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Topical Review

Experimental Therapies in the Murine Model of Globoid Cell Leukodystrophy

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ABSTRACT

BACKGROUND: Globoid cell leukodystrophy or Krabbe disease, is a rapidly progressive childhood lysosomal storage disorder caused by a deficiency in galactocerebrosidase. Galactocerebrosidase deficiency leads to the accumulation of galactosylsphingosine (psychosine), a cytotoxic lipid especially damaging to oligodendrocytes and Schwann cells. The progressive loss of cells involved in myelination results in a dysmyelinating phenotype affecting both the central and peripheral nervous systems. Current treatment for globoid cell leukodystrophy is limited to bone marrow or umbilical cord blood transplantation. However, these therapies are not curative and simply slow the progression of the disease. The Twitcher mouse is a naturally occurring biochemically faithful model of human globoid cell leukodystrophy that has been used extensively to study globoid cell leukodystrophy pathophysiology and experimental treatments. In this review, we present the major single and combination experimental therapies targeting specific aspects of murine globoid cell leukodystrophy. **METHODS:** Literature review and analysis. **RESULTS:** The evidence suggests that even with the best available therapies, targeting a single pathogenic mechanism provides minimal clinical benefit. More recently, combination therapies have demonstrated the potential to further advance globoid cell leukodystrophy treatment by synergistically increasing life span. However, such therapies must be designed and evaluated carefully because not all combination therapies yield such positive results. **CONCLUSIONS:** A more complete understanding of the underlying pathophysiology and the interplay between various therapies holds the key to the discovery of more effective treatments for globoid cell leukodystrophy.

Keywords: Krabbe disease, globoid cell leukodystrophy, galactocerebrosidase, lysosomal storage disease, gene therapy, bone marrow transplantation

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Globoid cell leukodystrophy

Globoid cell leukodystrophy (GLD), also known as Krabbe disease, is a rare autosomal recessive lysosomal storage disorder that results from the deficiency of the enzyme galactocerebrosidase (GALC). GALC removes galactose from the terminal end of galactosylated sphingolipids, including galactosylsphingosine (psychosine), a cytotoxic lipid that is exclusively degraded by GALC. The accumulation of psychosine in GALC-deficient cells leads to the clinical sequelae observed in patients with GLD.

Because oligodendrocytes are particularly susceptible to psychosine buildup, patients with GLD exhibit demyelinating phenotypes. In infantile GLD, the most common form of GLD, symptom onset typically occurs at 5 months of age followed by a rapid disease progression. Patients experience intractable seizures, central nervous system (CNS) and peripheral nervous system (PNS) involvement, sensory loss (blindness and deafness), profound neuroinflammation, and rapid psychomotor deterioration. Death typically occurs at 2-4 years of age.

Currently, the only treatment for GLD is bone marrow or umbilical cord blood transplantation, which has been revealed to slow the progression of GLD but not to cure the disease. Although post-transplant patients have a more indolent course, progressive neurological deterioration still occurs, and the quality of life is diminished. Furthermore, transplantation is clinically effective only when given before

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symptom onset.[1,2] Although newborn GLD screening is available in some states, most patients in states without newborn GLD screening are diagnosed after symptom onset and thus would be poor candidates for transplant. Because of these limitations, there is a great need for more effective therapies.

The Twitcher mouse

The Twitcher (Twi) mouse is a spontaneously arising murine model of GLD that has a mutation at position 1017 of the *GALC* gene, creating a premature termination codon that results in nonsense-mediated decay of the messenger RNA.[3] Clinical progression and biochemical and histologic features of the Twi mouse closely align with those of human GLD patients. Symptom onset occurs at postnatal day 21, followed by a rapid progression of disease. Affected mice experience demyelination of the central white matter tracts and peripheral nerves, weight loss, hind limb ataxia, kyphosis, and severe tremor. Death occurs at approximately postnatal day 36.[4] Because it closely mimics the human disease, the murine model has been invaluable for the study of both the characteristics and the potential treatments of GLD.

Here we present a thorough but not exhaustive review of the major single (Table 1) and combination (Table 2) therapies that have been attempted in the treatment of murine GLD. We also discuss their putative mechanisms and their respective strengths and weaknesses.

Single-modality therapies for murine GLD

Cell-mediated therapies

Bone marrow transplantation (BMT) from a congenic enzymatically normal donor mouse was thought to be effective for GLD because it provided a continuous source of cells (microglia and/or macrophages) that could migrate into the brain and secrete the deficient enzyme. *In vitro* experiments have shown that secreted GALC can be taken up by enzyme-deficient oligodendrocytes, Schwann cells, and astrocytes via lysosomal enzyme-targeting receptors at the cell surface.[5-7] Although this process, known as cross-correction, can facilitate the distribution of functional enzyme to mutant cells throughout the body, the extent to which this occurs differs greatly for different tissues. Significantly, GALC enzyme levels in the CNS only increased to 15% of normal donor levels after complete hematopoietic engraftment.[8] Incomplete correction of the CNS enzyme deficit may be the reason why Twi mice treated with BMT experience only a minor increase in life span to approximately 80 days. In addition, BMT provides no significant improvement in behavioral assays measuring limb strength,[9-11] in spite of histologic evidence of peripheral nerve remyelination.[8,12,13]

It has been hypothesized that hematopoietic stem cells (HSCs) overexpressing GALC may more effectively treat murine GLD. Attempts to overexpress GALC in murine HSCs were unsuccessful because of transgene toxicity specific to HSCs but not their differentiated progenitors. More recently, GALC overexpression was induced in differentiated HSC progeny by using an HSC-specific micro RNA to inhibit GALC expression in HSCs but not their progeny.[14] Transplantation of the transduced HSCs into a murine model of GLD with less than 5% residual GALC activity increased life span to approximately 88 days compared with approximately 50 days for untreated mutant mice and approximately 63 days for mutants receiving nontransduced GALC+/+ donor bone marrow. Although the clinical benefits of donor hematopoietic GALC overexpression are mild, this study demonstrates the feasibility of autologous BMT using

TABLE 1.
Single Therapies for Murine Globoid Cell Leukodystrophy

Therapy	Putative Mechanism	Life span of Treated GALC−/− Mutants	References
Bone marrow transplant	Cross-correction	GALC+/+ donor: 80 days*	8,9,12,13
	Immunomodulation	GALC-overexpressing GALC−/− donor: 88 days*	14
Mesenchymal stem cells	Immunomodulation Replacement of damaged neuronal cells	40 days	23-27
Enzyme replacement therapy	Short-term replacement of deficient GALC activity	Single-dose ICV administration: 50 days*	31
		Weekly IP administration: 47 days*	35
Viral-mediated gene therapy	Long-term replacement of deficient GALC activity	AAV2/5-GALC IC administration: 63 days	40
		AAV2/5-GALC IC + IT administration: 71 days	10
		AAVrh10-GALC administration: 120 days*	42
NSC-directed gene therapy	Cross-correction	Recombinant retroviral-GALC transduced NSCs: 45 days	29
	Replacement of damaged neuronal cells	Recombinant lentiviral-GALC transduced NSCs: 53 days*	30
L-cycloserine	Substrate reduction therapy	57 days*	45
Anti-inflammatory therapies	Immunomodulation	Ibudilast: N/R†	52
		Indomethacin: 65 days	53

Abbreviations:
GALC = Galactocerebrosidase
IC = Intracranial
ICV = Intracerebroventricular
IP = Intraperitoneal
IT = Intrathecal
NSC = Neuronal stem cell
NR = Not reported

* Mean life spans. All other life spans are represented by the median.
† Exact life span of treated Twitcher mice not reported but was not significantly different compared with untreated mutants.