Bethanys Hope Foundation
Research Laboratory Program

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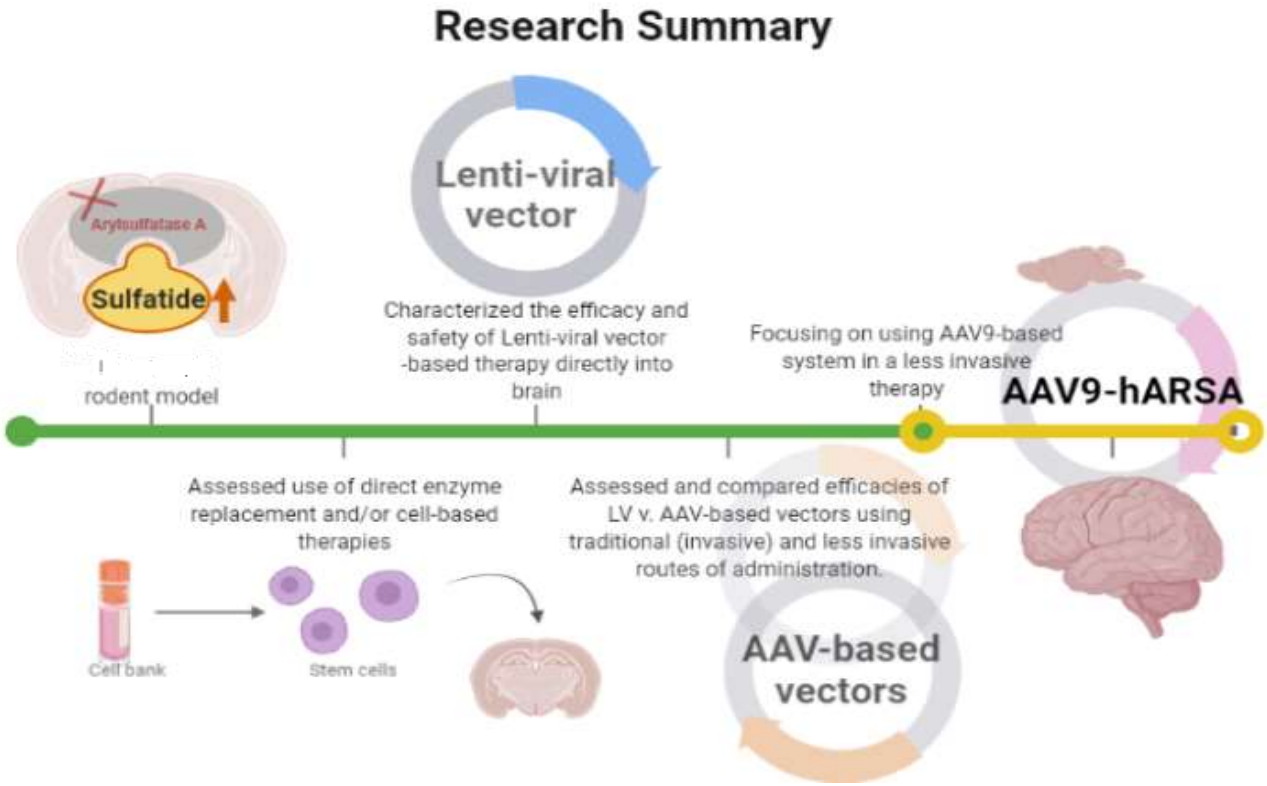
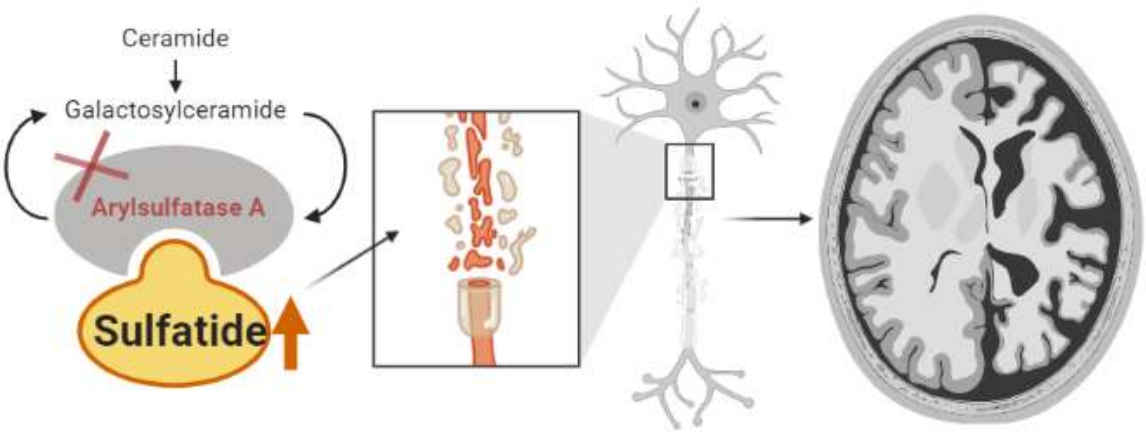


***Green** = completed*
***Yellow** = in progress*

Disease Background

Metachromatic leukodystrophy (MLD) is a rare, fatal genetic disease of thenervous system disease that mainly affects children. At a young age (1-3 years), children develop difficulties with walking, speech, cognitive abilities, and vision. MLD is caused by the absence of an enzyme, known as arylsulfatase A (ARSA) that functions in the recycling centers of the cell (known as lysosomes). ARSA breaks down sulfatide, an important lipid that has a role in the development and function of white matter (an insulating tissue that allows nerve signals to travel throughout the body) in the brain and peripheral nerves. All research is oriented towards creating effective therapies, one must consider three aspects: how a disease starts, how it progresses within the body, and how we perceive it progressing outside the body (clinically). We know that MLD is caused by a deficient ARSA enzyme and that this causes an abnormal accumulation of sulfatide within the cell. Its abnormal metabolism is detrimental to the function of these systems, which is why children affected by MLD develop neurological symptoms over time.



Therapy Development Challenges

The blood brain barrier which protects the brain from exposure to potentially harmful substances in blood is a major impediment to treating MLD and some other neurological diseases. MLD most often affects children and it is important to develop a therapy that is safe and provides long term benefits.

Our Work and Approach

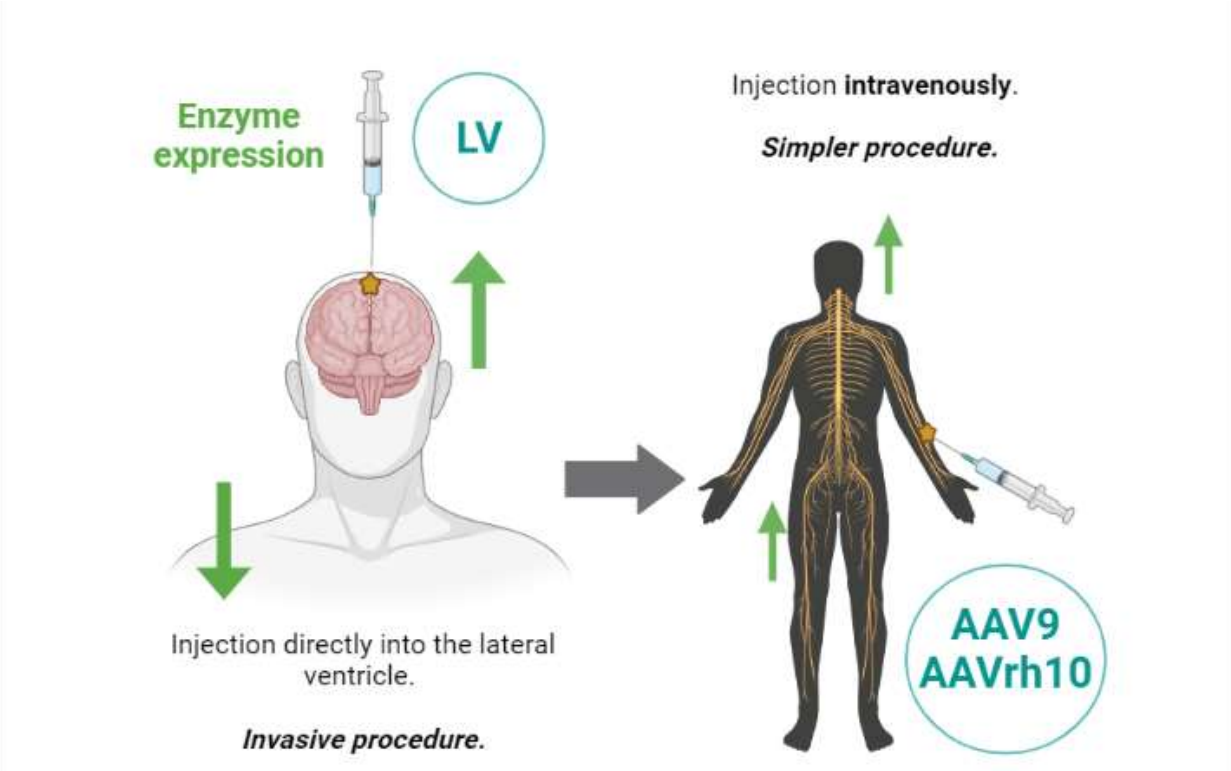
We have shown that providing a correct version of the gene that codes for the enzyme ARSA using virus derived vectors can allow cells to produce the correct form of the enzyme, restoring natural sulfatide metabolism. This research used patient-derived skin cells (fibroblasts) and cells from the mouse model of MLD.

Our efforts started with the lenti-viral vector (LV; LV-hARSA) system, which integrates into the DNA and codes for a functional ARSA. Surgical delivery of LV-hARSA into the lateral ventricle of adult MLD mouse brains results in the widespread transduction of cells within the choroid plexus and ependymal cells throughout the CNS and stable expression of ARSA for months. Detailed studies to identify the location of the integrated ARSA gene within those chromosomes have been completed as part of a safety assessment and demonstrated a clinically favourable integration site profile. Further safety studies have been successfully completed using both the mouse model of MLD and cultured cells obtained from patients with MLD. These studies have not identified vector-related toxicity. However, the biggest drawbacks of this approach are the invasiveness of the vector administration and the inability to treat the peripheral nervous system.

(Current Work): Adeno-Associated Vector (AAV)—Based Approach

Despite the initial focus on the central nervous system symptoms alone, we increasingly recognize the prevalence of the peripheral nervous system symptoms and their impact on disease progression. AAV-based systems have been an attractive option for gene therapy because they can infect several different cell types (including peripheral nervous system), have little risk of pathogenicity, and do not integrate into the DNA (like LVs do). As a result, we investigated adeno-associated viral vector-based gene therapies (AAV9-hARSA and AAVrh10-hARSA). Similar AAV vectors are used in clinical trials for other diseases. Additionally, Other research groups have shown their administration to treat neurological diseases using less invasive routes such as an intravenous injection rather than directly into the lateral ventricle, like we have been

doing with LV. So, to assess their utility in MLD, we surgically delivered (to remain consistent with our LV work) AAV9/AAVrh10-hARSA into the lateral ventricle of adult MLD mice. We also performed separate experiments where we injected the mice intravenously (less invasive procedure) with the same vectors.

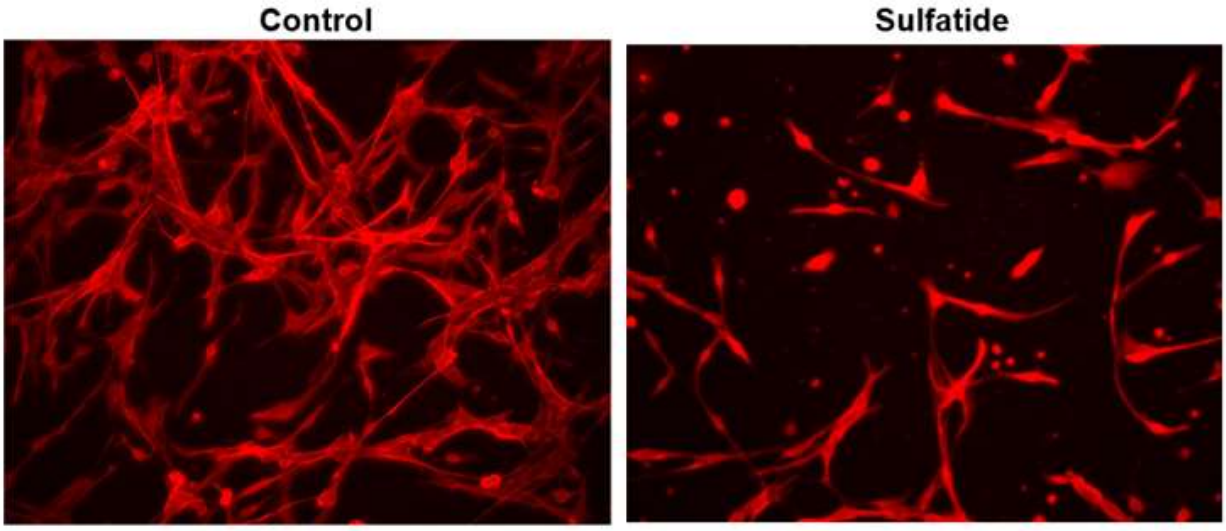


We modified our approach from a more invasive (directly into brain) one to a less invasive (intravenous injection) procedure.

Our results with the AAV vectors showed that treatments were effective at reducing the sulfatide accumulation inside the body. This is promising as these vectors (AAV9) may enable us to develop a therapy that more broadly treats MLD (across the central and peripheral nervous systems) and is administered using less invasive and inherently safer routes of administration—both of which can contribute to improved patient outcomes.

Through the Looking Glass: Differences Across Tissues &Cells in MLD

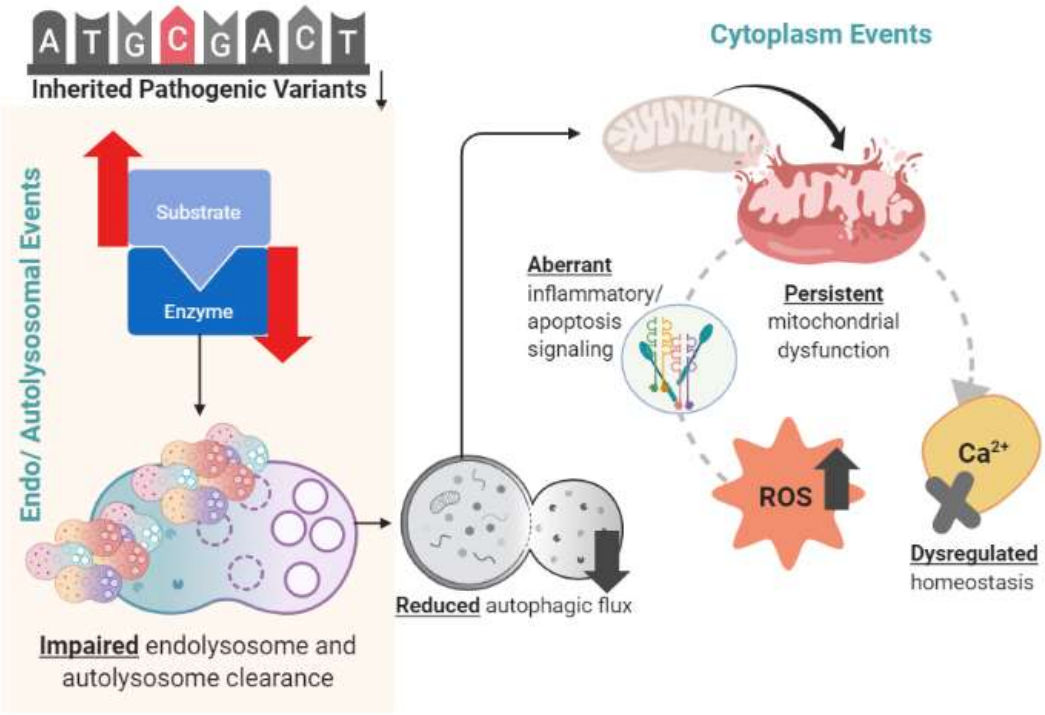
In MLD, new sulfatide is constantly made and accumulates due to the absence of ARSA. Our previous work has already found that cells isolated from the brains of ARSA$^{-/-}$ mice are sensitive to sulfatide exposure.



Immunohistochemical staining of primary neural cell cultures from MLD mice with Anti –GFAP identifying astrocytes. Stained with anti - GFAP Alexa Fluro 568. This indicates that astrocyte cells in the MLD mouse brain are a primary target of sulfatide induced cell death.

Efforts at correcting MLD have had varied success and only impacted brain-associated symptoms. Destruction of peripheral nerves continued in patients suggesting that sulfatide accumulation has different consequences across tissue and cell types. Research in similar diseases has shown improper lysosomal accumulation causes other cellular organelles to function inappropriately. The collective dysfunction in multiple cellular components is thought to contribute to eventual cell death. Some components examined include mitochondria (cellular energy-producing centers) and immune responses to stress such as cytokine secretion (the ability of the cell to release signalling molecules to recruit outside help) and phagocytosis (a process used to engulf and degrade abnormal and/or foreign particles). A greater understanding of the disease process of MLD at a cellular level may lead to the identification of therapeutic targets.

A proposed cascade of events in lysosomal storage diseases and MLD:



Looking Forward

We are currently in the process of evaluating the safety and efficacy of AAV based intravenous therapy to prepare a clinical trial application.