear translocation of activating transcription factor-4 (ATF4) and continued activation of the unfolded protein response (UPR) and ER stress (Schiffmann and Elroy-Stein, 2006; van der Voorn et al., 2005). The ISR-related changes are specific to both mouse and human VWM astrocytes and ATF4-regulated transcriptome correlates with the disease severity (Abbink et al., 2019; Dooves et al.,

2016). Another proposed mechanism in VWM is mitochondrial dysfunction that affects the oxidative phosphorylation pathway, resulting in energy deficiency. Astrocytes isolated from VWM mice show an increase in both the number and size of mitochondria to compensate for energy deficits (Raini et al., 2017). Thus, eI2FB mutations in VWM cause astrocyte specific effects with non-cell autonomous effects on OL and axonal health in the CNS, in addition to some non-CNS effects (ovarian dysfunction).

Aicardi Goutières Syndrome (AGS)

In Aicardi-Goutières Syndrome (AGS), mutations in genes regulating nucleic acid metabolism (*TREX1*, *RNASEH2A*, *RNASEH2B*, *RNASEH2C*, *SAMHD1*, *ADAR1*, and *IFIH1/MDA5*) cause intracellular accumulation of nucleic acids. This results in activation of type I interferon (IFN) innate immune pathway and upregulation of downstream IFN stimulated genes (ISG) (Crow et al., 2006; Dai et al., 2019; Gao et al., 2015; La Maestra et al., 2018; van Heteren et al., 2008; Xiao et al., 2019). AGS is an autoinflammatory disorder with evidence of glial cells as the driver of CNS pathology. Clinically, AGS is characterized by neurologic dysfunction accompanied with systemic signs and symptoms of chronic inflammation, such as hepatitis and chilblains (Jorge and Bugiani, 2019). While variable, neuroimaging often reveals bilateral calcifications within the basal ganglia. Most individuals with AGS demonstrate sustained, elevated IFN levels in their blood and CSF.

Post-mortem pathology indicates lymphocytosis and astrogliosis, along with severe demyelination and thrombotic microangiopathy (Fazzi et al., 2013; Jorge and Bugiani, 2019; La Maestra et al., 2018). Astrocytes and microglia are thought to be the primary cells that produce IFN-α within the CNS in response to a viral infection (Sase et al., 2018; van Heteren et al., 2008). In addition, CSF and post-mortem samples also detect an increase in the cytokine CXCL10 in AGS patients and co-labeling studies confirm astrocytes as a source of IFN-α and CXCL10 (van Heteren et al., 2008).

Transgenic mice over expressing IFN-α under a GFAP promoter develop neuropathology similar to AGS post-mortem brains (Campbell et al., 1999). The mouse models of AGS display some features of the disease, however they fail to recapitulate the CNS damage seen in humans (La Maestra et al., 2018). *TREX1* typically manifests with a severe neurological phenotype but *Trex1* null mice present with no evidence of neurological inflammation albeit inflammatory myocarditis from IFN overproduction, (Nundel and Marshak-Rothstein, 2019). Similarly, other AGS mouse models do not display the neurological phenotype, although can result in early embryonic lethality (Bartsch et al., 2018; Rabe, 2013; Roesch and Schwartz, 2013; Wang et al., 2017; Wu, 2013). The embryonic brain tissue (E12.5) from mutant *Adar1* or *Adar1* KO mice shows a robust upregulation of IFN and ISG expression (Heraud-Farlow et al., 2017). The inducible *Adar1* knockout mouse helps circumvent the

lethality issue and upon tamoxifen treatment results in elevated IFN-α levels in the brain and spinal cord (Pestal et al., 2015; Yang et al., 2014). Thus, this work provides a mouse model to tease out CNS involvement in AGS.

Primary astrocytes isolated from the *RNaseH2B*$^{\Delta GFAP}$ mice demonstrate cellular defects, including DNA damage, premature senescence, and increase in ISG expression although no *in vivo* phenotype is noted (Bartsch et al., 2018; Rabe, 2013; Wang et al., 2017). AGS patient-derived human cells have become a helpful tool, especially for AGS mutations without any CNS phenotypes in mice (Ferraro et al., 2019a; Ferraro et al., 2019b; Masneri et al., 2019). Human iPSCs with *TREX1* deficiency differentiate normally into neural precursor cells (NPCs), neurons, and astrocytes. TREX1-deficient neurons exhibit an increase in cell death due to ssDNA accumulation, which is further exacerbated upon co-culturing with *TREX1*-deficient astrocytes due to IFN-α secretion (Thomas et al., 2017). While a few studies show potential involvement of astrocytes in mediating neuroinflammation in AGS, much work needs to be done to establish their role and underlying mechanisms. The blood brain barrier (BBB) is compromised in AGS, leading to an influx of peripheral immune cells. Astrocyte endfeet along with endothelial cells help maintain BBB; hence astrocyte dysfunction potentially affects health of endothelial cells and BBB integrity. This in turn may mediate infiltration of immune cells. Thus, AGS has a complex and evolving landscape as new evidence gathers pertaining to role of glial cells such as astrocytes and microglia.

Megalencephalic Leukoencephalopathy with subcortical Cysts (MLC)

MLC is an autosomal recessive disorder due to mutations in *MLC1* or *GLIALCAM (HEPACAM)* genes that encode cell junction and adhesion proteins. Recessive mutations in both genes disrupt membrane localization of their proteins with indistinguishable disease phenotypes. The dominant heterozygous mutations in *GLIALCAM* also initially manifest similar to recessive form, but over time the white matter abnormalities can self-resolve (Bugiani et al., 2017).

The onset of MLC1 starts shortly after birth with increasing macrocephaly that stabilizes after the first year. MRI and diffusion-weighted imaging reveals white matter signal abnormalities, cerebral swelling due to increased water content in the white matter, with the development of subcortical cysts in the anterior temporal region and variably throughout the frontal and parietal regions (Bugiani et al., 2017; Dubey et al., 2015; Ridder et al., 2011). Individuals with MLC develop progressive cerebellar ataxia, cognitive decline, spasticity, sometimes with epileptic seizures, and cerebellar white matter atrophy after swelling subsides. Pathological forebrain specimens reveal small fluid-filled vacuoles present within myelin sheaths and astrocytic endfeet (Bugiani et al., 2017; Dubey et al., 2015).

MLC1 is primarily present on astrocyte processes adjacent to the blood brain barrier and Bergmann glia in the cerebellum (Bugiani et al., 2017; Duarri et al., 2011; Dubey et al., 2015; Ridder et al., 2011; Teijido et al., 2007). While the exact function of MLC1 remains largely unknown, amino acid sequence analysis elicits a weak similarity to potassium channels, ABC-2 type transporters, and sodium/galactoside symporters. This indicates that MLC1 plays a homeostatic role in cell volume regulation and ion transportation. While *GLIALCAM* follows a similar expression pattern to *MLC1*, it is also expressed in OLs and axons (Bugiani et al., 2017; Favre-Kontula et al., 2008). GlialCAM is a cell adhesion molecule and protein chaperon that colocalizes with MLC1 in astrocyte-astrocyte junctions at astrocytic endfeet and targets MLC1 to these junctions (Jeworutzki et al., 2012; Lopez-Hernandez et al., 2011; Ridder et al., 2011). GlialCAM has been identified as an auxiliary subunit of the chloride channel ClC-2, localized to cell junctions in certain cell types, suggesting a role for ClC-2 in MLC pathology (Jeworutzki et al., 2012).

The availability of post-mortem brain tissue from MLC patients is scarce and does not allow for functional studies and understanding the pathomechanisms (Brignone et al., 2019; Dubey et al., 2015). Mouse models have been helpful and display similar disease manifestation to humans. *Mlc1*-null *(Mlc-Egfp)* mice present normally at birth with little myelin, but progressively develop megalocephaly and intramyelinic vacuolization throughout the white matter (Dubey et al., 2015). Astrocytes in proximity to blood vessels from *Mlc1*-null have abnormally thick processes with swollen morphology that appears before the onset of myelin vacuolization. *Glialcam*-null (*Glialcam-LacZ*) mice present with a similar disease phenotype and notably, astrocytes from these mice do not express MLC1. Interestingly, there is also a decrease in expression of chloride channel ClC-2 with an increase and redistribution of the water channel aquaporin-4. The interactions between these channels demonstrates disruption of ion-water homeostasis in MLC (Bugiani et al., 2017). Primary astrocytes with MLC1 knockdown results in impaired cell volume regulation in response to hypotonia through the potassium siphoning pathway, leading to intramyelinic edema (Dubey et al., 2015; Ridder et al., 2011). The expression pattern of *MLC1* in human and mouse astrocytes correlates with myelination and potentially explains early manifestation of white matter edema, followed by stabilization and eventual decrease in edema. While the mouse models depict early astrocyte dysfunction and swelling, this is not reported in the human tissues (Bugiani et al., 2017; Dubey et al., 2015). Thus, while currently there is no treatment, there may be a therapeutic time-window available with potential interventions targeting astrocytes and water-ion homeostasis pathways.

OL-associated leukodystrophies

The leukodystrophies discussed in this section present with mutation-specific susceptibility of OLs causing deficits in OL development, hypomyelination and/or demyelination and associated axonal pathology (Figure 2).

Pelizaeus-Merzbacher disease (PMD)

PMD is an inherited X-linked recessive dysmyelinating disease characterized by early-onset nystagmus, hypotonia, ataxia, gait disturbance, and cognitive impairment, with onset in early childhood (Boulloche and Aicardi, 1986). As PMD is X-linked, boys present with a severe neurologic phenotype while heterozygous females are typically asymptomatic or present with mild to moderate disease (Hodes et al., 1995; Lu et al., 2017; Trofatter et al., 1989). PMD is caused by duplications, deletions, and missense mutations in the *PLP1* gene, which encodes for proteolipid protein (PLP) and its alternative splice form DM20 (Lu et al., 2017). The PLP protein is a major myelin protein and expressed abundantly in both central and peripheral nervous system.

Mutations in *PLP1* can cause a broad phenotypic spectrum of symptoms, and thus PMD is classified based on the severity of the disease and clinical features as: i) connatal PMD with a missense mutation in *PLP1* coding region or splice site and severe phenotype; ii) classic PMD due to duplication of wild type *PLP1* with slow progression and mild phenotype; iii) *PLP1*-null syndrome with null alleles and phenotypes ranging from mild to moderate; and iv) mild spastic paraplegia (SPG2) caused by a *PLP1* deletion or null mutation (Woodward, 2008). The individuals affected with *PLP1*-null syndrome or SPG2 have milder phenotypes and show normal myelination despite lack of PLP1/DM20 protein (Garbern et al., 2002). MRI imaging shows an absence of myelin based on T1-weighted images with no signal in white matter and diffuse atrophy based on T2-weighted high intensity signal in cerebral hemispheres, cerebellum and brainstem (Koeppen and Robitaille, 2002; Nezu et al., 1998; Sumida et al., 2016). There is no clear association observed between genotype, clinical phenotype and MRI phenotype.

PMD was named after Pelizaeus and Merzbacher; specifically, Merzbacher first described the post-mortem findings in 1910, as the absence of myelin sheaths specific to CNS with some myelin present around blood vessels (Koeppen and Robitaille, 2002). Neuropathological evaluation on brain sections of PMD patients indicate an incomplete demarcation of gray and white matter, ill-developed internal capsule and corpus callosum, and lack of myelin on axons of CNS. Additionally, there is a profound loss of OLs with some axonal damage (Hudson et al., 1989; Inoue et al., 2001; Koeppen and Robitaille, 2002; Koeppen et al., 1987).

The post-mortem human tissue, along with the cellular and mouse models, shed some light on PMD pathogenesis. The *jimpy* mouse models developed with X-linked point mutations in *Plp1 gene*

range from mild to severe phenotype. The *jimpy mice* have an intronic point mutation *and jimpy-myelin synthesis deficient (Jimpy^{msd})* mice have a point mutation causing single amino acid substitution as seen in PMD-affected individuals. Their phenotype models the connatal severe PMD; as these mice display early onset of tremors at postnatal day 12 (P12), with progressive tremors, seizures and mortality by 3-4 weeks (Gow and Lazzarini, 1996). Both these mutations result in mislocalization of the PLP protein in ER and DM20 in Golgi, causing ER stress and activation of UPR in OLs (Clayton and Popko, 2016; D'Antonio et al., 2009; Gow and Lazzarini, 1996; Southwood et al., 2002). The *jimpy-rumpshaker (Jimpy^{rsh}*) mouse is a model for SPG2, the mildest form of PMD, manifesting with mild tremors and ataxia but almost no seizures and normal survival rates (Griffiths et al., 1990) (Mayer et al., 2015). High expression of CHOP, the protein induced by ER stress, is observed in PMD human post-mortem and mouse brain samples (Southwood et al., 2002). To test if UPR and CHOP pathways play a critical role in the severe form of PMD, Southwood *et. al.* generated double transgenic mice by crossing the *Jimpy^{rsh}* mice and CHOP-null mice (Southwood et al., 2002). Instead of rescuing the PMD phenotype, these mice exhibit a severe disease course with OL loss, suggesting that CHOP plays a critical role in protecting OLs (Southwood et al., 2002).

Transgenic mice overexpressing PLP recapitulate features of the classic PMD, including dysmyelination and OL death (Kagawa et al., 1994; Readhead et al., 1994). This occurs due to increase in PLP1 dosage causing accumulation of PLP1 in the late endosomes and lysosomes, eventually resulting in OL death (Readhead et al., 1994; Simons et al., 2002). *Plp1*-null mice do not develop any discernable symptoms; although they exhibit SPG2-like mild symptoms with ultrastructural abnormalities specifically in small diameter axons (Boison and Stoffel, 1994; Griffiths et al., 1998). This suggests that the null syndrome is due to loss-of-function of PLP that causes disruption of axon-OL interaction. The *PLP-ISEdel* (ISE; intronic splicing enhancer) mice is a knock-in model of PMD patient variant, which results in diminished *Plp1* alternative splicing and a decreased amount of PLP protein. While the adult mice display motor deficits, microglial and astrocytic activation, there are no deficits in myelination (Bachstetter et al., 2013; Hobson et al., 2002).

Studies in iPSC derived OLs from individuals with PMD with a wide spectrum of mutations (point mutations, duplication, triplication and deletion of *PLP1*) demonstrate heterogeneity of the disease. While majority of the mutations result in defects in OPC proliferation, they are able to differentiate into OLs. However, the OLs appear abnormal with stunted processes and branching. An underlying mechanism is the retention of PLP1 in the perinuclear space indicative of ER stress indicating a defect in cellular homeostasis (Nevin et al., 2017; Numasawa-Kuroiwa et al., 2014). In another study, iPSC-derived OLs from individuals with PMD undergo cell death (ferroptosis) due to characteristic features of lipid peroxidation, abnormal iron metabolism and hypersensitivity to free iron, which is reversed in

presence of an iron chelator (Nobuta et al., 2019). Since OL death is the primary cause of PMD, cell-based therapy and other treatment strategies targeting the cellular pathways above are discussed in a later section.

Pelizaeus-Merzbacher-like disease (PMLD)

Based on clinical and radiographic similarity to PMD, there is a class of disorders referred as Pelizaeus-Merzbacher-like diseases (PMLD). Recessive mutations in Gap Junction Protein Gamma-2 (*GJC2)* gene encoding Cx47 protein are denoted as Pelizaeus-Merzbacher-like disease type 1 (Orthmann-Murphy et al., 2009). PMLD1 is phenotypically similar to PMD and presents with severely impaired motor development in the first year of life with gait difficulties, trunk instability, spasticity, pendular nystagmus, and cognitive impairment (Abrams et al., 2014). Hereditary spastic paraplegia type 44 (SPG44) is a milder form of PMLD1 with a late-onset, progressive spastic gait disorder and associated with changes in white matter in the brain based on MRI imaging (Orthmann-Murphy et al., 2009).

Connexins (Cxs) are a family of integral membrane proteins that assemble to form gap junctions (GJs) and allow diffusion of ions and small molecules between adjacent cells (Abrams et al., 2014). Over-expression studies of Cx47, a major OL specific connexin have been pivotal in understanding effects of Cx47 mutation on function, and providing a genotype-phenotype correlation (Abrams and Scherer, 2012; Abrams et al., 2014). PMLD1-associated mutations cause Cx47 retention in the ER, disabling Cx47 to form a functional channel with Cx43, the major astrocyte Cx (Gotoh et al., 2014; Orthmann-Murphy et al., 2009). In SPG44-associated Cx47 mutation, they can form normal gap junction plaques, but their conductance voltage is altered resulting in a mild disease phenotype (Abrams et al., 2014; Orthmann-Murphy et al., 2009). Thus, Cx47 may have coupling-dependent and independent functions and SPG44 mutation results in only loss of coupling-dependent function (Abrams et al., 2014). PMLD1 mouse models with the orthologous $Cx47^{M282T}$ mutation display impaired motor function with a decrease in OL gap junctional coupling and myelination in the corpus callosum during early development (Blackstone, 2015; Tress et al., 2011). Adult $Cx47^{M282T}$ mice do not show substantial myelin deficits; however, when they are deprived of Cx32 (another major OL connexin), the mice exhibit severe myelin defects and die within 6 weeks after birth. Similarly, *Cx32* and *Cx47* double knockout mice present with profound CNS demyelination, axonal injury, tremors, seizures, and premature death by 2 months of age (Morrison et al., 2013). This provides evidence that PMLD and SPG44 occurs due to loss of Cx47 channel function that impairs glial coupling in the white matter (Blackstone, 2015; Tress et al., 2011).

Allan-Herndon-Dudley syndrome (AHDS) is a X-linked recessive multisystem genetic disorder, also classified as a PMLD due to its similarities with PMD and other hypomyelinating leukodystrophies. Mutations in the *MCT8/SLC162A* gene that encodes the monocarboxylate transporter 8 (MCT8) protein cause AHDS. Males present with severe intellectual disability, spastic paraplegia, extrapyramidal movement disorders and severe hypotonia, whereas most female carriers have normal neurodevelopmental function due to random X-chromosome inactivation (Charzewska et al., 2016; Vaurs-Barriere et al., 2009). Affected males can also have peripheral thyrotoxicosis, in contrast to CNS thyroid deficiency, which can be associated with poor weight gain and other systemic complications.

MCT8 plays a critical role in the transport of triiodothyronine (T_3) and thyroxine (T_4) across the BBB, which is critical for neuronal migration, dendritic outgrowth, synapse formation, and myelination (Bernal and Nunez, 1995; Charzewska et al., 2016). *MCT8* mutations result in high levels of T_3 and low levels of T_4, causing toxicity to multiple organ systems and serving as a diagnostic marker for AHDS (Charzewska et al., 2016). The mouse models show subtle behavioral changes and abnormal T_3 and T_4 serum levels but do not manifest any neurological symptoms (Dumitrescu et al., 2006; Trajkovic et al., 2007; Wirth et al., 2009). However, morpholino-ASO based knock down of *mct8* in zebrafish models establishes the first vertebrate model with defects in brain development (Vatine et al., 2013). MCT8-deficient iPSC neurons differentiate and mature normally, although they exhibit a reduction in TH uptake. This indicates that it is MCT8 transporter deficiency rather than decline in TH levels that may be the underlying cause of the neurological phenotype in AHDS. Given the BBB regulates the entry of TH into the brain, a model of MCT8-deficient BBB with human iPSC-derived microvascular endothelial cells demonstrates that T_3 transport across the BBB is MCT8-dependent and is in fact the driving cause for decrease in TH levels in the brain (Vatine et al., 2017).

Autosomal Dominant Leukodystrophy (ADLD)

Autosomal Dominant Leukodystrophy (ADLD) is a late-onset progressive neurological and demyelinating disorder. This disorder presents between the fourth and sixth decades of life and is characterized by gait abnormalities, muscle weakness, spasticity, and autonomic symptoms including bowel dysfunction, impotence in males, and orthostatic hypotension (Coffeen et al., 2000). ADLD is associated with duplication of *LMNB1* gene or deletion of upstream regions of the *LMNB1* promoter (Padiath et al., 2006). *LMNB1* gene encodes Lamin B1 which is a part of nuclear lamina, a dense fibrillary network inside the nucleus of most cells. Nuclear lamina provides mechanical support to the nucleus and regulates crucial cellular events such as DNA replication, cell division, transcription, DNA repair, and epigenetic regulation of euchromatin and heterochromatin (Dechat et al., 2010). The nuclear lamina is composed of lamins and nuclear lamin-associated membrane proteins. Lamins are

categorized as either Lamin A-type (lamin A, C) or B-type (lamin B1, B2), and lamin B1 is important for gene expression, chromatin structure, and nuclear stability (Finlan et al., 2008; Malhas et al., 2009; Vergnes et al., 2004).

MRI findings show ADLD affected individuals have higher intensity T2-signal in cerebral white matter extending to the motor cortex, internal capsule, and medulla oblongata. Overtime, an apparent atrophy of cerebrum, cerebellum and corpus callosum occurs in some individuals (Bergui et al., 1997; Melberg et al., 2006). Brain pathology of ADLD individuals reveal spared oligodendrocytes but show sparse, abnormally beaded and thickened astrocytic processes (Lin et al., 2011; Sundblom et al., 2009).

While ADLD is the only known nuclear lamina disease that results in abnormal myelination, the mechanistic pathways remains to be explored (Padiath et al., 2006). Mice with global over-expression of lamin B1 (*Laminb1*^{BAC}) and OL-specific over-expression of lamin B1 (*Plp-LMNB1*) show a progressive age-reliant phenotype with kyphosis, limb paralysis and reduced survival. Further, *Laminb1*^{BAC} and *Plp-LMNB1* adult mice show abnormal myelin architecture, thinner myelin, decreased PLP1 expression and axonal degeneration reminiscent of phenotypes seen in ADLD-affected individuals. Surprisingly, the number of OLs remain the same with no detection of OL death, suggesting that lamin B1 over-expression does not affect OL survival (Heng et al., 2013). However, cell-specific over-expression of Lamin B1 in neurons and astrocytes in mice do not show discernable phenotypes indicating secondary involvement of these cell types in ADLD (Heng et al., 2013). Thus, this study shows that Lamin B1 over-expression induces a cell-autonomous deficit in OLs. In another independent study, authors demonstrate downregulation of several different lipid synthesis genes in *Plp-LMNB1* mice, thus elegantly establishing a link between lamin B1 and disruption of lipid synthesis (Rolyan et al., 2015). The authors also show an increase in epigenetic histone modifications and suggest an association between decrease in gene expression of lipid synthesis pathway and increase in histone modification (Rolyan et al., 2015). Therefore, myelin loss in ADLD could be attributed to the lipid dysregulation and disruption of myelin composition. Furthermore, *in vitro* studies with ADLD fibroblasts illustrate an increase in *LMNB1* expression with defects in genes regulating cellular response to oxidative stress and RNA splicing of *Plp1* gene, which may in turn contribute to the demyelinating phenotype in ADLD-affected individuals (Bartoletti-Stella et al., 2015; Columbaro et al., 2013).

Hypomyelination with Atrophy of Basal Ganglia and Cerebellum (H-ABC)

Heterozygous and often *de novo* mutation of the tubulin beta class IVA (*TUBB4A*) gene results in a spectrum of disease ranging from Hypomyelination with Atrophy of Basal Ganglia and Cerebellum (H-ABC) to mild adult onset Dystonia type 4 (Curiel et al., 2017; Simons et al., 2013). H-ABC is the

most common subtype and is characterized by presentation in the toddler years with dystonia, progressive gait impairment, as well as speech and cognitive deficits (Hersheson et al., 2013). Characteristic neuroimaging features include hypomyelination and atrophy of the caudate and putamen with cerebellar atrophy, which is consistent with pathologic specimens demonstrating with loss of dorsal striatal areas and cerebellar granular neurons, along with axonal swelling and diffuse paucity of myelin (Curiel et al., 2017; Simons et al., 2013; van der Knaap et al., 2007).

TUBB4A protein is an isoform of β-tubulin, which heterodimerizes with α-tubulin and assembles into microtubules (Simons et al., 2013). Microtubules, which are cytoskeleton of the cell, are essential to the proper development of neurons and OLs for cellular transport of key proteins. *TUBB4A* mutations disrupt microtubule dynamics and transportation (Curiel et al., 2017). Our group has previously overexpressed different *TUBB4A* mutations *in vitro* in both glia and neurons and examined a mutation-specific effect on these cells. Particularly, $TUBB4A^{D249N}$ over-expression affects OL formation and maturation along with neuronal branching and survival when compared to over-expression of $TUBB4A^{WT}$ (Curiel et al., 2017). The *taiep* rat exhibits a spontaneously occurring *Tubb4a* mutation (p.Ala302Thr). Although this mutation has not yet been reported in individuals, it shows an accumulation of microtubules in OLs that ultimately results in demyelination (Curiel et al., 2017; Duncan et al., 2017). Work from our group involves characterization of a novel mouse with the canonical $Tubb4a^{D249N}$ (p.Asp249Asn) mutation, which recapitulates the human disease with loss of OPCs and OLs causing hypomyelination and profound loss of cerebellar granular and striatal neurons (Sase et al., 2020). A study on iPSC-derived neurons with heterozygous knockout of *TUBB4A* mutations, including $TUBB4A^{D249N}$, demonstrates deficits in mitochondrial motility and transport (Vulinovic et al., 2018). While there is no current treatment for H-ABC, the studies focused on modeling of the disease will advance our understanding about the disease mechanisms and help identify potential therapeutic targets.

Krabbe Disease

Krabbe disease, is a progressive and fatal lysosomal storage disorder that is a result of biallelic mutations in the β-galactosidase (GALC) gene. β-galactosidase is a lysosomal enzyme involved in the hydrolysis of galactosylceramide, a major lipid in the myelin membrane. While most individuals typically have an early infantile disease, the disease presentation can also occur later in life (Bascou et al., 2018; Lee et al., 2019). Clinically, affected individuals in infancy show irritability, hypersensitivity, psychomotor arrest, and spasticity. This rapidly progress in neurologic deterioration and seizures, often resulting in death before 2 years of age (Lee et al., 2019; O'Sullivan and Dev, 2015). MRI imaging show hyperintensities indicating abnormalities in periventricular white matter. The human pathology consists

of characteristic demyelination and axonal degeneration in both CNS and PNS. A hallmark feature is activated macrophages and microglia transforming into multinucleated globoid phagocytes with lysosomes, and hence this disease is also called as globoid cell leukodystrophy (GLD) (LeVine and Brown, 1997; Meisingset et al., 2013; Nicaise et al., 2016). CSF levels of GALC has been used as a diagnostic measure but there is no correlation between GALC activity and disease severity.

Krabbe disease is spontaneously found in a number of species, including different breeds of dogs, domestic cats, sheep, rams, primates, and the twitcher (*Twi*) mouse (Bradbury et al., 2018; Lee et al., 2019). *Twi* mouse is the most widely used animal model for Krabbe disease and has naturally occurring point mutation that results in a premature stop codon, resulting in no residual GALC activity (Meisingset et al., 2013). The '*quaking*' mouse is caused due to an autosomal recessive trait leading to dysmyelination without myelin degradation, globoid cell formation, metachromatic lipids, and/or inflammation (LeVine and Brown, 1997). The canine model of Krabbe disease is hereditary and is the only naturally occurring disease model that results from a missense mutation in the *GALC* gene, with disease progression that closely recapitulates the human disease. The strengths and weaknesses of the canine and murine models are complementary as the canine model is more suited as a long-lived model for pre-clinical testing and evaluation of diagnostic measures such as magnetic imaging techniques and tissue biopsies. Comparatively, the murine models are better for experiments requiring a large number animals for testing *in vivo* therapies due to their small size, ease of maintenance, and rapid reproduction (Kobayashi et al., 1980; Suzuki and Suzuki, 1983; Wenger, 2000). In addition to animal models, cellular models using rodent and human cells have helped tease out the cell-autonomous effects and cross-talk across different cells in this disease (Meisingset et al., 2013).

GALC mutation results in the accumulation of galactolipids called psychosine, a cytotoxic lipid intermediate and the OL cell death is hypothesized to primarily be induced by psychosine-mediated toxicity (Lee et al., 2019; Misslin et al., 2017). As OLs support axonal integrity and function, their loss also results in axonal degeneration. In addition to OLs, psychosine also causes astrogliosis, microglial activation, and formation of globoid cells from microglia or monocytes, which accumulate around blood vessels and in demyelinated regions (Giri et al., 2006; Nicaise et al., 2016; O'Sullivan and Dev, 2015). GALC is also responsible for the metabolism of sphingolipids, including ceramide, sphingosine, and sphingosine 1-phosphate (S1P). S1P and its family of receptors (S1PR) regulate a number of intracellular pathways, including astrocyte migration and promotion of OL differentiation and survival (O'Sullivan and Dev, 2015). The mechanistic pathways associated with psychosine-mediated toxicity in OLs include TNFα, IL-6, iNOS, protein kinase C (PKC), NF-κB, cytochrome c, and direct activation of apoptotic pathways (Giri et al., 2006; Misslin et al., 2017). Psychosine-mediated activation of

phospholipase A2 produces lysophosphatidylcholine, directly triggering cell death pathways in OPCs and mature OLs causing demyelination (Giri et al., 2002; Giri et al., 2006; LeVine and Brown, 1997).

Inflammation is thought to be a critical factor in Krabbe disease pathology contributing to OL death, evident by presence of multinucleated globoid phagocytes with an increase in release of TNF-α, IL-6 and inducible nitric oxide synthase (iNOS) in mouse models and individuals with Krabbe disease. Microglia can adapt to different activation states such as a resting state (M0), pro-inflammatory state (M1, also known as "classic activation") and anti-inflammatory state (M2, also known as "alternative activation") (Nicaise et al., 2016). Shifts between the states of activation are referred to as polarization because microglia exhibit different morphologies and functions in these states. The pro-inflammatory M1-polarized microglia typically become round cells and cytotoxic to neurons and OLs, while M2-polarized microglia become ramified and conduct functions such as phagocytose cellular debris, reconstruct the extracellular matrix, and promote neurite outgrowth (Nicaise et al., 2016). The *Twi* mice have extensive demyelination, astrogliosis and an increase in both activated M1 and M2 polarized microglia, which can stimulate astrocytes and increase the release of cytokines and chemokines, thereby markedly exacerbating inflammation (Meisingset et al., 2013; Nicaise et al., 2016). While classification of microglia as M1 and M2 is an over-simplification, microglia may exhibit various in-between transition states. In addition, it does little to describe the unique microglial activation or phenotype of globoid cells found in Krabbe disease. Therefore, a third class called M3 microglia has been proposed specifically for psychosine activated microglia giving rise to the novel M3 psychosine-activation state (Nicaise et al., 2016). Due to the prevalence of aberrantly formed microglial globoid cells as well as multiple forms of activated microglia, these glial cells have been largely implicated in Krabbe disease and hence this disease could also be classified as a microglia related leukodystrophy.

Microglia-related leukodystrophies

As the role of microglia is often overlooked in leukodystrophies, recent studies elucidate its role in several diseases. The leukodystrophies in this section are focused on mutations in genes primarily affecting microglia and/or microglia involvement as a primary cause of the pathogenesis (Figure 3).

Adult-onset Leukoencephalopathy with Axonal Spheroids and Pigmented Glia (ALSP)

Adult-onset Leukoencephalopathy with Axonal Spheroids and Pigmented Glia (ALSP) is a late-onset neurodegenerative disease associated with heterozygous autosomal dominant mutations in colony-stimulating factor 1 receptor (CSF1R). ALSP is characterized by prominent and progressive cognitive dysfunction, including behavioral changes, executive dysfunction, and neuropsychiatric symptoms. Later, individuals experience a progressive decline in motor function, including ataxia and

parkinsonism. Brain imaging shows classic features of white matter abnormalities, enlargement of lateral ventricles, cortical atrophy thinning of the corpus callosum, and brain calcifications (Konno et al., 2018). Histological examination of brain tissue with ALSP shows extensive myelin degeneration with axonal spheroids, pigmented macrophages and reduced number of microglia in cortical gray and white matter areas (Adams et al., 2018; Konno et al., 2018; Oosterhof et al., 2017).

CSF1R is involved in development of a multitude of cell types, including microglia, NPCs, and neurons in the CNS, as well as other peripheral immune cells. This receptor exhibits the canonical shape of other platelet-derived growth factor (PDGF) family members. CSF1R is stimulated by two ligands, colony-stimulating factor-1 (CSF-1) and interleukin-34 (IL-34), which are both essential in regulating the growth and activity of macrophages. While their functions are similar, these ligands have differential spatiotemporal expression, with IL-34 only acting locally and CSF-1 acting both locally and in circulation. Upon binding to CSF-1 or IL-34, CSF1R maintains macrophage survival, proliferation, and chemotaxis by phosphorylating multiple downstream proteins (Stanley and Chitu, 2014). Thus, in the case of ALSP, mutation of this receptor disrupts microglial survival and proliferation.

To elucidate the molecular mechanisms of *CSF1R* mutations in microglia, zebrafish models have been helpful for their ease of *in vivo* imaging and shared microglial transcriptomes with humans (Oosterhof et al., 2017). Zebrafish homologs *csf1ra* and *csf1rb* have different developmental impacts, where *csf1ra*$^{-/-}$ show greater loss of microglia in early larvae development and *csf1rb*$^{-/-}$ mutants show loss of microglia during the adult stages of development. In addition, 5-month adult zebrafish with *csf1ra*$^{-/-}$*;csf1rb*$^{+/-}$ mutations exhibit fewer microglia on the dorsolateral side of the optic tectum, however microglia accumulation occurs in the underlying brain regions. Mutant zebrafish with both *csf1ra* and *csf1rb* deficiency (named *csf1r*^{DM} for double mutant) show little to no microglia cell population (Oosterhof et al., 2018). RNA seq analysis in *csf1r*^{DM} mutants confirm that microglia are the primary affected cell type establishing their critical role in Csf1r signaling (Oosterhof et al., 2017; Oosterhof et al., 2018). Specifically, there is downregulation of genes involved in neurodevelopment and neuronal differentiation, as well as upregulation in chemotaxis and immune response genes (Oosterhof et al., 2018). In a model of neuronal ablation, the typical response involves activation and proliferation of local microglia. However, the *csf1ra*$^{-/-}$ and *csf1ra*$^{-/-}$*;csf1rb*$^{+/-}$ mutants display a significant decrease and delay in microglia proliferation. This suggests, an aberrant distribution and density of microglia occurs during recruitment in the *Csfr1* mutants. Although zebrafish models have been recognized as an efficient model for studying the effect of *CSF1R* mutations on microglia, there is no demyelination observed in this model (Oosterhof et al., 2018).

Csf1r$^{-/-}$ mice show near complete eradication of microglial cells, widened cerebral ventricles, cerebrovascular defects, reduced amount of OPCs and shorter lifespan as seen in human patients

(Erblich et al., 2011; Hagemeyer et al., 2017; Nandi et al., 2012). Haploinsufficient Csfr1$^{+/-}$ mice recapitulate many aspects of ALSP including motor and cognitive deficits, white matter abnormalities and axonal spheroids albeit with increased microglial density (Chitu et al., 2015). Pharmacological blockade of CSF1R decreases OPC proliferation and differentiation in OLs (Hagemeyer et al., 2017). On the other hand, stimulation of CSF1R elicits expression of CD11c class of microglia, which are thought to be neuroprotective and help in disease amelioration and remyelination in mouse model for multiple sclerosis (Wlodarczyk et al., 2018). The above studies implicate that CSF1R modulates microglia density, regional distribution and plays a role in myelination, serving as potential mechanisms underlying this leukodystrophy.

Polycystic Lipomembranous Osteodysplasia with Sclerosing Leukoencephalopathy (PLOSL)

Polycystic Lipomembranous Osteodysplasia with Sclerosing Leukoencephalopathy (PLOSL; also known as Nasu-Hakola disease) is a late-onset autosomal recessive disease, characterized by painful bone cysts with fractures and early dementia (Bianchin et al., 2004; Xing et al., 2015). The genetic cause of PLOSL is the loss-of-function or deletion of one of two genes, *TYROBP* and *TREM2*, which respectively encode DNAX-activating protein 12 (DAP12) and triggering receptor expressed on myeloid cells-2 (TREM2) proteins (Xing et al., 2015). Similar to ALSP, imaging studies reveal bilateral basal ganglia calcification and white matter abnormalities (Errichiello et al., 2019; Konno et al., 2018). In the later stages of disease, individuals with PLOSL experience seizures, profound dementia, pathological bone fractures and death within the fifth decade of life (Bianchin et al., 2004; Xing et al., 2015). Histological analysis of PLOSL demonstrates a significant loss of axons and myelin, fibrillary gliosis, axonal spheroids, as well as vascular abnormalities and microglial activation within the frontal and temporal white matter (Bianchin et al., 2004; Xing et al., 2015).

TYROBP and TREM2 proteins are highly expressed in microglia and osteoclasts, and both cells originate from the hematopoietic lineage (Bianchin et al., 2004). In microglia, these proteins are crucial for recognizing neuronal debris and amyloid deposits for phagocytic function; whereas in osteoclasts, they play an essential role in bone-remodeling homeostasis, including osteoclastogenesis (Xing et al., 2015). While the exact molecular underpinnings of TREM2-DAP12 dysregulation are unknown, it is postulated that loss-of-function in the TREM2-DAP12 signaling pathway decreases clearance of axonal debris, which triggers an increase in microglial activity, and contributes to myelin loss (Sasaki, 2017). Thus, this disease qualifies as a microgliopathy with secondary effects on OLs.

The primary mouse models for PLOSL, both *Trem2* knockout and *Dap12* knockout mice, establishes that both these proteins co-localize with common microglia/macrophage markers in the brain (Xing et al., 2015). *Trem2 KO* mice show circulating immune cells and upregulation of

proinflammatory cytokines, while *Tyrob* KO mice have increased auto-antibodies and defective natural killer (NK) and T cells. A recent study reports the presence of auto-antibodies and a decrease in NK cells in patients with PLOSL, suggesting that this disease may be more complex and involves an auto-immune component in addition to CNS inflammation and bone-related pathology (Errichiello et al., 2019).

X-linked adrenoleukodystrophy (X-ALD) and Metachromatic leukodystrophy (MLD)

X-linked adrenoleukodystrophy (X-ALD) and Metachromatic leukodystrophy (MLD) are both demyelinating diseases caused due to dysfunction in the peroxisomal and lysosomal lipid degradation pathway, respectively (Bergner et al., 2019). X-ALD is a peroxisomal storage disease caused by a gene mutation for ATP-binding cassette protein subfamily D member 1 *(ABCD1)*, which typically shuttle very long chain fatty acids (VLCFA) to the peroxisome for degradation, ultimately resulting in destruction of cerebral white matter (Bergner et al., 2019). It is characterized by adrenocortical insufficiency and cerebral demyelination, along with progressive myelopathy (adrenomyeloneuropathy AMN). Histopathological examination of patients with X-ALD shows demyelination in white matter of the parieto-occipital regions and the corpus callosum, as well as loss of myelin and axons in the corticospinal, gracile and spinocerebellar tracts of the spinal cord (Engelen et al., 2014). While mouse model of X-ALD have accumulation of VLCFA, they do not recapitulate all clinical phenotypes observed in humans. The *Abcd1*$^{-/-}$ mice develop a gait disorder secondary to myelin and axonal loss in the spinal cord and sciatic nerve, but are otherwise unaffected (Pujol et al, 2002). The *Abcd1*$^{-/-}$; *Abcd2*$^{-/-}$ double knockout mice exhibit a more severe disease phenotype with earlier onset but no adrenocortical insufficiency or cerebral disease, albeit cerebellar degeneration, which is atypical in X-ALD. (Pujol et al, 2004; Engelen & Kemp, 2014).

MLD is a lysosomal storage disease caused by biallelic mutations in arylsulfatase A (*ARSA*). In MLD, impaired recycling of sulfatides results in accumulation of non-degraded sulfatides and the subsequent destruction of cerebral white matter (Bergner et al., 2019). Clinically, MLD can present as late-infantile, juvenile, or adult variants showing slower disease progression with age. Individuals with late-infantile MLD have a shortened life expectancy, with symptoms of progressive peripheral and central neuropathy. The adult form presents with cognitive and behavioral changes, while the juvenile form bridges the clinical characteristics of both the late infantile and adult forms.

Classic neuroimaging findings demonstrate hyperintense signal in corpus callosum and radiating stripes of normal signal within abnormal white matter. Histological analysis shows sulfatide accumulation in astrocytes, neurons, OLs and Schwann cells, which ultimately leads to demyelination (van Rappard et al., 2015). Knockout mice for *Arsa A* (*Asa*$^{-/-}$), resemble the late-infantile form of MLD

due to the severe phenotype and exhibit auditory deficits, ataxia, tremors, and hypotonic paresis. Pathological examination of the nervous system shows loss of acoustic ganglion neurons, altered Purkinje cell morphology, reduced myelinated axons of the optic nerve and corpus callosum, astrogliosis secondary to demyelination and the two-year old adult mice showed activated microglia. Notably, while there is deficiency in sulfatide metabolism, there is no evidence of widespread demyelination until two years of age. At that point, there is accumulation of sulfatides in astrocytes and microglia that appear phagocytic along with neurons, suggesting early involvement of these glial cells. As these mice do not display progressive demyelination, they make an opportune model for elucidating the mechanism of the early stages of MLD (Hess et al, 1996).

Human pathological studies shed more light into the contribution of microglia in X-ALD and MLD based on the use of resident brain microglia-specific markers Tmem119 and P2ry12 instead of traditional phagocyte markers Ki-M1P and Iba1. In X-ALD patients, a stark decrease in microglial markers is observed without ongoing OL loss, indicating early loss of microglia in prelesional areas and these microglia markers reappear later along with astrogliotic scarring. In contrast, while microglia specific markers are present early in MLD, a progressive loss of both microglia and phagocytic markers occurs before OL loss in pre-lesional white matter. Unlike as in X-ALD, the resident microglia neither re-adopt the microglial markers nor morphology later in MLD (Bergner et al., 2019). In summary, the changes in morphology and cell death in microglia precedes OL degeneration and demyelination implying their role in pathogenesis of both X-ALD and MLD (Eichler et al., 2008).

Therapeutic strategies for leukodystrophies

Currently medical management of existing symptoms through physiotherapy procedures, psychomotor stimulation and treatment of seizures remains the standard of care for individuals with leukodystrophies (Batla et al., 2011; Dash et al., 2015; Kohler et al., 2018; Kohlschutter and Eichler, 2011). Thus, there is an urgent unmet need for targeted therapies towards these diseases. A number of therapeutic strategies discussed below hold promise in ameliorating disease pathology based on preclinical models, which can or have extended to clinical trials for specific leukodystrophies.

Pharmacological Interventions

Understanding the molecular mechanisms of leukodystrophies will help target affected pathways and design novel therapeutic strategies. A range of neurological disease utilize traditional pharmaceuticals and small molecules are becoming an emerging therapeutic modality in leukodystrophies (Helman et al., 2015a; Helman et al., 2015b; Patil and Maegawa, 2013). Small molecule intervention is particularly advantageous in the treatment of neurological disorders, as they

are able to cross the BBB to affect the CNS without requiring any invasive procedure; a major limitation for many therapies (Patil and Maegawa, 2013). Examples of pharmacological targets using small molecules are chaperone to rescue misfolded proteins, proteostasis regulators for enzyme enhancement, molecules regulating pathogenic pathways, OL maturation and modulators of neuroinflammation (Helman et al., 2015a; Helman et al., 2015b; Patil and Maegawa, 2013).

AGS shares a common signaling pathway with autoimmune rheumatologic disorders and there is great interest in repurposing some of these drugs. Broad-based immunomodulatory therapies have shown mixed success for AGS (Crow et al., 2019; D'Arrigo et al., 2008; Orcesi et al., 2008). As AGS mutations trigger an IFN-α response, inhibiting cGAS or JAK/STAT, the downstream signaling pathway of IFN activation is a potential therapeutic strategy (Meesilpavikkai et al., 2019; Xiao et al., 2019). Accumulation of endogenous retroelements in AGS overdrive the IFN response and an attempt to block synthesis of these retroelements with a combination of reverse transcriptase inhibitors (RTIs) rescued AGS-related phenotype in *Trex1*$^{-/-}$ mice (Achleitner et al., 2017; Beck-Engeser et al., 2011). Importantly, a pilot clinical trial in AGS patients using RTI reduces IFN and ISGs in both serum and plasma but does not alleviate the neurological impairments (Crow et al., 2019; Rice et al., 2018; Thomas et al., 2017).

For PMD and VWM, the underlying pathogenesis is linked to ER stress and preclinical studies demonstrate reducing ER stress can help rescue disease phenotype. The use of small molecules targeting ER stress extend OL survival *in vitro* in iPSC-derived OL, PMD-oligocortical spheroids and *in vivo* in *Jimpy* mice (Elitt et al., 2018; Nevin et al., 2017; Yool et al., 2000). However, this OL survival still fails to restore myelination suggesting other strategies may be necessary to remediate this phenotype (Elitt et al., 2018). Long-term treatment with FDA-approved guanabenz in VWM mouse models improves both the cerebellar myelin pathology and eIF2B activity (Dooves et al., 2018; Tsaytler et al., 2011). VWM also involves dysregulation in ISR pathway, and ISR inhibitors (ISRIB) are able to improve both white matter pathology and motor development in VWM mice, making ISRIB a viable clinical target for VWM (Abbink et al., 2019). Treatment with agonists of Sigma-1-Receptor (S1R) protein, which is diminished in VWM astrocytes, also rescues mitochondrial dysfunction and cell death due to ER stress (Atzmon et al., 2018). For PMD, curcumin and cholesterol-enriched diet treatment in mice alleviates the clinical and pathological phenotype to some extent; however, neither of these treatment strategies are considered promising for human translation (Epplen et al., 2015; Saher et al., 2012; Yu et al., 2012). Treatment with drugs such as Diiodothyropropionic Acid (DITPA), an MCT8-independent thyroid (TH) analog promotes OPC differentiation and myelination in AHDS disease caused by *MCT8* mutations in zebrafish models (Lee et al., 2017).

Together, some of these disease-modifying drugs could effectively target signaling pathways and ameliorate pathology. Additionally, as these compounds typically target multiple pathways, it will help shed light on the pathogenesis and mechanism of specific leukodystrophies.

Antisense oligonucleotide (ASO) therapy

ASOs are short, synthetic oligonucleotides ranging from 18 to 30 base pairs in length designed to alter expression of target mRNA. Mechanistically, ASOs modify the mRNA expression by altering splicing; by employing RNase H enzyme to degrade the mRNA and steric hindrance of ribosomal activity. Several chemical modifications can be added to ASOs to avoid their susceptibility to nuclease degradation and increase their half-life; such as 2'-O methyl (2'OMe), 2-methoxyethyl (2'-Moe), locked nucleic acids, phosphorodiamidate morpholino oligomer (PMO) and peptide nucleic acids (Bennett and Swayze, 2010; Schoch and Miller, 2017). FDA-approved ASOs for diseases like Duchene muscular atrophy and spinal muscular atrophy have shown promising outcomes in clinical trials (Sardone et al., 2017).

In AxD, a single intracerebroventricular (ICV) administration of ASOs against the α and δ isoforms have been effective in suppressing the *Gfap* transcript in *Gfap*$^{+/R236H}$ mice, resulting in a significant reduction of *GFAP*, microglial activation and allowing clearance of Rosenthal fibers (Hagemann et al., 2018). Similarly, in PMD, a single dose administration of morpholino ASOs can correct the splicing defect in the *PLP-ISE*^{tg} mice and decrease microglial and astrocytic activation (Tantzer et al., 2018). Recent work by the Tesar group, a leading group in PMD, conducted ASO delivery in the *jimpy mice,* a severe PMD mouse model. The results show remarkable reversal of hallmark disease features such as motor dysfunction, abnormal respiratory function, loss of OL numbers with a further increase in myelination and extending life span from 3-4 weeks to 8-months (Elitt et al., 2020). Canavan's disease, another leukodystrophy which occurs due to mutation in *ASPA* gene results in brain accumulation of amino acid N-acetyl L-aspartate. ASOs administered through the cisterna magna route for Canavan's disease in a preclinical mouse model results in reversal of ataxia, Purkinje cell atrophy and cerebellar thalamic vacuolation (Hull et al., 2020). While high concentrations of ASOs could cause an inflammatory response due to its design chemistry, ASOs hold strong hope for future clinical trials specifically for leukodystrophies with toxic gain-of-function mutations.

Gene Therapy

Gene therapy approaches involve introduction of exogenous genetic material into cells to compensate for a mutant gene resulting in loss or gain of function. A common approach for gene therapy includes use of vector such as retroviruses (lentivirus) or adeno-associated virus (AAV).

Currently, AAV is considered a gold standard for CNS diseases due to its good safety profile, long-term expression and ability to penetrate CNS in order to target glia and neurons. There are several serotypes of AAV (AAV1 to AAV9) based on their capsid design suited for cellular tropism and route of administration (e.g. ICV and cisterna magna). The success of gene therapy in any disease is based on the choice of vector, modification of viral capsids and the route of administration to achieve optimal compensation of the target gene (Gray et al., 2010). There is robust evidence from preclinical studies that gene therapy can rescue pathology and phenotype in leukodystrophies. For MLD, ICV administration of AAV5 human *ARSA* (AAV.rh.10-hARSA) in *Mld* mouse model at pre-symptomatic stage restores the enzyme resulting in reversal of glucolipid storage in CNS, microglial activation, neuronal degeneration and motor deficits (Sevin et al., 2007). The use of AAV.rh.10-h*ARSA* in the same mouse model further improves the transduction efficiency in neurons and OLs even at advanced stage of disease compared to AAV5 vectors (Piguet et al., 2012). Injecting AAV.rh.10-hARSA in non-human primates demonstrated a safe and efficacious profile and is currently being tested for a MLD clinical trial in France (NCT01801709, ClinicalTrials.gov) (Zerah et al., 2015).

In the case of MLC disease, administration of AAV GFAP-MLC1(specific for astrocytes) in adult *Mlc1*$^{-/-}$ mice restores the adhesion molecule-glialCAM activity and localization of the chloride channel ClC-2 in Bergmann glia with decrease in cerebellar vacuolation (Sanchez et al., 2020). In the canine model of Krabbe disease, delivery of AAV.rh.10-cGALC delays the onset of clinical symptoms, attenuates neuropathy and extends lifespan; thus it is a promising therapeutic avenue (Bradbury et al., 2018). Work from the Inoue group using AAV-mediated gene therapy establishes the proof-of-concept that knockdown of *PLP* in mice overexpressing *Plp1* can be a potential cure for PMD (Li et al., 2019). This treatment helps preserve mature OLs, restore myelin, and improve both survival rates and neurological phenotypes. Despite the associated side-effects (e.g. activation of immune system, liver-associated toxicity) with AAV-mediated gene therapy, gene therapy is a hopeful approach for leukodystrophies especially where loss-of-function occurs.

Cellular replacement therapy

Bone marrow, and hematopoietic stem cell transplantation

Bone marrow transplant (BMT) and hematopoietic stem cell transplant (HSCT) offers treatment potential for some leukodystrophies that manifest with a component of peripheral immune system involvement, enzyme deficiency or replacement of defective genes. Treatment with BMT involves transplantation of a matched donor's bone marrow while HSCT requires transplantation of a healthy donor's hematopoietic stem cells from either bone marrow, umbilical cord or peripheral blood. While HSCT has not undergone the scrutiny of a clinical trial, but in the absence of approved therapies, it is

currently the only available treatment for ALD, MLD, and Krabbe patients. HSCT is only beneficial prior to the onset of fulminant symptoms. In pre-symptomatic Krabbe patients, HSCT increases life expectancy and reduces inflammation, thus providing a novel source of functional GALC (Graziano and Cardile, 2015; Nicaise et al., 2016; Weinstock et al., 2020; Wenger and Luzi, 2015). Despite being poorly understood, the mechanism of HSCT is believed to work through cross-correction, in which donor-derived cells transfer missing enzymes to *GALC*-deficient cells (Mikulka and Sands, 2016). However, in the conditional *Galc*-deficient Krabbe mouse model targeting the PNS, donor-derived cells inefficiently cross-correct neuronal and peripheral glial cells. This argues against the notion of cross-correction and instead, authors suggest that HSCT reinstates GALC-expression in macrophages and microglia, thus, reducing the accumulation of psychosine and disease phenotype (Weinstock et al., 2020).

HSCT helps prevent progression of the cerebral disease phenotype in pre-symptomatic X-ALD affected children (Engelen et al., 2014). In X-ALD, HSCT is effective and attenuates cerebral demyelination and reduction of plasma VLCFA (Cartier et al, 2009). BMT can also reduce lipid peroxidation and protein damage seen in plasma of X-ALD patients (Rockenbach et al, 2012). Similarly, HSCT was beneficial for early stages of MLD than aggressive form, although, most patients eventually experienced neurologic decline (Boucher et al., 2015). HSCT using umbilical cord was effective for treating PMD disease in two-affected boys leading to improvement in neurocognitive testing and myelination (Schiller et al., 2019; Wishnew et al., 2014). While proven to have potential beneficial, BMT or HSCT can have serious complications such as graft versus host reactions (for allogeneic grafts), graft rejection, organ damage and so on, which can be occasionally life threatening. Therefore, a combinatorial treatment option is needed to treat complicated neurological disease such as leukodystrophies.

Glial cells as therapy

The replacement of glial cells, in addition to neurons, has also become a conceivable therapeutic avenue for neurological diseases. Studies in rodent models have set a precedent for cell replacement therapy to proceed further towards clinical trials (Leferink et al., 2018). The dysmyelinated mouse model called the *shiverer (shi)* mice have no functional OLs or myelin present and hence serve as a great system for testing the ability of the transplanted cells to myelinate (Kondo and Duncan, 2016; Kondo et al., 2005; Wang et al., 2013). Both rodent and human NSCs upon transplantation successfully engraft in the host tissue and differentiate into functional OLs that can remyelinate the brains of *shi* mice (Osorio et al., 2017; Uchida et al., 2012; Yandava et al., 1999). OPCs can also remyelinate upon transplantation in the *shi* mice; although in comparison to NPCs, they possess limited proliferative

capacity. The success of human NSC transplantation in rodents advanced towards testing of safety and efficacy for PMD, resulting in a phase I clinical trial. Individuals with PMD display neurological improvements and a mild increase in MRI-assessed myelination upon receiving NSC transplantation with no known side effects (Gupta et al., 2012). This pilot trial was not powered to demonstrate clinical benefit but the safety profile reported in these studies enable researchers to move towards future trials.

In Krabbe disease, transplanted OPCs in the *Twi* mice are unable to rescue the myelination defect, hence NSC transplantation was attempted based on the success of PMD studies (Kondo and Duncan, 2016; Marteyn et al., 2016). The NSCs survive in the *Twi* mice and are resistant to psychosine, that typically cause OL cell death. In addition, NSCs restore GALC levels and increase myelination, thereby extending the survival 2-3-fold in the *Twi* mice. However, the pluripotency of NSCs and extensive time needed for their differentiation still remains a hurdle in the rapidly progressing disease course of Krabbe disease (Allewelt et al., 2018; Kondo and Duncan, 2016; Matthes et al., 2015; Taylor et al., 2006).

Strategies to replace astrocytes through transplantation of healthy astrocytes in neurodegenerative diseases have shown promise for Amyotrophic Lateral Sclerosis, Alzheimer's and Parkinson's disease in animal models (Almad and Maragakis, 2012). A recent study explored astrocyte replacement for VWM by transplantation of glial progenitor cells, which integrate and differentiate into astrocytes, resulting inamelioration of the VWM phenotype in the mouse model (Leferink et al., 2018). While the murine models have been helpful to dissect the molecular mechanisms for leukodystrophies, they often lack the neurologic phenotype seen in human disease (Leferink et al., 2018; Zhou et al., 2019). Complementary to the animal models, human iPSCs have facilitated modeling of leukodystrophies and it is feasible that iPSC approaches may serve as a cell-based therapy to repair glial-driven diseases in the near future.

Multimodal approaches and other treatment approaches:

As with most neurological diseases, leukodystrophies involve multiple cellular pathologies and therefore applying combinatorial therapy instead of unimodal therapy has offered positive therapeutic outcomes. Enzyme replacement therapy (ERT) using recombinant ARSA in MLD mice reduced sulfatide storage during early stages of disease progression (Matthes et al., 2015). However, the promising multimodal approach of ex vivo gene correction involving lentiviral-based gene therapy combined with autologous HSCs transplantion back into patients for MLD restored normal ARSA enzyme activity in the CNS and halted disease progression in pre-symptomatic patients (NCT01560182, ClinicalTrials.gov) (Biffi et al., 2013). For Krabbe disease, unimodal therapy of ERT decreases psychosine levels and moderately extends life-span in *twi* mice but fails to reverse

neuroinflammation and demyelination (Matthes et al., 2015). Using multi-modal therapy of BMT with ERT in the same model significantly extends life-span further (~59 days), reversing neuroinflammation and demyelination (Qin et al., 2012). Similarly, combinatorial approach using AAV2/5 GALC gene therapy with BMT extended life span (~104 versus 52 days) and shows enhanced rescue of motor deficits compared to AAV2/5 GALC gene therapy alone (Lin et al., 2005). Thus, combined treatment approaches are a viable alternative to treat complex leukodystrophies.

There are currently no therapeutic strategies available for the neurological symptoms of PLOSL. Few clinical cases report improvement of extremity fractures through surgical procedures and stress the importance of seizure management and molecular analysis, though none address the characteristic microglial dysfunction (Arikan et al., 2014; Koseoglu et al., 2018). Future therapeutics will depend on more case studies of this rare microgliopathy to elucidate the mechanism of TREM2-DAP12 pathway in mouse and cell models.

CONCLUSIONS

Leukodystrophies are increasingly recognized as a disease group in which glial cells are the primary players. With the list of diseases continuously growing, in parallel with advances in laboratory models; there is a new appreciation that glial cells are not just “support” cells and they play a critical role in the health and diseases of the CNS (Stadelmann et al., 2019). There are a number of outstanding questions in understanding fundamental mechanisms of these neurogenetic diseases. These unresolved issues encompass understanding which glial cells are primarily driving the disease pathology as well which molecular pathways are involved and can be targeted as efficient therapeutic strategy. Comprehension of the distinct contributions from both peripheral and central nervous system are still being elucidated, as some of these leukodystrophies also have major involvement of peripheral immune cells, as in AGS and peripheral glia such as Schwann cells in Krabbe Disease. Thus, focusing and developing more glia-inclusive therapies will be beneficial to leukodystrophies as well as other neurological diseases.

Figure 1. Astrocyte-related Leukodystrophies:

1. AxD: Mutation in the *GFAP* gene, causes accumulation of Rosenthal fibers in astrocytes and a cascade of events that inhibits OPC proliferation and OL formation. It also activates microglia that release inflammatory cytokines and chemokines causing myelin degeneration; **Treatment for AxD** aims to downregulate GFAP with ASO mediated antisense therapy; **2. VWM:** Mutation in the *eIB2B* gene leads to ER stress and UPR in astrocytes. These astrocytes secrete hyaluronan, that inhibits OL maturation and differentiation; **Treatments for VWM** to improve WM pathology includes drug inhibition

of ISR and cell replacement therapy; **3. AGS:** Mutations in *TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR1, IFIH1,* and *MDA5*, cause nucleic acid accumulation and activation of IFN pathway. Astrocytes in AGS release IFN-α and CXCL10, contributing to auto-immune inflammation and demyelination; **Treatments for AGS** seek to reduce IFN-α and downstream ISGs by means of JAK inhibitor (Baricitinib) and block accumulation of nucleic acid with RTI treatment; **4. MLC:** Mutations in *MLC1* and/or *GLIALCAM* affects localization of MLC1 and GLIALCAM on astrocytic processes adjacent to the BBB. This results in loss of MLC1 function disturbing ion-water homeostasis, leading to accumulation of subcortical cysts and formation of intramyelinic edema, which ultimately results in atrophy of myelin; **Treatment for MLC** aims to improve MLC1 localization and function through gene therapy.

Figure 2. Oligodendrocyte-related Leukodystrophies.

1. Severe PMD: *PLP1* mutation causes accumulation of PLP protein in the ER, triggering UPR and ER stress, signaling cell-autonomous apoptotic pathways in OLs; **Classic PMD:** *PLP1* duplication causes over-expression of *PLP1* in endosomes, and turns on apoptotic pathways in OLs; **Treatments for PMD** have shown restoration of myelin and preservation of mature OLs with cellular transplantation (UCBT, HSCT, OPC/NPC grafts), a curcumin-cholesterol diet and antisense therapy; **2. ADLD:** Mutation in *LMNB1* causes over-expression of Lamin B1, which inhibits the lipid synthesis pathway and disrupts the myelin architecture albeit intact OLs; **Treatment for ADLD** seeks to downregulate LMNB1 through antisense therapy using ASOs; **3. AHDS:** Mutation of *MCT8/SLC162A* results in a dysfunctional MCT8 transporter that is unable to transport thyroid hormones T3 and T4 across the BBB. This causes decreased T3 activity in the brain and subsequent abnormal CNS development, including apoptotic pathways in OLs and impaired myelination; **Treatments for AHDS** aim to normalize T3 levels and improve OPC differentiation and myelination using gene therapy and pharmacological treatment with DITPA; **4. H-ABC:** A tubulin mutation, specifically of *Tubb4a,* disrupts MT function which affects OPC and OL health leading to cell death; **Treatment for H-ABC** seeks to downregulate TUBB4A through potential antisense therapy using ASOs; **5. Krabbe Disease:** Deficiency of the enzyme GALC leads to toxic accumulation of psychosine and galactose. Psychosine activates cell death pathways in OPCs and OLs, ultimately leading to demyelination; **Treatments for Krabbe** include AAV-mediated gene therapy, BMT, ERT, and HSCT to upregulate the expression of GALC enzyme.

Figure 3. Microglia-related Leukodystrophies.

1. Krabbe: Deficiency of the enzyme GALC leads to toxic accumulation of psychosine and galactose. Psychosine turns on cell death pathways in OPCs and OLs and also activates microglia. The activated

microglia display characteristic morphology of globoid, multinucleated phagocytes and secrete cytokines, ultimately leading to OL toxicity and demyelination; **Treatment for Krabbe Disease** include AAV-mediated gene therapy, BMT, ERT, and HSCT to upregulate the expression of GALC enzyme; **2. ALSP:** Mutation in *CSF1R* affects microglial proliferation and reduces microglia number affecting OL survival and causing demyelination due to unknown mechanisms; **Treatment for ALSP** involves elimination or repolarization of tumor-associated macrophages (TAM) with immunomodulatory drugs that inhibit CSF1R; **3. PLOSL:** Mutations in the *TREM2* and *DAP12* genes result in dysfunction of both osteoclasts and microglia. This disrupts the ability of activated microglia turned phagocytes to recognize cellular debris, leading to decreased clearance of myelin and axonal with ongoing myelin loss; **Treatments for PLOSL** are not yet known; **4. X-ALD:** Mutation in *ABCD1* gene fails to transport VCLFA in peroxisomes. This build-up of VCLFA leads to oxidative stress and microglia death, which eventually causes OL death and myelin loss; **Treatments for X-ALD** mostly includes transplantation strategies such as HSCT, HCT, and BMT to prevent progression of the disease phenotype; **5. MLD:** Mutation in the *ARSA* gene results in accumulation of sulfatides in multiple cell types, including OLs, astrocytes, neurons, Schwann cells, and phagocytes. This sulfatide accumulation activates microglia along with OL toxicity and ultimately leads to demyelination; **Treatments for MLD** seek to stabilize disease progression through HSCT, UCBT, ERT, BMT, and gene therapy.

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Disease	Mutation	Hallmark features	Affected Cells	Mechanism	Models	Treatments
Alexander's Disease (AxD)	GFAP	↑ Rosenthal fibers	**Astrocyte**, OL	↓ Homeostatic regulation	*Mouse:* *Gfap*$^{R236H/+}$, *Gfap*$^{R76H/+}$, *Gfap*^{Tg};*Gfap*$^{R236H/+}$ *iPSC:* Astrocytes	*Pre-clinical Model:* ASO therapy *Patients:* Clinical trials with ASO therapy
Vanishing White Matter Disease (VWM)	EIF2B1-5	Foamy OL, astrocyte death	**Astrocyte,** OL	ER stress, Mitochondrial dysfunction, ISR, UPR	*Mouse:* *eIF2B5*$^{R191H/R191H}$ *eIF2B4*$^{R484W/R484W}$ *iPSC:* Astrocytes	*Pre-clinical Model:* Drug inhibition of ISR, Cell replacement therapy *Patients:* No known treatment
Aicardi-Goutières Syndrome (AGS)	TREX1 RNASEH 2A/2B/2C SAMHD1 ADAR1 IFIH1/ MDA5	↑ IFN-α/ IFN-I in CSF in absence of viral infection	**Astrocyte**, Microglia	↑ Nucleic acids, ↑ IFN-I, ↑ CXCL10 cytokines	*Mouse:* *Trex1*$^{-/-}$, *Trex1*$^{D18N/D18N}$, *Adar1lox/lox;CreER*$^{+}$, *RnaseH2B*$^{-/GFAP}$, *RnaseH2H2*$^{-/EmxI}$, *Samhd1*$^{-/-}$ *iPSC:* Astrocytes, Neurons	*Pre-clinical Model:* RTI treatment *Patients:* Clinical trials with JAK inhibitor (Baricitinib)
Megalencephalic Leukoencephalopathy with subcortical Cysts (MLC)	MLC1, GLIALCAM	↑ Subcortical cysts, ↑ Fluid-filled vacuoles in myelin sheaths and astrocytic endfeet	**OL**, Astrocyte	MLC1 mislocalization, Impairment of ion-water homeostasis, ↑ Intramyelinic edema	*Mouse:* *Mlc1-null, Glialcam-LacZ*	*Pre-clinical Model:* Gene therapy *Patients:* No known treatment

Pelizaeus-Merzbacher Disease (PMD)	PLP1	↑ PLP1 accumulation, OL toxicity	**OL,** Astrocyte; Microglia	PLP mislocalization, ER stress, UPR, ↑ CHOP levels	*Mouse:* *Jimpy^{msd}, Jimpy^{rsh}, CHOP-null/rsh, 4e-Plp, Plp1-null, PLP-ISEdel* *iPSC:* OLs	*Pre-clinical Model:* Curcumin, Cholesterol-enriched diet, ASO therapy *Patients:* Clinical trial for human NSC therapy
Globoid Cell Leukodystrophy (GLD); "Krabbe Disease"	GALC	Psychosine-accumulation, globoid cell formation ↓ GALC	**OL, Microglia,** Astrocyte	↑ Psychosine	*Mouse:* *Twitcher*, *Quaking* *Other:* Canine model	*Pre-clinical Model:* AAV, viral-mediated gene therapy, BMT, ERT *Patients:* BMT, HSCT
Autosomal Dominant Leukodystrophy (ADLD)	LMNB1	Over-expression of lamin B1	**OL,** Astrocyte	Dysfunction in lipid synthesis	*Mouse:* *Lamin1^{BAC}, Plp-LMNB1*	*Pre-clinical Model:* ASO therapy *Patients:* No known treatment
Hypo-myelination with Atrophy of Basal Ganglia (H-ABC)	TUBB4A	Cerebellar atrophy, Hypo-myelination and atrophy of caudate and putamen	**OL**	Abnormal microtubule dynamics	*Mouse:* *Tubb4a^{D249N}* *Rat:* *taiep* *iPSC:* Neurons	*Pre-clinical Model:* No known treatment *Patients:* No known treatment
Pelizaeus-Merzbacher-like disease (PMLD1)	Cx47	Similar to PMD, without PLP1 mutation	**OL,** Astrocyte	↓ Functional Cx47/Cx43 channels	*Mouse:* *Cx47^{EGFP}, Cx47$^{EGFP/-}$, Cx47^{M282T}, Cx32$^{-/-}$;Cx47$^{-/-}$*	*Pre-clinical Model:* No known treatment *Patients:* UCBT, HSCT, OPC/NSC Grafts
Allan-Herndon-Dudley Syndrome (AHDS)	MCT8/ SLC162A	Abnormal OL maturation	**OL**	Disruption of T3 & T4 transport across BBB, X-linked multisystem toxicity due to ↑ T3 and ↓ T4	*Mouse:* *Mct8$^{-/y}$, Mct8$^{-/+}$* *Zebrafish:* *mct8$^{-/-}$* *hESC:* OPCs, OLs *iPSC:* Neural cells	*Pre-clinical Model:* DITPA and clemastine treatment in zebrafish, Gene therapy *Patients:* DITPA treatment
Adult-onset Leuko-encephalopathy with Axonal Spheroids and Pigmented Glia (ALSP)	CSF1R	Axonal swelling and degeneration, ↓ Microglia, ↓ OPCs	**Microglia,** OL	Disruption of microglial survival and proliferation	*Mouse:* *Csf1r$^{-/-}$* *Zebrafish:* *csf1ra$^{-/-}$, csf1rb$^{-/-}$, csf1ra$^{-/-}$;csf1rb$^{-/-}$, csf1r^{DM}*	*Pre-clinical Model:* No known treatment *Patients:* Potential CSF1R Inhibition via immune-modulatory drugs
Polycystic Lipo-membranous Osteodysplasia with Sclerosing Leuko-encephalopathy (PLOSL); "Nasu-Hakola Disease"	TREM2, DAP12	↓ Myelin, ↓ Axons, Fibrillary gliosis, Abnormal fronto-temporal WM	**Microglia,** OL	↓ Clearance of axonal debris, Lipid bilayers in cystic bone lesions	*Mouse:* *Trem2$^{-/-}$, Dap12$^{-/-}$*	*Pre-clinical Model:* No known treatment *Patients:* No known neurologic treatment
X-linked Adreno-leukodystrophy (X-ALD)	ABCD1	De-myelination of parieto-	**Microglia,** OL	↓ Shuttling of activated VLCFA to	*Mouse:* *Abcd$^{-/-}$, Abcd1$^{-/-}$;Abcd2$^{-/-}$*	*Pre-clinical Model:* No known treatment

		occipital WM and CC, ↓ Myelin and ↓ Axons in spinal cord		peroxisome	*Cell line:* BV2 microglia	*Patients:* HCT, BMT, HSCT
Metachromatic Leukodystrophy (MLD)	ARSA	Demyelination due to ↑ sulfatides in OL, Schwann cells, phagocytes, astrocytes, & neurons	**Microglia**, OL, Astrocyte	↑ Sulfatides	*Mouse:* *ASA*$^{-/-}$, *Double transgenic ASA*$^{-/-}$	*Pre-clinical Model:* ERT, Gene therapy *Patients:* HSCT, UCBT, BMT

ASO, Anti-sense oligonucleotide; OL, oligodendrocyte; OPCs, oligodendrocyte progenitor cells; WM, white matter; S1R, Sigma-1-Receptor; ISR, Integrated Stress Response; RTI, Reverse Transcriptase Inhibitor; IFN-α/IFN-I, Type I interferon; NPCs, neural progenitor cells; ER, endoplasmic reticulum; BMT, Bone marrow transplantation; HCT: Hematopoietic cell transplantation; HSCT, Hematopoietic stem cell transplantation; ERT, enzyme replacement therapy; NSC, neural stem cells; UPR, unfolded protein response; UCBT, Umbilical cord blood transplantation; T3, triiodothyronine; T4, thyroxine; DITPA, Diiodo-thyropropionic Acid; PTU, propylthiouracil; LT4, L-thyroxine; BBB, Blood brain barrier; VLCFA, very long chain fatty acids

Table 1: Leukodystrophies Classified by Affected Glial Cell Type