PreIND Meeting

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Meeting format: Teleconference

Briefing Document

AAV9/SUMF1 GENE THERAPY FOR TREATMENT OF SUBJECTS WITH MULTIPLE SULFATASE DEFICIENCY

Sponsor

[redacted]

Confidential

Submitted By

[redacted]

Table of Contents

1. Summary 8
   1.1. Description of the Genetic Disorder 8
   1.2. Clinical and Pathological Presentation of MSD 9
   1.3. Current Standard of Care 10
   1.4. Approach and Rationale 10
   1.5. Therapeutic Limitations 11
2. Introduction 11
   2.1. Product Summary 11
   2.2. Chemical Name and Structure 12
   2.3. Proposed Indication 12
   2.4. Dosage Form, Route of Administration, and Dosing Regimen 12
   2.5. Target Product Profile 12
3. PreIND Meeting 15
   3.1. List of Specific Objectives 15
      3.1.1. Nonclinical 15
      3.1.2. Chemistry, Manufacturing and Controls 15
      3.1.3. Clinical 15
   3.2. Proposed Agenda 16
   3.3. List of Sponsor Attendees 16
   3.4. List of Requested FDA Attendees 16
4. Preclinical Studies (Ongoing and Proposed) 17
   4.1. Preclinical Mouse Model for MSD 18
      4.1.1. Development of an Alternate Model Sumf1-/- KO mice stock JR 31625 20
      4.1.2. Limits Imposed by the Available Models 20
   4.2. Biology of the SUMF1/FGE enzyme (comparability of mouse to human) 20
   4.3. Viral Vector Use for AAV9/SUMF1 Gene Therapy 22
   4.4. Preclinical Study Design 24
   4.5. AAV9/SUMF1 Formulation and Administration Protocol 25
   4.6. Preliminary Preclinical Data 26
      4.6.1. AAV9/SUMF1 Improves Survival in Sumf1-/- mice (stock JR 30711) 26

[redacted]

4.6.2. Tissue Sulfatase Activity 27
4.6.3. Reduction in GAG Storage 28
4.6.4. Behavioral Testing 28
4.6.5. Neuroinflammation 28
4.7. Safety Studies 28
4.7.1. Pilot Safety/Survival Studies 28
4.7.2. Biodistribution 29
4.7.2.1. scAAV9/CBh-GFP – Mouse IT Biodistribution 29
4.7.2.2. scAAV9/GAN – Rat IT Biodistribution 31
4.7.2.3. scAAV9/JeT-GANopt – Human IT Biodistribution 31
4.8. Proposed Pivotal IND-enabling Studies 31
4.8.1. Pivotal GLP-Compliant Toxicology Study 31
4.9. Preclinical Summary 34
5. Overview of the Biological Product and Manufacturing Components 35
5.1. Description of Components Used in Manufacturing 35
5.1.1. Vector, Packaging and Helper Plasmid DNA 35
5.1.2. Plasmid DNA Manufacturing, Testing and Qualification 35
5.1.3. Expi293F™ (cGMP Banked) Master Cell Bank 35
5.1.3.1. Origin and History of HEK293 (Expi293F™) Production Cells 36
5.1.3.2. Testing and Specification of Expi293F™ Cell Bank 36
5.2. AAV9/SUMF1 Vector Manufacturing 36
5.2.1. Introduction 36
5.2.2. Overview of rAAV Manufacturing at the UTSW Facility 36
5.2.2.1. Initiation and Expansion of Suspension Expi293F™ SWCB Cultures 37
5.2.2.2. Assembly of Transfection Plasmid Cocktail Mix 37
5.2.2.3. Transfection of Bioreactor Culture 37
5.2.2.4. Harvesting Cell Culture from Bioreactor (to be updated) 37
5.2.2.5. POROS-AAV9 Resin Affinity Chromatography 39
5.2.2.6. Empty/Full Separation by IEX 39
5.2.2.7. Final Formulation by TFF and Sterile Filtration 39
5.3. Quality Testing Program for AAV9/SUMF1 39
5.4. AAV9/SUMF1 Product Stability Testing 40

[redacted]

5.4.1. In Vitro Potency Assay 40
5.5. Storage, Transportation, Formulation and Retention of AAV9/SUMF1 Clinical Lot 41
5.6. Facility Location 41
5.7. Facility Operations 41
5.7.1. Gene Therapy Vector Manufacturing Facility Records 41
5.8. Facility Layout 41
5.9. Facility Description 43
5.10. Categorical Exclusion from Environmental Assessment 45
6. Proposed Phase I/II Clinical Trial 45
6.1. Investigators at the University of Texas Southwestern 45
6.2. Clinical Collaborators and Subject Matter Experts 46
6.3. Synopsis of Clinical Trial I/II 47
6.4. Draft of Informed Consent for the Trial 51
6.5. Justification for Clinical Studies Dose 51
6.6. Brain Volume Dependent Dosing for Phase I/II 52
6.7. Dosing Device for Phase I/II Clinical Trials 52
6.8. Protocol for Gene Therapy 52
6.8.1. Screening/Baseline Measures Prior to Vector Injection (Day -28 to Day -7) 54
6.8.2. Pre-infusion visit (Day -1) 55
6.8.3. Day of Gene Transfer (Day 1) 56
6.9. Post Therapy Monitoring 58
6.9.1. Early Inpatient Monitoring (up to 48 hours of AAV9/SUMF1 dose) 58
6.9.2. Follow up monitoring (may be revised to eliminate visits at day 270 and 540) 59
6.9.2.1. In patient follow up (Day 7, 30, 60, 90, 180, 270, 360, 540 and 720) 59
6.9.2.2. Outpatient follow up (Day 14±3) 60
6.9.3. Long Term Monitoring 61
6.10. Outcome measures 61
6.11. Statistical Analysis 64
6.12. Proposed Clinical Development Plan 64
7. References 64

[redacted]

List of Figures

Figure 1: Sumf1-/- strain JR 31625 has improved survival compared to JR 30711. 19
Figure 2. Similarities in protein sequence between different species..... 21
Figure 3. Study plan, duration and readouts for Sumf1 and wild-type phenotypes 23
Figure 4. AAV9/SUMF1 therapy improves survival of Sumf1-/- mice (stock JR 30711). 27
Figure 5. AAV9/GFP biodistribution in WT mice. 30
Figure 6. AAV9/GAN biodistribution in WT rats. 32
Figure 7. Biodistribution of scAAV9/hGANopt in a human subject at 8 months post-injection..... 32
Figure 8. Flow chart of AAV9/SUMF1 cGMP manufacturing. 38
Figure 9. Layout of GMP manufacturing facility at UTSW for AAV9/SUMF1 production. 42
Figure 10. Gowning room. 44
Figure 11. Prep room. 44
Figure 12. ISO5 rated biological safety cabinet in downstream processing room. 45

List of Tables

Table 1: Published SUMF1 mutations and phenotypes 8
Table 2: Overview of MSD impact on organ systems 9
Table 3: Clinical presentation of MSD subtypes and severity 10
Table 4: Summary of the translational strategies 10
Table 5: AAV9/SUMF1 target product profile. 13
Table 6: Summary of non-GLP murine cohorts 17
Table 7: Summary of the published Sumf1 knockout phenotype 18
Table 8: JR 31625 Sumf1 strain animal assignment for natural history study 19
Table 9: AAV9/SUMF1 dose levels tested on the non-GLP preclinical studies 25
Table 10: Study activity schedule for non-GLP studies 25
Table 11: JR 30711 Sumf1 strain animal assignment for non GLP studies 26
Table 12: Summary of neuronal histopathology following AAV9/SUMF1 treatment 29
Table 13: Summary of published AAV9-CSF biodistribution studies 29
Table 14: Biodistribution study design for AAV9 dosed IT in mice 29
Table 15: Biodistribution study design for AAV9 dosed IT in rats 31
Table 16: Proposed lot release criteria for AAV9/SUMF1 vectors for toxicology studies 33
Table 17: Treatment groups for safety assessment of AAV9/SUMF1 in rats 34
Table 18: *Release tests and specifications for suspension cell* 36
Table 19: Proposed lot release for clinical grade AAV9/SUMF1 vector 39
Table 20: cGMP drug product stability testing program 40
Table 21: Standardized neuropsychological assessments and self-report/proxy-report measures 50
Table 22: Relationship between preclinical study doses and proposed human IT dose 51
Table 23: Dose extrapolation based on age and brain volume 52
Table 24: Visit and monitoring schedule (May revise to eliminate visits at day 270 and 540) 53

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Abbreviations	
BSC	Biological Safety Cabinet
CBER	Center for Biologics Evaluation and Research
CMO	Clinical Manufacturing Organization
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CRO	Clinical Research Organization
DNA	Deoxyribonucleic Acid
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
FGE	Formyglycine Generating Enzyme
GAN	Giant Axonal Neuropathy
GCP	Good Clinical Practice
GFP	Green Fluorescent Protein
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
ICH	International Conference on Harmonisation
IND	Investigational New Drug
IRB	Institutional Review Board
IT	Intrathecal
ITR	Inverted Terminal Repeats
ICV	Intracerebroventricular
IV	Intravenous
KO	Knock-out
LSD	Lysosomal Storage Disease
MCB	Master Cell Bank
Mg	Milligrams
MSD	Multiple Sulfatase Deficiency
NCT	National Clinical Trial
NMR	Nuclear Magnetic Resonance
OMIM	Online Mendelian Inheritance in Man
PCR	Polymerase Chain Reaction
rAAV	Recombinant Adeno-Associated Virus
RT-qPCR	Real-Time Quantitative Polymerase Chain Reaction
SAE	Serious Adverse Event
SAN	Salt Active Nuclease
scAAV	Self-complementary adeno-associated virus
SWCB	Suspension Working Cell Bank
USP	United States Pharmacopeia
T2	Transverse relaxation time
TFF	Tangential Flow Filtration

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1. Summary

The overall goal of this requested meeting is to obtain guidance from the Food and Drug Administration (FDA) on a proposed investigational new drug (IND) for multiple sulfatase deficiency (MSD; OMIM # 272200), a terminal, autosomal recessive pediatric genetic ultra-rare neurometabolic disease. Our aim is to deliver a copy of *Sulfatase Modifying Factor 1* (*SUMF1*) gene via a recombinant adeno-associated virus (rAAV) vector to individuals affected by MSD.

1.1. Description of the Genetic Disorder

The 9-exon *SUMF1* gene encodes formylglycine-generating enzyme (FGE), which is required for post-translational modification and activation of sulfatase enzymes [1]. As such pathogenic mutations in the *SUMF1* gene impact the function of all 17 human sulfatase enzymes [2, 3]. FGE modifies a common active site cysteine residue into C-alpha-formylglycine. Without this post-translational modification, sulfatase activity is absent [1]. The correlation between different specific *SUMF1* mutations, alteration in enzyme activity, and clinical presentation has not been fully elucidated [4]. Some of the variety of mutations reported in the literature and the associated disease phenotype are listed in table 1. However, the genotype-phenotype association is not well understood.

Table 1: Published SUMF1 mutations and phenotypes

Genetic change	Mutant protein	Exon impact	Disease phenotype	Ref
661delG	fs, trun;	5	Neonatal, severe	3, 17
777C>G	N259K		Neonatal, severe	19
725+1G>C; 776A>G; 1018T>C	P202_R242del; N259S;Y340H	5, 6, 9	Neonatal, severe	16
979C>T	R327X		Neonatal, severe	8
IVS3+5-8del; 979C>T	A149_A173del ; S327X	3 , x	Neonatal, very severe	17
536G>C	W179S	4	Late infantile, mild	15
529G>C; 748delC	A177P; L250fs	4, 6	Late infantile, mild	15
788G>T	G263V		Late infantile, mild	8
836C>T	A279V	6	Late infantile, mild	15, 16
519+4A>G; 893C>A	A149_A173del, A175del; A298E	3, 7	Late infantile, mild	16
520_954dup	V174_P318dup	4 to 7	Late infantile, mild	16
337G>A; 519+5_519+8del	E113K; A149_A173del	2, 3	Late infantile, severe	6
463C>T	S155P		Late infantile, severe	8
739G>C	G247R		Late infantile, severe	
1033C>T	R345C		Late infantile, severe	8
1045C>T	R349W	9	Late infantile, severe	15, 17
132_133insG; 1045C>T	V45fsX75; R349W	1, 9	Late infantile, severe	16
706C>T; 1045C>T	R236X; R349W	5, 9	Late infantile, severe	16
463T>C; 1029G>T	S155P; R343S	3, 9	Juvenile	16
IVS3+5-8del; 1076C>A	A149_A173del ; S359X	3 , 9	unspecified	3, 17
1006T>C; 1046G>A	C336R; R349Q	9	Severe	17
243delC; 836 C>T	fs, trun; A279V	6	unspecified	17

fs – frame shift; dup – duplication; del – deletion

[redacted]

1.2. Clinical and Pathological Presentation of MSD

Individuals affected by MSD exhibit a constellation of neurologic and somatic features that overlap with known inherited single sulfatase disorders (i.e., metachromatic leukodystrophy (MLD) and five mucopolysaccharidoses (MPS) subtypes, X-linked ichthyosis and X- linked chondrodysplasia punctata). In addition all other sulfatases without known clinical correlation also contribute to the complex and variable phenotype found in individuals with MSD [4]. Some of the organ-specific clinical manifestations of the disease are summarized in table 2.

Table 2: 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

*Adapted from [4]

There are four clinical subtypes of MSD based on the predominant symptoms and ages of onset as outlined in table 3 [5, 6, 7, 8]. The neonatal subtype is characterized by severe mucopolysaccharidoses-like symptoms occurring in the first months of life and usually leads to early death before 1 year of age. The late infantile forms include a severe and mild form that onset before or after 2 years of age, respectively. The late infantile forms are characterized by progressive neurodegeneration, such as that observed in metachromatic leukodystrophy; however, individuals may also demonstrate MPS-like somatic symptoms. The juvenile subtype is characterized by a later onset and attenuated symptoms. Although this is the "mildest" form of MSD, individuals with juvenile MSD are affected by severe neurologic impairment by early childhood and premature death. While the existence of an adult-onset form of the disease has been postulated, no genetically confirmed adult-onset individuals have been reported in the literature.

In summary, all clinical subtypes of MSD present in early childhood and experience severe, progressive central nervous system (CNS) dysfunction. Additionally, most Individuals also are affected by extensive somatic involvement, and unfortunately, all affected individuals die by early adulthood mostly due to secondary problems as a result of MSD symptoms.

Table 3: Clinical presentation of MSD subtypes and severity

MSD subtype	Onset (years)	Signs and symptoms
Neonatal	First months of life	Most severe form often fatal before 1 year of age; neuronal deterioration, seizures, motor deficits, delayed growth and development; dry/scaly skin, skeletal abnormalities, stiff joints, facial deformities; hearing loss, malformation of heart, enlarged liver and spleen
Severe late infantile	< 2	Most common form; Normal cognitive development early on; Rapidly progressive psychological and motor deterioration; neuronal deterioration; scaly skin, skeletal and facial abnormalities; milder form shows lesser number of the symptoms listed above
Mild late infantile	> 2	
Juvenile	2 – 4	Rarest form; Normal cognitive development; attenuated psychological and motor deterioration starts later, and progress is slower; scaly skin

*Adapted from [5, 6, 7, 8]

1.3. Current Standard of Care

There are currently no specific treatments available for this disorder. Individuals affected by MSD are managed by supportive care, consultation with medical professionals from multiple disciplines, physical therapy, and pharmacological interventions to alleviate symptoms [4]. Gene therapy may represent a reasonable and promising approach to provide a meaningful and long-term therapeutic benefit for this population in the near future.

1.4. Approach and Rationale

Our group and other investigators have utilized recombinant adeno-associated viral vector type 9 (AAV9) directed at the CNS in ongoing human clinical trials for gene therapy (clinical trial.gov identifiers NCT02122952, NCT02362438, NCT02725580, NCT02716246, NCT03315182). These vectors are non-pathogenic, non-replicating, and transduce non-dividing cells. The recombinant vectors are however incapable of coding viral proteins or actively integrating with the host genome making them an ideal vector currently available for the gene delivery (reviewed by [9, 10]). Additionally, AAV9 can be purified in large quantities at high concentrations for potential use in delivering a functional copy of a gene to cells with aberrant, disease causing mutations.

In disorders of neurologic origin direct CNS administration is utilized to achieve broad transgene distribution in the CNS. We have advanced this approach with intrathecal (IT) administration of AAV9 vector for the treatment of Giant Axonal Neuropathy (GAN). The laboratory of Dr. Steven Gray, in partnership with Hannah's Hope Fund, initiated a first-in-human Phase I gene therapy clinical trial for GAN using this approach, in collaboration with Dr. Carsten Bonnemann at the US National Institutes of Health Clinical Center (NCT02362438) in 2015. The key components of our strategy are to utilize an approach similar to our previously developed GAN gene therapy (IND 16003) and are summarized in table 4.

Table 4: Summary of the translational strategies

Strategy	Rationale
***Sumf1* -/- mice**	Best translational model currently available that mirrors the human neonatal-onset MSD phenotype for efficacy testing.

AAV9 vector	Safe and efficacious neurotropic vector available for delivery of the transgene to the CNS. Approved by the agency for many ongoing gene therapy clinical trials including GAN from our research group.
Gene therapy	Promising and rational approach for an effective, long term treatment of MSD.
Intrathecal dosing	Targets the main organ system (central nervous system) afflicted by the disease pathology for maximum exposure. Secretion of some sulfatases from the transduced cells into the circulation may be capable of improving the pathology in peripheral tissues that may not have the transgene copy (cross-correction). Note that we are also evaluating the combination of an IV dose.
Activity of sulfatases	Translatable assay measures potential improvement of enzymatic activity of sulfatases in tissue samples and body fluids from experimental mice.
Glycosaminoglycan and Sulfatide storage	Assay quantifies the Sulfatides and Glycosaminoglycans (GAGs) in specimens such as neuronal and visceral tissue, as well as body fluids.
Behavioral testing	Tests measure motor, learning and memory improvements following gene therapy.
Neuroinflammation	Microglial activation and astrocytosis in neuronal tissues

1.5. Therapeutic Limitations

Since the maximum safe dose will not result in transduction of 100% of the neuronal tissue, a complete rescue of the condition is not expected. However, improvements in the pathology from the transduced cells is enough to provide a significant benefit to the individual. *SUMF1* expression and the consequential secretion of activated sulfatases (that are not membrane-bound) from transduced cells, may reach non-transduced cells and cross-correct some of the sulfatase deficiencies [18]. There is a reasonable conceptual rationale to utilize the maximum safe dose possible in these cases.

The timing of the proposed treatment is expected to be critical to a successful intervention. The disease onset at a very early age alters the brain structure and associated function during neurologic development. In the most severe cases the mutation is fatal in MSD individuals before they are one year old. The ability of the therapy to reverse damage to the nervous system may be limited. The fast progressing pathology and the severity of the symptoms provide a rationale for an earliest possible, rational and safe intervention for maximal efficacy of the therapy. The nature of the disease also warrants a rational investigational drug trial design, considering there are no other options for individuals affected by MSD.

2. Introduction

2.1. Product Summary

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

2.2. Chemical Name and Structure

AAV9/SUMF1 is a recombinant serotype 9 adeno-associated virus (AAV) encoding a codon-optimized human *SUMF1* transgene (*hSUMF1opt*). The final product consists of AAV9 capsids that are packaged with the self-complementary AAV genome comprising a mutant AAV2 inverted terminal repeat (ITR) with the D element deleted, the "CBh" promoter (796 kb CMV enhancer, chicken beta actin promoter, synthetic intron [11]), codon-optimized human SUMF1 DNA coding sequence (1122 bp), the simian virus 40 polyadenylation signal (143 bp), and WT AAV2 ITR. The CBh promoter is identical to the construct utilized and characterized in rodents, pigs, and non-human primates [12, 13]. The full sequences of the plasmid will be provided in the IND.

2.3. Proposed Indication

Treatment of MSD, an autosomal recessively inherited neurometabolic disorder caused by loss-of-function or null mutations in the *SUMF1* gene.

2.4. Dosage Form, Route of Administration, and Dosing Regimen

Final Formulation: The final product will consist of a solution of AAV9/SUMF1 in phosphate-buffered saline with 5% D-sorbitol. The final dose and volume are subject to change based on the results of preclinical studies, in consultation with the Center for Biologics Evaluation and Research (CBER).

Route: We propose a single lumbar IT administration of AAV9/SUMF1 to broadly treat the central nervous system as well as peripheral organs. Choice of IT dose administration is the based on the following rationale. IT route achieves the maximal concentration at dosing in CSF and consequently maximum possible transduction of the CNS, the organ system severely afflicted by the condition. Further, IT minimizes the exposure of the immune system to AAV9 vector limiting the virus mostly to CNS space. The proposed dose escalation study will involve 2 cohorts. We expect the degree of efficacy to be dose responsive, but we do not expect a maximum feasible dose (MFD, approximately $1x10^{15}$ vg IT) to completely rescue the disease. This is because the SUMF1 protein is widely expressed and the MFD will not transduce 100% of cells across the CNS [18]. However, there could be some non-cell-autonomous benefit of SUMF1 gene expression via secreted sulfatases. Our objective is to use the highest safe dose possible. We anticipate that the first cohort will receive an IT dose of $5x10^{14}$ vg per human subject (age ≥ 6 months old). Following review of the safety and preliminary efficacy data from the first cohort in the phase I trial, the second cohort will receive 2-fold higher IT dose of approximately $1x10^{15}$ vg.

Device for Proposed Human Therapy: For IT administration, a Pajunk Atraumatic Sprotte Needle will be inserted percutaneously at the lumbar level into the IT space of the spinal column. A volume of CSF approximately equal to the infusion volume is withdrawn from lumbar thecal sac. The AAV vector solution will be loaded into a 20 mL BD syringe, connected to the needle with 60" Marquette Medical IV extension tubing and Braun Discofix 4-way stopcock. The vector solution is then infused at a rate of 1 mL per minute, using a Medfusion 3500 pump. Subject will remain in a 15-degree Trendelenburg (head down) position for 1 hour following vector administration. For subjects 4 years and older, a 10 mL infusion volume will be used. For subjects < 4 years old, the dose will be scaled lower according to approximate brain size (table 23).

2.5. Target Product Profile

The proposed target profile (TPP; table 5) has been compiled based on the available data including those expected based on the current knowledge from the field and publications. The profile will be revised and annotated as preclinical, CMC and clinical data become available.

Table 5: AAV9/SUMF1 target product profile.

Milestone (meeting/submission)	Date	TPP Submitted? Y/N	TPP Version Date	TPP Discussed? Y/N
Pre-IND	09/18/2018	Y	9/18/2018	Y
IND Submission	Projected May 2019			
EOP1				
EOP2A				
EOP2/Pre-Phase 3				
Pre-BLA				
Other (specify)				

Product Properties	Minimum Acceptable Result	Ideal Result
Primary Indication (inclusion criteria)	Treatment for symptomatic Multiple Sulfatase Deficiency	Treatment and preventing onset of Multiple Sulfatase Deficiency
	Intend to provide evidence in support efficacy from clinical trial (protocol #) *Improved survival in preclinical animal models of the disease (preIND section: 4.6)*	
Delivery Mode	Intrathecal infusion only (inpatient under anesthesia)	Intrathecal infusion only (outpatient)
	Intend to provide evidence to support the route of administration in clinical trials *Preclinical testing supports the route for clinical investigation*	
Delivery Device	Pajunk Atraumatic Sprotte Needle, BD syringe Marquette Medical IV extension tubing and Braun Discofix 4-way stopcock with a Medfusion 3500 pump	Pajunk Atraumatic Sprotte Needle, BD syringe Marquette Medical IV extension tubing and Braun Discofix 4-way stopcock with a Medfusion 3500 pump
	Previous clinical trial (IND 16003) from our group used same device	
Dose Formulation (strength and potency)	Frozen solution in vials at $1x10^{14}$ vg/mL concentration for formulation in by the clinical pharmacy	Frozen solution in pre-filled syringes ready for use
	Previous clinical trial (IND 16003) from our group used similar formulation	
Dose (scaled per age)	$0.5x10^{15}$ - $1x10^{15}$ vg per dose in patients ≥ 4 years, scaled down for younger.	$1x10^{15}$ vg per dose in patients ≥4 years, scaled down for younger.
	Actual dose calculations will be based on the CSF volume per the age of patient (table 23). Intend to provide data to support the actual dose from the phase I/II clinical trials outcome.	
Description (Ingredients, physical and chemical characteristics)	AAV9/SUMF1 is a recombinant AAV capsid with an functional copy of optimized human SUMF1 gene with a synthetic promotor and	AAV9/SUMF1 is a recombinant AAV capsid with an functional copy of optimized human SUMF1 gene with a synthetic promotor and

	polyadenylation sequence in PBS and 5% D-sorbitol solution (clear).	polyadenylation sequence in PBS and 5% D-sorbitol solution (clear).
	See section 2.2	
Storage and handling	Frozen ≤ -60˚C until dose formulation	Frozen ≤ -60˚C until dose formulation
	See section 5.5	
Patient Population (male or female including pediatric)	≥ 2 years with loss-of-function (inactivating) mutations	All ages (including newborns) with loss-of-function and null mutations
	Intend to provide evidence of populations to include based on clinical trial efficacy and safety data	
Treatment Duration	Single dose	Single dose
	Intend to provide evidence of lasting impact from the clinical trial. Preclinical data shows improved survival (section 4.6.1)	
Clinical pharmacology (factual summary)	Increase in SUMF1 enzyme activity in CSF and serum over baseline.	Improved life-span, motor, cognitive abilities and quality of life comparable to healthy individuals.
	To be populated based on clinical outcomes. Some of the MOA, PK, absorptions, biodistribution, biotransformation, metabolites, half-life, elimination, binding, uptake and BBB passage data in literature.	
Clinical studies		
	To be populated based on clinical outcomes. Proposed safety and efficacy trials; primary and secondary benefits, outcomes.	
Patient counseling		
	To be populated based on clinical outcomes. Precautions with activity (eg – driving), using of other medications, associated adverse reaction; patient package insert, etc.	
Risk/Side Effect	Devoid of treatment-related SAEs or clinically-significant immune responses to AAV9 or SUMF1	Devoid of treatment-related SAEs or clinically-significant immune responses to AAV9 or SUMF1
	To be populated based on clinical outcomes.	
	To be populated based on clinical outcomes and preclinical GLP safety study.	
Contraindications (see exclusions)	Not for pregnant and nursing mothers, other organ impairment that are a limitation.	Not for pregnant and nursing mothers, other organ impairment that are a limitation.
	To be populated based on clinical outcomes and preclinical GLP safety study.	
Non-clinical toxicology	none known (to be determined)	none known (to be determined).
	To be populated based on outcomes from GLP tox study	
Warnings and precautions	To be administered under physician supervision in clinical setting only	To administered under physician supervision in clinical setting only
	Clinical SAE or ARs will determine the instructions to be listed here based on observations during clinical trials. Lab tests following any adverse reactions and Literature references for ARs based on class of the drug will be added. To be populated based on clinical outcomes and preclinical GLP safety study.	
Adverse reactions	none known (to be determined)	none known (to be determined).
	To be added based on AR profile from proposed/planned clinical trial or related call of therapeutics. For each system list severity, frequency, etc.	
Overdose or accidental dose		
	Signs, symptoms, complications of overdose and recommended general treatment procedures.	

[redacted]

Drug abuse and dependence	Not a controlled substance that would result in abuse or dependence	Not a controlled substance that would result in abuse or dependence
	Intended for single use only.	
Drug interactions	none known (to be determined)	none known (to be determined).
	List if any for this class.	

3. PreIND Meeting

The requested meeting with the agency is to discuss the overall AAV9/SUMF1 development program and a path to initial clinical trials for the treatment of MSD.

3.1. List of Specific Objectives

Agreement from FDA is sought on the following questions:

3.1.1. Nonclinical

a. In this briefing package, we list non-GLP pilot efficacy (section 4.6), and toxicology (section 4.7) studies, which will support (and continue to test) the long-term safety of AAV9/SUMF1. We proposed a study design for a supplementing GLP toxicology study (section 4.8) to be conducted prior to the submission of the IND. **Does the agency agree that the proposed GLP rat toxicology study, along with our non-GLP mouse studies, are sufficient to support the safety of AAV9/SUMF1 in the proposed Phase I/II clinical trial?**

b. **Does FDA/CBER agree that the completed and planned efficacy studies provide sufficient evidence of benefit to justify the initiation of a Phase I/II pediatric clinical trial?**

c. Biodistribution following IT administration of scAAV9 vector has been well characterized and published extensively in small and large animal models. Section 4.7.2 lists the publications (table 13), mouse (4.7.2.1), rat (4.7.2.2) and human (4.7.2.3) data. **Does the agency agree that sufficient information already exists regarding AAV9 vector biodistribution to support the use of AAV9/SUMF1 in the proposed Phase I/II clinical trial?**

3.1.2. Chemistry, Manufacturing and Controls

a. **Does the agency agree that the proposed cGMP manufacturing process to be performed at UTSW (figure 9) is suitable to support the Phase I/II clinical study and product development?**

b. **Are the assays and strategy proposed for release testing and product characterization acceptable (table 18, 19)?**

c. **Does the Agency agree with the proposed stability program (table 20) for AAV9/SUMF1 to support the proposed clinical trial?**

d. We propose to explore the development of an *in vitro* potency assay **(**section 5.4.1**)** for release and to monitor the stability of the drug product but can't comment at this time whether it will be sufficiently accurate or precise to use for dose calculations. Therefore, we are planning to dose AAV9/SUMF1 according to a quantity of vector genome-containing viral particles. **Is this plan acceptable?**

3.1.3. Clinical

a. **Does the agency agree with the general design and enrollment criteria of the Phase I/II trial, the dose escalation strategy and route of administration as summarized in the protocol synopsis (**section 6.3**)?**

b. **Are the proposed safety and exploratory efficacy outcome measures, based on the known cause and natural history of the disease, acceptable to the Agency?**

c. Since the ratio of central nervous system mass to whole body mass changes with age, the dose

[redacted]

will be scaled by age correlated to brain mass ([48, 49, 50], not body weight based. **Does CBER agree with both the rationale for dosing based on age (≥ 4 years old), or by approximate brain size (table 23) for subjects < 4 years old?**

3.2. Proposed Agenda

Introductions
Discussion of questions submitted
Discussions of issues identified by the Agency
Summary of conclusions reached at the meeting

3.3. List of Sponsor Attendees

Name	Title	Affiliation
[redacted]	[redacted]	[redacted]

3.4. List of Requested FDA Attendees

[redacted]

4. Preclinical Studies (Ongoing and Proposed)

Table 6: Summary of non-GLP murine cohorts

Primary Outcome	Mice/group		Age/ Disease status	Route	*Sumf1*	AAV9/SUMF1 Dose level	Dose/mouse (vgx10^{11})
	Male	Female					
Efficacy AAV9/SUMF1 Stock 30711	≥5	≥5	P1 Neonate	-	+/-	-	-
				ICV	-/-	Vehicle	-
						Maximum*	2.8
Efficacy AAV9/SUMF1 Stock 31625	≥5	≥5	P1 Neonate	-	+/-	-	-
				ICV	-/-	Vehicle	-
						Maximum*	2.8
	≥5	≥5	P7 Symptomatic	IT	-/-	Vehicle	-
						low	1.25
						high	5
	≥5	≥5	P14 Symptomatic	IT	-/-	Vehicle	-
						Low	1.25
						High	5
				IT+IV	-/-	Vehicle	-
						**High+	5+50
Primary Outcome	**Mice/group**		**Age/ Disease status**	**Route**	***Sumf1***	**Dose Level**	**Dose/mouse (vgx10^{11})**
	Male	**Female**					
Safety B6 WT	7	7	P1 Healthy	-	+/+	-	-
				ICV		High	2.8
	7	7	P28 Healthy	-	+/+	-	-
				IT		High	5
				IT+IV		**High+	5+50

*Maximum feasible dose for ICV route only. **mice were administered IT and IV on the same day

[redacted]

The UNC Gene Therapy Center undertook the preclinical development of the Investigational Drug, *scAAV9/CBh-hSUMF1opt-SV40pA*, in the laboratory of Steven Gray at the University of North Carolina at Chapel Hill (UNC-CH). The supporting *in vivo* studies in the strains described below are being performed under supervision of Cathleen Lutz at The Jackson Laboratory. Ongoing *in vivo* studies are focused on the efficacy AAV9/SUMF1 in the MSD mouse model, as well as preliminary safety assessments in WT mice. Previous AAV9 supporting biodistribution studies are provided. Pivotal GLP safety studies in rats are proposed.

4.1. Preclinical Mouse Model for MSD

Settembre et. al, generated a *Sumf1* knock out mouse model [14, 18]. 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*-KO mice. These mice display severe developmental, neurological, behavioral and histopathological deficits as summarized in table 7. 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 neonatal 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 in the model. 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.

Table 7: Summary of the published Sumf1 knockout phenotype

Character	B6;129P2-*Sumf1*$^{Gt(RST760)Byg}$/ClearJ [Ref. 14, 18] Stock # 30711
Mutagenesis	Insertion of β-Geo gene-trap cassette in intron 3 of *Sumf1* gene
***Sumf1* protein**	Null
Tissue expression	Fusion transcript with first three exons of Sumf1 fused with β-Geo mRNA
Mortality (normal lifespan ~ 2.1 years)	Starts in first week of life with only 10% surviving past 3 months age; Median survival is about 30 days (note that at Jackson labs these mice have a median survival of 1 day).
Growth and appearance	Smaller compared to wildtype mice; slow overall growth, flattened facial features, short limbs, smaller skull, starting at 2 weeks age severe kyphosis, spinal vertebral and joint deficits
Hind limb clasping	starts at 1-month age
Seizures/tremors	starts at 1-month age in the limbs and head
Sulfatase activity	Absence of arylsulfatase A, B, C, and E (ARSA, ARSB, ARSC, and ARSE), sulfamidase (SGSH), iduronate-2-sulfatase (IDS), N-acetylglucosamine-6-sulfatase (G6S), and N-acetylgalactosamine-6-sulfatase (GAL6S) activity in mouse embryonic fibroblasts
Glycosaminoglycan (GAG) storage and vacuolation	Progressively massive GAG accumulation in macrophages of heart, brain, lungs, liver, kidney, blood vessels and other tissues give the cells a vacuolar appearance in 3-month mice
Neuroinflammation microgliosis (CD68), astrogliosis (GFAP)	Severe neuroinflammation throughout the CNS; massive macrophage infiltration and diffuse CD68 and GFAP immunoreactivity; significant and progressive Purkinje cell loss
Apoptosis	Significant apoptosis in liver at 1 month and cerebral cortex at 2 months age
Behavioral phenotype	Severe motor, learning and memory deficits in mice surviving to 4 months of age

A colony of *Sumf1-/-* mice was established at the Jackson Laboratory, Bar Harbor, ME under the direction of Dr. Lutz. Proof-of-concept and therapeutic interventions with AAV9/SUMF1 were executed in mice from the colony. These mice have the same allele as those shown in table 7. The cohorts of mice established had higher and much earlier mortality with a median survival of 1 day, with 40% of the mice that did survive past one day living to approximately 14 days of age. This is a more severe phenotype compared to that reported in published literature [14, 18]. The mice at The Jackson laboratory had been backcrossed multiple times to a C57BL/6J background as compared to the 129 background as reported in the publications, which might explain the difference in phenotype severity.

Table 8: JR 31625 Sumf1 strain animal assignment for natural history study

Genotype 31625	Route*	Age (PND)	Dose (x10^{11} vg/mouse)	Mice/group		
				Assigned/dosed	Dead	Live
+/+	-	1	-	14	0	14
-/-	-	1	-	25	18	6

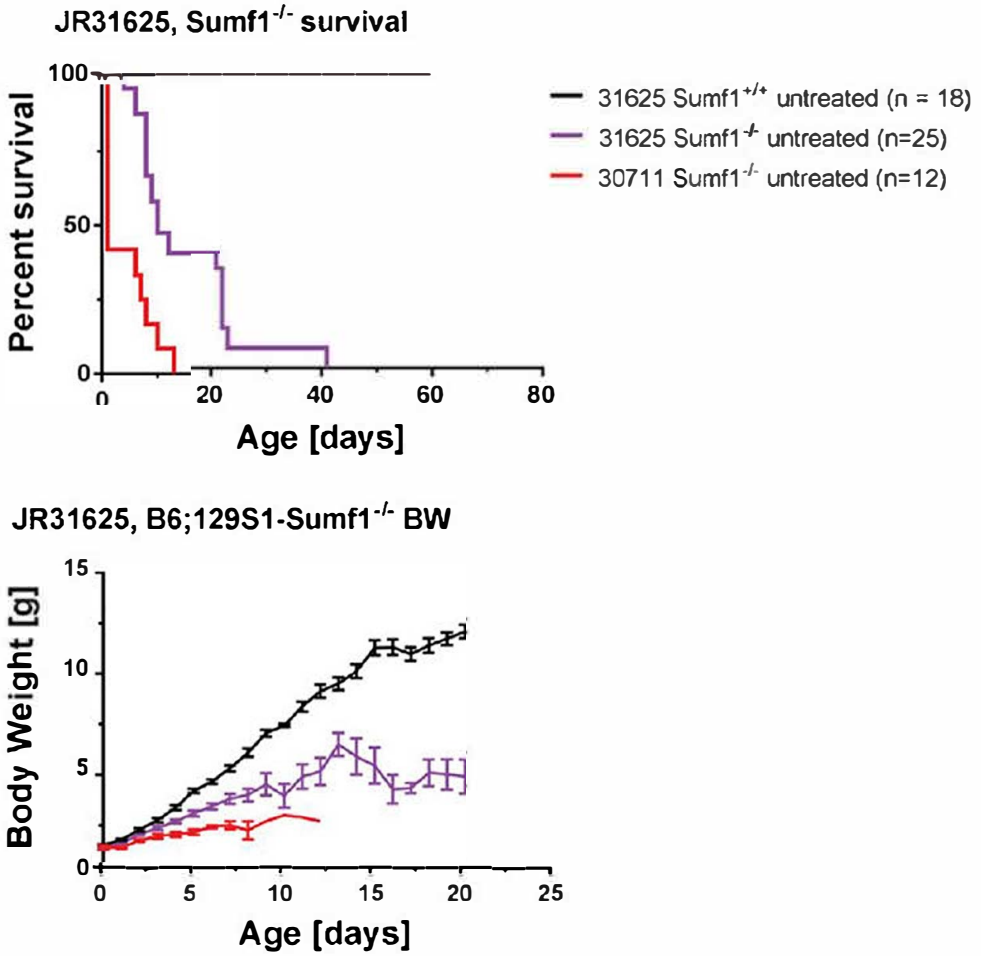


Figure 1: Sumf1-/- strain JR 31625 has improved survival compared to JR 30711.
Top panel: Survival curve for the newly developed JR 31625 strain (129 Sumf1-/- untreated). Stock JR 30711 (Sumf1-/-) is included as a reference. Bottom panel: Weight gain in these cohorts.

4.1.1. Development of an Alternate Model *Sumf1-/-* KO mice stock JR 31625

Since the *Sumf1-/-* KO 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 lab developed an alternate mouse model by introducing 129S1/SvlmJ genetic background into the 30711 stock which could be considered as >90% C57BL/6J. The new stock (JR31625) 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 been enrolled in a natural history study to better characterize the phenotype (table 8).These cohorts have a mean survival of 10 days, comparatively better than the JR 30711 (figure 1). All these mice are able to survive until 5 days of age, and approximately 40% survive to 20 days. 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. We consider the availability of this model a good development for evaluation of our product. However, there are no later onset models for evaluation of our product at this time.

4.1.2. Limits Imposed by the Available Models

The published mouse model [14, 18] that was maintained at The Jackson Laboratory displayed a phenotype that was much more severe than the original publication. The reason for this earlier mortality is not entirely clear but is suspected to be related to a genetic background resulting from inbreeding. The median survival of the homozygous mutants was 1 day at Jackson laboratories (stock # 30711), compared to a 30-day median survival reported by the research group in the publication. The mouse recapitulates the general disease features, but the severity models the neonatal form of the disease, whereas our target patient population is the late infantile patients. A model for the late infantile form of the disease does not exist. Any phenotype evaluation in the untreated cohorts will be limited to clinical signs, survival, molecular and histopathology analysis due to the very short lifespan. Since the untreated KO mice die early, efficacy has been defined as relative improvement in survival, increased sulfatase activity and reduction in the accumulation of sulfatase substrates in AAV9/SUMF1-treated KO mice. Equivalence of neurologic and cognitive abilities (compared to WT) will be assessed in these cohorts to assess the degree of therapeutic impact. Wild-type mice will be used to compare the performance of the treatment where untreated KO mice are not available due to their short lifespan.

The age of disease onset in individuals with *SUMF1* mutations is at birth or within the first few years [5, 6, 7, 8]. Earliest possible intervention is expected to be the most beneficial, due to the rapid neurodegenerative properties of the disease. The preclinical models we have are an early lethality phenotype, so our intervention window is limited. Survival and adverse events will be a measure of therapeutic benefit and safety endpoints for risk analysis following the treatment. Mice *in extremis* will be euthanized for humane reasons per IACUC guidelines and the "survival" will be scored as the time until mice reach pre-defined humane endpoint for euthanasia.

4.2. Biology of the SUMF1/FGE enzyme (comparability of mouse to human)

Sulfatases are a conserved family of enzymes catalyzing hydrolysis of ester sulfates [38, 40]. In humans there are 17 sulfatases localized to various subcellular regions where they metabolize specific substrates [2] such as glycosaminoglycans (GAGs), sulfolipids and steroid sulfates [21] among others.

[redacted]

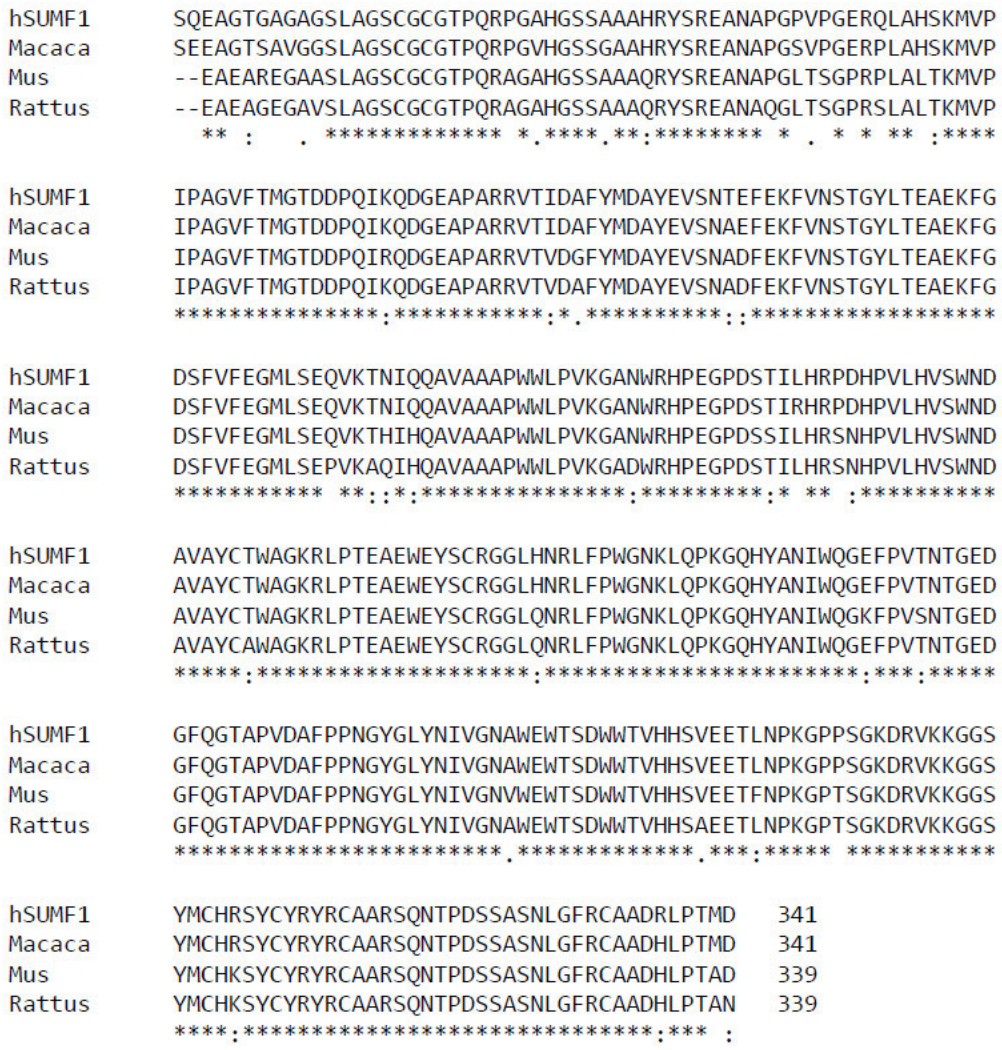


Figure 2. Similarities in protein sequence between different species.
Human (homo) SUMF1 protein sequence compared to the mouse (mus; 90.27%), rat (rattus; 90.56%) and monkey (macaca; 96.77%) retain high level of amino acid identity. The N-terminal signal peptide from the sequence was removed prior to the comparison. The asterisk (*) annotates a fully conserved amino acid residue, colon (:) annotates strongly similar residues and period (.) annotates weakly similar residues. Amino acids that are not conserved are not annotated.

Post-translational activation of these sulfatase enzymes is dependent upon modification of a conserved catalytic domain cysteine within a conserved amino acid sequence recognized by FGE in every sulfatase [20]. *SUMF1*-encoded FGE is the only enzyme capable of performing this modification in mammals [39]. When *SUMF1* is mutated, impacting FGE function, the activity of sulfatases is severely impaired [2, 3, 39]. Residual sulfatase activity depends on stability and activity of mutant FGE. Impaired or absent sulfatase activities result in lysosomal storage of substrates resulting in cell pathology as a lysosomal storage disorder and additional dysfunction of non- lysosomal sulfatases [39].

SUMF1 has been conserved through evolution retaining high level of homology across species [40]. The enzyme's stability and activity highly depend on disulfide bridges within the protein and cysteine residues in the active site. These residues are identical throughout species and allow similar fold and function of any *SUMF1* homologue [39, 40, 41]. *SUMF2*, a highly similar paralogue of *SUMF1*

gene, lacks catalytic activity and is not able to activate sulfatases [42]. Overexpression of SUMF1 in cell and animal models and in combination with sulfatases did not result any pathophysiology [18].

We used a codon-optimized (amino acids do not change) human *SUMF1* sequence for AAV9-mediated delivery. The *hSUMF1opt* was compared to the mouse, rat and monkey using the Clustal sequence alignment program. The comparison of the sequences with the signal peptide removed indicates a high-level of identity between the sequences as shown in figure 2. Based on the high degree of conservation, we propose that it is highly unlikely that SUMF1 will have an altered biological activity in rodents versus primates.

4.3. Viral Vector Use for AAV9/SUMF1 Gene Therapy

Phase I and II clinical trials demonstrated therapeutic benefits for several neurologic diseases involving replacement of defective genes using AAV vectors (Reviewed by [22]). These include restoration of vision for extended periods in Leber's congenital amaurosis (NCT02781480) and curbing further neurodegeneration in the brain of metachromatic leukodystrophy (NCT01801709) and improving motor function in spinal muscle atrophy (NCT02122952) [23] among other ongoing trials.

Our group and other researchers investigated global transgene CNS delivery in rodents, pigs, and non-human primates (NHPs). Compared to other AAV vector serotypes and routes of administration, IT (lumbar) administration of AAV9 vectors into the cerebrospinal fluid (CSF) achieves widespread distribution of the transgene to neurons and glia throughout the spinal cord and brain at a translationally-relevant dose [24, 25, 26, 27]. In pigs, a 50-100% transduction was achieved in the spinal cord motor neurons along with transduction of neurons and glia in the brain at a dose of $1.7x10^{11}$ vg/kg [28]. In Cynomolgous monkeys, a dose of $2\text{-}3x10^{12}$ vg per animal (~$0.5x10^{11}$ vg/kg) of AAV9/GFP vectors evenly distributed GFP-positive cells in a spatially homogenous manner throughout the entire brain [29]. Similar results have been observed in cats, dogs, and NHPs [30, 31, 32, 33].

AAV serotype 9 vector is capable of widespread transduction (tissue tropism), including the central nervous system (CNS) and somatic system (body) following intravenous or intracerebrospinal fluid administration. The CNS and systemic tropisms of AAV9 make it ideal for treating lysosomal storage diseases with global organ disease manifestations. The transgene is a *hSUMF1* codon-optimized construct (*hSUMF1opt*); where the DNA sequence is modified such that the final amino acid sequence is unchanged but nucleotide sequence is altered for easier detection along with potentially stronger expression. The transgene consists 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. The CBh promoter and SV40 polyA are utilized for their ability to both be small in size as well as drive strong expression allowing for packaging into a self-complementary (sc) AAV vector [11]. 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 [35, 36].

Research grade vector lots were produced for preclinical non-GLP studies. A lot will be produced for GLP toxicology studies, which will use a manufacturing process matched to the proposed GMP clinical vector:

1. **UNC Vector core**: The research grade vector was produced in suspension HEK293 cells using a mixture of chromatography and density gradient purification. It was used in cohorts of WT and *Sumf1*$^{-/-}$ mice to assay efficacy and long-term safety of the gene therapy.

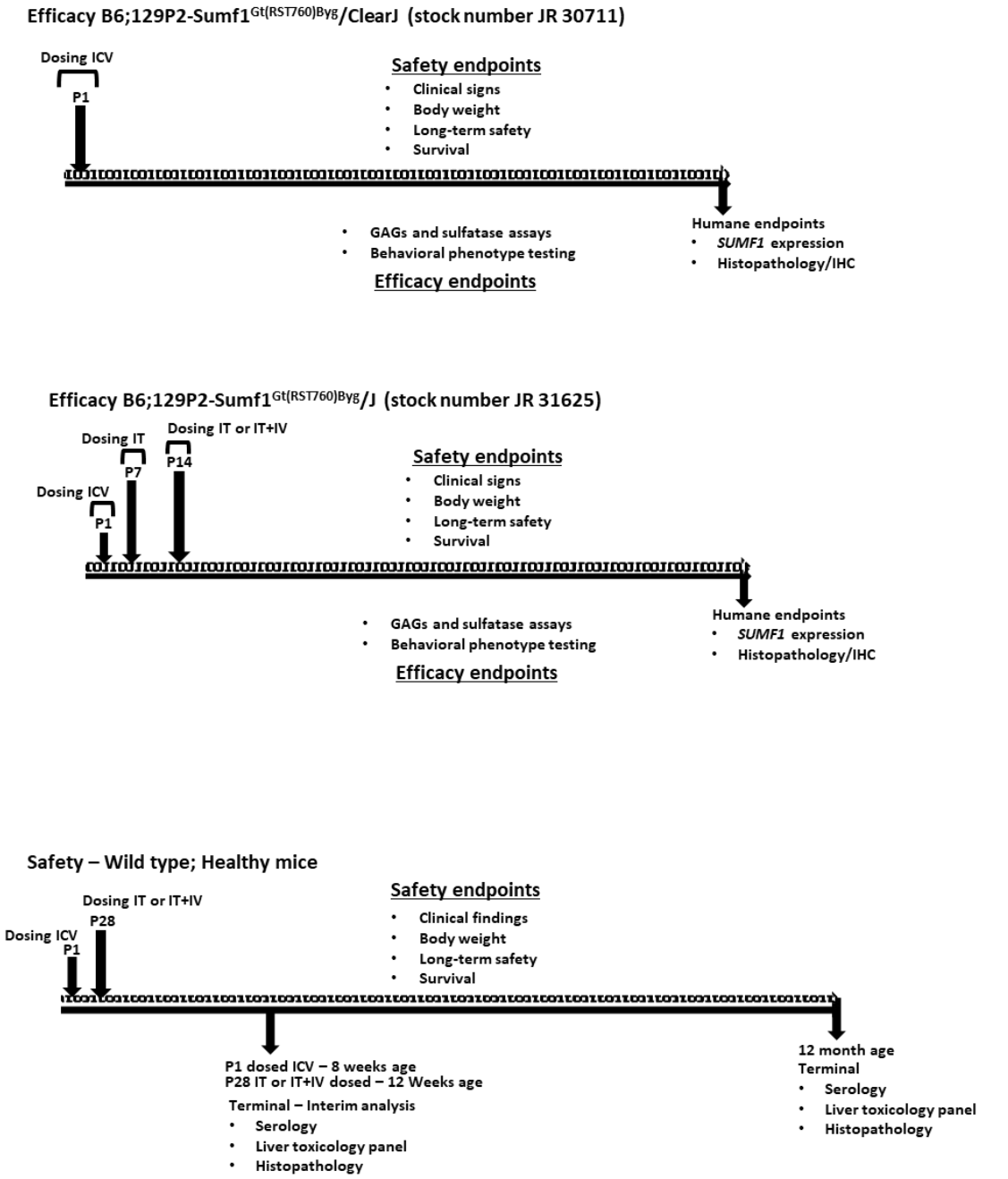


Figure 3. Study plan, duration and readouts for Sumf1 and wild-type phenotypes
Top panel: AAV9/SUMF1 proof-of-concept therapeutic evaluation in mouse model JR 30711. The KO mice in this strain have a mean survival of 1 day, ICV is the only route being investigated in these cohorts. Middle panel: AAV9/SUMF1 therapeutic efficacy evaluation in mouse model JR 31625. The KO mice in this strain have a mean survival of 9 days. ICV at P1 (PND1) is being evaluated as POC. IT at P7 (PND7) and P14 (PND14); IT+IV route of administration in PND14 mice were evaluated. Bottom panel: In wild-type mice AAV9/SUMF1 administration via ICV, IT or IT+IV will be evaluated for safety.

2. **UTSW:** Qualified research grade vector of AAV9/SUMF1 for toxicology and cGMP lots for clinical use. These will use an overall highly similar process based on HEK293 suspension cells, which is similar to the vector made at UNC but will not utilize density gradients in the purification process.

[redacted]

The equivalence of the "research grade" AAV9/SUMF1 vector lots made at UNC and UTSW will be analyzed by injecting the same doses of each vector in WT mice and measuring the biodistribution of the vectors to the heart, liver, brain, and spinal cord after 1 week. Equivalency of *in vivo* biodistribution patterns, biopotency, and titers will be performed by SDS-PAGE, qPCR, and silver stain analysis.

4.4. Preclinical Study Design

A representation of the non-GLP preclinical study design is presented in figure 3 to gather the proof-of-concept and long-term safety data (table 6) to support AAV9/SUMF1 gene therapy clinical trials.

1. **Animal species:** *Mus musculus*

Mouse models: Different models of mice are being used for the preclinical testing to assay the safety and efficacy of AAV9/SUMF1. Studies in the mice were initiated as pups become available from the active ongoing breeding program to fill the numbers in each cohort.

I. **Strain - JR 30711 -B6;129P2-Sumf1$^{Gt(RST760)Byg/ClearJ}$** – Mean survival up to PND1
II. **Strain - JR 31625 - STOCK Sumf1$^{Gt(RST760)Byg/J}$** – Mean survival up to approximately PND10

Both strains are being used for testing. JR 30711 intervention is limited to PND1. JR31625 intervention is at PND1 and PND7. Another cohort will be tested at PND14 is possible, to evaluate a combination IT+IV therapy. The following genotype for each strain will be used for the analysis.

a. **Sumf1+/-:** Heterozygous (+/-) mice are healthy.
b. **Sumf1-/-**: Homozygous (-/-) mice display severe phenotype and relevant model for the human MSD disease.
c. **Wild type:** Homozygous (+/+) C57BL/6 mice are healthy.

2. **Age at intervention:**
   a. **PND1 (Neonatal intervention; Proof-of-Concept and Safety)**: The data from this cohort is expected to provide a proof-of-concept for the therapy, demonstrating the highest efficacy and allowing for evaluation of the long-term safety. The route of administration for this cohort will be intracerebroventricular, as a proof-of-concept, although for the clinical trial we have no plans to use this route of administration. Note that these mice receive a dose of $2.8x10^{11}$ vg (approximately $2.8x10^{14}$ vg/kg).
   b. **PND7, PND14 (Delayed intervention; Efficacy and Safety)**: This cohort will represent intervention following the detection of MSD disease signs in the mice, using the IT route of administration. At PND14, a combination IT+IV dosing strategy will be evaluated even though this is not proposed for clinical translation at this time.
   c. **PND1, PND28 (Healthy; Safety)**: This wild-type cohort of will provide long-term safety data in healthy animals from exogenous overexpression of *SUMF1* from AAV9/SUMF1 gene therapy.
3. **Dosing groups in cohorts:** Dosing and assessments in each cohort will include a combination of the following groups.

   Efficacy Cohorts:
   a. AAV9/SUMF1-injected *Sumf1* deficient (-/-) to determine safety and efficacy
   b. Un-injected/Vehicle-injected *Sumf1* deficient (-/-) will represent the natural course of the disease
   c. Un-injected/ Vehicle-treated *Sumf1* heterozygotes (+/-) will serve as healthy controls

   Pilot toxicology cohorts:
   d. AAV9/SUMF1-injected WT mice (+/+) to determine safety of therapy from overexpression.

e. Vehicle-injected WT mice (+/+) to serve as controls for overexpression to determine safety of therapy.

4. **Animal randomization and cohort assignment**: The animals will be randomly assigned to the study cohorts following the determination of genotype. When genotyping turnaround time is NOT met, animals are enrolled in untreated and vehicle groups.
5. **Dose levels:** Mice in each group will receive a fixed single dose of AAV9/SUMF1. The dose levels tested and the manufacturer information in the cohorts are listed in table 9. The maximum feasible IT dose (~$5x10^{11}$ vg total by IT administration) corresponds to our target high clinical dose ($1x10^{15}$ vg), and we anticipate this dose to be more effective than the 4-fold lower dose that will be evaluated in parallel.
6. **Actual Dose Consideration:** It is not expected that AAV9 will bind to the needle/syringe used in these experiments, but this will be tested directly by comparing the qPCR-based titer of the vector before and after passage through the device in a mock injection setup.

Table 9: AAV9/SUMF1 dose levels tested on the non-GLP preclinical studies

		Dose			
Level	**Route**	**Volume µL**	**Concentration vgx10^{11}/µL**	**per mouse vgx10^{11}**	**Clinical dose * equivalent vgx10^{15}**
Low	IT	5	0.25	1.25	0.25-0.5
High	IT	5	1	5	1-2
Maximum	ICV (neonatal)	2	1	2	not directly comparable
High+**	IT+IV	5+50	1	5+50	not directly comparable

*CSF volume in adult mice is 0.035 mL to 140 mL in human (≥ 4 year or older), a 4000-fold difference.
**exploratory study to evaluate the utility of a combination of IT and IV routes of administration.

7. **Study activities:** Following the dose administration the mice will be assessed for safety and efficacy of the AAV9/SUMF1 gene therapy per the testing schedule (table 10). Safety of the therapy will be determined by monitoring clinical signs, survival and change in body weight, and histopathology. Efficacy will be determined via behavioral, clinical, neurological, tissue histology and molecular analysis. Wild type mice over expressing the transgene will be monitored for adverse clinical signs, morbidity, mortality or other signs of toxicity.

Table 10: Study activity schedule for non-GLP studies

***Sumf1* strain**	**Dose age**	**Clinical signs**	**Body weight**	**Behavioral tests (weeks)**	**Terminal endpoints (months)**
JR 30711	P1	Morbidity, mortality and adverse events will be recorded	Daily	8, 24, 52	12 or Humane
JR 31625	P1				
	P7				
	P14				
Wild type	P1	Detailed*	Twice weekly to 2 months, then monthly	n/a	2**
	P28				12

* Mortality/morbidity, body condition, clinical signs and group health compared to control; **Interim collections are from 4 mice/group at 8 weeks postdose.

4.5. AAV9/SUMF1 Formulation and Administration Protocol

Prior to injection, the AAV9/SUMF1 vector is formulated in a vehicle containing 1x PBS with 350 mM NaCl and 5% sorbitol. A single dose of the vector formulation or vehicle is administered to the mice either intravenously (IV) or intracerebroventricular (ICV) using a ½ cc insulin syringe, or IT using a Hamilton®

syringe. The dose level, injection volume, concentration and route of administration information are presented in table 9.

ICV injections are qualified per the following criteria:

a. Pups at P1 or P4 will be cryoanesthetized and injected by hand.
b. JAX technicians lead the dosing efforts for the project. Evaluation and experience with this dosing technique are available for review at Jax facility.
c. Injected pups will be placed in the cage with the dam and be monitored for clinical signs or distress. Animals identified as meeting a pre-defined human endpoint will be euthanized and tissue will be collected for evaluation.

IT injections are qualified per the following criteria:

a. Tail flick is observed when the needle enters the IT space
b. Mice are monitored post-injection for a return to normal ambulation
c. JAX technicians lead the dosing efforts for the project. Evaluation and experience with this dosing technique are available for review at Jax facility.
d. Animals will be monitored after dosing for impairment in breathing, poor body condition, posture, coordination, mobility or any motor function impairment. These clinical signs if noticed in the first 24 hours of lumbar puncture are attributable to a spinal injury and the mice will be taken off the study and euthanized.

IV injections are qualified per the following criteria:

a. Injection site will be monitored for blanching or bleb resulting from perivascular injection.
b. Mice are monitored immediately post-injection for a return to normal ambulation.
c. Technicians performing these dosing have multiple years of experience.
d. Animals will be monitored after dosing for clinical signs of impairment in breathing, poor body condition, posture, coordination or mobility. If clinical signs do not subside with supportive treatment in the first 24 hours and the mice will be taken off the study and euthanized.

4.6. Preliminary Preclinical Data

Phenotype with early mortality limits our ability to obtain behavioral data from untreated *Sumf1* KO mice. Data where possible is being collected from the dosed cohorts. Some of the preliminary survival and body weight data is presented in this section.

Table 11: JR 30711 Sumf1 strain animal assignment for non GLP studies

Sumf1	Route*	Age (PND)	Dose (x10^{11} vg/mouse)	Mice/group			
				Assigned/ dosed	Interim analysis	Dead	Live
+/+	-	1	-	14	4*	0	14
+/+	ICV	1	2	18	4*	0	14
-/-	ICV	1	2	9	-	5	4
-/-	ICV	1	-	12	-	12	0

*These mice were sacrificed at a planned endpoint (8 weeks post-injection) for histology.

4.6.1. AAV9/SUMF1 Improves Survival in *Sumf1-/-* mice (stock JR 30711)

The number of mice assigned to each group for the studies conducted so far in this strain are listed in table 11. The survival data for this cohort and the body weights are presented in figure 4. The untreated *Sumf1* KO cohort had 60% mortality on PND1 and a 100% lethality by Day 13. Mice that received AAV9/SUMF1 on PND1 had significantly better survival with no deaths on PND1 and over 40% of mice surviving beyond 150 days, so far. Mortality in this cohort occurred within the first 25 days, all the

[redacted]

mice that lived >1 month are alive at the 150-day mark. The treated mice gained weight gradually with a growth curve similar to their wild-type littermates, but at a slower pace. No signs of tremors or seizures were detected, either signs of kyphosis and bone deformities.

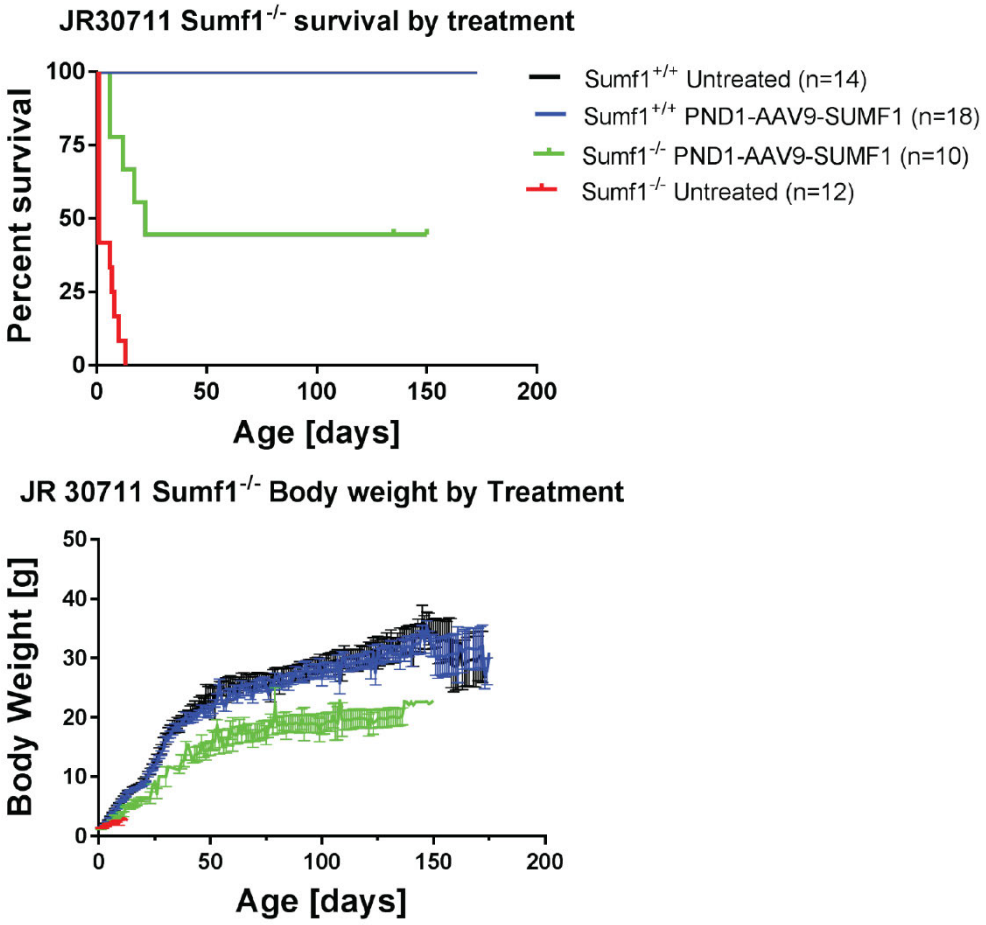


Figure 4. AAV9/SUMF1 therapy improves survival of Sumf1-/- mice (stock JR 30711).
Sumf1-/- KO and Sumf1+/+ mice received a single dose of AAV9/SUMF1 via ICV on PND1. Control cohorts did not receive any dosing. Top panel: Survival curve for mice in each cohort. Bottom panel: Mean body weight of each cohort. Body weight of mice that were alive at the time of data collection have been included. Legend: Listed in the top corner of the figure applies to both panels.

4.6.2. Tissue Sulfatase Activity

Sulfatase enzymatic activity was previously measured in tissue homogenates from mice [14, 18] and in the patient fibroblasts [17, 57]. Brain, spinal cord, liver, kidney, heart, lung, spleen, pancreas, gonad and muscle will be collected and snap frozen for measurement of arylsulfatase A (ARSA), arylsulfatase C (ARSC), and iduronate-2-sulfatase (IDS)] sulfatase activities. It should be noted that ARSA and IDS are secreted and may be measure 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.

Our expectation is that the functional copy of *Sumf1* delivered via AAV9/SUMF1 therapy will result in the increased expression of the enzyme that in turn activates the sulfatases via enzymatic action. The tissues isolated from AAV9/SUMF1 treated *Sumf1-/-* are expected to have improved sulfatase enzymatic

activity compared to untreated cohorts. The measured activity of sulfatases in the tissue lysates is expected to increase in a dose dependent manner.

4.6.3. Reduction in GAG Storage

Pathological events in MSD are due to the lack of sulfatase activity resulting in accumulation of GAGs in the lysosomes resulting in vacuolation of the cells [17, 57, 58]. The increase in cellular activity of sulfatases following AAV9/SUMF1 therapy in *Sumf1-/-* mouse tissue along with the increase in secreted sulfatases are expected to reduce the GAG storage. The reduction in accumulation is expected to improve the organ function and thus reduce the pathology for excess GAG storage.

4.6.4. Behavioral Testing

The behavioral impairment in *Sumf1-/-* mice are similar to the those seen in animals with other lysosomal disorders [18, 59, 60]. The motor and cognitive capacities in the treated cohorts will be tested via Open field and Morris water maze testing to assess the therapeutic benefit. Any deficits in peripheral organ function would be identified via the performance of mice in the open field. Water maze test will identify cognitive deficits in the treated cohorts. It should however be noted that these tests would likely be done after 6 weeks of age in these mice, untreated controls may not be available for comparison.

4.6.5. Neuroinflammation

Massive macrophage infiltration is seen in the neuronal tissues of *Sumf1-/-* mice [14, 18]. Immunoreactivity of inflammatory (CD68) and astrogliosis (GFAP) markers are upregulated in these mice. An effective AAV9/SUMF1 therapy is expected to reduce the inflammation in the neuronal tissue of *Sumf1-/-* mice.

4.7. Safety Studies

These studies will test the safety and identify potential adverse clinical signs of IT route of the AAV9/SUMF1 administration and the transgene expression. A summary of the non-GLP and GLP studies being conducted are presented in this section.

4.7.1. Pilot Safety/Survival Studies

The non-GLP studies outlined in figure 3 are designed to identify any major long-term safety concerns for the experimental therapy. Mice are monitored for general morbidity, changes in weight, survival and clinical signs following the treatment. Terminal blood and tissue samples will be collected for histopathology and serological assessment. Any adverse events will be investigated and recorded. Mice that exhibit neurologic symptoms or general malaise will be evaluated and appropriate supportive or therapeutic interventions will be offered per IACUC and veterinary guidance. Blood and tissue samples will be collected from mice that are euthanized for humane reasons. A detailed necropsy will be performed to investigate or identify the reason for the ailment by a trained technician or veterinary staff, when possible. The necropsy report results will be summarized and included in the final reports.

The preliminary data collected from these treated *Sumf1+/+* mice is encouraging, as no adverse effects have been noted during in-life, so far in terms of body weight. The growth rate and survival were not different in treated and untreated *Sumf1+/+* cohorts (figure 4). An interim necropsy and histopathological analysis by a blinded, qualified veterinary pathologist at 8 weeks postdose in these cohorts showed no signs of pathology or toxicity in the neuronal tissue (preliminary data in table 12).

Table 12: Summary of neuronal histopathology following AAV9/SUMF1 treatment

Tissue	Sumf1$^{+/+}$ untreated*	Sumf1$^{+/+}$ PND1 AAV9/SUMF1*
Brain	Normal.	Normal.
Spinal Cord-Lumbar	Normal. Dorsal root ganglions are normal.	Normal. Dorsal root ganglions are normal.

* N=4, 2 females/2 males. Necropsy at 8 weeks postdose

4.7.2. Biodistribution

AAV serotype 9 was chosen for its ability to widely disseminate through the spinal cord and brain, providing neuronal and DRG tropism. Compared to other routes of administration, the IT route favorably directs the vector biodistribution to CNS [18, 33, 34, 35]. In order to lower the manufacturing burden and reduce the overall AAV9/SUMF1 exposure to the individual, we propose to utilize the IT route of administration in the proposed human trial. Since biodistribution studies have already been carried out following intra-CSF AAV9 vector administration by multiple laboratories (table 13), a rigorous biodistribution is not proposed to support AAV9/SUMF1.

Supporting data to gauge the biodistribution of AAV9 dosed intrathecally is presented below in the 2 sets of non-GLP studies conducted in our lab.

Table 13: Summary of published AAV9-CSF biodistribution studies

Species	Reference	Total AAV9 dose (vg)	AAV9 dose* (vg/Kg)	Vector dose per mL CSF** (vg)
Mouse	24	$2.5x10^{9}$	$1.1x10^{11}$	$1x10^{11}$
	51	$1x10^{10}$	$5x10^{11}$	$4x10^{11}$
	52	$7.3x10^{11}$	$3.3x10^{13}$	$2.9x10^{13}$
	53	$4x10^{11}$	$1.8x10^{13}$	$1.6x10^{13}$
Cat	30	$1x10^{12}$	$1x10^{12}$	$2.3x10^{11}$
Dog	31	$2x10^{13}$	$2x10^{12}$	$1.6x10^{12}$
	54	$1.8x10^{13}$	$1.8x10^{12}$	$1.4x10^{12}$
Pig	12	$8.6x10^{11}$	$4.3x10^{10}$	$4.3x10^{10}$
	55	$3x10^{12}$	$1.5x10^{11}$	$1.5x10^{11}$
NHP	51	$5.5x10^{12}$	$1.45 x10^{12}$	$4.6x10^{11}$
	32	$1.8x10^{13}$	$7x10^{12}$	$1.5x10^{12}$
	33	$1.8x10^{13}$	$7x10^{12}$	$1.5x10^{12}$
	55	$2.5x10^{13}$	$7x10^{12}$	$2.1x10^{12}$
	52	$2x10^{13}$	$1x10^{13}$	$1.7x10^{12}$
	54	$2x10^{13}$	$3.3x10^{12}$	$1.7x10^{12}$

*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. References [34, 37, 44, 45]. **Note**: Doses were per animal or by weight.

4.7.2.1. scAAV9/CBh-GFP – Mouse IT Biodistribution

Table 14: Biodistribution study design for AAV9 dosed IT in mice

Genotype	Route*	Age (months)	Sex	Dose ($x10^{11}$ vg/mouse)	Number Dosed	Endpoint
WT	IT	2	F	4.15	9	3 months (4 weeks postdose)

*IT injections were via lumbar puncture, a 5 µL dose in vehicle (350mM phosphate-buffered saline, 5% sorbitol).

[redacted]

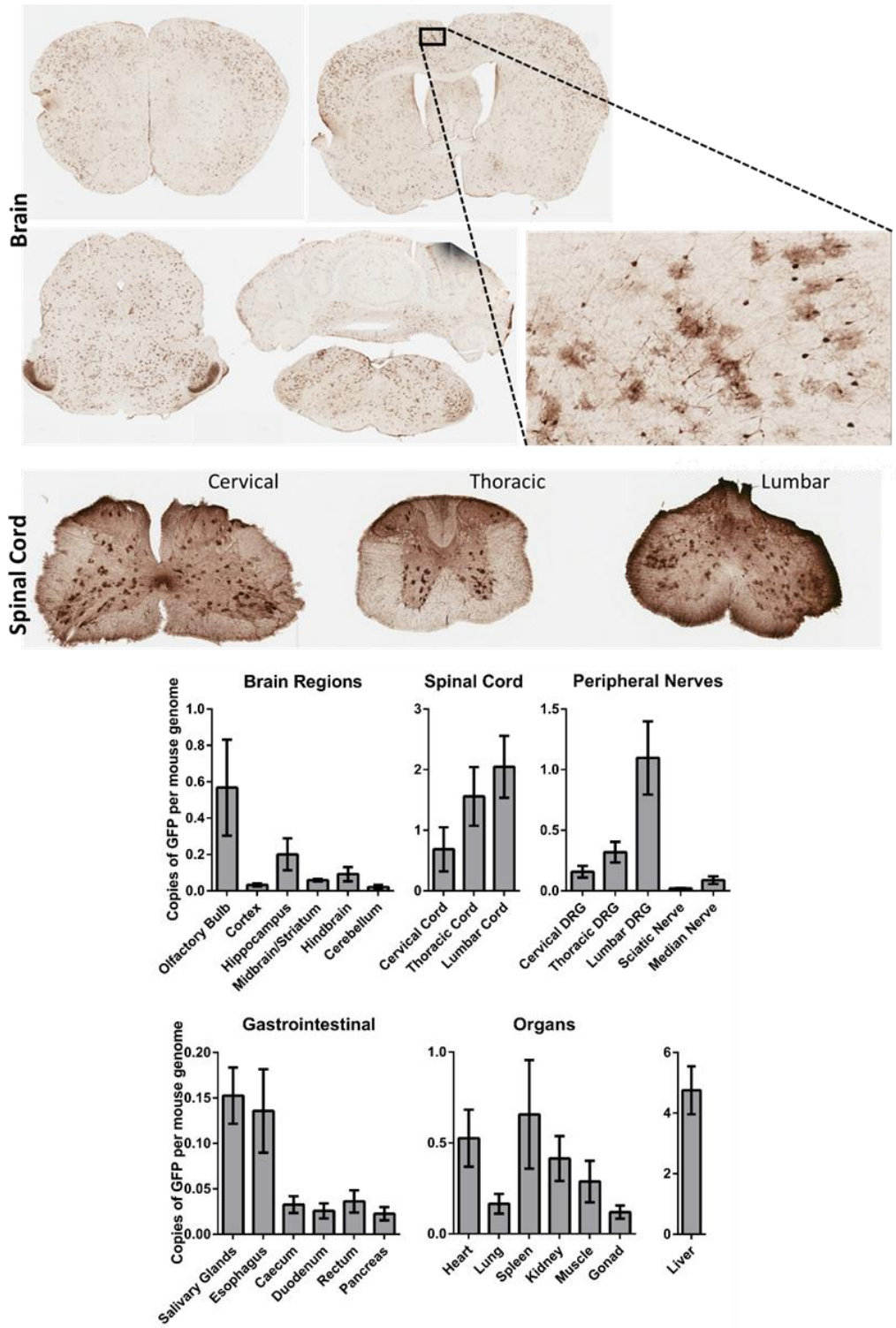


Figure 5. AAV9/GFP biodistribution in WT mice.
Eight-week-old WT C57BL/6 mice were given a single lumbar IT injection of scAAV9/CBh-GFP vector at a dose of 4.15x10^{11} vg per mouse. At 4 weeks post-injection immunohistochemistry of the brain and spinal cord was conducted to visualize the spatial distribution of GFP expression (top panel; n=4), and qPCR was conducted to quantify the biodistribution of the vector across the nervous system and peripheral tissues (bottom panel; n=5). Bars are the mean, +/- SEM.

4.7.2.2. scAAV9/GAN – Rat IT Biodistribution

4.7.2.3. scAAV9/JeT-GANopt – Human IT Biodistribution

4.8. Proposed Pivotal IND-enabling Studies

4.8.1. Pivotal GLP-Compliant Toxicology Study

Species: We propose to conduct the toxicology study in CD rats because of the large knowledge base of normal lab values and previous histological studies using AAV. Using small rodents allows n=5 of each sex per group, providing a clear picture of dose- and time-dependent toxicities. We propose to initiate treatments for this study with juvenile rats (approximately 8 weeks of age). This age was chosen to utilize rats as young as practical, but not too young as smaller animals could introduce technical difficulties with the accuracy of IT injections.

Vector: We will perform pivotal toxicology using an AAV9/SUMF1 vector manufactured under comparable conditions and reagents as the GMP vector intended for the clinical study, although it will be manufactured outside of a GMP-compliant facility. The vector will be formulated at a concentration of $\geq 1 \times 10^{14}$ vg/mL in 1x phosphate-buffered saline with 5% added sorbitol (identical to the clinical vector), which we have found to provide suitable long-term stability. This formulation is utilized in the clinical trial for Giant Axonal Neuropathy that Dr. Gray is involved with (IND # 16003). Lot-release criteria for vectors to be used in safety studies have been established, and assay methodology details will be provided in the

[redacted]

IND (Table 16). All vectors will be stored in aliquots at ≤ –60°C and thawed on the day they are to be used. The titers of all preclinical and clinical lots will be compared using the same methodology in parallel to ensure equivalent dosing.

Table 16: Proposed lot release criteria for AAV9/SUMF1 vectors for toxicology studies

Criteria	Specification to meet or exceed
Sterility	No growth observed in 3 test media, 14 days
Mycoplasma	Not detected
Endotoxin	LAL (Endosafe) <10 EU/mL
Potency	Infect Lec2 cells, assay SUMF1 expression by qPCR-based quantitation of mRNA, record results
Genomic structure	Identity of packaged DNA (between ITRs) confirmed by sequencing
Purity	SDS-PAGE, showing 3 bands for VP1, VP2, and VP3 at a ratio of 1:1:10 with minimal other bands visible
Identity	Western blot using anti-AAV9 antibodies; presence of VP1, VP2, and VP3 bands
Appearance	Transparent and colorless
pH	Test strip, pH 6.5–7.5
Concentration	qPCR, ≥ 1×10^{14} vg/ml
In vitro adventitious virus	3 cell lines, no cytopathic effect
Replication-competent AAV	Limiting dilution on 293T cells in presence of adenovirus helper virus, no AAV replication
Presence of host cell DNA	qPCR; record results
Presence of host cell protein	Record results
Empty:full ratio of capsids	Transmission electron microscopy or analytical centrifugation; ≥50% full capsids
Residual plasmid DNA	qPCR; record results
Binding	Binding to the needle and syringe (for toxicology study) assess by qPCR before and after passage through the device
Precipitation	Vector will be frozen and thawed, assessed for precipitation by qPCR of the supernatant following centrifugation.

Doses: We have established a target dose of 2.0×10^{12} vg per rat with the following rationale: The anticipated most effective dose from the MSD mouse studies is 5×10^{11} vg per mouse, which is essentially a maximum feasible dose given IT injection volume constraints. The maximum feasible dose in rats (using a 20 µL IT injection volume) is 2×10^{12} vg per rat, which correlates to the 5×10^{11} vg mouse dose. We plan to bracket this target dose with lower and higher doses of 5×10^{11} vg and 6.0×10^{12} vg, respectively. The 6.0×10^{12} vg dose is achieved by utilizing a higher-than-normal injection volume of 60 µL (~25% of CSF volume instead of ~7%).

[redacted]

Actual Dose Consideration: It is not expected that AAV9 will bind to the needle/syringe used in these experiments, but this will be tested directly by comparing the qPCR-based titer of the vector before and after passage through the device in a mock injection setup.

Timing of sacrifice: We propose sacrifice at 7, 28, and 90 days with the following rationale: We expect a maximal SUMF1 activity at 7 days post-injection in mice, and expression was stable from 28 days onward. The 90-day time point will investigate any signs of long-term adverse effects.

Control groups: A cohort of rats will be administered vehicle in place of AAV9/SUMF1.

Study procedures: This study will be performed by a GLP-compliant contractor, with the study cohorts outlined in table 17.

Table 17: Treatment groups for safety assessment of AAV9/SUMF1 in rats

Group	No. of Rats (male + female)	Dose (x10^{11}Vg)	Volume (µL)	Time points (days)
A	30	Vehicle	60	7, 28, 90
B	30	5	20	7, 28, 90
C	30	20	20	7, 28, 90
D	30	60	60*	7, 28, 90

* or maximum feasible dose.

Readouts and Assessment: Each time point per group will consist of 5 males and 5 females. Animals will be examined twice daily for mortality/moribundity. A detailed clinical examination and weight acquisition will be performed weekly. Food consumption will be assessed qualitatively weekly. Ophthalmology examination will be done at pretest and on all survivors at termination and recovery. Electrocardiograms will be done on all animals pretest, and on all surviving animals predose and postdose on Day 1, prior to the terminal necropsy, and prior to the recovery necropsy. For clinical pathology: hematology, coagulation, clinical chemistry, and urinalysis evaluations on all animals pretest, Day 2 and all survivors prior to the terminal and recovery necropsies.

Rats (n=5 of each sex per time point) will be sacrificed by barbiturate treatment, and cardiac puncture is used to collect blood for complete blood count and serum chemistry tests. The rats will be examined by a board-certified veterinary pathologist for any gross abnormalities, which will be recorded and excised. The following organs will be removed and weighed: liver, kidney, heart, and lungs. Samples of the following organs will be taken for histopathological examination and quantitation of any abnormal findings: adrenal gland, brain (cortex, cerebellum), colon, diaphragm, duodenum, epididymis, esophagus, gross lesions, heart, ileum, kidney, liver, lung/bronchi, >2 lymph nodes, skeletal muscle, sciatic nerve, ovary, pancreas, spinal cord, spleen, testis, and uterus. A board-certified veterinary pathologist will perform a blinded evaluation of hematoxylin and eosin-stained sections for each organ. Duplicate samples of the same tissues will be retained and archived frozen.

4.9. Preclinical Summary

The data presented indicates a vast improvement in survival following AAV9/SUMF1 administration, which is quite remarkable given the rapid disease progression in the severe JR30711 mice. The improved survival is likely from resolution of underlying pathology as evidenced by lack of physical deformities (besides facial dismorphometry) and clinical signs including seizures in these mice. Given the initial limitations imposed by the mouse model, our data indicates the vast potential of gene transfer to treat this disease, but we have not yet formally established a minimally effective dose. The doses presented assume that anything less than a maximum feasible dose will have reduced efficacy, based on the known biodistribution pattern of AAV9 to the brain. Our planned dosing experiments at P7 and P14

in stock JR-31625 cohorts will establish the dose-responsiveness of the treatment. Our planned molecular assays and behavioral testing will provide greater clarity to predict appropriate efficacy outcome measures for the trial design. Given the rapid progression of the disease and the unmet need to treat MSD patients, our goal is to rapidly translate a safe and effective therapy to human populations of MSD via AAV9/SUMF1 therapy.

5. Overview of the Biological Product and Manufacturing Components

Biological Substance: AAV9/SUMF1 is an adeno-associated virus serotype 9 (AAV9) vector engineered to encode the human *SUMF1* gene for expression of active human SUMF1 enzyme.

Biological Product: *In vivo* preclinical studies demonstrated function and enzymatic activity of AAV9/SUMF1 vector. The vector for clinical use will be evaluated for purity and potency before release for human injection.

Research grade AAV9/SUMF1 vector for preclinical testing was manufactured at University of North Carolina Vector Core (UNC-VC). The vector for clinical use will be manufactured at a cGMP facility at UTSW and testing will be performed in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR part 58, EU Good Laboratory Practice regulations, (EMEA GMP, Rules and Guidance for Pharmaceutical Manufacturers and Distributors, Annex 13), and applicable ICH Q7 standards. Prior to use, the vector titer for clinical use will be determined by quantitative polymerase chain reaction (qPCR) using a transgene specific *SUMF1* primer and probe set, as well as an additional primer and probe set directed to the AAV ITR.

The sterile AAV9/SUMF1 biological product will be provided in capped vials for aseptic delivery. The product will contain AAV9/SUMF1 in a 2-mL vial; it will be stored and shipped at ≤ -60°C. The product will be an aqueous solution in pre-sterilized vehicle (phosphate buffered saline containing 5% W/V D-sorbitol).

5.1. Description of Components Used in Manufacturing

5.1.1. Vector, Packaging and Helper Plasmid DNA

Three plasmids will be used for manufacturing the AAV9/SUMF1 vector. The vector plasmid (pSJG-CBh-hSUMF1opt-SV40pA), and the packaging/helper plasmids pAAV9 and pALD-X80 (which provide the AAV and Adenoviral helper functions needed for AAV vector production in mammalian cell culture). Plasmid pSJG-CBh-hSUMF1opt-SV40pA contains a beta actin promoter driving the expression of human SUMF1 and a simian virus 40 polyadenylation sequence.

5.1.2. Plasmid DNA Manufacturing, Testing and Qualification

The plasmid DNA for use in clinical vector manufacturing will be produced using standard methods for E. coli fermentation and plasmid purification by a contracted manufacturing organization. Testing and qualification of plasmid DNA lots will be performed prior to release for GMP manufacturing of the vector and will include assays of sterility, endotoxin content, identity and purity.

5.1.3. *Expi293F™* (cGMP Banked) Master Cell Bank

AAV9/SUMF1 will be manufactured using Expi293F™ cells (Vendor catalogue number 100044202; licensed from Thermo-Fisher, Life technologies, Carlsbad, CA), a fully documented cGMP-banked cell line from the vendor, to support the Phase I/II clinical study. Expi293F™ are a commercially available variant of HEK-293 cell line adapted for high-density suspension culture. These cells are transfection compatible and adapted to grow in serum-free suspension culture medium. Cell culture and other GMP records will be maintained during any additional derivations. Frozen cells will be supplied by the vendor in a vial

[redacted]

containing 1.1 mL of the cells at $1x10^7$ viable cells/mL in Expi293 Expression Medium and 10% DMSO. The cells will be expanded and then frozen down in 200 vials as aliquots to generate a Suspension Working Cell Bank (SWCB). A number of vials from this cell bank will be subsequently thawed and used during process development studies or to create a scalable suspension-based manufacturing, and then directly for cGMP manufacturing.

5.1.3.1. Origin and History of HEK293 (*Expi293F™*) Production Cells

The 293-cell line is established from primary embryonal human kidney that was transformed with recombinant human adenovirus type 5 [46, 47]. The adenovirus gene, E1A, transactivates viral promoters resulting in very high levels of viral proteins in these 293 cells. The Expi293F™ (Life technologies, Carlsbad, CA) cell line is a variant of the 293-cell line.

5.1.3.2. Testing and Specification of *Expi293F™* Cell Bank

A certificate of analysis will be provided by the supplying vendor assuring the compatibility of this cell for cGMP use.

5.2. AAV9/SUMF1 Vector Manufacturing

5.2.1. Introduction

Process development is under way to finalize the manufacturing process. The following sub-sections provide an overview of the proposed manufacturing process and are presented as a flow chart in figure 8, but are subject to change.

5.2.2. Overview of rAAV Manufacturing at the UTSW Facility

The rAAV manufacturing process is an animal component-free system with the exception of the suspension Expi293F™ cell line itself. Use of the suspension Expi293F™ cell line allows for scalable production of rAAV using suspension cell bioreactors.

Table 18: Release tests and specifications for suspension cell

Test	Specification	Result
Safety/sterility		
Sterility	No Growth/No inhibition	No Growth/No inhibition
In Vitro Adventitious	Negative	Negative
In Vivo Adventitious Virus	Negative	Negative
Mycoplasma (PTC)	Negative	Negative
Specific Pathogen Tests		
Pan AAV	Negative	Negative
HIV-1	Negative	Negative
HIV-2	Negative	Negative
HTLV I & II	Negative	Negative
HBV	Negative	Negative
HCV	Negative	Negative
CMV	Negative	Negative

EBV	Negative	Negative
HHV-6 A	Negative	Negative
HHV-6 B	Negative	Negative
HHV-8	Negative	Negative
Parvo B19	Negative	Negative
SV40	Negative	Negative
Identity And Function		
Identification of Cell Line Species	Human	Human
Determination of Cellular Morphology and Growth	Anchorage-independent, Logarithmic growth	Anchorage-independent, Logarithmic growth
Subcellular Components and Detection of Viral Particles	Normal subcellular components, Negative for viral particles	Normal subcellular components, Negative for viral particles
Viability	≥ 70% Viable	≥ 70% Viable
F-PBRT Assay	Negative	Negative

5.2.2.1. Initiation and Expansion of Suspension *Expi293F™* SWCB Cultures

The SWCB of suspension Expi293F™ will be produced by thawing a vial of MCB; culturing and expanding the cells for a few days within a single-use stir-tank bioreactor; and then freezing down a SWCB. Before use, each SWCB lot must pass the manufacturer criteria. Two vials of SWCB will be thawed and pooled for use within a manufacturing run. The cells will be cultured and expanded in single-use stir-tank bioreactors. The lot release tests and specifications for the suspension Expi293F™ are shown in table 18.

5.2.2.2. Assembly of Transfection Plasmid Cocktail Mix

The transfection cocktail mix will be assembled using established plasmid ratios of Ad helper plasmid:AAV helper plasmid:vector plasmid, and PEIpro®-HQ (Polyplus-transfection® SA) in serum-free media.

5.2.2.3. Transfection of Bioreactor Culture

Two days post-seeding the disposable bioreactor liner, cell culture counts and viabilities will be assessed. The cells will be expanded to 85% of the final cell culture volume. After an incubation at room temperature, the transfection cocktail will be pumped into the bioreactor. Several hours post-transfection serum-free media will be pumped in at a volume of 10% of the final volume of cell culture.

5.2.2.4. Harvesting Cell Culture from Bioreactor *(to be updated)*

About 48-60 hours post-transfection, a solution of serum-free media, NaCl (final concentration up to 250 mM), and mild detergent Triton X-100 (final concentration no more than 0.5% v/v) will be pumped into the bioreactor to gently lyse the cells and release intact AAV particles into the supernatant. This mixture will then be passed through polypropylene depth filters to clear the cellular debris and will then be passed through TFF and concentrated 10-20X and buffer exchanged against a 10 nm filter. Salt active nuclease (SAN) is then added to the cell lysate and incubated at room temperature. SAN will be removed during further downstream processing (i.e. FPLC). The final details of the manufacturing process are pending the results of pilot process development studies.

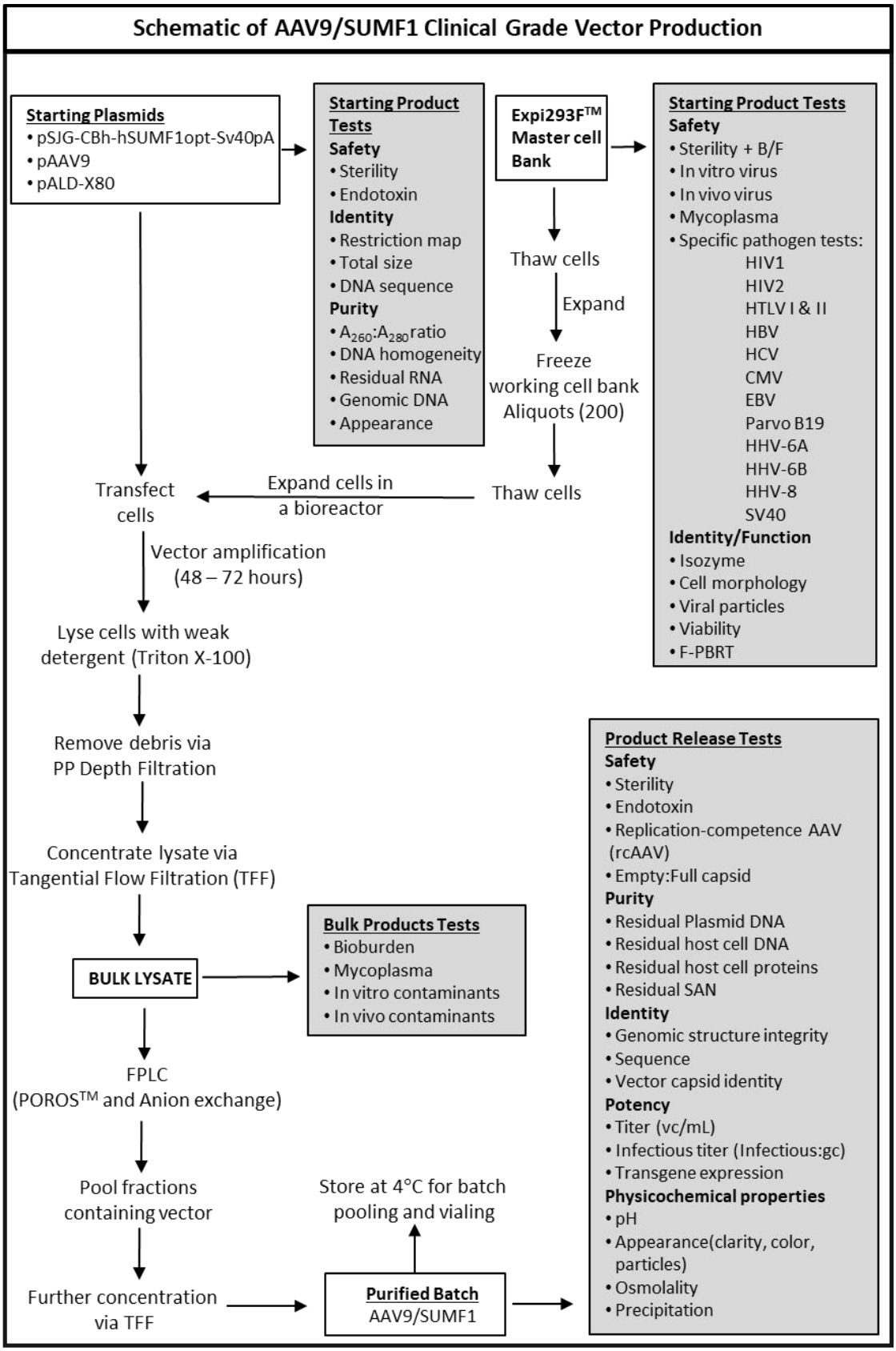


Figure 8. Flow chart of AAV9/SUMF1 cGMP manufacturing.

[redacted]

5.2.2.5. POROS-AAV9 Resin Affinity Chromatography

POROS™ CaptureSelect™ AAV9 affinity Resins (catalogue number A27354; ThermoScientific™, Waltham, MA, USA) are 50-µm diameter, rigid, polymeric affinity chromatography resin beads for purification recombinant adeno-associated virus subtypes with high purity. These affinity resins will enable single-step purification and yield at higher flow rates for improved throughput. Affinity-based chromatography using the POROS™ resin will be conducted using a Fast Protein Liquid Chromatography (FPLC) purification step.

5.2.2.6. Empty/Full Separation by IEX

An additional purification will be performed using an anion-exchange chromatography to remove additional contaminants and to separate empty AAV particles. The purified rAAV will be enriched for full particles by ion exchange (IEX) chromatography.

5.2.2.7. Final Formulation by TFF and Sterile Filtration

The recombinant AAV9/SUMF1 will be formulated in phosphate-buffered saline containing 5% D-sorbitol using TFF. The IEX eluate will be diafiltered against 5 volumes of final formulation buffer and either concentrated or diluted to the desired concentration based on pre-process titers. The final formulated vector will be sterile filtered at 0.22 µm. Terminal filtration ensures sterility of the AAV9/SUMF1 final product.

Final details of vector design, formulation, clinical-grade manufacturing, lot-release assays, stability studies, and potency assay will be presented in the IND submission.

5.3. Quality Testing Program for AAV9/SUMF1

Each lot of AAV9/SUMF1 vector will be tested prior to product release. A one-time analysis of the complete sequence of the AAV9/SUMF1 vector (not including ITR elements) will be performed. The sequence will then be compared to the sequence database to confirm that no unexpected open reading frames are present in the vector. Final product qualification and characterization testing will include assays of sterility, endotoxin, purity, identity, activity/potency, infectious titer, vector genome titer, capsid particle titer, appearance and genome structure.

The plasmids used for raw material will be well characterized products made with animal-free reagents with their own lot releases. Preliminary lot release criteria for clinical-grade vector to be supplied will meet or exceed the specifications listed in table 19. The analysis listed will be performed at UTSW GMP facility or an independent contract testing laboratory and the certification will be provided.

Table 19: Proposed lot release for clinical grade AAV9/SUMF1 vector

Test	Method Reference	Specification
Bulk Lysate		
Mycoplasma	TBD	Not detected
Bioburden	TBD	Negative
In vitro assay for the presence of viral contaminants	TBD	Not detected
In vivo assay for viral contaminants	TBD	Negative
Bulk Product (Purified Batch)		
Genomic structural integrity	PCR and restriction digest	Predicted pattern observed
Sequence	At least 4-fold coverage w/o total inverted terminal repeats	Match expected

[redacted]

Test	Method Reference	Specification
Residual host cell DNA	qPCR	For information only
Residual host cell proteins	TBD	For information only
Residual plasmid DNA	qPCR	<100 pg per 10^9 particles
Residual SAN	ELISA	<1 pg per 10^9 particles
Viral titer	In-house qPCR	For information only
Final Product		
Transgene expression	Infection of cultured cells followed by SUMF1 mRNA production	To be determined
Viral capsid purity and identity	SDS-PAGE/densitometry	Detection of VP 1,2,3 > 90% band intensity
Infectious titer	Infectious center assay	For information only
Ratio infectious titer to genome copy titer	Calculation	For information only
Replication-competent AAV (rcAAV)	Cell culture and PCR	For information only
Sterility	TBD	Negative
Bacterial endotoxin test	TBD	< 0.2 EU/dose
Osmolality	TBD	Report value
pH/Appearance	In-house	pH 6.5–8 Clear and colorless
Empty:full capsid ratio	TBD	> 50% DNA-containing particles
Precipitation	In-house	< 50% precipitation
Viral titer	In-house qPCR	≥ 10^{14} vg/mL

5.4. AAV9/SUMF1 Product Stability Testing

The tests listed in table 20 will be performed at each time point using the methods listed. These tests will be performed at UTSW cGMP facility or a certified CRO.

Table 20: cGMP drug product stability testing program.

Assay	Method	Time points (months)
Vector Genome Titer	qPCR	0, 6, 12, 24, 36, 60
Infectious Titer	TCID50	0, 6, 12, 24, 36, 60
Viral Protein Purity	SDS-PAGE/RP-HPLC	0, 6, 12, 24, 36, 60
Transgene Expression/Activity	RT-qPCR for mRNA expression after Lec2 transduction	0, 6, 12, 24, 36, 60
Precipitation	Re-titer supernatant after centrifugation	0, 6, 12, 24, 36, 60
Sterility	TBD	0, 6, 12, 24, 36, 60

5.4.1. In Vitro Potency Assay

A potency assay will be developed to quantify transgene expression following transduction of cells *in vitro*. We plan to infect Chinese Hamster Ovary derived Lec2 cells (which are permissive to AAV9) with standardized quantities of the AAV9/SUMF1 vector, then measure *SUMF1* expression via *SUMF1* mRNA levels.

5.5. Storage, Transportation, Formulation and Retention of AAV9/SUMF1 Clinical Lot

AAV9/SUMF1 lots will be stored in a locked and monitored ≤ -60˚C freezer with controlled access. UTSW or other clinical site will fill an Investigational Product Request Form and a Drug Shipment Request Form that will be sent to cGMP director. This will initiate transfer of the required number of product vials from the GMP storage depot location to the designated pharmacy recipient at the designated clinical site, once all essential documents have been provided by the site. All shipments of investigational product will be sent with a temperature measurement device included.

The drug will be received at the Pharmacy Service and stored in a locked ≤ -60˚C freezer. Removal of sealed vials containing clinical grade AAV9/AAV9 vector will be logged into the Clinical Vector Log book that is kept in a separate location. Upon withdrawal of a sample, the logbook will be annotated with the specific Vial # and Lot # of the reagent. The responsible person will then transport the vial on cold packs. Preparation of dose solution will occur in a sterile environment. The exterior of the sample vial will be wiped thoroughly with disinfectant solution (70% ethanol) and provided to authorized personnel for intraCSF injection to the individual via lumbar puncture.

5.6. Facility Location

[redacted]

5.7. Facility Operations

[redacted]

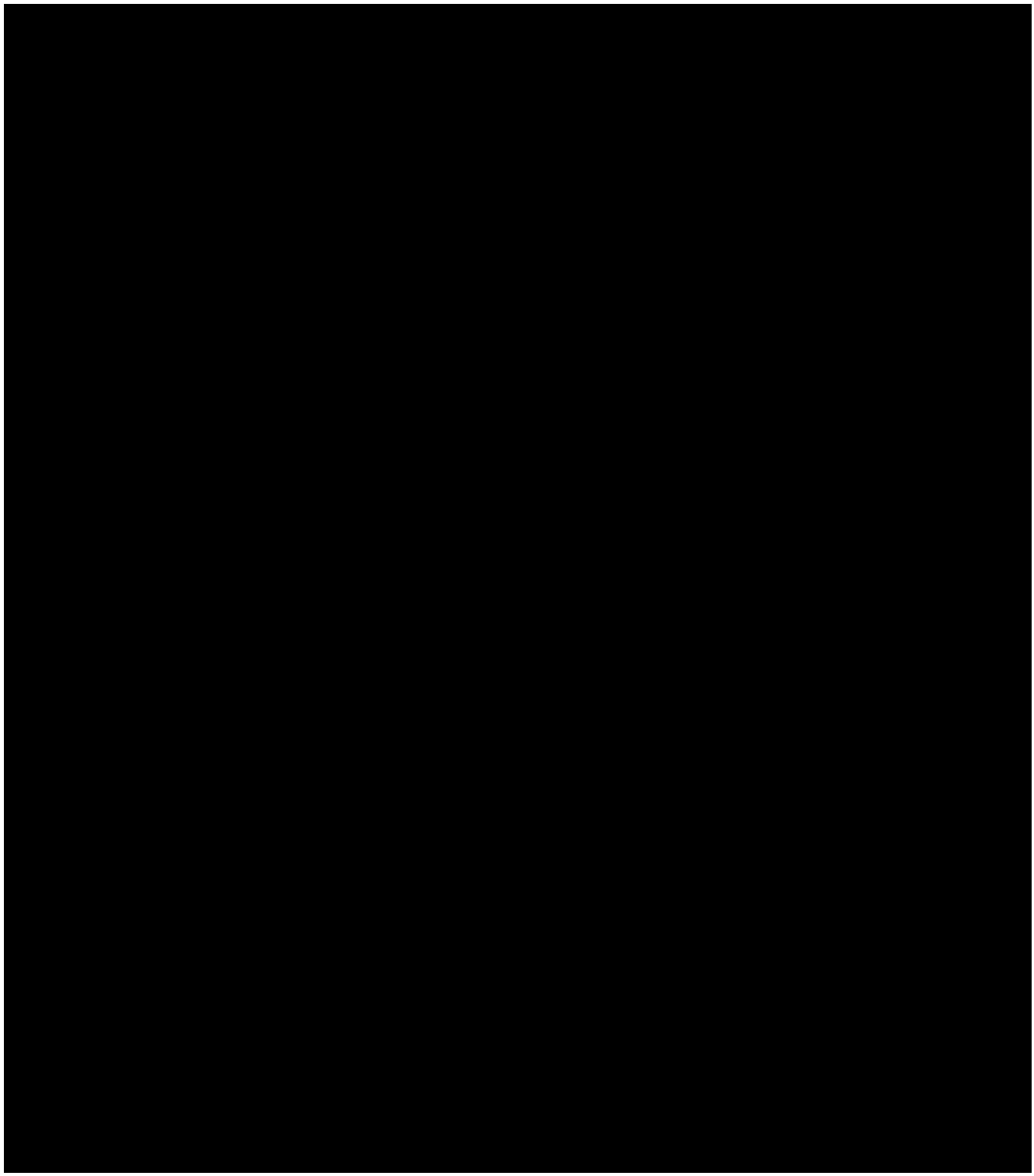
5.7.1. Gene Therapy Vector Manufacturing Facility Records

[redacted]

5.8. Facility Layout

[redacted]

[redacted]



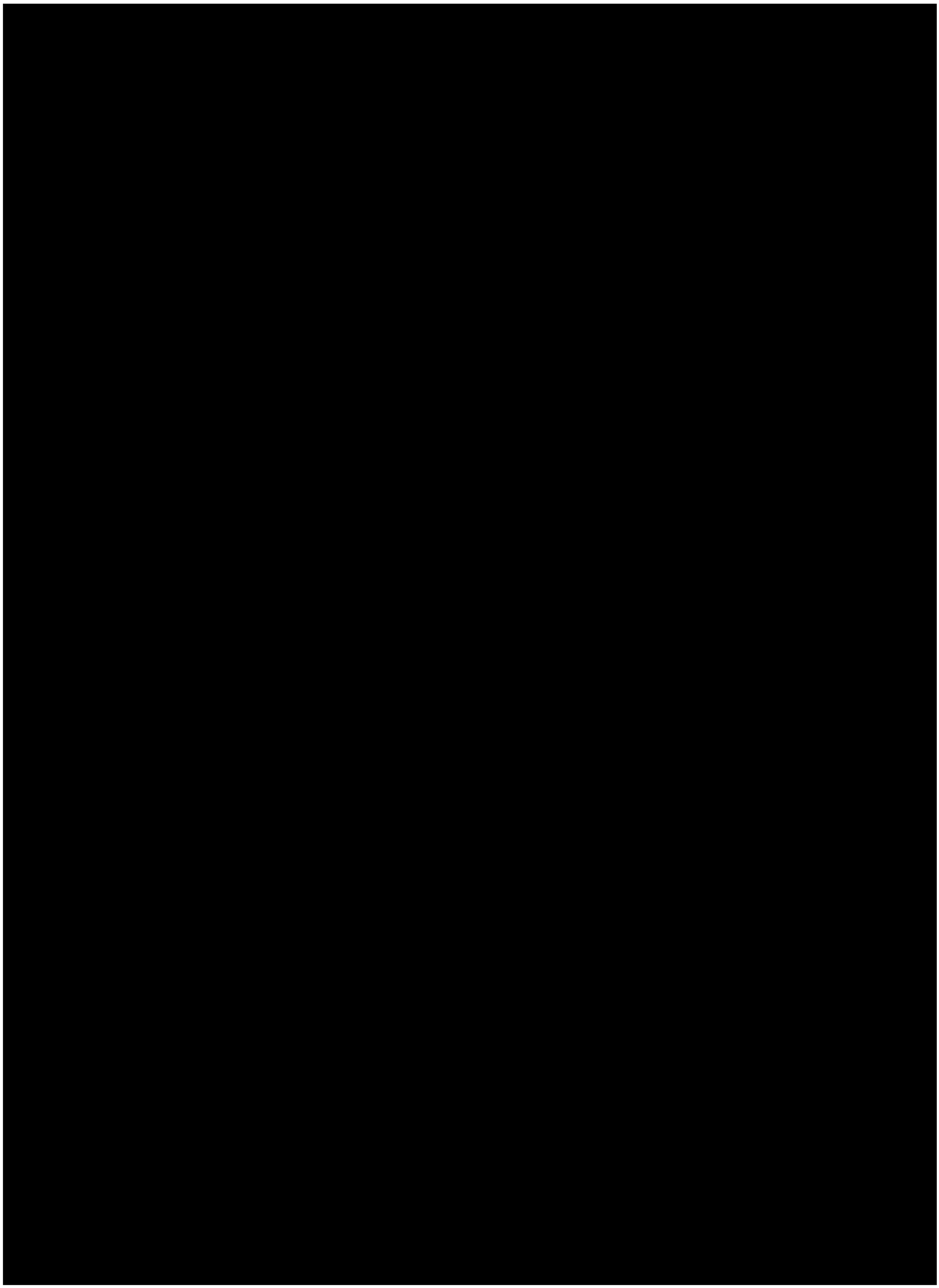
5.9. Facility Description

[redacted]

[redacted]

[redacted]

[redacted]



[redacted]

5.10. Categorical Exclusion from Environmental Assessment

We claim a categorical exclusion from the requirement for preparation of an environmental assessment as per 21 CFR 25.31 (e). The production and testing of AAV9/SUMF1 vector will confirm to BioSafety Level-1 containment procedures whereby all liquid waste is decontaminated either with bleach or via sterile filtration against a 0.2-micron filter prior to discarding and all solid waste is bagged and sealed. All solid waste will be autoclaved before disposal. The laboratories where cells are grown and tested are in compliance with federal, state, and local requirements for hazardous waste.

6. Proposed Phase I/II Clinical Trial

The phase I clinical trial will be an open-label dose escalation study. Two cohorts will undergo gene transfer by IT AAV9/SUMF1 administration.

6.1. Investigators at the University of Texas Southwestern

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

6.2. Clinical Collaborators and Subject Matter Experts

[redacted]

[redacted]

[redacted]

6.3. Synopsis of Clinical Trial I/II

Title	Phase I/II gene transfer clinical trial of AAV9/SUMF1 for treatment of subjects with MSD disease.
Number of Subjects	[redacted]
Clinical Study Phase	Phase I/II trial
Number of Centers	[redacted]
Study Objectives	Primary outcome is safety Secondary outcome is initial efficacy
Study Design	Open-label, single dose, dose-escalation clinical trial of AAV9/SUMF1 injected intrathecally.
Inclusion Criteria	[redacted]
Exclusion Criteria	[redacted]

[redacted]

	[redacted]
Study Procedures	Proposed Human Therapy: In Cohort 1 and 2, MSD subjects will have a spinal needle inserted percutaneously at the lumbar level into the IT space of the spinal column. A volume of CSF approximately equal to the infusion volume is withdrawn from lumbar thecal sac. The vector solution is then infused at a rate of 1 mL per minute. Subject will remain 15-degree Trendelenburg (head down) for 1 hour following vector administration. Dosing volume will be calculated per the table 23, depending on final vector product concentration and subject cohort. A tapering course of prophylactic enteral prednisolone will be administered. The procedure will be performed in the Radiology Fluoroscopy suite with an anesthesiologist or qualified physician present to administer sedation as needed. Subjects will stay in Pediatric ICU overnight. Physiologic monitoring in accord with the standards set by the American Society of Anesthesiologists will be utilized for all subjects while they are receiving analgesia/anesthesia and until they have fully recovered from its effects. Vital signs (BP, HR, RR, T, O2 saturation, telemetry) will be checked hourly while hospitalized after anesthesia.

[redacted]

Primary Outcome	Determination of safety based on the development of unacceptable toxicity. Unacceptable toxicity at any dose level will be defined as the occurrence of two or more unanticipated Grade III or higher treatment-related toxicities.
Secondary Outcomes	• Change in sulfatase enzyme activity over baseline in the serum and CSF • Reduction in urine and CSF sulfatides and glycosaminoglycans • MSD Disease specific measures or ratings are not available. Motor and cognitive functioning will be assessed standardized neuropsychological assessments and self-report/proxy-report measures, see table 21 for complete battery. • Change in brain, spinal cord, liver, and spleen MRI parameters, including volumetric change (% gray matter volume, % ventricular volume, total brain volume, and total liver and spleen volumes), and MRS. These imaging parameters will be used to assess disease progression and safety of the gene transfer vector over time. • Change in respiratory function as measured by pulmonary function tests and end tidal CO2 • Change in apneic or hypopneic events on polysomnography • Change in nerve conduction velocities on electromyography and nerve conduction studies • Change in hearing function as measured by auditory brainstem evoked responses
Study Duration	Initial screening and evaluation will be performed up to 4 weeks before the administration of AAV9/SUMF1. We will evaluate short-term safety over a two-year period. Individuals will be tested at baseline (-28 to -1 days) and return for follow up visits on days 7, 14, 30, 60, 90, 180, 270, 360, 540 and 720 for active monitoring. After the 24-month visit, they will be followed according to an annual monitoring plan.
Sample Size	This is a dose escalation study in six (6) – nine (9) subjects consisting of two cohorts. Cohort 1 will receive an IT infusion; Cohort 2 will receive escalating IT dose. The stated doses are for subjects ≥4 years of age and will be scaled downward for younger subjects. • Cohort 1 (IT, Low dose): 10 mL at 5×10^{14} vg total (n=3) • Cohort 2 (IT, High dose): 10 mL at 1×10^{15} vg total (n=3-6) Dose escalation will only occur after review of safety information with the Data Safety Monitoring Board (to be identified).
Statistical Analysis	This is a Phase I/II trial, with safety as the primary measure. Dose escalation will occur based on evaluation of dose-limiting toxicity as typically applied in a 2x3 design. Secondary outcomes include potential efficacy measures. Data of post-gene transfer monitoring will be compared with baseline data and analysed by paired t-tests with a significance level of $p = 0.05$.
Long-term follow-up	Safety follow-up will continue over a two-year period that incorporates the active phase of the protocol. Individuals will then transfer to an annual monitoring program where data will be collected from annual standard care visits for up to 15 years.

[redacted]

Table 21: Standardized neuropsychological assessments and self-report/proxy-report measures

Phenotyping Domain	Measure	Age Range	Relevant Scores	Estimated Time to Complete	
				Child	Parent
Global Cognitive	Leiter International Performance Scale, 3rd Ed	3+	Nonverbal IQ standard score, scaled scores for cognitive subtests, growth scores, percentiles	45-50 min	
	Reynolds Intellectual Assessment Scales, 2nd Ed	3-94	Verbal Intelligence Index standard score, t-scores for verbal subtests, percentiles	10-15 min	
	*Mullen Scales of Early Learning	Birth-68 months; may be given out of age range	Early Learning Composite standard score, t-scores for domains, age score that can be used to calculate Ratio IQ	20-30 min	
Language	Expressive One-Word Picture Vocabulary Test, 4th Ed	2+	Standard score, percentile, age equivalent	15-25 min	
	Receptive One-Word Picture Vocabulary Tests, 4th Ed	2+	Standard score, percentile, age equivalent	15-25 min	
	NEPSY, 2nd Ed	3-16	Scaled score for Word Generation subtest	5 min	
Memory	Child and Adolescent Memory Profile	5-21	Scaled scores for Lists and Objects subtests, Screening Index standard score, percentiles	20-30 min	
Motor	NIH Toolbox Early Childhood Motor Battery or NIH Toolbox Motor Battery	3-6, 7+	Standard scores for Dexterity, Grip Strength, Standing Balance, Endurance, Gait Speed subtests	20 min	

[redacted]

	Beery-Buktenica Developmental Test of Visual-Motor Integration, 6th Ed	2-99	Standard score, percentile, age equivalent	10-15 min	
Adaptive Functioning	Vineland Adaptive Behavior Scales, 3rd Ed	Birth-90	Standard score for Adaptive Behavior Composite, standard scores for Communication, Daily Living Skills, Socialization, and Motor domains, *V*-scale scores for subdomains, age equivalents, percentiles		45-60 min structured interview
Quality of Life	Pediatric Quality of Life Inventory	2-18	Standard scores for Physical, Emotional, Social, School Functioning	5 min	5 min

**Mullen Scales of Early Learning will only be administered if participants are unable to demonstrate understanding of subtests required for a Full IQ after administration of entry level teaching trials on the Leiter International Performance Scale, 3rd Ed*

6.4. Draft of Informed Consent for the Trial

This document will be submitted at the time of IND submission.

Table 22: Relationship between preclinical study doses and proposed human IT dose

Species	Injection Volume (mL)	Weight (kg)	CSF Volume (mL)	Dose as % of CSF Volume	Min. Total Dose (vg)	Min. Dose per CSF Volume (vg/mL)	Max. Total Dose (vg) ***	Max. Dose per CSF Volume (vg/mL)
Preclinical:								
Mouse	0.005	~0.025	0.035	14	$1.0x10^{11}$	$3.0x10^{12}$	$2.4x10^{11}$	$7.0x10^{12}$
Rat	0.02	0.25	0.25	8.0	$7.5x10^{11}$	$3.0x10^{12}$	$1.8x10^{12}$	$7.0x10^{12}$
NHP*	1.0	3-6	12	8.3	$3.6x10^{13}$	$3.0x10^{12}$	$7.0x10^{13}$	$7.0x10^{12}$
Clinical:								
Human (≥4 years)**	10	~40	140	7.1	$4.0x10^{14}$	$3.0x10^{12}$	$1.0x10^{15}$	$7.0x10^{12}$

For CSF Volumes, we used the following references: [34, 37, 44, 45]
* Provided for reference. Additional NHP studies are not proposed.
** For human subjects <4 years old, a dosing strategy is outlined in table 23.
*** Approximate maximum feasible dose provided for reference, if needed.

6.5. Justification for Clinical Studies Dose

This will be a single administration, dose-escalation study, involving 2 cohorts. We anticipate that a maximum feasible dose ($1x10^{15}$ vg) will provide a significant benefit to the subjects, but insufficient to fully rescue all aspects of the disease. Pending the final results of preclinical dose-response studies in the MSD model, our objective is to use the highest safe dose possible. We anticipate that the first cohort will receive an IT dose of $5x10^{14}$ vg per human subject (age ≥ 4 years old; table 23). Following review of the

[redacted]

safety data from the first cohort in phase I trial, the second cohort will receive 2-fold higher IT dose of approximately $1x10^{15}$ vg. The 2nd cohort dose escalation studies will be based on the outcomes of the first cohort. We are exploring the benefit of an additional intravenous dose given on the same day as the IT dose, and this will be guided by the preclinical studies. All these clinical doses would be within the range of doses considered safe in the toxicology studies.

6.6. Brain Volume Dependent Dosing for Phase I/II

The study agent, AAV9/SUMF1 vials, will be formulated as a concentrated stock in phosphate-buffered saline containing 5% D-sorbitol and stored at ≤ -60C until the day of the administration procedure(s). The solution will be thawed within 4 hours of administration and diluted to the appropriate final dosage concentration and volume using phosphate-buffered saline with 5% D-sorbitol. The concentration would be kept constant, but a lower dose would be administered intrathecally to younger subjects (according to brain volume) by reducing the injection volume (table 23).

Individuals affected by MSD will have a spinal needle inserted percutaneously at the lumbar level into the IT space of the spinal column. A volume of CSF approximately equal to the infusion volume is withdrawn from lumbar thecal sac. The vector solution is then infused at a rate of 1 mL per minute. Subject will remain 15-degree Trendelenburg (head down) for 1 hour following vector administration.

Table 23: Dose extrapolation based on age and brain volume

Age (years)	Brain Volume* (approx. cm^3)	Infusion volume (mL)	Total IT Dose ($x10^{14}$ vg)
7+	1312	10	5 to 10
4	(same as above)	10	5 to 10
3	1180	9	4.5 to 9
2	1080	8.2	4.1 to 8.2
1	955	7.3	3.7 to 7.3
0.5	525	4	2 to 4
Newborn	400	3	1.5 to 3

Brain volume will be determined with the baseline MRI. The infusion volume for children <4 years old will be calculated as follows: ([MRI brain volume]/1312 cm^3) x 10 mL = infusion volume. The concentration of the injection solution will be kept constant at $5x10^{13}$ vg/mL or $1x10^{14}$ vg/mL, so the total vg dose will be scaled according to the volume injected. *References [48, 49, 50].

6.7. Dosing Device for Phase I/II Clinical Trials

A Pajunk Atraumatic Sprotte Needle will be inserted percutaneously at the lumbar level into the IT space of the spinal column. A volume of CSF approximately equal to the infusion volume is withdrawn from lumbar thecal sac. The AAV vector solution will be loaded into a 20 mL BD syringe, connected to the needle with 60" Marquette Medical IV extension tubing and Braun Discofix 4-way stopcock. The vector solution is then infused at a rate of 1 mL per minute, using a Medfusion 3500 pump. We will seek a letter of cross reference from IND 16007, which employs the same device. It did not significantly bind AAV9.

6.8. Protocol for Gene Therapy

The current anticipated assessment protocol is as follows. This Phase I/II clinical trial is a single dose, dose-escalation study of self-complementary, recombinant AAV9 carrying a human *SUMF1* gene (AAV9/SUMF1), by IT vector administration delivered to individuals with MSD disease at the University of Texas Southwestern in cohorts 1 and 2. Vector infusion will take place in the inpatient setting within the pediatric intensive care unit (PICU) or interventional radiology suite as clinically indicated. Participants will be evaluated over a 24-month period.

[redacted]

Table 24: Visit and monitoring schedule (May revise to eliminate visits at day 270 and 540)

Visit/ Screen¥	[redacted]
Day	
Informed Consent	
Informed Consent Review	
Vitals	
Medical History	
Physical Exam	
Con Meds	
AE Review	
Screen Labs*	
Safety Labs**	
EKG	
PFTs	
Neurologic Exam	
PT Evaluation	
Eye Exam	
Skin Exam	
Lumbar Puncture	
CSF Analysis***	
Neuropsych	
MRI	
EMG/NCS	
PSG	
BAER	
AAV NAb Titers	
Clinical Assessment	
PROs	
C-SSRS	

[redacted]

[redacted]

6.8.1. Screening/Baseline Measures Prior to Vector Injection (Day -28 to Day -7)

Screening/Baseline measures from subjects will be obtained prior to gene transfer.

The following evaluations will be conducted during their Screening Visit:

- Medical history
- A complete physical examination
- Concomitant medication history
- Vital signs (temperature, respiratory rate, heart rate, blood pressure)
    - Height, weight and head circumference (FOC)
        - The weight obtained at screening.
- Ophthalmology examination including visual acuity, visual fields, optical coherence tomography, intraocular pressure, and fundus photography
- Hearing evaluation with brainstem auditory evoked responses (BAER)
- Electrocardiogram (EKG)
- Echocardiogram
- Pulmonary function tests (PFTs) with end tidal CO_2
- Polysomnography (PSG)
- Skin evaluation with Ichthyosis Severity Index [43]
- Safety-related laboratory studies:
    - Complete blood cell count (CBC) and differential with smear
    - Electrolytes
    - Blood urea nitrogen (BUN)
    - Creatinine
    - Aspartate aminotransferase (AST)
    - Alanine transaminase (ALT)
    - Gamma-glutamyl transpeptidase (GGT)
    - Alkaline phosphatase
    - Serum total and direct bilirubin
    - Serum total protein
    - Albumin
    - Prothrombin time (PT) and INR
    - Activated partial thromboplastin time (PTT)
    - Serum glucose
    - Serum calcium
    - Amylase
    - Lipase
    - Urinalysis
    - Blood for DNA isolation
    - Alpha-fetoprotein
    - Hepatitis (A, B, C) and HIV by PCR
    - Plasma biobanking
    - Blood DNA and RNA biobanking
- Disease-related laboratory studies:
    - Leukocyte sulfatase activity levels
    - Urine glycosaminoglycans (GAG) and sulfatides substrate levels
- Biological fluid samples for vector shedding (urine, saliva, feces, and plasma)
- ELISpots for T-cell responses to AAV9 and SUMF1 protein

[redacted]

- ELISA for detection of total antibodies to AAV9
- Confirm diagnosis of MSD via gene sequencing and leukocyte sulfatase activity levels (deficiency demonstrated in 3 or more sulfatases)
- Global disease burden and motor assessment:
    - NIH Toolbox Early Childhood Motor Battery or NIH Toolbox Motor Battery
    - Beery-Buktenica Developmental Test of Visual-Motor Integration, 6th Edition
    - Peds QL (Quality of Life Assessment)
    - Passive range of motion and functional motor evaluation by a physical therapist with expertise in pediatric neurodegenerative diseases
- Global cognition, development, and adaptive function:
    - Leiter International Performance Scale, 3rd Ed
    - Reynolds Intellectual Assessment Scales, 2nd Ed
    - Mullen Scales of Early Learning or Leiter International Performance Scale, 3rd Edition
        - Mullen Scales of Early Learning will only be administered if participants are unable to demonstrate understanding of subtests required for a Full IQ after administration of entry level teaching trials on the Leiter International Performance Scale, 3rd Ed
    - Expressive One-Word Picture Vocabulary Tests, 4th Edition
    - Receptive One-Word Picture Vocabulary Tests, 4th Edition
    - NEPSY, 2nd Ed
    - Child and Adolescent Memory Profile
    - Vineland Adaptive Behavior Scales, 3rd Edition
- Administered standard assessments (cognitive assessments) will be video-recorded, throughout the trial.
- X-ray spine scoliosis series
- X-ray cervical spine flexion and extension

6.8.2. Pre-infusion visit (Day -1)

Subjects enrolled based on the screening data will arrive to the hospital within 24 hours prior to gene transfer.

They will undergo the following evaluations:

- Medical History
- Adverse events
- A physical exam
- Vital signs (temperature, respiratory rate, heart rate, blood pressure)
    - Weight and head circumference (FOC)
- Concomitant medications will be reviewed
- Labs will be obtained, including
    - CBC and differential
    - Electrolytes
    - Serum total protein
    - Albumin
    - PT, PTT, INR
    - Creatinine
    - BUN
    - AST, ALT, serum GGT

[redacted]

- Alkaline phosphatase
- Amylase
- Lipase
- Serum total and direct bilirubin
- Serum glucose
- Serum calcium

[redacted]

6.8.3. Day of Gene Transfer (Day 1)

Phase I will enroll 6 individuals in two cohorts. The first cohort of 3 individuals will be initiated into the study on a rolling basis and undergo low-dose IT AAV9/SUMF1 gene transfer.

Subjects will be admitted to the hospital on the day of gene transfer. Prior to vector infusion, the subject will undergo the following evaluations:

- Medical History
- Adverse events
- A physical exam
- Concomitant medications
- Vital signs (temperature, heart rate, respiratory rate, blood pressure, pulse oximetry)
- Brain MRI (3T) with MR Spectroscopy and Diffusion Tensor Imaging
- Cervical, thoracic, and lumbar spine MRI
- Liver and spleen MRI for total volume
- Lumbar puncture
  - CSF sulfatase activity levels and glycosaminoglycan (GAG) substrate levels
  - Protein, glucose and cell count and differential
- Electromyography and nerve conduction study (EMG/NCS)
- A photograph will be taken of the chosen injection site prior to and after vector administration.

(Note: MRI, LP, BAER, and EMG/NCS will be performed while the individual is under a single sedation event under the direction of a qualified anesthesiologist or pediatric intensive care unit (PICU) physician)

Gene Transfer Procedures:

An independent assessment will be performed by anesthesia staff per local procedures and in accordance with clinical guidelines for multiple sulfatase deficiency (4) with vitals collected for clinical purposes to ensure the subject is cleared for sedation.

[redacted]

If the subject appears inadequately hydrated in the judgment of the Principal Investigator, bolus(es) of 10-20 mL/kg normal saline may be given during the time between subject check-in and gene transfer. The lowest level of sedation required will be used. Subjects will be continued on their usual diet until eight hours prior to gene transfer, after which they will have no solid food; clear liquids will be allowed up until NPO as per institutional guidelines for sedation, based on age. They will resume their usual diet after they have returned to pre-sedation baseline.

The study agent, AAV9/SUMF1 vials, will be formulated as a concentrated stock in phosphate buffered saline containing 5% D-sorbitol and stored at ≤ -60°C until the day of the administration procedure(s). The solution will be thawed within 4 hours of administration and diluted to the appropriate final dosage concentration and volume using phosphate-buffered saline with 5% D-sorbitol.

Gene transfer will be performed under sterile conditions within the Pediatric Intensive Care Unit or interventional radiology suite as appropriate.

AAV9/SUMF1 will be delivered one time intrathecally through a spinal needle.

IT administration:

The IT injection will be delivered in a constant volume (scaled with age and/or brain volume according to table 23. A Pajunk Atraumatic Sprotte needle will be inserted percutaneously at the lumbar level into the IT space of the spinal column. A volume of CSF approximately equal to the infusion volume is withdrawn from lumbar thecal sac and is sent for standard evaluation (CSF cell count and differential, glucose, protein, culture). The AAV vector solution will be loaded into a 20 mL BD syringe, connected to the needle with 60" Marquette Medical IV extension tubing and Braun Discofix 4-way stopcock. The vector solution is then infused at a rate of 1 mL per minute, using a Medfusion 3500 pump. We will seek a letter of cross reference from IND 16007, which employs the same device. Subject will remain 15-degree Trendelenburg (head down) for 1 hour following vector administration to promote distribution throughout the CSF space.

Dosing:

The stated doses are for subjects ≥4 years of age and will be scaled downward for younger subjects as appropriate.

- Cohort 1 (IT, Low dose): 5 X 10^{14} vg total in a volume of 10 mL (n=3)
- Cohort 2 (IT, High dose): 1 X 10^{15} vg total in a volume of 10 mL (n=3-6)

Subjects will be closely monitored for side effects during the infusion: Heart rate, respiratory rate, pulse oximetry, temperature, and blood pressure will be measured before the infusion, every five minutes during the infusion, immediately after the infusion, and repeated at 15 minutes post-infusion for 1 hour, then hourly for the next 4 hours, then 4 hours thereafter until discharge.

Infusion reactions:

Infusion reactions will be graded and reported according to CTCAE v.4 criteria:

Grade 1: Transient flushing or rash, drug fever <38 degrees C (<100.4 degrees F); intervention not indicated

Grade 2: Rash, flushing, urticaria, dyspnea, drug fever >38°C:
Intervention or infusion interruption indicated; responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics); prophylactic medications indicated for ≤24 hrs.

Grade 3: Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates)

Grade 4: Life-threatening consequences; urgent intervention indicated

[redacted]

Grade 5: Death

Infusion will be terminated for evidence of an allergic reaction of **grade 2 or greater**. Under CTCAE v.4 criteria, **anaphylaxis** is by definition grade 3 (Symptomatic bronchospasm with or without urticaria; allergy-related edema/angioedema, hypotension), and would result in infusion termination and systemic treatment.

Subjects will remain in ICU bed following gene transfer and remain admitted to the hospital for 48 hours after gene transfer. Vital signs will be obtained every 15 minutes for the first hour post-infusion, then hourly for 4 hours following the injection and then every 4 hours until discharge. Transfer out of the PICU may be undertaken after the initial 24 hours of post-infusion monitoring, if there are no medical safety concerns in the opinion of the Principal Investigator.

6.9. Post Therapy Monitoring

A Data and Safety Monitoring Board (DSMB) will review all labs and safety data from the first individual at the 30-day period before dosing the second individual. Similarly, safety labs will be reviewed from the first 2 individuals (at least 30 days follow-up each) before planned lumbar puncture to ensure no contraindications to the procedure (coagulopathy) and to ensure no evidence of SAEs that would prompt review.

Thirty days following the last dosing within cohort one, data will be reviewed by the DSMB to determine if cohort two will be enrolled utilizing the same dose, a lower dose or a higher dose according to the dose escalation plan.

6.9.1. Early Inpatient Monitoring (up to 48 hours of AAV9/SUMF1 dose)

The subject will be evaluated by the Principal Investigator or designee on the morning of day following AAV9/SUMF1 administration for adverse events, vital signs and repeat physical examination and laboratory studies for assessment of safety will be collected. [redacted].

First 24 hours:

- Medical history
- Adverse events
- Concomitant medications
- Physical examination
- Vital signs (temperature, heart rate, respiratory rate, blood pressure, pulse oximetry, and telemetry)
- Labs will be obtained, including
    - CBC and differential
    - Electrolytes
    - Serum total protein
    - Albumin
    - PT, PTT, INR
    - Creatinine
    - BUN
    - AST, ALT, serum GGT
    - Alkaline phosphatase
    - Amylase, lipase
    - Serum total and direct bilirubin

[redacted]

- Serum glucose
- Serum calcium
- Urinalysis

- Samples for vector shedding (plasma, urine, saliva and feces)

[redacted]

24-48 hours:

- Medical history
- Adverse events
- Concomitant medications
- Physical examination
- Vital signs (temperature, heart rate, respiratory rate, blood pressure, pulse oximetry, telemetry)
- Samples for vector shedding (plasma, urine, saliva and feces)
- Disease-related laboratory studies:
  - Serum/leukocyte sulfatase enzyme activity levels
  - Urine glycosaminoglycans (GAGs) and sulfatides
- ELISA for detection of serum antibodies to AAV9

The subject will be evaluated by the Principal Investigator or designee on the morning of Day 2 for repeat physical examination. Oral prednisone or prednisolone will be administered as outlined in the peri-gene transfer immunosuppression regimen above. On the second day after gene transfer (Day 2), the subject will be discharged from the hospital, after administration of prednisone/prednisolone, if the physical examination and laboratory studies do not indicate a need for further inpatient level care.

6.9.2. Follow up monitoring (may be revised to eliminate visits at day 270 and 540)

6.9.2.1. In patient follow up (Day 7, 30, 60, 90, 180, 270, 360, 540 and 720)

Subjects will return for follow up visits on after treatment ±3 days. At each visit, the following assessments (or a portion) will be performed per the schedule in table 24.

- Medical history (except 7)
- Adverse events
- Concomitant medications (except 7)
- Physical examination
- Vital signs (temperature, heart rate, respiratory rate, blood pressure)
  - Height, weight, and head circumference
- Echocardiogram (except 7)
- Safety-related laboratory studies:

[redacted]

    - Complete blood cell count (CBC) and differential with smear
    - Electrolytes
    - Blood urea nitrogen (BUN)
    - Creatinine
    - Aspartate aminotransferase (AST)
    - Alanine transaminase (ALT)
    - Gamma-glutamyl transpeptidase (GGT)
    - Alkaline phosphatase
    - Total protein
    - Serum total and direct bilirubin
    - Prothrombin time (PT) and INR
    - Activated partial thromboplastin time (PTT)
    - Serum glucose
    - Serum calcium
    - Amylase
    - Lipase
    - Urinalysis
    - Alpha-fetoprotein
    - Plasma biobanking
- Samples for vector shedding (plasma, urine, saliva and feces)
- Disease-related laboratory studies:
    - Serum/leukocyte sulfatase enzyme activity levels
    - Urine glycosaminoglycans (GAGs) and sulfatides
- ELISA for detection of serum antibodies to AAV9
- ELISpot for T cell responses to AAV9 and SUMF1
- Brain MRI under anesthesia (30, 180, 360, and 720)
- Lumbar puncture under anesthesia (30, 180, 360, and 720)
    - CSF analyses
        - Sulfatase enzyme activity levels
        - Glycosaminoglycan (GAG) substrate levels
        - Protein, glucose, cell count and differential, culture
- Vector shedding analysis on 1, 7, 14 and 60 days post dosing with AAV9/SUMF1 may be performed on DNA isolated from positive biological fluids (plasma, urine, saliva and feces) only if two previous consecutive positive samples were positive for the presence of viral DNA for each specimen type.

6.9.2.2. Outpatient follow up (Day 14±3)

Lab work on the days listed will be directed at safety. Subjects unable to travel to the hospital will have their blood drawn by a contracted home care service.

- AAV titers
- Safety-related laboratory studies:
    - Complete blood cell count (CBC) and differential with smear
    - Electrolytes
    - Blood urea nitrogen (BUN)
    - Creatinine
    - Aspartate aminotransferase (AST)
    - Alanine transaminase (ALT)

[redacted]

- Gamma-glutamyl transpeptidase (GGT)
- Alkaline phosphatase
- Total protein
- Serum total and direct bilirubin
- Prothrombin time (PT) and INR
- Activated partial thromboplastin time (PTT)
- Serum glucose
- Serum calcium
- Amylase
- Lipase
- Urinalysis
- Alpha-fetoprotein
- ELISpot for T cell responses to AAV9 and SUMF1
- Plasma biobanking

6.9.3. Long Term Monitoring

We will follow the most recent FDA guidelines with regard to long-term subject follow-up following gene transfer. As indicated by the guidelines, our proposed vector has a very low probability of gene transfer-related adverse events. We will, however, evaluate short-term safety over a two-year period that incorporates the active phase of the protocol. Following the active two-year follow-up phase of the study, subjects will then transfer to a long-term monitoring program where data will continue to be collected from annual visits with their standard care physician up to 15 years following AAV9/SUMF1 therapy.

Communication will be established with each subject's local standard-care physician, and participation will be requested from them as part of this long-term monitoring program. When feasible, subjects will be asked to continue to be followed at the University of Texas Southwestern Medical Center either in-person or via telemedicine approach. The following information will be requested to be collected annually on standard case report forms (CRFs) and submitted to UTSW as part of the annual monitoring program:

- Review of any medical issues, pain, or discomfort
- Physical exam and review of body systems
- Medications
- Survey of behavioral and day to day function.
- Repeat motor, cognitive, and quality of life measures

If newly identified risks are associated with our product, or if the subjects suffer any adverse events during this period, we will initiate a long-term follow-up according to the FDA guidelines.

6.10. Outcome measures

The study is a phase I/II clinical trial inclusive of safety and efficacy outcome measures

A. Safety is a primary outcome for this clinical gene transfer trial. Stopping criteria within a certain dose cohort are based on development of unacceptable toxicity defined as the occurrence two or more Grade III or higher unanticipated treatment-related toxicities.

B. Biopotency outcome measures: Although tools have been developed for natural history assessment, because of the lack of treatment and the rarity of the disease, to date, no specific outcome measures have been established specifically for evaluation of treatment response in

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MSD disease. We therefore propose to use as secondary efficacy measures in this trial improvements in expression of hallmarks of MSD disease: CSF and serum leukocyte sulfatase activity levels. Specifically, we anticipate that the CSF and serum sulfatase activity levels will increase in response to gene transfer. This ratio will be compared to that of pre-treatment CSF and serum leukocyte sulfatase enzyme activity. Significant changes in post treatment CSF and serum leukocyte sulfatase enzyme activity levels represent the therapeutic target and are likely to have clinical relevance. Additionally, levels of glycosaminoglycan excretion in the urine will be measured as a marker of substrate accumulation. We anticipate the levels of urinary glycosaminoglycans to decrease post-treatment. Serum and CSF leukocyte sulfatase enzyme activity levels and urine glycosaminoglycan levels will be performed at a laboratory that has established and performed these assays under CLIA-certified procedures (Greenwood Genetic Center).

C. Efficacy outcome measures: Because of the lack of prior treatment trials and the rarity of the disease, to date, no specific outcome measure has been formally established as the optimal measure for therapeutic evaluation. Based on (1) the characteristic clinical presentation of MSD patients, (2) our pre-clinical data in MSD mice and (3) published data, where the cognitive and behavioral assessments have been shown to be able to measure disease progression, we propose the following exploratory outcome measures up to 24 months.

I. Explore a changes in serum sulfatase enzyme activity over baseline as a possible biomarker

II. Explore a change in CSF sulfatase enzyme activity over baseline as a possible biomarker

III. Explore the stabilization, reduction, or normalization of urinary excretion of GAGs and sulftatides compared to baseline and natural history of the disease.

IV. Explore measuring a reduction in CSF levels of GAGs compared to baseline, as a possible biomarker.

V. Stabilized or improved motor function or arrest of motor deterioration at 180, 360 and 720 days after treatment based on NIH Toolbox Motor Battery.
    a. MSD is characterized by a marked decline in motor skills and eventually total loss of ambulation. The NIH Toolbox Motor Battery is validated for children ages 3 years and older and measures dexterity, grip strength, standing balance, endurance, and gait speed. If all measures are completed, the test takes approximately 20 minutes.

VI. Improved cognitive ability or arrest of cognitive deterioration at 180, 360 and 720 days after treatment, as measured by the following:
    a. MSD is characterized by a progressive loss of cognitive and social skills. Patients eventually lose all verbal communication skills and become intellectually disabled and completely dependent on caregivers. As it is likely we will capture participants at variable levels of disease progression, the following measures are designed to accommodate a broad range of cognitive and adaptive skill levels. See table 21 for further detail on the specific measures selected.

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VII. Slowing of brain atrophy as measured by decreased rate of gray matter volume loss, total brain volume loss, or ventricular volume increase, compared with natural history of disease at 180, 360 and 720 days.
    a. As MSD patients experience clinical progression, the total brain volume decreases as there is a compensatory increase in ventricular volume. This can be evaluated using volumetric analysis and whole brain gray matter density measurements to compare rate of change from baseline.

VIII. Reduction, normalization, or stabilization of liver and spleen volume at 180, 360 and 720 days.
    a. The liver and spleen enlarge in patient with MSD as clinical progression worsens. We aim to measure the volume of these organs via MRI overtime.

IX. Stabilization or improvement in respiratory function as measured by pulmonary function tests and end tidal CO_2 at 30, 60, 90, 180, 270, 360, 540, and 720 days.
    a. Secondary to neurodegenerative changes in muscle strength/tone and skeletal changes, MSD patients are at an increased risk of progressive respiratory insufficiency and a significant contributor to morbidity and mortality. We will monitor end tidal CO2 as a marker of respiratory insufficiency in all participants as well as PFTs in patients over the age of 6 years old and capable of participating in the testing.

X. Stabilization or improvement in sleep apnea as measured by polysomnography at 360 and 720 days.
    a. Both obstructive sleep apnea (related to skeletal changes and hypotonia) and central sleep apnea (related to brainstem dysfunction and dysautonomia) have been reported in MSD and have a significant impact on quality of life of both the patient and the family/caregivers. We will perform formal polysomnography annually and collect parent report of sleep at all clinical visits.

XI. Stabilization or improvement in hearing function as measured by brainstem auditory evoked responses at 180, 360, and 720 days.
    a. Progressive hearing impairment is felt to be common in MSD and can have a negative impact on language and communication function. We propose to objectively quantify hearing function by measuring BAER.

XII. Stabilization or improvement in peripheral neuropathy as measured by EMG/NCS at 180, 360, and 720 days.
    a. A progressive peripheral neuropathy has been described in MSD and contributes to loss of motor function as well a potential source of neuropathic pain. We propose to measure conduction velocities on nerve conduction studies to quantify the degree of sensorimotor neuropathy over time.

XIII. Reduction or stabilization of scores on the Ichthyosis Severity Index at 30, 60, 90, 180, 270, 360, 540, and 720 days.
    a. Dry skin is a common finding in MSD and contributes to morbidity and quality of life of MSD patients. We will utilize the Ichthyosis

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Severity Scale to objectively quantify the degree or severity of skin changes over time. This scale involves rating erythema and presence of scales/dry skin in 4 body regions on a 5-point Likert scale (0 to 4) with a maximum score of 32 indicating the most severe erythema and scaling. This scale can be completed by the physician in the office.

6.11. Statistical Analysis

The statistical analysis plan will be developed in conjunction with a biostatistician, who has collaborated with the University of Texas at Southwestern on research, as the overall clinical study design and outcome measures are finalized.

6.12. Proposed Clinical Development Plan

Future development plans are contingent on determining the safety of AAV9/SUMF1 in individuals with MSD disease as proposed in this phase I/II trial. If the safety profile is satisfactory, it is the sponsor's intent to continue development of the product to prolong survival and halt disease progression in individuals with MSD disease. If a suitable commercial partner is identified, this may include the submission of a Biologic License Application once appropriate data have been collected.

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