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Preclinical studies in Krabbe disease: A model for the investigation of novel combination therapies for lysosomal storage diseases

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Krabbe disease (KD) is a lysosomal storage disease (LSD) caused by mutations in the *galc* gene. There are over 50 monogenic LSDs, which largely impede the normal development of children and often lead to premature death. At present, there are no cures for LSDs and the available treatments are generally insufficient, short acting, and not without co-morbidities or long-term side effects. The last 30 years have seen significant advances in our understanding of LSD pathology as well as treatment options. Two gene therapy-based clinical trials, NCT04693598 and NCT04771416, for KD were recently started based on those advances. This review will discuss how our knowledge of KD got to where it is today, focusing on pre-clinical investigations, and how what was discovered may prove beneficial for the treatment of other LSDs.

KRABBE DISEASE: INTRODUCTION AND PATHOGENESIS

Lysosomal storage diseases (LSDs) are family of over 50 diseases caused by genetic defects in lysosomal genes. While each individual LSD is rare, together they are relatively common with a combined estimated incidence rate of 1 in 2,300–7,700 live births.¹ These genetic defects, usually leading to expression of defective lysosomal enzymes, typically result in accumulation of the defective enzyme's substrate because it cannot be catabolized. The symptoms exhibited in LSDs vary significantly depending on the specific genetic defect but there are some commonalities such as developmental delay/regression, vision loss, hepatosplenomegaly, and premature death.¹ At present, there are no cures for any LSD and the existing treatments mostly only delay symptom development. This is particularly true for LSDs that affect the central nervous system (CNS). Additionally, many of the current treatments, such as hematopoietic stem cell transplantation (HSCT), for LSDs pose risks of their own.¹ This review will focus on Krabbe disease (KD) as an introduction to potential therapeutics for other LSDs.

KD is a leukodystrophy and LSD first described by Knud H. Krabbe in 1916.² In the last century there have been countless breakthroughs in our understanding of the underlying pathology as well as treatment, but there is still no cure. KD is an autosomal recessive disease caused

by mutations in the *galc* gene that result in reduced activity of the lysosomal hydrolase, galactosylceramidase (GALC).

KD is typically divided into three subtypes based on age of onset and severity of symptoms: (1) infantile (symptom onset before 3 years of age; some physicians and investigators further divide it into early infantile and late infantile), (2) juvenile (onset after 3 years of age), and (3) adult onset (onset after 18 years of age).³ A retrospective analysis of KD cases in the United States estimates an incidence of 1 in 310,000 live births.⁴ Newborn screening (NBS) data from New York State estimate an incidence of 1 in 394,000 live births.⁵ It should be noted that these estimates are from hospitalization and NBS data, respectively, and both of these data collection methods are biased toward identifying infantile KD. Given that, the actual incidence may be higher because juvenile- and adult-onset patients may not have been detected in these studies. Additionally, there is some disparity between different ethnic groups; for example, the Druze population in the Middle East has an estimated incidence of 1 in 100 live births.⁶

GALC is responsible for the catabolic removal of galactose from sphingolipids containing galactose, such as galactosylceramides.^{7,8} GALC deficiency renders the body unable to metabolize several galactosphingolipids, which leads to their subsequent accumulation. This process is illustrated in Figure 1.^{7,9} Elevated levels of

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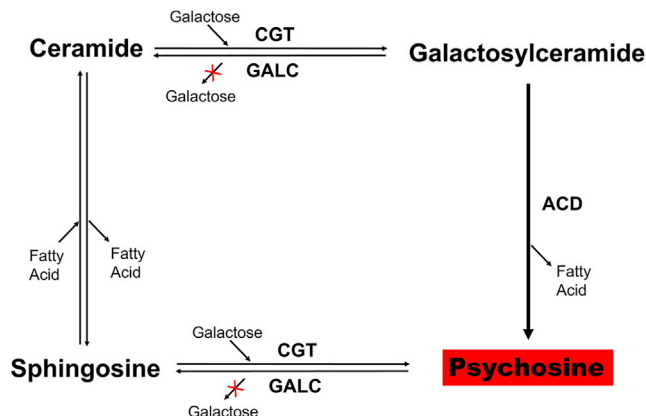


Figure 1. Main metabolic pathways involving GALC

This figure outlines some of the relevant molecules and enzymes in KD. It is important to note that most of these reactions are normally reversible using differing enzymes; for example, ceramide galactosyltransferase (CGT) catalyzes addition of galactose to ceramides to form galactosylceramides, and GALC normally catalyzes the removal of galactose from galactosylceramides. Additionally, many of these enzymes catalyze reactions with other substrates, such as sphingosine and psychosine. One exception is the irreversible catalytic removal of the fatty acid group from galactosylceramides by acid ceramidase (ACD).

galactosylceramides cause the characteristic globoid cell reaction in macrophages that gives rise to KD's formal name, globoid cell leukodystrophy (GLD).¹⁰ However, it should be mentioned that KD is different from many other LSDs in that the main substrate of the GALC enzyme, galactosylceramides, does not reach the levels of accumulated substrate seen in other LSDs.^{9,11}

Galactosylsphingosine (commonly referred to as psychosine) is a sphingolipid that is catabolized by GALC, such that it is nearly undetectable in unaffected people and animals.^{11–15} High concentrations of psychosine are cytotoxic and trigger a cascade of pathogenic mechanisms, primarily by disrupting cell membranes as well as activating apoptotic pathways.^{16–20} Psychosine accumulation has been confirmed in KD patients, the Twitcher (TWI) mouse model of KD, new gene edited mouse KD models, and the canine model of KD.^{11,14,15,21} Miyatake and Suzuki first proposed what is now known as the psychosine hypothesis in 1972 to address the relatively low levels of galactosylceramides and high levels of psychosine. This hypothesis suggests that, because of the metabolic block preventing the normal breakdown of galactosylceramides into galactose and ceramides, galactosylceramides are instead deacylated into psychosine, which cannot be catabolized due to GALC deficiency.¹³ Most of the psychosine hypothesis has been confirmed, the one exception being that psychosine was originally thought to be generated through aberrant sphingolipid synthesis.¹² However, it was recently shown that the main source of psychosine is through catabolic de-acylation of galactosylceramides by acid ceramidase with the anabolic pathway possibly playing a minor role.²²

Myelinating cells, oligodendrocytes, and Schwann cells generate the aforementioned sphingolipids in significantly higher quantities than

other cell types, thus they are particularly vulnerable to the metabolic blockade caused by the lack of GALC.^{11,23} The main pathological characteristics of KD are global diffuse demyelination (both CNS and peripheral nervous system [PNS]) and the appearance of multinucleated globoid cells.^{2,10,24} There are, however, many other pathological processes occurring in KD, such as disordered cell membrane dynamics (particularly within lipid rafts), disrupted signal transduction pathways, axonopathy, synaptic complications, overactivation of the proteasome degradation pathway, and problems regulating apoptosis.^{16–18,25–30} Together, these pathological processes give rise to the clinical signs observed in KD patients.

All of the research into KD pathology, NBS, treatments, and more by numerous independent investigators has provided the foundation of two recently started phase 1/2 clinical trials investigating adeno-associated virus (AAV)-based gene therapy for KD: NCT04771416 and NCT04693598.

KD: CLINICAL PRESENTATION

Untreated infantile KD is typically divided into an asymptomatic stage, followed by three progressively worsening symptomatic stages. Affected infants typically have a short asymptomatic period where they seemingly develop normally. Stage 1 is characterized by mostly nonspecific symptoms such as difficulty feeding, vomiting, hypersensitivity, hyperirritability, and fever. Stiffness, developmental delay or regression, and seizures are less common but possible in this stage. The main laboratory finding at this stage is increased cerebrospinal fluid protein levels. Stage 2 symptoms include severe motor and mental deterioration with hypertonicity and hyperactive reflexes. Optic atrophy and tonic/clonic seizures are less common symptoms at this stage. At stage 3, the infant has very limited mobility, is decerebrate, and is blind. Untreated infants rarely live beyond 2–3 years.^{3,31}

Later-onset subtypes of KD, including juvenile and adult onset, have more variable presentations. Often, the patients are clinically normal prior to symptom onset. Common presenting symptoms include visual deficits, weakness, balance problems, and cognitive impairment. Not only are symptoms variable but the age of onset and disease course are also variable, even between siblings with the same mutation.^{32,33}

KD: GENETICS AND ANIMAL MODELS

The *galc* gene was localized to chromosome 14 by linkage analysis and was subsequently sequenced and cloned.^{34–36} Full sequencing of the *galc* gene led to identification of at least 147 mutations and many single-nucleotide polymorphisms.^{37,38} Given the relatively large number of variants of unknown significance (VUSs) and the variable nature of the disease, it has been difficult to establish a clear genotype-phenotype correlation. A notable exception is a 30-kb deletion that encompasses exons 11 through 17, which correlates to a loss of the entirety of the smaller 30-kDa subunit and part of the larger 50- to 52-kDa subunit of the GALC protein, leading to an infantile presentation in individuals homozygous for this mutation.^{38–41} Patients with juvenile- and adult-onset KD are typically compound heterozygotes, usually possessing one copy of the *galc* gene containing the 30-kb deletion, and

one copy with mutations that retain greater GALC functionality than mutations that more frequently lead to infantile KD.^{33,42–44}

There are many animal models of KD, such as mouse, dog, and rhesus macaque.^{45–48} These models have allowed research into its pathogenesis as well as possible treatment options.^{49–53} The most commonly used animal models include the TWI mouse and canine KD models, both of which closely model infantile KD.^{49,51,52,54–58} TWI are a naturally occurring KD model possessing a spontaneous nonsense mutation in the *galc* gene leading to nonsense-mediated mRNA decay and a complete lack of functional GALC enzyme.^{45,59–61} TWI exhibit psychosine accumulation analogous to KD patients. Similar to KD patients, TWI show no signs of disease for the first weeks of life. TWI reach their maximum mass of 8–12 g around postnatal day (PND) 30, then continuously decline until their death at ~PND40, analogous to failure to thrive in KD infants. Around PND20, muscle atrophy and weakness begin to develop, particularly in the hind limbs, ultimately leading to paralysis at approximately PND35–40. TWI begin to display the eponymous tremor or twitch around PND30. The histological features of disease in TWI include diffuse, global demyelination (both CNS and PNS), globoid cell formation, axonopathy, and neuroinflammation characterized by astrogliosis and microgliosis.^{45,61–64}

The *trs* mouse is another KD model; they harbor a H168C missense mutation leading to 10%–20% wildtype (WT) mouse GALC activity. *trs* mice accumulate psychosine slightly slower than TWI. The median survival of *trs* mice is approximately 50 days, and they exhibit remarkably similar disease signs to TWI but delayed by about 10 days. Finally, the *trs* mice display similar levels of demyelination to TWI but have fewer infiltrating macrophages.⁶⁵ An inducible *galc* knockout (KO) mouse was developed using a *galc* gene flanked by loxP sites and the Cre/enzyme replacement therapy (ERT) system to examine the effect of *galc* loss at different time points. It was found that very early (PND0) *galc* KO led to disease progression essentially identical to TWI, but, as *galc* KO was introduced later, disease onset was delayed and disease severity was reduced.^{66,67} A lysosomal membrane tethered GALC mouse, GALCLAMP1, was created to investigate cell-autonomous expression of GALC. GALCLAMP1 mice can also be induced to delete *galc* to recapitulate the TWI phenotype because the *galclamp1* gene construct is flanked by loxP sites. Furthermore, GALCLAMP1 can be crossed with mouse lines that express Cre only in certain cell types to selectively delete *galclamp1* expression in specific cells and determine the unique role of *galc* in only those cells.⁶⁸ CRISPR-based gene editing has also been used to generate a severe infantile-like murine line carrying the T513M mutation and an adult-onset model murine line carrying the G41S mutation. The T513M mice exhibit disease progression almost identically to TWI as assessed by median survival, weight, tremor, and a multitude of behavioral tests. Pathologically, T513M mice are also extremely similar to TWI in that GALC activity is severely reduced and psychosine is elevated compared with WT. Since the G41S mice model adult-onset KD, they do not present pathological abnormalities until ~PND200 and, even then, display much milder disease signs and pathology than TWI.²¹

The canine model of KD is another naturally occurring model that was first reported in 1966 in the West Highland terriers and Cairn terrier breeds.⁴⁶ This model has reduced GALC activity due to a spontaneous missense mutation, Y158S, leading to elevated psychosine levels throughout the nervous system.^{52,58,69–71} The KD canine phenotype typically manifests at 4–6 weeks of age, with the first disease signs being hind limb weakness, forelimb dysmetria, and tremor. The signs progressively worsen, such that, by 12 weeks of age, they display hind limb ataxia and reach humane endpoint by 16 weeks of age due to disease burden.^{52,72,73} Histologically, KD canines display myelin loss, astrogliosis, microgliosis, and globoid cell accumulation similar to the human disease.^{52,58,72,74}

KD: TREATMENTS AND SCREENING

Currently, the standard-of-care treatment for pre-symptomatic infants with KD is HSCT.⁷⁵ HSCT can utilize either bone marrow transplantation (BMT) or umbilical cord blood transplantation (UCBT). BMT was first found to increase survival in TWI from PND40 to PND80 days in 1984 but it resulted in only a minor increase in GALC activity and a small decrease in psychosine accumulation within the brain.^{49,76,77} In addition to providing a source of GALC enzyme, it is thought that BMT may also play a role in reducing neuroinflammation, as shown by a significant reduction in astrogliosis and microgliosis.⁷⁸ BMT was first attempted in infantile- and juvenile-onset patients in 1998.⁷⁹ BMT attenuated KD pathology in the juvenile-onset patients and significantly slowed the development of symptoms in infantile KD. BMT increased leukocyte GALC activity from a nearly undetectable level to approximately 3 nmol/h/mg protein (normal: >0.8 nmol/h/mg protein) within a few months of the treatment.^{31,79} This effect was sustained for at least 3 years. Additionally, BMT reduced abnormal signal intensity on magnetic resonance imaging (MRI), indicating improved myelination.⁷⁹

A subsequent study of UCBT for infantile KD examined 11 pre-symptomatic patients and 14 symptomatic patients. All pre-symptomatic patients and six of the 14 symptomatic patients were alive at the end of a 3-year follow-up period with a median survival of 30 months for symptomatic patients, compared with untreated early infantile KD patients with a median survival of 13–18 months.^{80,81} UCBT had little effect on symptomatic patients. UCBT delayed the development of many standard KD symptoms in the pre-symptomatic treatment group, such that they had improved vision, cognitive skills, and peripheral nerve conduction, compared with untreated KD children. Most of the pre-symptomatic treatment group patients displayed improved language skills and gross motor skills, and ~50% showed normal hearing.⁸² Long-term follow-up of 16 UCBT and two BMT early infantile KD patients treated prior to symptom onset showed that 13 were alive after a 15-year follow-up period with a median survival of 11 years. All patients had normalized leukocyte GALC activity ranging from 1.1–4.4 nmol/h/mg (pre-transplant levels ranged from 0–0.12 nmol/h/mg). The majority showed stable MRI findings, approximately average language skills, could walk with assistance, and slightly below-average cognitive development. However, many exhibited symptoms, including spasticity, eating

difficulties, vision impairment, as well as bone and joint involvement.⁷⁵ These studies show that, while HSCT provides a significant benefit to infants compared with untreated KD patients, it has limitations in preventing disease progression.

While HSCT provides significant benefit for KD patients, it is associated with both acute and chronic complications that pose a risk to patients. Patients must complete an immunosuppression regime for graft-versus-host disease (GVHD) prophylaxis prior to the procedure. Acute and chronic GVHD are associated with potential life-threatening complications. HSCT graft failure leads to minimal effectiveness and symptoms comparable with those of untreated KD.^{75,79,82} Altogether, while HSCT is not a cure for KD, it increases survival and improves quality of life for patients, but it has the potential for procedure-related complications such as GVHD.

HSCT has also been performed in other LSDs with varied levels of success. One of the most successful cases, in addition to KD, is metachromatic leukodystrophy (MLD). MLD is a demyelinating disorder similar to KD caused by mutations in arylsulfatase A (ARSA). Similar to the therapeutic effect observed in KD, HSCT attenuates clinical symptoms and extends median lifespan of treated MLD patients into teenage years.⁸³ Additionally, HSCT is the standard-of-care treatment for mucopolysaccharidosis-1 (MPS1). MPS1 is an LSD caused by mutations in the *IDUA* gene, which is responsible for making the α -L-iduronidase enzyme. HSCT slows MPS1 progression and significantly extends life expectancy beyond untreated patients.^{84,85} Given the therapeutic efficacy of HSCT for KD and other LSDs, combined with the minimal number of currently available safe and effective treatments, HSCT will likely continue to be the standard of care for many of these diseases.

As mentioned previously, HSCT is most successful when performed prior to development of symptoms.⁸² Furthermore, HSCT has the highest chances of ameliorating KD symptoms when performed as early as possible.⁸⁶ These time constraints make treatment more complicated because they necessitate the ability to identify KD patients as early as possible. Historically, patients were diagnosed with KD either through symptom development, and thus would not benefit from HSCT, or family history.^{75,79,82} One potential way of solving this issue is through NBS programs. New York State was the first state to start testing newborns for KD in 2006; since then, seven more states have added KD to their NBS testing panel. Additionally, KD is currently undergoing evidence review to be added to the Recommended Uniform Screening Panel (RUSP), which would recommend every state add KD to their NBS testing panel.^{5,87} NBS programs take dried blood spot (DBS) samples from infants in the first days of life and then analyze them for indications of a given disease. In the case of KD, the exact tests performed on the samples vary by state, but the most common first-tier test examines GALC activity. Babies that display low GALC activity are then moved onto second-tier testing, which again varies by state but is usually *galc* genotyping or psychosine quantification. The families are notified at this point and the diagnosis is then confirmed with additional testing, such as MRI and nerve conduction tests.^{4,5,87}

It should be noted that the outcomes of the first KD patients identified through NBS in New York were mixed due to a multitude of factors, such as second-tier testing taking too long and patients not being promptly referred to specialty transplant centers. In the first 9 years, five infants were diagnosed with KD and four underwent HSCT. Of these four patients, one is overall doing well with some motor deficits, one has severe disabilities (received HSCT at 2 months), and two died due to transplant-related complications.^{5,86} Given these results, new protocols have been put in place to expedite second-tier testing and have confirmed cases transferred to transplant centers as fast as possible. The current goal of NBS programs is to have the results back by the seventh day of life. The infant should then be moved to a specialty center for confirmation of the KD diagnosis. Finally, the infant is referred to a transplant center for final evaluations and to have the procedure performed. The goal is to have the transplant completed by the 30th day of life to ensure the highest probability of success.⁸⁶

KD: PRECLINICAL INVESTIGATIVE THERAPIES

Many different therapies have been attempted for KD over the years. A notable, but not exhaustive, list includes stem cell transplantation, substrate reduction therapy (SRT), ERT, anti-inflammatory therapy, chaperone therapy, gene therapy, and various combination investigative therapies.^{49–51,53,54,57,62,78,88–100} The effects of these investigative therapies on TWI survival, GALC activity, psychosine levels, phenotypic changes, and histologic changes are outlined in [Table 1](#) (single-modality treatment) and [Table 2](#) (combination treatments).

One of the most important early findings from these investigations was that GALC overexpression can lead to secretion and uptake of GALC by neighboring cells; this phenomenon is known as cross-correction. Cross-correction was first demonstrated in 1968 when cultured fibroblasts from Hurler and Hunter disease patients were shown to attenuate the biochemical defect in the other cells when mixed together. The same held true when either Hurler or Hunter disease fibroblasts were mixed with fibroblasts from healthy patients.¹¹³ This phenomenon was then demonstrated specifically for KD after TWI nerves were grafted into healthy animals; this led to a reduction in pathology over time.¹¹⁴ It was then shown that, when GALC-overexpressing cells secreted GALC into media, neighboring cells endocytosed the secreted enzyme.^{115,116} Finally, TWI oligodendrocyte precursor cells (OPCs) were transplanted into myelin basic protein (MBP)-deficient shiverer mice, where they migrated away from the injection site, matured into oligodendrocytes, and synthesized nearly normal levels of myelin. These transplanted TWI oligodendrocytes were shown to have functional GALC, likely endocytosed from their neighboring GALC-expressing cells.¹¹⁷ This was an important finding because it reinforced the concept that therapies do not need to reach every cell in the body to be effective; GALC could naturally redistribute to many cells not directly targeted by the therapy.

SRT typically utilizes a small-molecule drug to inhibit the enzyme responsible for synthesis of the accumulating substrate, thus slowing

Table 1. Single-modality investigative therapies in TWI

Therapy	Median survival increase (days)	Brain G and P	Phenotypic change	Pathology
1×10^7 HSC i.p. on PND10 ⁴⁹	40	NA	slight delay in motor problems	no change
4×10^5 HSC i.p. on PND10 ⁷⁷	10	increased G to 15% WT	improved weight gain	NA
75 mg/kg LCS SQ every other day starting PND5 ⁵⁰	13	NA	delayed weight loss 2 weeks	decreased GC and AC no change in M
1.9×10^7 vg Adenovirus-GALC i.c.v. on PND0 ⁹⁷	3	increased G to 15% WT decreased P by 45%	delayed Twitching 3 days. improved weight gain 30%	no change
6 mg/kg GALC i.p. ⁵¹ every other day starting PND10	7	increased G to 7% WT decreased P by 20%	improved weight gain and mobility	no change
2.6×10^9 vg AAV2-GALC i.c. or 1.4×10^{10} vg AAV5-GALC i.c. on PND3 ⁹⁸	AAV2: 10 AAV5: 14	NA	improved weight gain and rotarod	decreased MP and improved M
1.2×10^{11} vg AAV1-GALC i.c.v. on PND1 ¹⁰¹	15	increased G to 5× WT	NA	normalized M, reduced MP, and reduced AC
1×10^8 vg lentivirus-GALC i.v. or i.p. on PND7. ¹⁰²	0	increased G to 10% WT	no change	no change
Single GALC injection i.c.v. on PND20 ¹⁰³	10	increased G to 5× WT decreased P but 17%	NA	NA
Anti-inflammatory therapy ^{92*}	5–15	NA	NA	reduced inflammatory markers 30–50%
GALC-expressing neural progenitors i.c. on PND2 ⁸⁸	7	qualitatively increased G	delayed tremor and hindlimb paralysis 5 days Improved weight	reduced GC and AC Improved M
2.4×10^9 vg AAV5-GALC i.c. on PND3 ¹⁰⁴	22	increased G to 4× WT Decreased P by 50%	delayed muscle atrophy 2 weeks Improved weight gain	improved M
7.5×10^5 NSC i.c.v. on PND2 ⁸⁹	5	increased G to 33% WT	improved gait	reduced GC, MP, MG, and AC. Improved myelination
4×10^5 MSC i.c.v. on PND3.5 ⁹⁰	5	no change in G	increased weight gain	decreased MP and MG
4×10^5 MSC i.c. on PND3.5 ¹⁰⁵	0	NA	improved rotarod performance	NA
2×10^{10} vg i.t. + 1.6×10^{10} vg i.c. AAV5-GALC on PND2.5 ¹⁰⁶	30	increased G to 5× WT	improved rotarod performance, moderately improved weight gain, reduced tremor, and no effect on wire hang	increased CD4 and CD8 T cells and decreased AC
4.5×10^9 vg i.c. + 7.6e9 vg i.v. AAVrh10-GALC on PND2 ⁹⁹	64	increased G to 4× WT	normalized motor ability	normal M reduced GC
0.15 mg/kg S202 i.p. 3×/week ⁹¹	20	decreased P by 85%	no effect on weight gain	normalized M. nonsignificant decrease in MP and AC
1×10^{11} vg AAVhu68-GALC i.c.v. on PND0 ¹⁰⁷	90	increased G to 2,1× WT	improved rotarod performance	NA
$\sim 2 \times 10^{12}$ vg AAVrh10 -GALC i.v. on PND10 (4×10^{14} vg/kg) ¹⁰⁸	240	increased G to $\sim 3.4 \times$ WT	normalized weight gain, wire hang, and gait	normalized MP and AC

HSC, hematopoietic stem cells; i.p., intraperitoneal; LCS, L-cycloserine; SQ, subcutaneous; vg, viral genomes; i.c.v., intracerebroventricular; i.c., intracerebral; MSC, mesenchymal stem cells; i.t., intrathecal; GC, globoid cells; AC, astrocytes; M, myelin; MP, macrophages; MG, microglia; NSC, neural stem cell; G, GALC activity; P, psychosine; NA, not available. *Study done on trs mice, not TWI.

the accumulation of that substrate. Up until recently, SRT for KD has primarily focused on the use of L-cycloserine, an inhibitor of serine palmitoyltransferase (previously called 3-ketodihydrospingosine synthetase/synthase), the first enzyme in the synthetic pathway of many ceramides and sphingolipids.^{118–120} Repeated subcutaneous in-

jections of L-cycloserine increased median survival in TWI by 13 days, increased body weight, reduced the number of globoid cells, and decreased astroglia.^{50,54} Unfortunately, L-cycloserine has a narrow therapeutic window since it leads to a reduction of many other critical lipids in addition to psychosine.^{118,119} Recently developed SRT

Table 2. Combination investigative therapies in TWI.

Therapy	Median survival increase (days)	Effect on brain G and P	Phenotypic improvement	Pathology
HSCT PND10-20 and SQ 50 mg/kg LCS ⁵⁴	85	NA	improved weight gain	decreased GC and AC
1.4 × 10 ¹⁰ vg AAV5-GALC i.c. and BMT on PND3 ¹⁰⁰	65	increased G to 4× WT	delayed motor problems increased weight	reduced MP, AC, and GC normalized M
BMT and 1 × 10 ⁷ vg lentivirus-GALC i.v. on PND1.5 ¹⁰⁹	35	increased G to 33% of WT reduced P	NA	reduced GC and improved M
BMT on PND3.5 and 2 × 10 ¹⁰ vg i.t. + 1.6 × 10 ¹⁰ vg i.c. AAV5-GALC on PND2.5 ¹⁰⁶	82	increased G to 1.5× WT reduced P by 66%	mostly normalized motor problems increased weight	Decreased CD4, CD8, and MG
5.33 ug i.t. + 1.6 ug i.c.v. GALC on PND2 and BMT i.v. on PND3 ⁷⁸	19	G increased to 2× WT at 24 h but decreased over time reduced P by 90%	delayed motor problems 10 days	moderate normalization of M
2 × 10 ¹⁰ vg i.t. + 1.5 × 10 ⁹ vg i.c. AAV5-GALC on PND2 BMT on PND3 25–50 mg/kg LCS 3/week starting PND5 ¹¹⁰	250	increased G to 6× WT but decreased to 2× over time reduced P to WT level	delayed motor problems 15 weeks	initially normalized M but multifocal demyelination later GC, AC, and MG reduced at p35 but elevated later
BMT on PND 9.5 and 2 × 10 ¹¹ vg AAV9-GALC i.t. on PND10.5 ⁵⁶	39	NA	NA	near normalization of M
BMT on p8/9 and 2 × 10 ¹¹ vg AAVrh10-GALC i.v. on PND10-12 ¹¹¹	300	increased G to WT but declined over time	normalized gait increased weight	normalized M decreased AC and MG
2 × 10 ⁶ vg lentivirus-GALC i.c. or 4 × 10 ⁵ NSC i.c.v. on p2 and 5 × 10 ⁶ BMT on PND7 ¹¹²	LV + BMT: 102 NSC + BMT: 173	increased G to 30% WT reduced P	increased weight	partially restored M AC initially reduced by increased over time
9 × 10 ⁹ vg i.c. + 8.25 × 10 ¹⁰ vg i.t. + 3.3 × 10 ¹¹ vg i.v. AAV9-GALC on PND0 and BMT on PND1 ⁵³	241	increased G to 14× WT but decline over time reduced P to WT level but increased over time	delayed motor problems 20 weeks increased weight	M normalized but focal demyelination later reduced AC and MG
1.5 × 10 ¹¹ vg i.t. + 6 × 10 ¹⁰ vg i.c. AAV9-GALC on PND0 BMT on PND1 25–50 mg/kg LCS 3×/week ⁵⁷	363	increased G to WT normalized P	normalized motor ability increased weight	decreased MG but increased over time
2 × 10 ¹¹ vg AAVrh10-GALC i.v. on PND10 BMT on PND10 ¹⁰⁸	311	increased G to WT level	NA	NA

LCS, L-cycloserine; SQ, subcutaneous; vg, viral genomes; i.c., intracerebral; i.v., intraventricular; i.t., intrathecal; i.c.v., intracerebroventricular; NSC, neural stem cells; GC, globoid cells; AC, astrocytes; M, myelin; MG, microglia; G, GALC activity; P, psychosine; NA, not available.

drugs target enzymes that are more proximal to psychosine synthesis, such as ceramide galactosyltransferase (CGT) or acid ceramidase, in an attempt to limit off-target effects.^{91,121} Intraperitoneal (i.p.) injections of the CGT inhibitor S202 in TWI increased median survival ~20 days and improved body weight. Furthermore, S202 reduced psychosine by >70% and normalized myelination when used at >0.15mg/kg. However, long-term treatment with such doses of S202 led to worsened nerve conduction velocity, poor performance on wire hang, and vacuolation in the CNS of WT mice. These side effects, which may be caused by collateral effects of CGT inhibition on the production of sulfatides and accumulation of ceramides, appear to be dose dependent.⁹¹ Another new SRT drug, 22m, inhibits acid ceramidase and has shown potential in reducing psychosine both *in vitro* and *in vivo*.¹²¹ An important observation from these studies is that the level of toxicity appears to correlate with the inhibitor

dose. This suggests that an appropriate dose might provide treatment conditions where side effects are minimal or negligent, while still exerting therapeutic effects on psychosine production.

SRT has been investigated for a number of other LSDs.¹²² As with KD, finding drugs that are effective but have minimal side effects has been difficult because many of the drugs target early steps within synthetic pathways. Additionally, drug discovery for CNS disorders is further complicated by finding drugs that will cross the blood-brain barrier (BBB).¹²³ Miglustat was the first SRT drug approved in humans for type 1 Gaucher disease (GD). Miglustat improved bone density, increased hemoglobin levels, and reduced liver and spleen volume.^{124–129} Miglustat's success in treating GD led to investigators using it to treat other LSDs with defects in common biochemical pathways, such as Niemann-Pick C (NPC). Miglustat treatment of NPC

patients led to an improvement in multiple symptoms of NPC, such as supranuclear gaze palsy and dysphagia.^{123,130} Eliglustat was later found to be superior to miglustat in treating GD and is now a first-line therapy.¹³¹ Two new drugs, venglustat and lucerastat, are currently being investigated predominately for the treatment of GD and Fabry disease.^{132,133} Looking forward, SRT has the potential to treat many LSDs, especially as new, more specific drugs are discovered.^{91,121}

ERT involves supplementing the defective enzyme using externally synthesized replacement enzyme. ERT using recombinant GALC delivered via i.p. injections every other day increased median survival in TWI by ~10 days. There was a minimal reduction of psychosine within the brain, suggesting that GALC cannot cross the BBB.⁵¹ Although a negative result, it was still an important finding since it showed that any successful therapy will need to be administered directly to the CNS or utilize technology to cross the BBB. Single dose intracerebroventricular (i.c.v.) administration of GALC enzyme increased TWI median survival by ~10 days. The minimal effectiveness of ERT is likely due to rapid enzyme turnover as shown by there being no detectable enzyme at a terminal time point.¹⁰³ KD patients would almost certainly require continuous GALC injections directly into the CNS for the duration of their life for ERT to work effectively. This severely limits the applicability of CNS-directed, ERT-based treatment for KD.

ERT has been attempted with varying levels of success in many other LSDs. Historically, as with KD, the replacement enzyme has not been able to sufficiently enter the CNS to significantly alter disease pathology or symptom development.^{1,125,131,134} ERT has been recommended mostly for non-neuropathic LSDs such as type 1 GD and Pompe disease; however, repeated i.c.v. administration of tripeptidyl peptidase 1 (TPP1) was recently approved for the treatment of CLN2 disease.^{125,135–137} For type 1 GD, long-term ERT normalized height, RBC counts, and bone density as well as reducing hepatosplenomegaly.¹³⁸ One method that has been attempted to circumvent the BBB is administering the replacement enzyme intrathecally (i.t.) or i.c.v. In the past, this treatment route has exhibited limited efficacy. For example, a clinical trial (NCT01510028) using repeated i.t. administered ARSA to treat MLD only showed slightly reduced disease symptoms and sulfatide accumulation.¹³⁹ More recently, there have been a few developments to improve ERT for CNS disorders. One system fuses the enzyme to antibodies targeting endogenous proteins (e.g., leptin, insulin, and transferrin) expressed in the lumen of brain capillary endothelial cells. Then, the enzyme-antibody-protein construct is taken into the endothelial cell via endocytosis. Finally, the enzyme-antibody-protein construct is exocytosed on the brain parenchyma side of the capillary. This process is known as transcytosis.^{140–145} An antibody-enzyme fusion protein, JR-141, containing the transferrin receptor antibody and iduronate-2-sulfatase was developed to treat MPS2. Following intravenous (i.v.) administration of JR-141 in a MPS2 mouse model, JR-141 was detected within the brain and was shown to decrease the accumulation of glycosaminoglycans.¹⁴⁶ Additionally, following the success of implantable CNS injec-

tion devices for ERT delivery in the canine model of neuronal ceroid lipofuscinosis (CLN2), a clinical trial indicated it significantly delayed symptom development, which led to its US Food and Drug Administration (FDA) approval.^{136,137,147} Implantable CNS injection devices have existed for some time, but using them for ERT has been limited due to the frequent injections, as often as every 2 weeks, and long injection time required for ERT. The frequent injections and long injection time significantly increase the risks of adverse events such as infection. However, a recent study developed guidelines that significantly reduced the risk of these adverse events.^{137,148} Given these recent advances in ERT for CNS LSDs, ERT could be an important component of their treatment in the future.

As a monogenic loss-of-function disorder, KD is an ideal candidate for gene therapy. A variety of viruses have been investigated for use in treating KD, including adenovirus, lentivirus, and AAV.^{53,55,57,58,97,98,102,108} To date, AAV appears to be the most promising viral vector for KD gene therapy.^{53,57,58,108}

The AAV capsid determines its cellular tropism and is, therefore, an important factor in therapy efficacy.^{149–153} Naturally occurring AAVs generally have a broad tropism but often still have a bias toward one specific cell type, with neuronal bias being the most common within the CNS.^{154–156} Each AAV's tropism varies depending on administration route, target species, age of infusion, and purification methods.^{154,157,158} CNS-directed, AAV-mediated gene therapy using vectors pseudotyped with either the AAV5 or AAV1 capsids carrying a *galc* transgene, initiated during the neonatal period, increased the median survival of TWI by 10–15 days.^{98,101} Addition of i.t. injections in conjunction with the intracerebral (i.c.) route further increased median survival by another ~15 days.¹⁰⁶ The greatest increase in lifespan, median survival of 280 days of age, in TWI attributed solely to AAV-mediated gene therapy was observed following high-dose i.v. AAVrh10. This approach significantly increased GALC activity in both the brain and sciatic nerve, reduced neuroinflammation, improved weight gain, increased strength, and normalized gait.¹⁰⁸

A study investigating gene therapy in the canine model of KD administered AAVrh10-GALC i.v. and i.c.v. at 3 days and 6 weeks, respectively. The therapy had a clear dose response and resulted in increased GALC activity, reduced psychosine, and improved myelination. However, correction was not complete, likely due to the treatment dosage being too low.⁵² A subsequent report from the same laboratory completed a dose-response study of AAV9-GALC delivered by intracisternal injection at pre-symptomatic (2 weeks) and post-symptomatic (6 weeks) time points. The second study found that delivery of high-dose AAV9-GALC extended survival over seven times that of untreated canines and corrected neurological dysfunction as shown by attenuated loss of myelin and significantly reduced psychosine.⁵⁸

Gene therapy has also been investigated in many other LSDs; see Ohashi et al. 2019 for a review focusing on this topic.¹⁵⁹ Like KD, many LSDs have shown significant improvements in median survival and disease pathology in animal models following gene therapy and are

now moving into clinical trials. For example, a mouse model of GM1 gangliosidosis, displayed near-complete correction of the β -galactosidase (β gal) deficiency following therapy with an AAV2 vector administered i.c.v.¹⁶⁰ Treating the GM1 gangliosidosis mouse model with an AAV9 vector administered i.v. increased β gal, significantly reduced GM1, improved behavioral performance on multiple assays, and increased median survival by 150 days.¹⁶¹ A similar therapeutic approach of i.v. AAV9 was used to treat a feline model of GM1 gangliosidosis. This treatment decreased GM1, nearly normalized β gal, reduced pathology, partially normalized myelination, and increased survival by almost 3 years.¹⁶² These results led to a phase 1/2 clinical trial using an AAV9 vector to treat GM1 gangliosidosis (NCT03952637). Any information that is gained from this and other clinical trials investigating AAV therapy for LSDs, such as which AAV serotype, treatment route, or dosing strategies are most efficacious, will be immensely useful for future LSD studies and clinical trials. For a more complete list of AAV-based gene therapy trials for LSDs currently underway, see clinicaltrials.gov.

Although each single-modality therapy has different targets and provides moderate benefit, they also each have their own limitations. The most promising therapies are those that employed combinations of previously discussed treatments.^{53,57,106,108,110} The first combination therapy attempted in TWI utilized BMT and L-cycloserine-mediated SRT. These therapies had a synergistic effect such that they increased median survival to 110 days, more than either achieved in isolation.⁵⁴ Similarly, combining BMT with i.c. AAV5-GALC increased median survival to 100 days and improved motor function.¹⁰⁰ Addition of an i.t. injection of AAV5-GALC further increased median survival an additional 25 days. This treatment protocol also increased GALC activity to five times WT levels in the brain and reduced psychosine to nearly WT levels in the brain and spinal cord. However, there were limited improvements in the wire hang assay, which may indicate this combination therapy did not adequately treat the PNS.^{100,106} Combining neonatal administrations of i.v., i.t., and i.c. AAV9-GALC with BMT increased median survival to 285 days. Additionally, the mice showed decreased signs of disease: increased body weight, improved locomotion, and improved performance on neurobehavioral tests. The mice exhibited improved myelination and supraphysiological GALC activity, and decreased psychosine accumulation.⁵³ Another AAV + BMT study administered the treatments later, BMT at PND8.5 and i.v. AAVrh10-GALC at PND11. This study found that the later treatment schedule led to better outcomes, including increasing median survival to 340 days, no tremor, normalized gait, improved weight, and near-normalized myelination.¹¹¹ The later dosing schedule may also better model the age of KD children when they should receive AAV therapy; they are currently treated with HSCT around 1 month old and potentially would be treated with AAV around the same time.⁸⁶ A triple therapy combining i.c. and i.t. AAV5, BMT, and L-cycloserine SRT increased the median survival to 290 days. This approach increased GALC activity to 3.5 times WT, reduced psychosine to nearly WT levels, reduced neuroinflammation, and effectively preserved myelin. To highlight the synergistic efficacy of SRT, this study was able to achieve

a similar median survival increase as an AAV + BMT-only study using 100-fold less AAV.^{110,111} A follow-up study of the triple therapy that changed the AAV capsid from AAV5 to AAV9 further increased median survival to 404 days.⁵⁷ Looking forward, future combination therapies utilizing newer SRT drugs, more effective AAV vectors, and improved HSCT protocols may further increase survival and reduce pathology due to improved specificity.^{91,121}

As with KD, combination therapies for other LSDs have been shown to be synergistic in reducing disease pathology and increasing median survival.^{163–166} Most of the combination therapies investigated for KD have also been studied in other LSDs, such as ERT + BMT, BMT + gene therapy, and SRT + BMT.^{163,165,166} These therapies had varying levels of success, but, like combination therapies for KD, they invariably were additive, and most showed some level of synergy. One previously discussed example, Eliglustat-based SRT, has already been shown to synergize with other treatment modalities in preclinical investigations.^{54,57,110,167} This indicates that SRT can be used in combination with other treatment modalities, such as HSCT and gene therapy, to treat LSD patients. Combination therapies allow for each treatment to address the weaknesses of the other treatment to ensure more complete therapeutic coverage.¹⁶⁴

AAV cellular dynamics and potential shortfalls

While AAV-based gene therapy both alone and in combination with other therapies appears to have enormous potential for the treatment of KD, there are still concerns that may limit its efficacy in clinic. Chief among these limitations is the fact that the most successful therapies still have a median survival of only 400 days in TWI, which is significantly lower than the WT mouse median survival of 700–900 days.^{57,168} Furthermore, there is evidence from multiple laboratories that the efficacy of the gene therapy treatment wanes over time. This was observed using several criteria, including decreasing myelination, worsening inflammation, declining GALC activity, and increasing psychosine.^{53,56,57,110} Additionally, many animals showed no signs of disease during the months immediately after treatment initiation, such that they were indistinguishable from WT animals, but began to exhibit progressively debilitating disease signs later in life.^{53,56–58,110,169} At present, the mechanism behind this age-related decline in therapeutic efficacy is unknown, although some clarity is emerging.

The majority of transcriptionally active recombinant AAV genomes exist as circular, non-replicating extrachromosomal episomes.^{170–173} We hypothesize that therapeutic efficacy declines over time because AAV episomes are not replicating in tandem when the host cell is proliferating. The Bongarzone lab recently presented evidence of this occurrence both *in vitro* and *in vivo*.¹⁶⁹ Myelinating oligodendrocytes and neurons are terminally differentiated and, therefore, should be permanently corrected by AAV. In contrast, other CNS cell types such as OPCs and neuronal precursors produced in the subventricular and hippocampal germinal zones and also residing throughout the CNS replicate throughout life to modify myelination in response to motor learning and contribute to neuronal regeneration.^{174–178} Based on this, we proposed that, since OPCs can replicate in the adult brain,

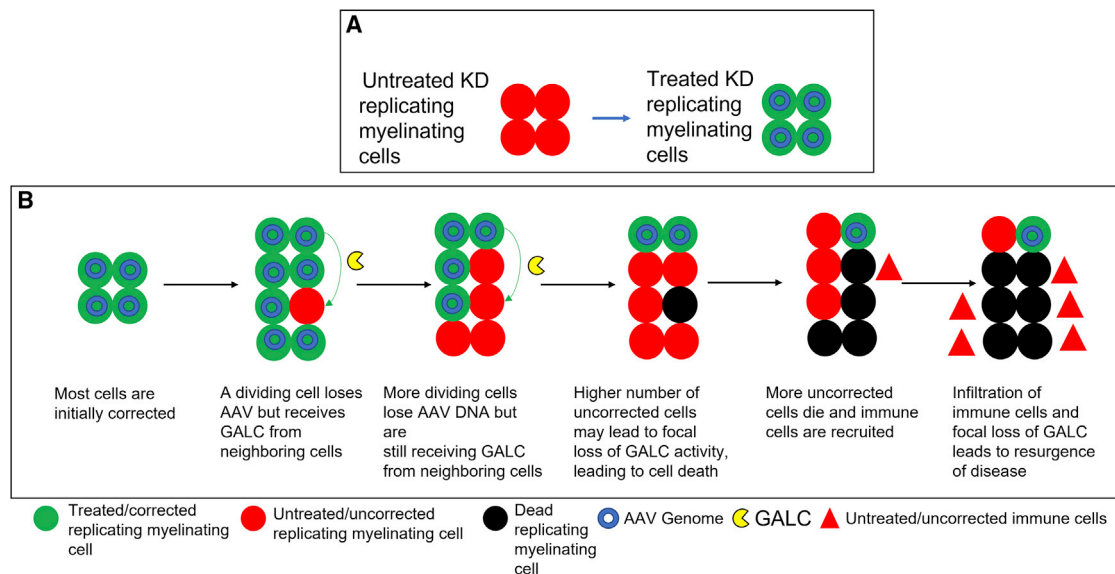


Figure 2. Proposed mechanism of episomal AAV dilution during myelinating cell proliferation

This figure proposes a theoretical mechanism of replication-induced dilution of episomal AAV DNA in proliferating myelinating cells starting from initial treatment in (A). (B) How replication leads to some proliferating myelinating cells not inheriting a copy of the therapeutic AAV DNA, but they initially receive GALC from neighboring treated cells. At some point, the ratio of untreated to treated cells becomes high enough that cross-correction can no longer supply sufficient GALC to all of the untreated cells, and the cells that lost the AAV DNA begin to die. Finally, uncorrected immune cells are recruited, culminating in the development of disease signs.

eventually a cell may not inherit copies of the therapeutic episome during cellular division. This would revert its phenotype to an untreated state. In this scenario, the “uncorrected” cell may initially show little or no signs of distress as it may endocytose GALC through cross-correction from neighboring treated cells. However, over time and with more cell divisions, there would be fewer OPCs that harbor the therapeutic transgene to provide sufficient GALC to effectively eliminate psychosine. At this point, areas containing an increasing number of uncorrected cells may activate repair mechanisms due to cellular damage, including recruitment of additional uncorrected repair/inflammatory cells, which would likely worsen the developing lesion. This hypothesized pathway is outlined in Figure 2 and supported by evidence including loss of *galc* expression and GALC activity as well as the presence of non-corrected OPCs within late-onset demyelinating lesions in the brain of long-surviving AAV-treated TWI. Notably, these late-onset demyelinating lesions have only been observed within the brain; none have been found in the spinal cord or peripheral nerves.^{53,110,169} However, this does not mean the spinal cord and peripheral nerves do not contain any pathology. The spine and sciatic nerves of long-term AAV9-GALC-treated animals exhibited significant decreases in GALC activity and significant increases in psychosine over time.⁵³ Additionally, the lack of demyelinating lesions in the peripheral nerves may simply mean that similar processes are occurring but develop at slower rates. There is evidence of a similar decline in AAV episome copy number and therapeutic efficacy over time for other AAV-treated LSDs.^{179–183} There are still many unanswered questions regarding this proposed pathway, including determining the timing and specific mechanisms of each step. Furthermore, a more complete understanding of the relation-

ship between the dose of AAV and the duration of protection against disease in the human brain is needed.

The observed decline in efficacy in animal models does not preclude the use of AAV-based therapies for the treatment of KD or other LSDs. There is a long history in preclinical AAV-based gene therapy research in KD showing a strong dose response from increasing survival by 10 days in the earliest experiments with 2.5×10^9 viral genomes (vg)/mouse to increasing survival by over 200 days with 6×10^{11} vg/mouse, as shown in Figure 3.^{52,53,56,58,98,99,108} We performed regression analysis of these experimental outcomes, which show the R^2 of AAV-only therapies is 0.64, indicating a reasonable correlation and dose response. The applicability of the R^2 is limited by the varying serotypes, injection routes, and other differences between investigators. The R^2 of AAV + BMT therapies is only 0.23, the lower R^2 for AAV + BMT is likely, at least partially, due to differing amounts of stem cells delivered to the animals and immune suppression protocols in different studies. An example from a single laboratory, Bradbury et al, demonstrated a clear dose response in their consecutive papers, indicating higher doses leads to reduced signs of disease and improved median survival in the canine KD model.^{52,58} Figure 3 also highlights the importance of AAV serotype and injection route. AAV9 and AAVrh10 had the largest increases in survival. Injection route also influenced treatment efficacy: i.c. and i.t. alone were generally the least efficacious, while i.v. alone, i.c. + i.t., and i.c. + i.t. + i.v. were generally the most efficacious.

Taking all of this into consideration, it may simply mean that AAV gene therapy needs further improvements, such as higher doses,

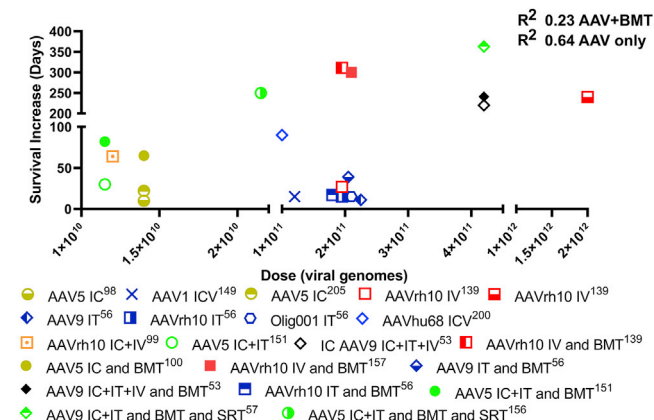


Figure 3. Preclinical AAV therapy efficacy in KD

This figure shows all preclinical AAV studies completed to date as dose (viral genomes) versus the increase in survival (days) from the treatment. The graph is organized by both route of administration (color: bronze, i.c.; blue, i.t./i.c.v.; red, i.v.; green, i.c. + i.t.; orange, i.c. + i.v.; and black, i.c. + i.t. + i.v.) and serotype (shape: X, AAV1; circle, AAV5; diamond, AAV9; square, AAVrh10; and hexagon, AAVOlig001). *All therapies centered around 2×10^{11} viral genomes (vg) but were separated in the graph for clarification.

multiple treatments, or new vectors with improved targeting of intended cell populations. Determining the relationship between the amount of AAV administered and expected median survival is critical. While it is very difficult to directly compare the dosage and survival from the different laboratories over the years because of differing AAV constructs, AAV serotypes, administration routes, timing of administration, use of immunomodulation, and other differences, the relationship does not appear to be linear.^{52,53,56,58,98,99,108} One alternative to simply increasing the dosage of the neonatal treatment is the possibility of redosing. While there is a concern about an immune reaction against the AAV in the second treatment, this could be mitigated by using a different AAV serotype for the second dose or potentially immune modulation prior to dosing. Certain AAV serotypes have been shown to have minimal cross reactivity, and serotype switching has already been shown to be possible.^{184–191} Corticosteroids are probably the most commonly used immunomodulatory treatments, but other modalities, such as B cell inhibition with rituximab, rapamycin (also known as sirolimus) inactivation of B and T cells, or plasmapheresis to remove anti-AAV neutralizing antibodies, are possible to minimize an immune response against the AAV capsid or transgene.^{192,193}

While there does not appear to be a direct linear relationship between AAV dose and survival, it generally appears that higher AAV doses lead to greater survival. This begs the question: why not continuously increase AAV dose to increase survival to that of WT animals? The answer to this is that high-dose systemic AAV therapy poses its own risks in the form of acute liver injury as well as pathology such as neuronal degeneration in the dorsal root ganglia.^{194–196} Another potential risk of high-dose AAV therapy is insertional mutagenesis leading to hepatocellular carcinoma.^{57,197,198} While the vast majority

of AAV genomes exist as extrachromosomal episomes, some may insert into the genomic DNA.^{170–173} The murine Rian locus appears to be particularly vulnerable to AAV insertion; at present it is unknown if the human Rian locus is similarly a target for AAV insertion.^{57,197} It appears that the Knudson hypothesis stating multiple genomic “hits” are required to develop cancer applies here.¹⁹⁹ One hit appears to be AAV insertion, with other hits possibly occurring from ionizing radiation used to condition animals prior to BMT, L-cycloserine-induced reduction of ceramides, or chronic liver injury from co-morbidities.^{57,198,200–202}

AAV FUTURE ADVANCES AND CLINICAL TRIALS

As mentioned previously, each AAV serotype has a unique tropism based on the capsid.^{149–156} There has been a significant amount of research into engineering AAV capsids for specific purposes, whether that be targeting specific cell types, detargeting off-target cell types, or increasing penetration through the BBB as reviewed in von Jonquieres et al. 2021.¹⁵⁴ One particularly relevant novel AAV capsid for KD and other LSDs that predominately affect oligodendrocytes is Olig001, which preferentially targets oligodendrocytes.²⁰³ This could potentially allow utilization of a lower total dose of AAV since a higher proportion of the virus will transduce oligodendrocytes. Alternatively, a neuron-specific AAV could be used to increase transduction of neurons in LSDs that primarily affect neurons.^{149–156,158} Additionally, transgene expression can be controlled using cell-specific promoters.¹⁵⁴ For example, the MBP promoter has been used to limit transgene expression to oligodendrocytes.²⁰⁴ Using engineered cell-specific capsids in conjunction with cell-specific promoters allows for precise targeting of transgene expression with minimal off-target effects.

All of the information acquired on KD genetics, pathology, and investigative therapies in the past 35 years have provided the foundation of two current trials. NCT04771416 is examining the efficacy of AAVhu68 (modified AAV9) administered into the cisterna magna and has already treated the first patient. This trial is based on work showing that the i.c.v. AAVhu68 increased median survival in TWI 3-fold, increased brain GALC activity to 200% WT, and improved rotarod latency to fall. Additionally, AAVhu68 was tested in the KD canine model; treated animals did not display any of the classical signs of disease throughout their lives. Furthermore, the canines displayed partially normalized myelination, normal auditory evoked potential, and reduced neuroinflammation. Finally, safety and tolerability of AAVhu68 was tested in non-human primates and found to be well tolerated.¹⁰⁷ NCT04693598 is studying HSCT combined with AAVrh10 administered i.v. based on preclinical studies discussed earlier.^{108,111} Since these clinical trials were only recently initiated, there are no data regarding efficacy yet. Whatever information is acquired from these trials will help not only KD patients but also those suffering from other LSDs as well by guiding the design of future clinical trials. In addition to these KD studies, there are currently ongoing AAV-based clinical trials for numerous LSDs such as GD, MLD, and GM1 gangliosidosis, as seen on clinicaltrials.gov.

Conclusions

Combination therapies using AAV gene therapy plus HSCT or SRT may provide an alternative to high-dose AAV therapies.^{53,57,100,106,110,112,205} Furthermore, the latest SRT drugs target more proximal enzymes to psychosine synthesis than L-cycloserine to limit off-target effects.^{91,121} Combination therapies could achieve similar efficacy to high-dose AAV therapies while using less AAV, thus avoiding the potential perils of high-dose AAV therapy. In tandem with combination therapies, engineered AAV serotypes allow for improved targeting and potentially further reduction of the required dose for effective treatment. In summary, investigative therapeutics for KD have significantly improved since the development of HSCT 35 years ago, particularly combination therapies. Looking to the future, the likely treatment for KD will be a combination of the current standard-of-care treatment, HSCT, and AAV, as well as possibly a third treatment modality such as ERT or SRT. This specific treatment combination used may even change depending on the subtype of KD (infantile, juvenile, or adult onset) or the specific mutation a patient may have, and this type of personalized medicine may further improve treatment outcomes. For example, an infantile KD patient homozygous for the 30-kb deletion may receive HSCT, gene therapy, and SRT, while a juvenile-onset patient may benefit from receiving only gene therapy, and an adult-onset KD patient may be managed with just ERT or SRT. This customized treatment plan could minimize the impact of KD for all patients, minimize treatment costs, and improve access to post-therapy follow-up.

The preclinical experiments and clinical trials using single and combination therapies for KD may also aid the development of more effective therapeutic strategies for other LSDs. Given that MLD is potentially the most similar disorder to KD with regard to pathology, symptoms, and treatment, we will use it as an example. At present, like KD, MLD is treated with HSCT, but this is not very effective in stopping disease pathology or symptom development. However, recently the European Medicine Agency approved a new gene-cell therapy using lentiviral transduced stem cells to treat MLD. This therapy significantly increased ARSA activity as well as improving motor function, verbal skills, and cognitive ability.²⁰⁶ Many of both the preclinical and clinical therapies being investigated for KD have a similar counterpart being investigated for MLD.²⁰⁷ For example, an ERT trial (NCT01510028) was completed recently and an AAV gene therapy trial (NCT01801709) is currently ongoing. Similarly, these same treatment modalities are currently being or will be investigated for other LSDs. The future of LSD treatment is advancing every day through earlier diagnosis, improved HSCT protocols, new SRT drugs, novel ERT protein constructs, and innovative AAV construct design.

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AUTHOR CONTRIBUTIONS

G.H. wrote the manuscript. G.H., A.M.B., M.S.S., and E.R.B. contributed equally to reviewing, editing, and final formatting. All authors read and approved the manuscript.

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A.M.B. is a beneficiary of a licensing agreement with Axovant Gene Therapies (royalties), has received income from Neurogene (consulting and honorarium), and is an inventor on a patent application (Gray SJ, Lykken E, Vite CH, Bradbury AM. Optimized GALC Genes and Expression Cassettes and Their Use. PCT/US2019/067727).

G.H. and M.S.S. report no conflicts of interest.

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