National Institutes of Health (NIH), National Center for Advancing Translational Sciences (NCATS)

Adeno-Associated Virus 9 Sulfatase Modifying Factor 1 (AAV9/SUMF1) for the Treatment of Patients with Multiple Sulfatase Deficiency (MSD)

Rare Pediatric Disease Designation Request

June 26, 2024

- CONFIDENTIAL -

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Covariance
ARSA	Arylsulfatase A
ARSB	Arylsulfatase B
ARSE	Arylsulfatase E
AAV	Adeno-Associated virus
AAV9	Adeno-Associated virus 9
AAV9/SUMF1	Adeno-Associated virus 9/Sulfatase modifying factor 1
CBER	Center for Biologics Evaluation and Research
CBh promoter	Chicken beta actin hybrid promoter
CNS	Central nervous system
CFR	Code of Federal Regulations
CSF	Cerebrospinal Fluid
Co-I	Co-Investigator
EKG	Electrocardiogram
FDA	U.S Food and Drug Administration
FDASIA	Food and Drug Administration Safety and Innovation Act
FGE	Formylglycine-generating enzyme
GAG	Glycosaminoglycan
GAG-NRE	Glycosaminoglycan non-reducing end
GALNS	N-acetylgalactosamine-6-sulfatase
GFAP	Glial fibrillary acidic protein
GMFC-MLD	The Gross Motor Function Classification in MLD
gnomAD	the Genome Aggregation Database
ICM	intracisternal magna
IDS	Iduronate-2-sulfatase
ICV	Intracerebroventricular
IND	Investigational New Drug Application
LAMP	Lysosome-associated membrane protein
intra-CSF	intra-Cerebral Spinal Fluid
ITR	Inverted terminal repeat
IT	Intrathecal
IV	Intravenous
KO	Knock-out
LSD	Lysosomal Storage Disorder
MLD	Metachromatic Leukodystrophy Disease
MPS	Mucopolysaccharidoses

MPSII	Mucopolysaccharidosis type II
MSD	Multiple Sulfatase Deficiency
NHP	non-human primates
ns	not significant
OTAT	Office of Tissues and Advanced Therapies
PND1	Postnatal day -1
PND7	Postnatal day-7
ROA	Route of Administration
sc	self-complementary
scAAV	self-complementary adeno-associated virus
SUMF1	Sulfatase-modifying factor 1
SGSH	N-sulfoglucosamine sulfohydrolase
USA	United States of America
USC	United States Code
WT	Wild Type

1 RARE PEDIATRIC DISEASE DESIGNATION REQUEST STATEMENT

Pursuant to 21 CFR 316.20, the National Institutes of Health, National Center for Advancing Translational Sciences (NCATS requests designation of Adeno-Associated virus 9 Sulfatase modifying factor 1 (AAV9/SUMF1) as a rare pediatric drug product for treatment of patients with Multiple Sulfatase Deficiency (MSD), resulting from the dysfunction of formylglycine-generating enzyme (FGE).

Multiple Sulfatase Deficiency (MSD) is a rare inherited lysosomal storage disorder (LSD) characterized by a decrease in the activity of all sulfatase enzymes. MSD is due to mutations in sulfatase modifying factor 1 (*SUMF1) gene*[1,2], which encodes FGE, a ubiquitously expressed enzyme essential for sulfatase activation[3,4]. Symptoms arise from the additive deficiency of all sulfatases, which includes metachromatic leukodystrophy (MLD) and six mucopolysaccharidoses (MPS) subtypes[5]. MSD is a progressive, multi-systemic pediatric disorder with a median age of death of 13 years. There are no FDA approved therapies for the treatment of MSD.

The estimated prevalence of MSD occurs in approximately 1 in 500,000 individuals[6], thereby categorizing MSD as a rare disease in accordance with the Orphan Drug Act.

2 ADMINISTRATIVE INFORMATION

2.1 Sponsor

National Center for Advancing Translational Sciences, National Institutes of Health
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2.2 Contacts

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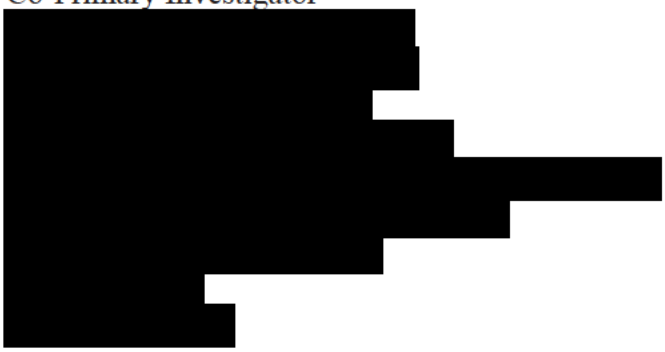
Co-Primary Investigator



2.3 Drug Name

2.3.1 Chemical Name - Drug Substance

Adeno-Associated Viral 9 Sulfatase Modifying Factor 1 (AAV9/SUMF1)

2.3.2 Generic/Trade Name - Drug Product

AAV9/SUMF1 is a recombinant serotype 9 Adeno-Associated Virus (AAV) encoding a codon-optimized human *SUMF1* transgene (*hSUMF1opt*). Detailed description will be included in **Section 4.1**.

Product Name	AAV9/SUMF1
Vector Name	scAAV9/CBh-*hSUMF1opt*-SV40pA
Serotype	AAV9 Serotype Capsid
Formulation	Delivered in 1x PBS with 5% D-sorbitol
Gene Insert	codon-optimized human SUMF1 transgene (Self-Complementary)

AAV2 mutant ITR	Synthetic CBh promoter	Human *SUMF1* gene	SV40 polyA	AAV2 ITR

2.4 Manufacturer's Name and Address

2.4.1 Drug Substance Manufacturer

Aldevron, LLC

4055 41st Ave South
Fargo, ND 58104
FEIN: 3015047170

2.4.2 Drug Product Manufacturer

ThermoFisher Scientific (Patheon Pharma Services)
Rue de la Marlette no14, B-7180 Seneffe (Zoning C)
Belgium
FEIN To Be Confirmed at IND

3 DESCRIPTION OF RARE DISEASE OR CONDITION, PROPOSED INDICATION, AND NEED FOR THERAPY

3.1 Description of Rare Disease or Condition

Molecular Etiology:

MSD arises exclusively from pathogenic variants in the sulfatase-modifying factor 1 (*SUMF1)* gene, which encodes the formylglycine-generating enzyme (FGE) [1,2]. FGE is required to post-translationally modify the active site cystine residue of all sulfatases to the active forymylglycine form[7]. Without this key step, newly synthesized sulfatases are not activated in the endoplasmic reticulum[8-10].

There is a clearly defined association between *SUMF1* mutations and disease phenotype[31,36]. In humans, biallelic *SUMF1* deletions or stop-gain alleles are associated with neonatal lethality, and all known affected children have residual enzyme activity[11]. Clinically, a *SUMF1* "genotype score" can be used to predict genotype severity, which strongly correlates with clinical severity[32]. Nonsense alleles result in the most severe clinical impact. Similarly, a S*UMF1* knock out mouse model[12,13] displays severe neurological, behavioral, and histopathological deficits consistent with neonatal MSD gene trap mutation in intron 3, B6;129P2-*SUMF1*$^{Gt(RST760)Byg}$/ClearJ (stock number 30711); null mutant for *SUMF1* with absent sulfatase activities.

There are two clinical subtypes of MSD, severe (infantile-onset) and attenuated, which both can be predicted by genotype[14]. All patients have two pathogenic *SUMF1* alleles located in trans. Patients with severe disease harbor at least one severe missense or nonsense allele. Conversely, attenuated disease is associated with two mild missense alleles. In the more common, attenuated subtype, children gain and then rapidly loose ambulation in the toddler years. Regardless of age at onset, the neurologic decline is rapid once ambulation is lost, similar to metachromatic leukodystrophy (MLD).[15].

Clinical Manifestations:

Initial symptoms of MSD can develop from infancy through early childhood, and presentation is widely variable[16]. Of the clinical subtypes, attenuated infantile MSD is less progressive than

its counterpart and is identified by the gain of ambulation and limited speech, which may have significant impairments later in childhood. In severe infantile MSD, disease progresses at a faster rate, resulting in significant changes such as dysmorphic features, skeletal changes, severe neurodegeneration, and shortened lifespan. Several clinical features found across all MSD subtypes include progressive neurologic regression, movement and tone abnormalities, musculoskeletal abnormalities, cardiovascular complications, upper airway obstruction, ichthyosis, and more[5,16] (Table 1).

Table 1. Overview of MSD impact on organ systems

Organ system	Clinical manifestation
Neurologic	Seizures, peripheral neuropathy, hydrocephaly and increased intracranial pressure, dysautonomia, developmental regression, dystonia, and tremor
Musculoskeletal	Poor bone health, spasticity, hypotonia, joint contractures, spinal stenosis, and dysostosis multiplex (a defined collection of radiographic abnormalities)
Ophthalmic	Glaucoma, cataract, corneal clouding, retinopathy, and optic nerve abnormalities
Dermatologic	Ichthyosis, hirsutism, and hyperpigmented plaques
Cardiovascular	Arrhythmias, hypertension, cardiac hypertrophy and valve abnormalities, and coronary artery disease
Respiratory	Sleep apnea, restrictive lung disease, and recurrent pneumonia
Gastrointestinal	Poor gut motility, hepatosplenomegaly, gastroesophageal reflux, and gall bladder disease
Metabolic	Metabolic acidosis
Oral and Otolaryngeal	Dental issues, hearing disorder, and recurrent ear infections

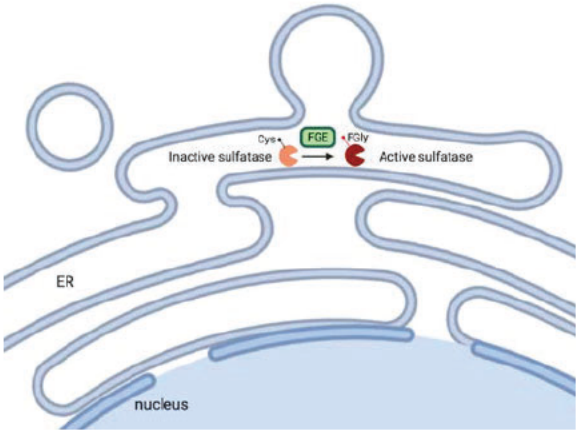
Clinical management for MSD patients involves robust multidisciplinary surveillance and standard of care for each individual symptom identified in patients. Common standard of care practices involves physical therapy for management of mobility and tone, alternative routes for nutrition (i.e. G-tube), standard therapeutic options for spasticity such as baclofen, and surgical care[16]. Without intervention, all forms of MSD result in a progressive loss of function and are ultimately fatal. There are no available targeted therapies for MSD, and thus all currently available interventions are considered palliative.

Pathophysiology:

The broad spectrum of clinical manifestations among patients can be attributed to the intricate pathophysiology of MSD. Since MSD can affect all 17 known human sulfatases, clinical presentation is compounded from each sulfatase deficiency within an individual[14,31]. The common early features include ichthyosis, organomegaly, dysostosis multiplex, and facial dysmorphism. Early neurologic features, include developmental delay, spasticity, and epilepsy[14]. There is an increasing burden of disease over time (Table 1)[14]. Disease symptoms at birth and early multisystemic involvement correlate with poor outcomes[14]. The spectrum of neurologic

disease includes a common, attenuated subtype with stereotyped progression in the toddler years, similar to late infantile MLD (**Figure 1**).

Figure 1. Pathogenesis of MSD. Multiple sulfatase deficiency is a rare, lysosomal storage disorder caused by decreased enzymatic activity of formylglycine generating enzyme (FGE), which is required for activation of the catalytic site of all sulfatases. Patients suffer from progressive neurologic and somatic symptoms due to decreased activity of all 17 known human sulfatases.



3.2 Proposed Indication and Use of AAV9/SUMF1

The proposed therapeutic use of AAV9/SUMF1 is for the treatment of patients with MSD, since the molecular construct encodes to restore FGE protein expression. Both severe and attenuated patients may derive benefit from this product. As mentioned, AAV9/SUMF1 is a recombinant serotype 9 AAV encoding a codon-optimized human *SUMF1* transgene (hSUMF1opt). In a well-characterized *Sumf1*$^{-/-}$ mouse model, AAV9/SUMF1 was administered via injection in JR31625 *SUMF1*$^{-/-}$ mice at both PND1 (ICV only) and PND7 (IT, IV, or IT+IV routes). Uninjected or Vehicle-treated WT littermates served as healthy controls. Treatment paradigms produced benefit as indicated by improved survival and behavior that was equivalent to WT littermates, including gross motor function, motor coordination, cognition, and memory. Restoration of sulfatase activity in tissues and a corresponding decrease in tissue GAGs, neuroinflammation and lysosomal markers was also seen. AAV9/SUMF1 treatment at PND7 also improved cardiac and vision function in a dose-dependent manner. These outcomes, along with previous work on the biodistribution of AAV9 and molecular analyses of *SUMF1* levels, indicate we are effectively targeting critically affected cell populations with this treatment strategy.

3.3 Reasons Why Such Therapy Is Needed

Children with leukodystrophies, including MSD, have a low health-related quality of life[18,19,20,21], and hospitalizations cost over $59 million per year[19]. There are no approved therapies for MSD, and standard of care is palliative symptom management[5]. Regarding MSD, its low prevalence yet

devastating symptoms creates several barriers for patients and families seeking diagnosis and care. Individuals with MSD suffer from neurologic regression and worsening somatic signs and symptoms including hepatomegaly, bone abnormalities, cardiac dysfunction, and hearing loss[7]. There is an urgent unmet need for disease modifying therapies as the ultra-rarity has hindered industry-sponsored therapy development.

The complex range of health problems encountered by families and individuals with MSD is further exacerbated by the necessity of multi-organ surveillance, multi-disciplinary care needs, and barriers to accessing medical specialists[14]. Furthermore, in a case report, Schittkowksi et al, 2023 reported the difficulties of performing ophthalmological functional assessments in patient due to severe visual disability. The lack of structured data collection and low prevalence of disease has created a chasm in which individuals with MSD are racing against rapid progression of the disease to catch multisymptomatic conditions and prevent further regression[22]. Based on preclinical data and mouse model studies, partial pathologic rescue using a single administration of an intra-cisterna magna (ICM) injection of AAV9/SUMF1 has the potential to stabilize neurologic function and improve the systemic burden of disease, benefitting symptomatic patients.

4 DESCRIPTION OF AAV9/SUMF1 AND SCIENTIFIC RATIONALE FOR USE

4.1 Description of AAV9/SUMF1

4.1.1 Drug Product Naming Convention

Adeno-Associated Virus 9 Sulfatase modifying factor 1 (AAV9/SUMF1)

4.1.2 Drug Product Physical, Chemical, and Pharmaceutical Properties

AAV9/SUMF1 is a recombinant serotype 9 AAV encoding a codon-optimized human *SUMF1* transgene (*hSUMF1opt*). The DNA sequence is modified such that the final amino acid sequence is unchanged, but nucleotide sequence is altered for easier detection and potentially stronger expression. The transgene consists of a *hSUMF1* DNA coding sequence of 1122 bp between a 796 bp chicken beta actin hybrid (CBh) promoter and a 143 bp simian virus 40 polyadenylation (SV40pA) signal (*hSUMF1* transgene sequence provided under **Appendix A**). The CBh promoter and SV40 polyA are small but drive strong expression allowing for packaging into a self-complementary (sc) AAV vector[23]. The CBh promoter is identical to the construct characterized in rodents, pigs, and non-human primates (NHPs)[24,25]. As loss of *SUMF1* affects a variety of cell-types, a ubiquitous promoter was selected. The upstream inverted terminal repeat (ITR; proximal to the promoter) is from AAV2, with the D element deleted to promote packaging of a sc genome. The downstream ITR (proximal to the polyA) is an intact WT AAV2 ITR. Self-complementary scAAV vectors are 10-100 times more efficient at transduction compared to traditional single-stranded AAV vectors[26,27]. The codon optimization of the *hSUMF1* transgene allows differentiation of transgenic SUMF1 at the nucleotide level from natural mouse, rat, NHP or human *SUMF1* by quantitative PCR and in situ hybridization methods such as RNAscope (**Figure 2A and Figure 2B**).

AAV2 mutant ITR	Synthetic CBh promoter	Human *SUMF1* gene	SV40 polyA	AAV2 ITR

Figure 2A. Diagram of the CBh-*hSUMF1opt*-SV40pA Vector Genome

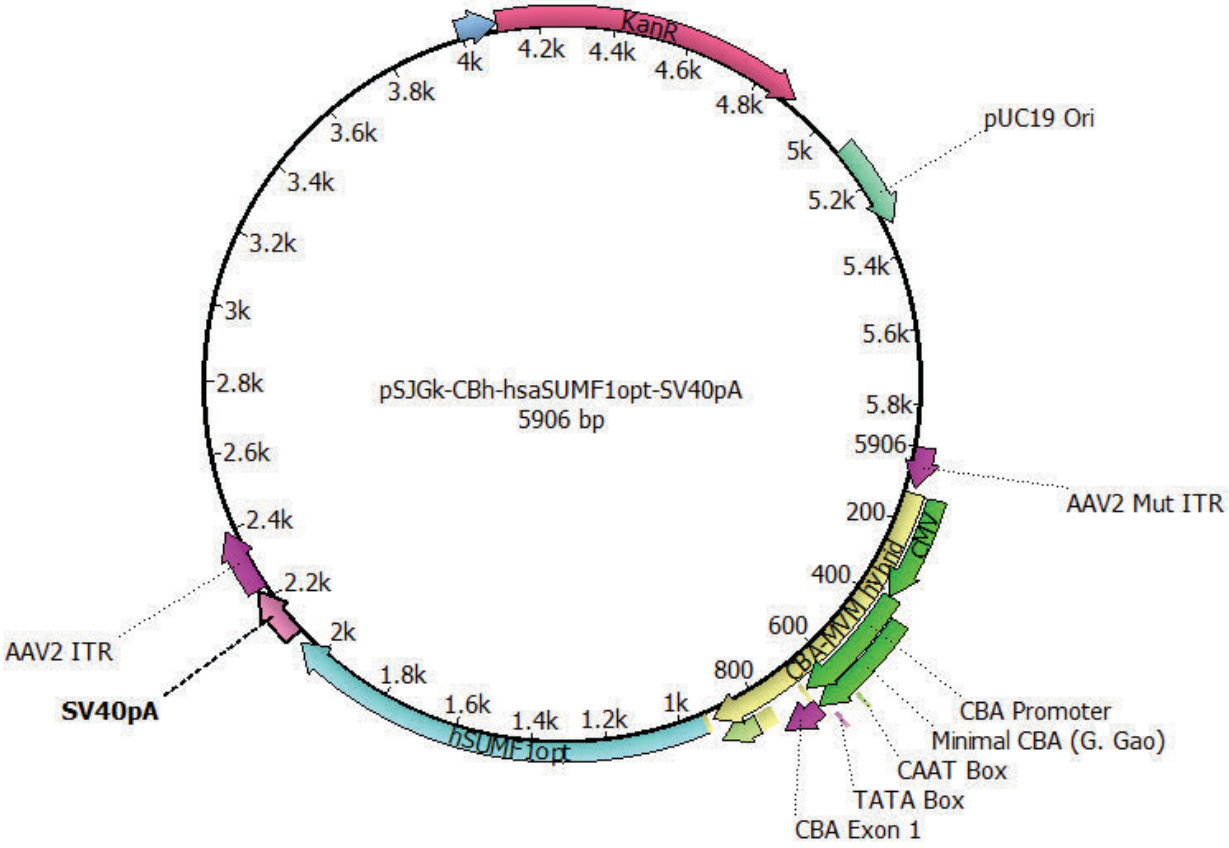


Figure 2B. Map of the pSJGk-CBh-*hSUMF1opt*-SV40pA production plasmid.

4.1.3 Drug Product Description, Mechanism of Action and Route of Administration

AAV9/SUMF1 is a biologic modality for introducing corrected DNA into MSD patients with biallelic pathogenic variants in the *SUMF1* gene. The gene is delivered using a recombinant AAV serotype 9, virus, which is non-infectious in humans but maintains its natural ability to deliver genetic material into cells. The AAV9/SUMF1 will introduce a normal copy of the human *SUMF1* gene to help restore the normal function of the FGE enzyme to post-translationally modify and activate all cellular sulfatases. AAV9/SUMF1 will be stored at -80°C and delivered to patients using the intracisternal magna (ICM) route of administration (ROA). The final formulation for the drug product has not been determined but the formulation for an initial batch for preclinical *in vivo* animal studies was 1x PBS with 5% D-sorbitol.

4.2 Scientific Rationale for the Use of *AAV9/SUMF1* in the Rare Disease or Condition

The rationale for *SUMF1* gene replacement is to restore FGE protein expression which will activate the target sulfatases that are produced normally in MSD patients. This will restore the activity of cellular sulfatases, including those that are associated with other monogenic disorders, including six forms of mucopolysaccharidoses (MPS) and metachromatic leukodystrophy (MLD)[16,30]. Leveraging trial experiences from MPS disorders and MLD, MSD also benefits from well-defined disease biomarkers that correlate with disease severity, and we anticipate we will be capable of demonstrating pharmacoresponsiveness. Specifically, the MPSII-related GAG non-reducing end (GAG-NRE) species discriminates severe from attenuated MSD cases and correlates with genotype-based severity predictions [36].

SUMF1 is ubiquitously expressed, but the majority of morbidity and mortality arises from cerebral dysfunction[14,30,31]. Therefore, preclinical studies have focused on brain-directed AAV therapy. In the *SUMF1* knock-out mouse, delivery of AAV9/SUMF1 to the cerebral ventricles or the lumbar intrathecal space improves survival, behavior, and biomarkers of disease. Median survival was extended to over a year, compared to approximately 10 days for untreated mice. Surviving treated mice performed equivalently to wild type mice on behavioral assessments of motor coordination, activity, and cognition/memory. With AAV9/SUMF1, a complete rescue of MSD is not expected, nor is it anticipated that lost neurologic function will be regained. The maximum safe dose will transduce <100% of neuronal cells, however, the cells that are transduced should be sufficient to provide prolonged benefit. Improved *SUMF1* expression in a subset of cells will allow for rescue of pathology in adjacent cells through secretion and cross-correction[13]. To this effect, the levels of secreted sulfatases are elevated in treated mice, which represent important biomarkers of treatment. Preclinical studies have not demonstrated negative consequences of nonuniform restoration of *SUMF1* expression, and overexpression-induced toxicity has not been observed in either MSD cell or mouse models[13,32]. Thus, we anticipate that partial pathologic rescue has the potential to stabilize neurologic function, benefitting symptomatic children.

4.2.1 Clinical Efficacy of AAV9/SUMF1

To date, clinical data have not been collected with AAV9/SUMF1 as a treatment option for patients with MSD. However, all data for subjects in the study will be interpreted in the context of an ongoing international prospective natural history study led by Drs. Ahrens-Nicklas and Adang. This study is funded by the United MSD Foundation (USA), MSD Action Foundation (Ireland), and NICHD R21HD106348. The natural history study was designed to mirror the proposed interventional trial, including procedures, laboratory testing, clinical evaluations, and neurodevelopmental assessments. As part of the natural history study, a minimum of 10 subjects will be enrolled and participate in two in-person study visits: baseline and at 12 months, similar to the proposed dosing and primary safety endpoint evaluation. Enrollment in the prospective natural history study is ongoing, with an anticipation study completion date by the end of 2024. In addition, the data from this interventional trial will be interpreted in the context of the ongoing retrospective natural history study (anticipated n>50 subjects)[31]. This project includes a detailed review of all clinical records and retrospective application of key clinical outcome measures including the Gross Motor Function Classification in MLD (GMFC-MLD) assessment, the proposed primary efficacy endpoint.

Furthermore, by leveraging trial and natural history data from MPS disorders and MLD, MSD also benefits from a strong understanding of disease biomarkers. Additionally, the study team has recently identified a urinary glycosaminoglycan (GAG) biomarker that correlates with disease severity subgroups. Specifically, the GAG non-reducing end (GAG-NRE) species that accumulates in MPSII can discriminate severe from attenuated MSD cases and correlates with genotype-based severity predictions[36].

4.2.2 Nonclinical Efficacy of AAV9/SUMF1

Settembre et. al, generated a *SUMF1* knock-out mouse model[12]. The mouse strain has a gene trap mutation in the intron 3, B6;129P2-*SUMF1*$^{Gt(RST760)Byg}$/ClearJ (stock number 30711) and is a null mutant for *SUMF1*. The sulfatase activities are completely absent in *SUMF1*-/- mice. These mice display severe developmental, neurological, behavioral, and histopathological deficits. While severe, the phenotype reported in these mice is consistent with the development of pathology from defects in *SUMF1*. This model is representative of the most severe form of MSD in human populations, the infantile presentation. The Jackson Labs have acquired this preclinical model, and it is being used to evaluate the safety and efficacy of the AAV9/SUMF1 therapy. Note that the colony at Jackson labs was found to have a more severe phenotype than what was reported in the literature, with a median lifespan of 1 day.

Since the *SUMF1-/-* mice stock JR 30711 did not survive longer it was not possible to test our product as an intervention at later stages in development. To address the issue Jackson Labs developed an alternate mouse model by introducing a 129S1/SvlmJ genetic background into the 30711 stock which could be considered as >90% C57BL/6J. The new stock (JR31625, *SUMF1-/-*) possesses a mixed genetic background B6;129 more similar to the initial reported stock. It is unknown which are the genetic modifiers present in 129S1/SvlmJ that can alter the severity of *SUMF1* deficiency. These KO mice have a mean survival of 10 days, comparatively better than the JR 30711. All these mice are able to survive until 5 days of age, and approximately 40% survive to 20 days, with a very small subset surviving into adulthood. The body weight gain in these mice is also better than the untreated JR 30711 stock suggesting a delayed onset of pathology. This provides us with an opportunity to evaluate our AAV9/SUMF1 therapeutic intervention at a later time.

AAV9

AAV serotype 9 was chosen for its ability to widely disseminate through the spinal cord and brain, providing neuronal tropism, as well as its spread to most peripheral organs. To lower the manufacturing burden, reduce the overall AAV9/SUMF1 exposure to the individual, and target the primary organ of interest, we propose to utilize the ICM route of administration in the proposed human trial. Biodistribution studies have already been carried out following intra-CSF AAV9 vector administration by multiple laboratories (**Table 2**). These published studies demonstrate the translational fidelity of the intra-CSF administration of AAV9 from rodents to larger animals. Dr. Gray's laboratory has conducted several recent, relevant studies to assess the biodistributions of AAV9 after administration into the CSF in mice[28], rats[29], and non-human primates[17] (comprehensively reviewed by Chen X, Gray SJ, et al. 2023). While most studies in the literature evaluated AAV9 distribution at relatively lower doses, these published and unpublished studies demonstrate the pervasive nature of high-dose CSF-delivered AAV9 to distribute across the CNS

and to peripheral organs of large animals. A recent high-dose study of AAV9/AP4M1 (equivalent to approximately $1x10^{15}$ vg total in a human) demonstrated pervasive biodistribution throughout the brain and peripheral organs of non-human primates, equivalent to the biodistribution seen in mice and rats[39]. In total, AAV9 has demonstrated the capacity to distribute a transgene across the brain in a disease-modifying manner for multiple disorders, especially for disorders that benefit from cross-correction such as MSD.

Table 2. Summary of published AAV9-CSF biodistribution studies

Species	Ref	Total AAV9 dose (vg)	AAV9 dose* (vg/kg)	Vector dose per mL CSF (vg)	Species	Ref	Total AAV9 dose (vg)	AAV9 dose* (vg/kg)	Vector dose per mL CSF (vg)
Mouse	25	$2.5x10^{9}$	$1.1x10^{11}$	$1x10^{11}$	**Pig**	21	8.6×10^{11}	4.3×10^{10}	$4.3x10^{10}$
	26	$1x10^{10}$	$5x10^{11}$	$4x10^{11}$		32	$3x10^{12}$	$1.5x10^{11}$	$1.5x10^{11}$
	27	$7.3x10^{11}$	$3.3x10^{13}$	$2.9x10^{13}$	**NHP**	26	$5.5x10^{12}$	$1.45x10^{12}$	$4.6x10^{11}$
	28	$4x10^{11}$	$1.8x10^{13}$	$1.6x10^{13}$		33	$1.8x10^{13}$	$7x10^{12}$	$1.5x10^{12}$
Cat	29	$1x10^{12}$	$1x10^{12}$	$2.3x10^{11}$		34	$1.8x10^{13}$	$7x10^{12}$	$1.5x10^{12}$
Dog	30	$2x10^{13}$	$2x10^{12}$	$1.6x10^{12}$		32	$2.5x10^{13}$	$7x10^{12}$	$2.1x10^{12}$
	31	$1.8x10^{13}$	$1.8x10^{12}$	$1.4x10^{12}$		27	$2x10^{13}$	$1x10^{13}$	$1.7x10^{12}$
						31	$2x10^{13}$	$3.3x10^{12}$	$1.7x10^{12}$

*CSF dosing was achieved via lumbar, intracisternal or intraventricular injection. Doses were normalized to body weights reported in the publications. CSF volumes in milliliters were: mouse – 0.035; cat – 4.4; dog – 12.5; pig – 20 and NHP – 12.

In vivo studies of AAV9/SUMF1

We tested AAV9/SUMF1 injection in the mixed background JR31625 *SUMF1-/-* mice at both PND1 (intracerebroventricular (ICV) only) and PND7 (IT, IV, or IT+IV routes). Uninjected or Vehicle-treated WT littermates served as healthy controls. The animals were randomly assigned to the study cohorts and all groups were monitored for growth, body condition, survival, and regular behavioral phenotyping, with an end point of up to 18 months. All of these treatment paradigms produced benefit as indicated by improved survival and behavior that was equivalent to WT littermates, including gross motor function, motor coordination, cognition, and memory. Restoration of sulfatase activity in tissues and a corresponding decrease in tissue GAGs, neuroinflammation and lysosomal markers was also seen. AAV9/SUMF1 treatment at PND7 also improved cardiac and vision function in a dose-dependent manner.

These outcomes, along with previous work on the biodistribution of AAV9 and molecular analyses of SUMF1 levels, indicate we are effectively targeting critically affected cell populations with this treatment strategy. Data from PND1 mice provided additional proof-of-concept for the therapy using CNS-directed delivery. Benefit was achieved even when AAV9/SUMF1 was delivered at a more advanced period of the disease course (PND7) (**Figure 3**).

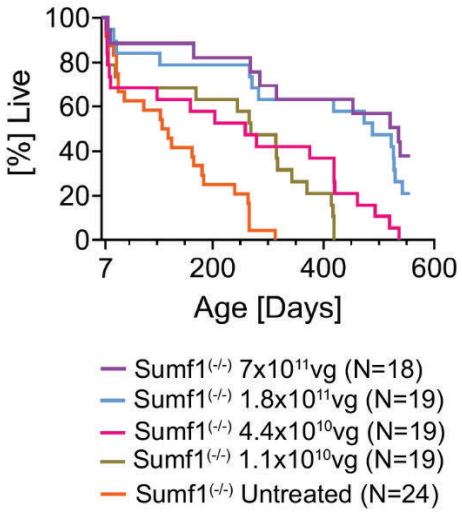


Figure 3. IT delivery of scAAV9/SUMF1 extends life span of Sumf1(-/-) mice in a dose-dependent manner. SUMF1-/- and +/+ mice treated at PND7 with vehicle or AAV9/SUMF1 were monitored until 18 months of age for survival.

Our results also indicate a clear dose-response of the treatment up to the maximum IT dose feasible in PND7 mice, justifying our selected doses for human translation. Previous reports showed the need to target systemic organs with gene therapy via an IV injection following CNS targeting via an ICV injection in order to achieve a significant benefit in *SUMF1*$^{-/-}$ mice[13]. With the improved vector design and increased dose, we found that CSF-delivery alone was sufficient for rescue and that a combination IT+IV dosing did not significantly increase treatment benefit over brain-directed alone at PND7. A summary of the nonclinical studies from mice is provided in **Table 3**.

Table 3. AAV9/SUMF1 dose levels tested in the non-GLP preclinical studies. *SUMF1*$^{-/-}$ mice on a B6/129S background were characterized by a median survival of 30 days and 10% of mice living >180 days[33]. Backcrossing these mice onto a C57BL/6J background (Jackson Laboratories stock number JR30711) resulted in a median survival of 1 day and a maximum life span of 13 days, so use of this strain was limited to PND1 injection of AAV9/SUMF1. Hybrid vigor was introduced by crossing heterozygous B6-*SUMF1*$^{-/+}$ mice with the inbred stock 129S1/J and heterozygous mice from this F1 generation were intercrossed to generate 129S1B6 mixed background *SUMF1*$^{-/-}$ homozygous mice (stock number JR31625). The JR31625 mice showed a median survival of 9-10 days with about 10 % of mice living more than 180 days.

Age at Treatment	Strain	Geno-type	Route/Dose (vg/mouse)	Median Survival	Key Outcomes/Conclusions
PND1	30711	WT	ICV/0	>440 days	
PND1	30711	WT	ICV/2.8E11	>440 days	• AAV9/SUMF1 was safe and well-tolerated • Behavior was normal at 9 and 12 months of age
PND1	30711	-/-	ICV/0	1 day	• Underweight relative to WT controls • Did not survive long enough for phenotyping.
PND1	30711	-/-	ICV/2.8E11	22 days	• Remain underweight relative to WT controls • 44% of treated mice survived >440 days • Behavior of surviving mice was comparable to WT at 9, 12 mo
PND1	31625	WT	ICV/0	>420 days	
PND1	31625	-/-	ICV/0	~10 days	• Underweight relative to WT controls • Unable to be assessed for behavior due to early lethality • Decreased sulfatase activity in brain and liver
PND1	31625	-/-	ICV/2.8E11	325 days	• Underweight relative to WT controls • 39% of treated mice survived >420 days • Normal behavior at 1.5, 6 and 9 months • Significant increase in brain sulfatase activity, small liver increase
PND7	31625	WT	IT/0	>550 days	
PND7	31625	WT	IT/7.0E11	>550 days	• AAV9/SUMF1 was safe and well-tolerated • Behavior normal at 1.5, 6 and 12 months of age
PND7	31625	WT	IT+IV/1.2E12	>550 days	• AAV9/SUMF1 was safe and well-tolerated • Behavior normal at 1.5, 6 and 12 months of age • Sulfatase activity in brain and liver similar to control WT
PND7	31625	-/-	IT/0	114*	• Underweight relative to WT controls • Progressive behavioral deficits from 1.5 to 6 months • Decreased heart rate and vision by 9 months • Decreased sulfatase activity in brain and liver • GAG accumulation in heart and liver • Lysosome accumulation and astrogliosis in brain
PND7	31625	-/-	IT/1.1E10	269	• Remain underweight relative to WT controls • Cardiac dysfunction at 9 months of age • Delayed vision degeneration after 9 months • Similar sulfatase activity as untreated -/- mice
PND7	31625	-/-	IT/4.5E10	259	• Remain underweight relative to WT controls • Similar sulfatase activity as untreated -/- mice
PND7	31625	-/-	IT/1.8E11	488	• Remain underweight relative to WT controls • Increased sulfatase activity • Decreased GAGs in heart

					• Similar lysosome accumulation and astrogliosis in brain as WT
PND7	31625	-/-	IT/7.0E11	535	• Remain underweight relative to WT controls • Behavior normal at 1.5, 6 and 12 months • Normal visual acuity and cardiac function at 12 months • Increased sulfatase activity • Decreased GAGs in heart and liver • Similar lysosome accumulation and astrogliosis in brain as WT
PND7	31625	-/-	IT+IV/1.2E 12	>550 days	• Remain underweight relative to WT controls • Behavior normal at 1.5, 6 and 12 months • Normal visual acuity and cardiac function at 12 months • Increased sulfatase activity • Decreased GAGs in heart and liver • Similar lysosome accumulation and astrogliosis in brain as WT

*median survival excludes those mice that died prior to PND7

Analysis of vector expression from mice treated at P7, obtained as mRNA copies of hSUMF1opt, showed a very wide distribution across multiple tissues with heart, liver and lung being the highest and kidney and spleen being the lowest expressed tissues. The expression levels were dose-dependent, where a single high dose IT was able to provide similar expression levels as combined dosing via IT/IV, while the single dose via IT at 1:4 dilution still achieved high expression levels in the brain, spinal cord tissues and more moderated efficiency in peripheral organs like heart, liver, and lungs **(Figure 4)**.

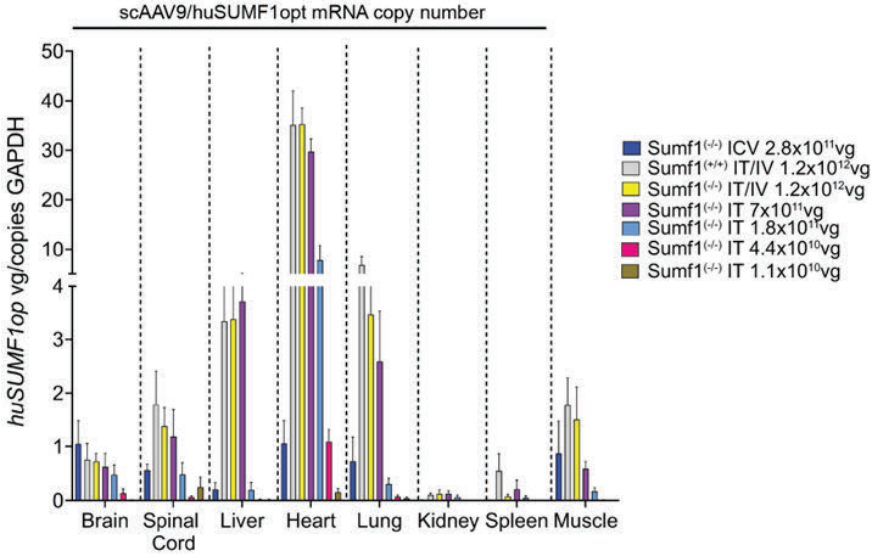


Figure 4. Robust *Sumf1* expression from AAV9/SUMF1 treatment. AAV9/SUMF1 vector copy number was determined at mRNA level by RT-qPCR, on brain, spinal cord, liver, heart, lung, kidney, spleen, and muscle. Vector cDNA is reported as the number of vector copies per copy of host *Gapdh*. Data represent mean ± SEM, N=5-9 by experimental group.

Behavioral impairments in *SUMF1-/-* mice are similar to the those seen in animals with other lysosomal disorders[13,33,34]. Mice treated with AAV9/SUMF1 at both PND1 and PND7 were assessed for motor coordination by rotarod, general activity by open field, and cognition-memory through spontaneous alternation test. At the time of testing for the PND1 cohort (9 and 12 months of age), the number of vehicle and untreated *SUMF1*$^{-/-}$ mice alive was too small to be assessed. Compared to WT controls, ICV-treated KO mice did not show significant differences in rotarod, open field and spontaneous alternation activity (data not shown). Similarly, KO mice receiving maximum doses of AAV9/SUMF1 by the IT only or IT+IV routes did not show significant differences from WT controls in behavioral tests at 6, 26, or 52 weeks of age. Together, these data support that AAV9/SUMF1 treatment normalizes behavioral phenotypes and extends lifespan (**Figure 3**). Next, we tested the potency of AAV9/SUMF1 therapy in a dose-response experiment using IT-only treatment. The highest dose and the 1:4 dose showed similar rescue of early lethality, while more diluted administrations were less effective in achieving a strong survival response (**Table 4**).

Table 4. AAV9/SUMF1 treatment extended lifespan of *SUMF1* -/- mice in a dose-dependent manner.

Treatment Group*	Dose ($vgx10^{11}$)	Mice (N/sex)	Genotype	Median Survival (Days)	Avg. BW at 556 days (g)	% Live at 556 days	*p-value versus High Dose
High Dose	7	18(10F/8M)	-/-	535	25.3 ± 1.2	38	
1:4	1.8	19(10F/9M)	-/-	488	23.6 ± 0.8	21	0.3438
1:16	0.45	19(10F/9M)	-/-	259	24.5 ± 0.0	0	0.0009
1:64	0.11	19(10F/9M)	-/-	269	14.9 ± 3.6	0	0.0004
Untreated	n/a	24(13F/11M)	-/-	114		0	<0.0001

*Mice enrolled at PND7. **Log-rank (Mantel-Cox) test. BW = body weight.

SUMF1$^{-/-}$ mice are reported to present signs of cardiac inflammation[12], and EKG analysis revealed a significant reduction in the heart rate of vehicle treated KO mice compared to WT controls. Both high dose combined IT+IV and IT alone treatment at PND7 restored the heart rate to normal levels in KO mice (**Figure 5A**). The level of restoration was dose-dependent, as KO mice treated with the low dose (1:64) had low heart rates comparable to vehicle treatment. Additionally, heart rate was normal in IT+IV treated WT mice, supporting that this treatment is well-tolerated.

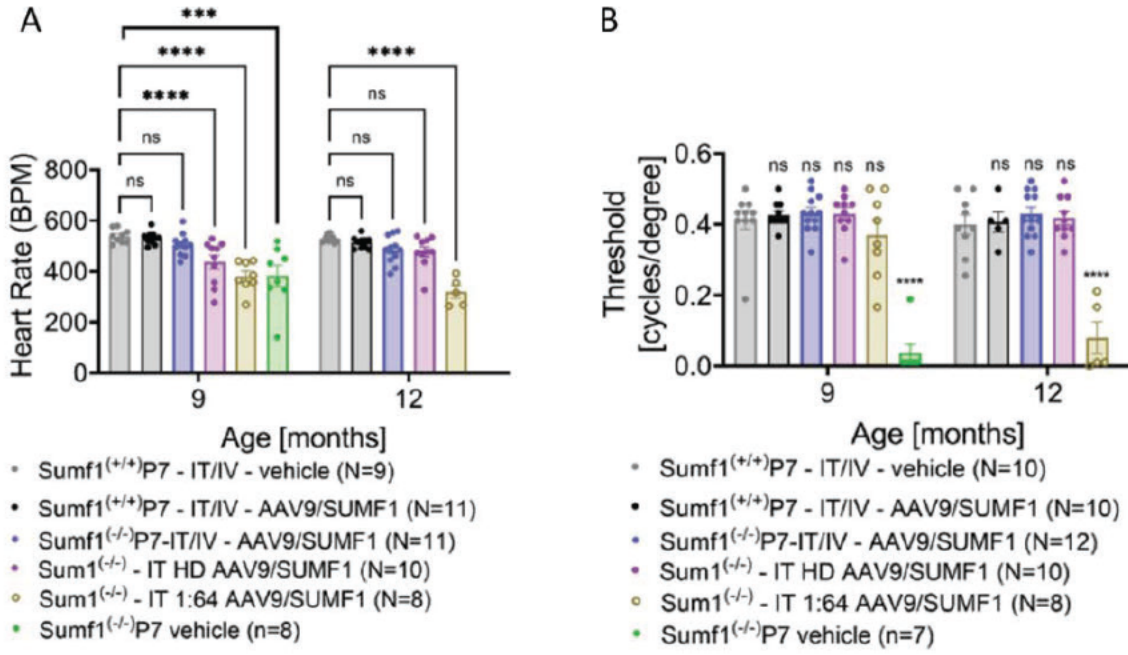


Figure 5. Dose-dependent AAV9/SUMF1 rescue of cardiac and vision deficits in SUMF1-/- mice. SUMF1-/- and +/+ mice treated at PND7 with vehicle or AAV9/SUMF1 were assessed at 9 and 12 months of age via EKG analysis of heart rate (A) and optokinetic threshold measurement for vision (B). ****p<0.0001, ns = not-significant, two-way ANOVA, Sidak's multiple comparison. KO mice were not assessed at 12 months due to early lethality.

Vision defects have also been reported in MSD-patients and other mouse models with deficiencies in sulfatases. A vision acuity test (optokinetic thresholds) identified a severe deficit in the small subset of vehicle-treated KO mice which survived until testing at 9 months old. All AAV9/SUMF1 treated KO mice showed normal visual acuity at 9 months old, even those treated at the 1:64 lowest dose (**Figure 5B**). However, at 12 months only the KO mice treated with high dose, either IT alone or IT+IV, still had normal visual acuity levels.

Sulfatase enzymatic activity was previously shown to be decreased in tissue homogenates from KO mice and in the patient fibroblasts[2]. At the endpoint of treatment, brain and liver were assessed for arylsulfatase A (ARSA), arylsulfatase B (ARSB), arylsulfatase C (ARSC), and arylsulfatase E (ARSE) activities **(Figure 6).** Brain was also assessed for iduronate-2-sulfatase (IDS) sulfatase and n-sulphoglucosamine sulphohydrolase (SGSH) activities. Liver was additionally assessed for N-acetylgalactosamine-6-sulfatase (GALNS) activity. It should be noted that ARSA and IDS are secreted and may be measured in the plasma/serum. ARSC is not secreted but localized to microsomes and can be detected in the tissue lysates along with ARSA and IDS.

The IT treatment with AAV9/SUMF1 resulted in a significant increased activity for all sulfatases tested in brain tissue, reaching levels between 20 – 30% of WT activity, while in liver levels reached between 10-20% of WT activity (**Figure 5**). Sulfatase analysis for ARSA, ARSB, ARSE, IDS, and SGSH, showed a clear dose-dependent efficacy in terms of restoration of sulfatase activity, with the high dose IT+IV and single IT (high dose) as the most effective followed by IT at 1:4 dilution (**Figure 6**).

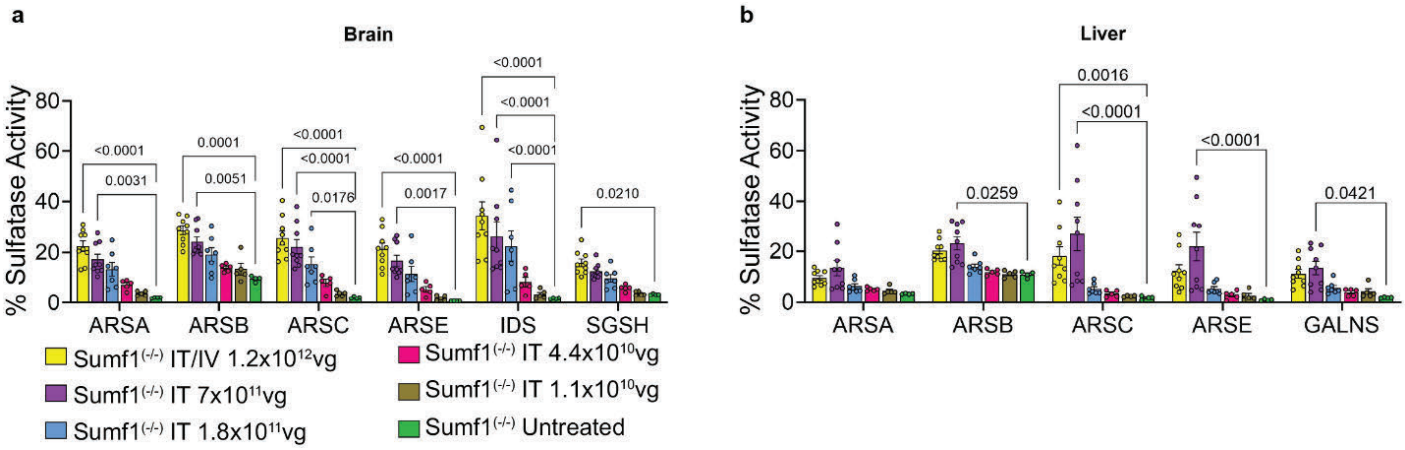


Figure 6. Dose-dependent increase of sulfatase activity in PND7 AAV9/SUMF1 treated SUMF1-/- mice. SUMF1-/- and SUMF1+/+ mice received a combination IT/IV injection or varying doses of a single IT injection of AAV9/SUMF1 or vehicle at PND7. Brain tissues were tested for sulfatase activities at 18 months post-injection and values reported as a percentage of the average sulfatase activity of SUMF1+/+ IV/IT vehicle treated mice. SUMF1-/- : *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001; two-way ANOVA, Dunnett's multiple comparison test versus SUMF1-/- I/ITV Vehicle. SUMF1+/+: ns = not significant, unpaired t-test.

As expected, upon treatment the increase in cellular activity of sulfatases, along with the increase in secreted sulfatases, reduced the GAG storage in the tissues assessed, including the liver. In the neuronal tissues of KO mice there is upregulated immunoreactivity of astrogliosis (GFAP) markers and increased lysosomal markers (LAMP), which was significantly decreased with treatment (**Figure 7**).

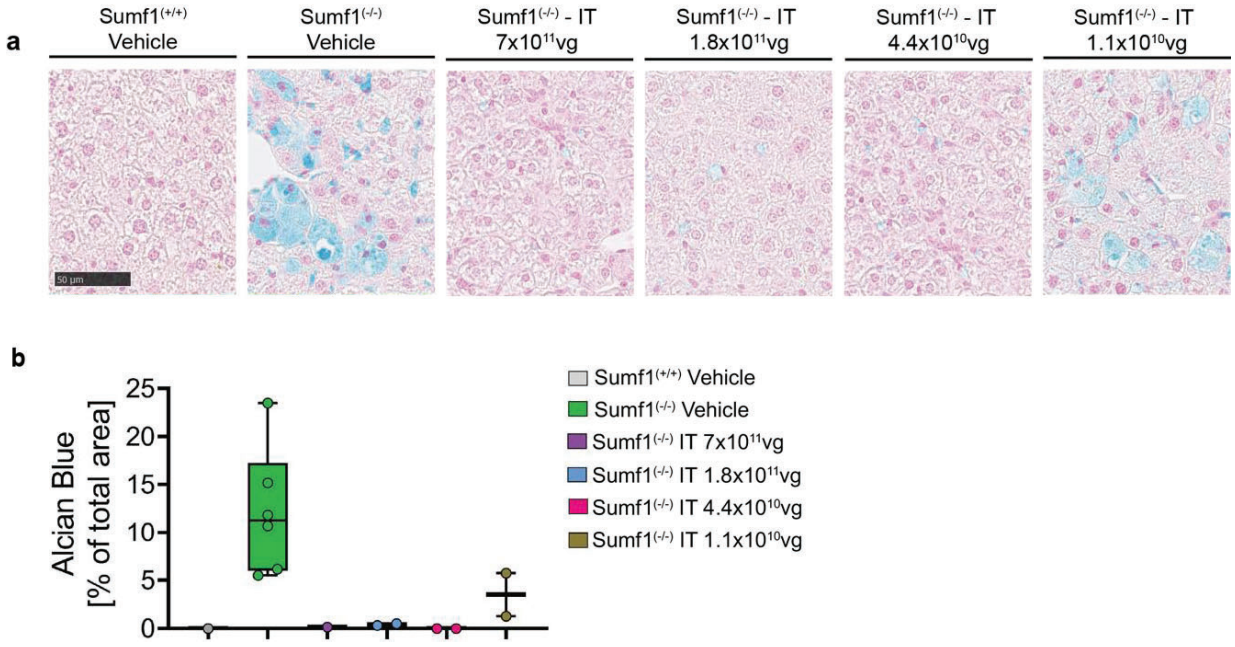


Figure 7. Decrease of liver GAGs in PND7 AAV9/SUMF1 *treated SUMF1*$^{-/-}$ mice. GAG tissue accumulation, was detected by Alcian blue staining on liver sections of mice IT injected with decreasing dose of AAV9/SUMF1, starting at high dose ($7x10^{11}$ vg/mouse), 1:4 dilution ($1.8x10^{11}$ vg/mouse), 1:16 dilution ($4.4x10^{10}$ vg/mouse), 1:64 dilution ($1.1x10^{10}$ vg/mouse). Sumf1$^{(-/-)}$ and Sumf1$^{(+/+)}$ vehicle-injected are included as control. (A) representative images of liver sections. Scale bar represents 50 µm. (B) Alcian blue quantification, represented as percentage of stained area.

5 ORPHAN DRUG STATUS

AAV9/SUMF1 is not designated as an orphan drug or product for the treatment of patients with MSD, and AAV9/SUMF1 is not designated as an orphan drug or product in the United States for any other indication.

6 PATIENT SUBSET CONSIDERATIONS AND MEDICAL PLAUSIBILITY OF THE CHOSEN SUBSET

7 REGULATORY STATUS AND MARKETING HISTORY

AAV9/SUMF1 is currently in late phase pre-clinical development. University of Texas Southwestern Medical Center held a Pre-IND meeting (Meeting ID# 11357; PTS# PS003895) with FDA, Center for Biologics Evaluation and Research (CBER), Office of Tissues and Advanced Therapies (OTAT), on September 18, 2018, was held to discuss completed pilot efficacy and toxicology studies and planned IND enabling toxicology studies. An IND submission is planned for the second quarter of 2025.

AAV9/SUMF1 is not currently marketed or approved for use in any country, including the United States. In accordance with section 526(a)(1) of the Federal Food, Drug, and Cosmetic Act [21 USC 360bb(a)(1)]; 21 CFR 316.20(b)(7); and 21 CFR 316.23(a), a marketing application has not previously been submitted to FDA for the same active moiety in AAV9/SUMF1for the same rare disease or condition prior to submission of the AAV9/SUMF1 orphan drug designation request.

8 DOCUMENTATION OF PATIENT POPULATION SIZE

MSD is an ultra-rare neurometabolic disorder, with approximately 150 cases of MSD described in literature as documented in recent meta-analysis[14], and 50 living individuals registered among patient-advocacy groups[16]. Data to estimate prevalence are limited. In a systematic cross-sectional survey of MSD, Cappucio et al, 2020, utilized data from Meikle et al, 1999, the Genome Aggregation Database (gnomAD), and estimated a disease carrier frequency of 1 in 355, and an estimated disease prevalence of approximately 1 in 500,000[6,35].

9 RARE PEDIATRIC DISEASE DESIGNATION JUSTIFICATION

Based on the evidence presented above, pursuant to Section 908 of FDASIA, and consistent with the Rare Pediatric Disease Priority Review Vouchers Guidance for Industry, the National Institutes of Health, National Center for Advancing Translational Sciences (NCATS requests that Adeno-Associated virus 9 Sulfatase modifying factor 1 (AAV9/SUMF1) be designated as a Rare Pediatric Drug Product for the treatment of Multiple Sulfatase Deficiency (MSD).

Based on the evidence presented above, pursuant to Section 908 of FDASIA[37] and consistent with the Rare Pediatric Disease Priority Review Vouchers Guidance for Industry[38] we believe that AAV9/SUMF1 qualifies as a Rare Pediatric Drug Product for the treatment of MSD. Specifically:

1) MSD is a serious or life-threatening disease in which the serious or life-threatening manifestations primarily affect individuals aged from birth to 18 years.

Most MSD patients are currently identified after symptom onset. Early symptoms include developmental delay, especially difficulties with speech. Patients can also have growth differences, recurrent respiratory infections, orthopedic complications, and other features commonly seen in MPS disorders[14]. Despite initiation of best-available supportive therapies, severely affected patients continue to experience serious or life-threatening complications of their disease. The majority of patients will manifest irreversible serious end- organ damage, including progressive neurologic dysfunction, during childhood and adolescence, and there is a need for better treatments to prevent, mitigate or ameliorate these disease-related complications. While AAV therapy will be administered to the CNS, it is known that in similar brain directed AAV programs, there is significant transduction of tissue outside of the brain including the liver and the spleen[40]. Therefore, there is a possibility that non-CNS symptoms of disease may be improved with therapy.

2) MSD is a rare disease, with an estimated prevalence of fewer than 50 patients in the US.

We additionally note that AAV9/SUMF1 is intended as a gene therapy specifically for gene replacement due to mutations in the FGE enzyme and has no foreseeable use in the treatment of any other disease or a different adult indication. AAV9/SUMF1 currently in late pre-clinical phases of testing at UT Southwestern, with planned progression to clinical trials within the next few years. The initial patient population will include children above 12 months of age.

We therefore request that AAV9/SUMF1 be designated as a Rare Pediatric Disease Product for the treatment of the MSD.

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APPENDIX A: NUCLEOTIDE SEQUENCE OF *HSUMF1OPT* TRANSGENE

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