Clinical Trial Protocol

A Phase 1/11 Open-label Intrathecal Administration of MELPIDA to Determine the Safety and Efficacy for Patients with Spastic Paraplegia Type 50 (SPGS0) caused by a Mutation in the AP4M1 gene.

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Sponsor Address:	6 Topham Rd Toronto, Ontario M4B 3K2 Canada
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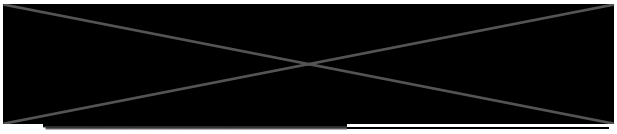
SIGNATURE PAGE AND REVISION HISTORY



8/28/2023

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Amendments: The following sections/appendices have been updated:

Section/Appendix	Description	Date
Amendment 1	Submitted with original IND application	22 JUL 2022
Amendment 2	Revision following 30 day review	16 SEP 2022
Amendment 3	Following IRB review	09 FEB 2023
Amendment 4	Revision of long term follow up	24 FEB 2023
Amendment 5	Addition of SAE	18 APR 2023
Amendment 6	Extension of inclusion age to 4 months-10years	9 AUG 2023

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List of Abbreviations

AAV	adeno-associated virus
Ab	antibody
AE	Adverse event
Ag	antigen
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
AP-4	Adaptor Protein complex
AP4M1	Adaptor protein complex, μ4
AST	Aspartate aminotransferase
ATG9A	Autophagy Related 9A
BCH	Boston Children's Hospital
BGH	Bovine growth hormone
BUN	Blood urea nitrogen
Ca	calcium
CBC	Complete blood counts
CDMO	Contract development and manufacturing organization
CK-MB	Creatine kinase - isotype MB
Cl	chloride
CMC	Chemistry, manufacturing and controls
CNS	Central nervous system
CO2	Carbon dioxide
Cr	Creatinine
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CTA	Clinical Trial Application
DAPI	4',6-diamidino-2-phenylindole
DNA	Deoxyribonucleic acid
DTI	Diffusion tensor imaging
EEG	electroencephalogram
EKG	electrocardiogram
ESR	Erythrocyte sedimentation rate
FDA	Food and Drug Administration
GFP	Green fluorescent protein
GGT	Gamma-glutamyl transferase
GLP	Good laboratory practice
h	Human

hAP4M1opt	Human optimized AP4M1
HSP	Hereditary spastic paraplegia
HC	Health Canada
HCT	hematocrit
HIV	Human immunodeficiency virus
HTLVI	Human T-Lymphotropic Virus Type 1
IND	Investigational New Drug
INR	International normalized ratio
iPSCs	Induced pluripotent stem cells
IRNHS	International registry Natural History Study
IT	Intrathecal
ITR	Inverted terminal repeat(s)
K	Potassium
kg	Kilogram
LFT	Liver function tests
LP	Lumbar puncture
MCV	Mean corpuscular volume
MOI	Multiplicity of Infection
MRI	Magnetic resonance imaging
Na	Sodium
NCS	Nerve conduction studies
NAb	Neutralizing antibody
NHP	Non-human primates
NIH	National Institutes of Health
opt	Optimized
OOPD	Office of Orphan Products Development
PACU	Post anesthesia care unit
PBMC	Peripheral blood mononuclear cell
PI	Principal investigator
PICU	Pediatric intensive care unit
PLT	Platelets
PPD	Purified protein derivative
ProBNP	Pro B-type Natriuretic peptide
PT	Prothrombin time
PTT	Partial Prothrombin time
SC	Self-complimentary
SPG50	Spastic Paraplegia 50
RNA	Ribonucleic acid

SAE	Serious adverse event
SUSAR	Suspected unexpected serious adverse reaction
TB	tuberculosis
TGN	Trans-Golgi network
Tn	troponin
US	Unites States of America
UTSW	University of Texas Southwestern Medical Center
VVC	Viralgen Vector Core, Spain
vg	Vector genome(s)
WT	Wild type

Title	A Phase 1/2 Open-label Intrathecal Administration of MELPIDA to Determine its Safety and Efficacy for Patients with Spastic Paraplegia Type 50 (SPG50) caused by Mutation in the AP4M1 gene.
Study Description	This will be a Phase 1/11, open-label, single dose clinical study of MELPIDA administered intrathecally (IT) through a lumbar puncture (LP) to subjects with confirmed pathogenic mutations in the AP4M1 gene.
Number of Subjects	3 Patients
Clinical Study Phase	Phase 1/11
Sponsor	CureSPG50
PI	Dr. Susan T. Iannaccone MD, FAAN
Co-PI	Dr. Kaitlin Batley, Dr. Veronica Bordes Edgar
Study Objectives	Primary outcome: determination of the safety and tolerability of MELPIDA in patients with SPG50, based on development of toxicity Secondary outcome: preliminary exploration of efficacy
Study Intervention	MELPIDA, a recombinant serotype 9 adeno-associated virus (AAV) encoding a codon- optimized human AP4M1 transgene
Study Dose	A single intrathecal infusion at IE14 vg/mL for a total dose of 1EI5 vg (for patients 4 years of age or older or based on Table 1 for patients younger than 4)
Study Population	Children with a confirmed mutation in the AP4M1 gene
Study Duration	The total study duration is 5 years post dosing. The participant will be tested at screening/baseline (-28 to -7 days), return for dosing, and then follow-up visits post-dosing on Days 7 (+/-2), 30 (+/-2), 60 (+/-2), 90 (+/-14), 180 (+/-14), 270 (+/-14), 360 (+/-14), 540 (+/-14), and 720 (+/-14) days, then annually for the last 3 years.
Inclusion Criteria	• Age 4 months to 10 years old • Body weight at or above 3rd percentile for age • Confirmed diagnosis of SPG50 disease by: • Genomic DNA mutation analysis demonstrating homozygous or compound heterozygous, confirmed pathogenic variants in the AP4M1 gene • Parent/legal guardian willing to provide written informed consent for their child prior to participation in the study • Subject able to comply with all protocol requirements and procedures For subjects 1 year or more: • Ability to stand for more than 5 seconds OR • Ability to take 5 steps independently or with a walker OR • Modified Ashworth Scale score 2 or below (Ankles).
Exclusion Criteria	• Inability to participate in study procedures (as determined by the site investigator) • Presence of a concomitant medical condition that precludes lumbar puncture (LP) or use of anesthetics • History of bleeding disorder or any other medical condition or circumstance in which lumbar puncture is contraindicated according to local institutional policy • Inability to be safely sedated in the opinion of the clinical anesthesiologist • Active infection, at the time of dosing, based on clinical observations

	• Concomitant illness or requirement for chronic drug treatment that in the opinion of the PI creates unnecessary risks for gene transfer • Inability of the patient to undergo MRI according to local institutional policy • Inability of the patient to undergo any other procedure required in this study • The presence of significant non-SPGS0 related CNS impairment or behavioral disturbances that would confound the scientific rigor or interpretation of results of the study • Have received an investigational drug within 30 days prior to screening or plan to receive an investigational drug (other than gene therapy) during the study. • Enrollment and participation in another interventional clinical trial • Contraindication to MELPIDA or any of its ingredients • Contraindication to any of the immune suppression medications used in this study • Clinically significant abnormal laboratory values (GGT, ALT, and AST, or total bilirubin > 3 x ULN, creatinine > 1.5 mg/dL, hemoglobin [Hgb] < 6 or> 20 g/dL; white blood cell [WBC] > 20,000 per mm3) prior to gene replacement therapy.
Study Design	This will be a first-in-human Phase 1/11, open-label, single dose clinical study of MELPIDA administered intrathecally through a lumbar puncture to subjects with confirmed pathogenic mutations in the AP4M1 gene.
Primary Endpoints	Incidence of unanticipated treatment-related adverse events, Grade 3 or higher
Secondary Endpoints	Stability or improvement in spasticity based on the Modified Ashworth scale (MAS) and/or Tardieu scale.
Exploratory Endpoints	• Bayley Scales of Infant and Toddler Development, 3rd Edition (Bayley-III) cognitive, language, fine motor. • Vineland Adaptive Behavior Scales, 3rd Edition (Vineland-3) • 10-meter walk test (item 3 on the Spastic Paraplegia Rating Scale) • Improvement of gait (three-minute walk test) • Spastic Paraplegia Rating Scale (SPRS) • Gross Motor Function Measure (GMFM)-88 • SPATAX-EUROSPA Disability Score • CPCHILD (Caregiver Priorities and Child Health Index of Life with Disabilities) for health-related quality of life • Clinical Global Impression of Overall Change by Physician (CGI)
Sample Size	N=3

1. INTRODUCTION AND BACKGROUND

MELPIDA is a gene therapy product being developed for the treatment of Spastic Paraplegia Type 50 (SPG50), which is one of a group of four genetic disorders (SPG47, SPG50, SPG51 and SPG52) comprising AP-4 related Spastic Paraplegia (AP4-SPG). Inherited in an autosomal recessive pattern, AP-4- SPG is caused by biallelic pathogenic variants in one of 4 genes that encode components of the heterotrimeric adaptor protein complex 4 (AP4). Mutations in any of the components result in disrupted AP-4 function, and result in a common, shared clinical phenotype (Behne et al., 2020; Ebrahimi-Fakhari et al., 2020; Ebrahimi-Fakhari et al., 2018).

Adaptor protein complexes such as AP-4 play key roles in signal-mediated trafficking of integral membrane proteins. They mediate vesicle formation and the cargo contained within these vesicles (Jamra et al., 2011). While the precise function of the AP-4 complex is not fully understood, recent data suggests it plays an important role in protein sorting through the Golgi, including regulation of trafficking of components required for autophagy (Davies et al., 2018).

Deficiency in AP-4 leads to progressive neurodegeneration.

AP-4-HSP is an ultra-rare autosomal recessive disease with ~156 patients identified worldwide, 59 of which have the SPG50 subtype. There are approximately 9 patients with SPG50 in North America (OMIM #612936) (source: Ebrahimi-Fakhari et al., 2020), ClinicalTrials.gov Identifier: NCT04712812. SPG50 is caused by biallelic pathogenic variants in the AP4M1 gene.

The AP4-deficiency syndrome (AP-4-HSP) is characterized by progressive spasticity, microcephaly, intellectual deficiency, dysmorphic traits, and growth retardation (Roubertie et al., 2018). Symptoms of AP-4-HSP begin in infancy, though patients are often not correctly identified and diagnosed until age 5 to 10 years. Patients experience progressive spastic paraplegia in the first decade of life, resulting in quadriplegia by adolescence or early adulthood with associated wheelchair dependence. There is also the presence of severe, progressive cognitive impairment. Epilepsy is an important co-morbidity present in the majority of cases (Ebrahimi-Fakhari et al., 2018). Only a few affected individuals have been identified to survive beyond age 30 year, though the extent of early mortality is yet to be fully elucidated (Ebrahimi- Fakhari et al., 2020).

Based on an AP-4-HSP natural history study currently in progress at Boston Children's Hospital (BCH), it is evident that disease severity ranges from child to child, but that most children fall into the severely affected (i.e., severe spasticity with paralysis and severe cognitive impairment) category. A small proportion of children, considered least severe, are able to speak in short sentences, walk with an abnormal gait, and have few to no seizures early on in the disease (less than 10 years of age). However, most children in this less severe category still experience progressive decline, ultimately losing the ability to walk and becoming quadriplegic between the ages of 10 and 20 years.

The majority of children with the SPG50 subtype of AP-4-HSP conform to a severe presentation, and are completely non-verbal, have microcephaly, never walk, have

epilepsy and are severely cognitively impaired by the age of 10. It is not known how patients are affected later in life as very few have been identified beyond the age of 30. SPG50 is thus a degenerative neurological disease, affecting both cognitive and motor capabilities. Importantly, there is significant care giver burden, as all patients eventually require complete support for all activities of daily living from family and/or caregivers. There are no treatments currently available for patients with SPG50.

Additional details on the disease pathophysiology and its progression are available in the Investigator's Brochure (Section 2).

2. RATIONALE FOR THE STUDY

SPG50 is a monogenetic disease caused by biallelic variants in the *AP4M1* gene. *APM41* encodes a subunit of the AP complex; mutations in APM41 result in failure to produce functional APM41 protein and impairment of the AP complex. Due to the small size of the *APM41* gene, and the loss of expression/function nature of AP4M1 mutations, SPG50 is ideally suited for gene replacement therapy. MELPIDA is a recombinant serotype 9 adeno-associated virus (AAV9) encoding a codon-optimized human AP4M1 transgene (Figure 1). 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 synthetic "UsP" promoter, codon-optimized human AP4M1 deoxyribonucleic acid (DNA) coding sequence, the bovine growth hormone (BGH) polyadenylation signal, and wild-type AAV2 ITR. As a gene therapy, MELPIDA is expected to provide a fully functional human AP4M1 cDNA copy to targeted neuronal and non-neuronal cells of the participant. Production of a fully functional AP4M1 subunit is hypothesized to halt neurodegeneration through the production of functional AP complex.

Figure 1 Schematic of MELPIDA

MELPIDA is an AAV9-based gene therapy vector that expresses the fully functional form of AP4M1 under the control of a synthetic promoter. MELPIDA will be delivered intrathecally and is designed to achieve stable, potentially life-long expression of AP4M1 in non-dividing cells. This clinical study is a first-in-human study designed to assess safety and tolerability of MELPIDA in SPG50 participants, as well as examine the clinical impact of the gene therapy on disease progression.

Numerous investigators have utilized recombinant AAV9 directed at the central nervous system (CNS) in on-going gene therapy clinical trials (clinical trial.gov identifiers NCT02122952, NCT02362438, NCT02725580, NCT02716246, NCT03315182). These vectors are non-pathogenic, non-replicating, and transduce non-dividing cells. However, the recombinant vectors are incapable of coding viral proteins or actively integrating with the host genome, making them ideal vectors for gene delivery. 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, targeted CNS-focused administration (via intrathecal administration) achieves broad transgene distribution throughout the CNS. An approach utilizing intrathecal (IT) administration of AAV9 was first advanced as a treatment of Giant Axonal Neuropathy (GAN). The laboratory of Dr. Steven Gray, in partnership with Hannah's Hope Fund, initiated the first intrathecal AAV9 gene therapy trial, which was a first-in-human Phase I gene therapy clinical trial for GAN, in collaboration with Dr. Carsten Bonnemann at the US National Institutes of Health Clinical Center (NCT02362438) in 2015.

There are no approved treatments for SPG50, leaving an unmet medical need for this serious, rare, progressive, and ultimately fatal neurodegenerative disease.

3. STUDY OBJECTIVES

3.1. Primary Objectives

The primary objective of this study is to evaluate the safety and tolerability of a single dose of MELPIDA administered intrathecally to children with SPG50 disease. Incidence of unanticipated treatment-related toxicities, Grade 3 or higher, will be determined from the collection of occurrence and severity of serious adverse events (SAEs).

3.2. Secondary Objectives

The secondary objectives will be efficacy of the drug. Efficacy will be determined by the stability or improvement in spasticity as assessed using the Modified Ashworth scale (MAS) and Tardieu scale. These assessments are summarized in the Table 3 and explained in Section 9.

3.3. Exploratory Objectives

Participants will undergo motor function, neuropsychological, and disease burden assessments every 3 months starting at screening/baseline to 24 months, then annually until 5 years post-dose. Additional assessments to evaluated as exploratory objectives include:

- Bayley Scales of Infant and Toddler Development, 3rd edition (Bayley-III)
- Vineland Adaptive Behavior Scales, 3rd Edition (Vineland-3)
- 10-meter walk test (item 3 on the Spastic Paraplegia Rating Scale)
- Improvement of gait (three-minute walk test)
- Spastic Paraplegia Rating Scale (SPRS)
- Gross Motor Function Measure (GMFM)-88
- SPATAX-EUROSPA Disability Score

- CPCHILD (Caregiver Priorities and Child Health Index of Life with Disabilities) for health-related quality of life
- Clinical Global Impression of Overall Change by Physician (CGI)

4. INVESTESTIGATIONAL PLAN

4.1. Study Design

This will be a Phase I/II, open-label, single dose clinical study of MELPIDA administered intrathecally (IT) through a lumbar puncture (LP) in subjects with confirmed pathogenic mutations in the AP4M1 gene and if old enough, clinical signs/symptoms of SPG50 disease.

As of August 2023, 3 subjects have been dosed. A single subject (aged 4y) received MELPIDA at 1E15 vg in March 2022 under a Canadian CTA, and 2 subjects have been dosed (February 2023 and May 2023) under the US IND. As of 1 June 2022 1 August 2023, 6 and 3 months after dosing, preliminary data indicate MELPIDA is well tolerated, with only mild adverse events noted.

A second subject that has been dosed with MELPIDA as part of this study, reported similar side effects after receiving the study drug:

- Vomiting
- Anemia
- Weight loss
- Neutropenia

4.2. Investigational Product

MELPIDA vials will be formulated as a concentrated stock in phosphate-buffered saline (PBS) containing 5% D-sorbitol and 0.001% Poloxamer 188 and stored at $\leq$ -60°C until the day of the administration. The solution will be thawed within 4 hours prior to administration and diluted to the appropriate final dosage concentration and volume using PBS with 5% D-sorbitol and 0.001% Poloxamer 188 (if necessary).

4.3. Packaging

MELPIDA is supplied as a 2 mL Daikyo CZ® vial containing 1.15 mL of a sterile clear solution. Each mL contains 1E14 vector genome-containing particles (vg) of MELPIDA in phosphate buffered saline (PBS) containing 5% sorbitol and 0.001% Poloxamer 188.

4.4. Labeling and Storage

MELPIDA and diluent are labeled with the lot/batch number, individual vial number, contents, and manufacture date, along with a warning that they are for investigational use

only. They will be stored at or below -80°C in Room Y4.204 on the campus of UTSW This is a locked, certified, and monitored freezer located within the UTSW Translational Gene Therapy Core, where the drug product will be stored.

4.5. Dose and Route

The participants will have a spinal needle inserted percutaneously at the lumbar level into the intrathecal space of the spinal column (L4/L5 interspace). A volume of CSF approximately equal to the infusion volume is withdrawn from the lumbar thecal sac. With the patient in the Trendelenburg position (head down), the vector solution is then infused at a rate of 1 mL per minute for a total of 10 mL for participants 4 years of age and older (see Table 1 for volume adjustments for younger participants). The participant will remain side-lying in the Trendelenburg position (head down) at 15 degrees for one (1) hour following administration, during which time the patient will be turned (from left to right side/right to left side) every 15 minutes. Dosing volumes will be calculated per Table 1, depending on final vector product concentration. It has been previously reported tha age related volumetric changes in brain white matter, grey matter and CSF, increase rapidly during the first 2 years after birth (Matsuzawa et al, 2001) and CSF volumes remain steady during this period. The procedure will be performed in a procedure unit with an anesthesiologist or qualified physician present to administer sedation as needed. Participants will be closely monitored in the Intensive Care Unit for 24 hours post procedure. An immune suppression regimen will be utilized. Prophylactic enteral prednisone or prednisolone and sirolimus will be administered to participant. Additional immunosuppression with tacrolimus will be administered as defined in the immune modulation protocol (Section 6.10).

Table 1 Dose Extrapolation Based on Age and Brain Size

Age (years)	Brain Volume (approx. cm3)	Infusion volume (mL)	Total IT High Dose (E14 vg)
4+	1312	10	10
3	1180	9	9
2	1080	8.2	8.2
1	955	7.3	7.3
0.5	525	4	4
Newborn	400	3	3

4.5.1. Dose Selection Rationale

4.5.1.1. Justification of Clinical Study Dose

Nonclinical studies have evaluated toxicity, safety, tolerability, expression and biodistribution of MELPIDA in various models including normal (C57BL/6J) mice, an Ap4m1 knock-out (KO) mouse model of SPG50, Sprague Dawley (SD) rats and non-human primates (NHPs). In vitro studies have also been conducted in patient derived fibroblasts.

These studies support the following conclusions:

- Fibroblasts from 2x patients with SPG50 transduced with MELPIDA (using an AAV2 capsid) restored autophagy related 9A (ATG9A) trafficking and hence AP4 function, at multiplicity of infection (MOI)s of 1E2 to 1E5 vg/cell. This study showed a dose- dependent reduction in ATG9A staining at the TGN, AP4El localization to the TGN and unchanged staining for TGN46 with phenotypic rescue in up to 77% of fibroblasts and no associated toxicity.
- An in vivo efficacy study is ongoing in WT, heterozygous and homozygous Ap4m1 knock out (KO) mice dosed intrathecally at post-natal day (PND) 7 to 10 or PND 90 with no treatment, vehicle, low (1.25E11 vg), mid (2.5E11 vg) or high (5E11 vg) doses of MELPIDA and assessed for potential phenotypic rescue. Interim results demonstrated that MELPIDA increased hAP4M1opt mRNA in all brain regions at 3 weeks post dosing, and improved impaired behaviors, induced minimal immune responses, and did not lead to elevation of serum markers of toxicity at 5- and 8-months post dosing.
- An in vivo 12-month non-GLP toxicology study was carried out in WT C57BL/6J mice dosed intrathecally at the age of 7 weeks with vehicle, low (1.25E11), or high (5E11) doses of MELPIDA. Results demonstrated that MELPIDA was generally safe and well tolerated. Dose-dependent hAP4M1opt mRNA expression was noted in all brain regions at 4 weeks post IT injection, with expression sustained up to at least 12 months post infusion, confirming that MELPIDA reached and achieved transgene expression at the targeted site of action. There were no effects on body weight, hematology or clinical signs; minimal effects on clinical chemistry were noted. Several male animals were found to have hepatocellular adenoma's which are expected in these mice as they age (up to 51% in males aged 9 to 15m).
- An in vivo 3-month GLP toxicology and biodistribution study in WT Sprague Dawley (SD) rats dosed intrathecally at the age of 7 weeks with 0 (vehicle), 0.36E12, 1.1E12, or 3.3E12 vg/rat of MELPIDA was well tolerated. Findings were limited to neurobehavioral effects such as increased excitability and activity and decreases in body weight at 3.3E12 vg, and microscopic findings in the lumbar dorsal nerve roots, lumbar dorsal root ganglion, cauda equina in the injection site, and peripheral nerves (sciatic/tibial nerves). Due to the nature of the neuronal degeneration noted in the lumbar dorsal root ganglion at $\geq$ 1.1E12 vg and the absence of recovery in this finding, it was considered adverse. Based on these results, the no-observed-adverse-effect level (NOAEL) was considered to be 3.6E11 vg.
- An in vivo 3-month GLP toxicology and biodistribution study was conducted in WT Cynomolgus monkeys (Macaca fascicularis) (Study CRL-5550014, Section 4.8.1.3) dosed intrathecally at the age of 2 to 4 years with 0 (vehicle) (N=2), 8.4E13 (N=2), or 1.68E14 (N=2) vg/MELPIDA. The following parameters and endpoints were evaluated: mortality, clinical observations, body weights, appetence, neurological examinations, nerve conduction velocity (NCV) evaluation, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), cytokines analysis, tissue bioanalysis, splenocyte analysis, organ weights, and macroscopic and microscopic examinations. Administration of MELPIDA was well tolerated.

Adverse findings at 1.68E14 vg included axonal or neuronal degeneration noted microscopically in the spinal cord (including the injection site), lumbar DRG, dorsal nerve roots, brain, trigeminal ganglion, and peripheral nerves (sciatic, sural, and tibial), with associated decreases in nerve conduction velocity and neurological effects. Based on these results, the no-observed-adverse-effect level (NOAEL) was considered to be 8.4E13 vg.

The findings from these various nonclinical studies provide the proof of concept to support the potential of benefit of MELPIDA to patients with SGP50.

Previous toxicology studies in rodents and large animals have indicated that the potential side effects emerging from the AAV9 capsids were minimal and manageable (Gougeon et al., 2021). Clinical experience for intrathecal AAV9 administration is emerging from several active human trials using AAV9-mediated gene replacement for treating CNS disorders initiated starting in 2015 (GAN, NCT02362438; CLN3, NCT03770572; CLN6, NCT02725580; MPS I, NCT03580083; SMA, NCT03461289, CLN7, NCT04737460, GM2, NCT04798235), with no serious safety concerns publicly disclosed from any of those trials. The primary anticipated complications from intrathecal AAV administration are likely to be anti-capsid immune responses, which appear to be manageable with transient immunosuppressive regimens (Gougeon et al., 2021; Ramsingh et al., 2018). Approximately 47% of humans are seropositive for AAV9, and naturally-occurring AAV9 is not known to cause any human disease (Boutin et al., 2010).

Vector distribution of MELPIDA after a single intrathecal administration measured in two studies was consistent with expected AAV9 biodistribution. Tissue transduction and expression of the biologically active gene product in vivo were demonstrated in both WT rats, and WT mice and Ap4m1 KO mice. Efficacy endpoints were measured only in the Ap4m1 KO mouse model, which demonstrated a dose response in expression of hAP4M1 mRNA across both sexes, with a dose of 5E11 vg providing maximal and near normalization of behavioral tests and a lower dose of 2.5E11 vg providing significant (albeit lower) benefit to the mice. Of note, the lowest dose of 1.25E11 vg in mice did not provide a clear behavioral benefit, indicating a minimally effective dose of 2.5E11 vg in mice.

Safety data was gathered in rodents and NHPs. All studies indicated MELPIDA was generally safe and well tolerated at all doses with some neurobehavioral effects such as increased excitability and activity and decreases in body weight in the rat GLP study at 12 weeks post dose at the highest dose of 3.3E12 vg, which corresponds to a human dose of 1.8E15 vg. Other toxicities of note included neuronal degeneration in the lumbar dorsal root ganglion at doses of $\geq$ 1.1E12 vg with no recovery. The NOAEL was considered to be 3.6E11 vg corresponding to a human dose of 2.0E14 vg. In the NHP non-GLP study, NCV was reduced in the sural nerve in one animal at the 1.68E14 vg dose, corresponding to a human dose of 2E15 vg.

Safety concerns in humans are (i) possible immunological issues and (ii) the potential for DRG toxicity. The immunological issues relate to the possibility of a cytotoxic lymphocyte response against an expressed foreign antigen and the likelihood of a deleterious immune response to the high intrathecal load of AAV9 capsid. The anti-

AAV9 response is theoretical, but dose- responsive pleocytosis has been reported (Gougeon et al., 2021; Abstract #637 by Bharucha- Goebel et al., 2019 at American Society of Gene and Cell Therapy) that was not associated with clinical symptoms. Pleocytosis was managed with an extended steroid regimen and justifies adding additional transient T cell suppression strategies (Chu et al., 2021). These findings support the incorporation of the immune management protocol proposed with MELPIDA treatment in the clinic. Additional risks include: hepatotoxicities, thrombotic microangiopathies (TMA), and neurotoxicities that include brain magnetic resonance imaging (MRI) findings of uncertain significance, and dorsal root ganglion (DRG) neuronal loss.

The toxicity noted within the dorsal root ganglia (DRG) in NHPs was a histological finding without any clinical/functional correlate (Hinderer et al., 2018; Hordeaux et al., 2020). It was noted with MELPIDA in the rat GLP study (CRL-5550008) and presumed in the non-GLP NHP study due to the slowing of NCV in the sural nerve at Day 92 after the highest dose of 1.68E14 vg. However, the clinical significance of this finding in humans remains unknown at the present time.

The proposed dose for these patients is 1E15 vg in 10 mL (for patients 4 years of age or older or based on Table 2 for patients younger than 4 years of age).

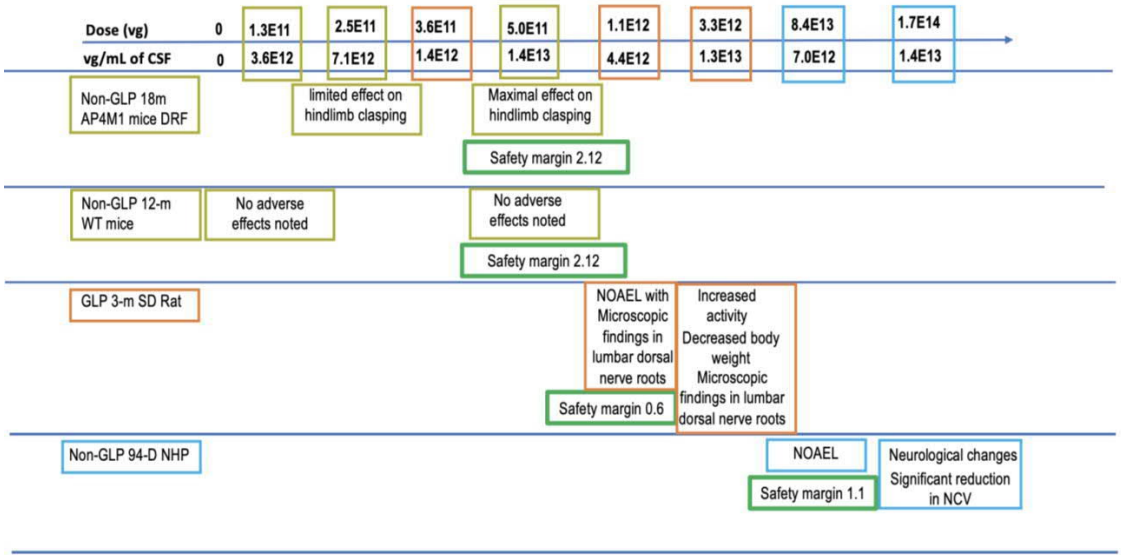
When considering the CSF volume in the various species, this corresponds to a dose equivalent of 7.0E12 vg per mL of CSF in humans, mice, rats and NHPs. The comparative absolute vg dose across species is 1E15 vg in humans, 8.4E13 vg in NHPs, 1.8E12 vg in rats, and 2.4E11 vg in mice (Table 2; Figure 2). The pharmacology studies in mice predict a benefit to patients at this dose, considering a dose of 2.5E11 vg in mice provided a clear behavioral benefit with a 5E11 vg dose providing a greater benefit. Of note, 1.25E11 vg (equivalent to 5E14 vg in a human) did not provide a clear behavioral benefit, justifying 1E15 vg as the minimally effective human dose.

Table 2 Relationship between Preclinical Study Doses and Proposed Human Intrathecal Dose

Species	Low Dose		Mid Dose		Upper Dose		CSF Volume (mL)	HED of NOAEL (vg/mL CSF)	Intended Human Dose		Safety Margin (vg/mL)
	(vg)	per CSF (vg/mL)	(vg)	per CSF (vg/mL)	(vg)	per CSF (vg/mL)			(vg)	(vg/mL)	
Mouse	1.3E11	3.6E12	2.5E11	7.1E12	5.0E11	1.4E13	0.035	1.4E13	1E15	6.6E12	2.12x**
Rat	3.6E11	1.4E12	1.1E12*	4.4E12*	3.3E12	1.3E13	0.25	4.4E12			0.6x*
NH	8.4E13*	7.0E12*			1.7E14	1.4E13	12	7E12			1.1x*
Human ≥ 4 years							140				

HED, human equivalent dose; CSF Volumes taken from the following references: Morgan et al., 2004; Sullivan et al., 1979; Pardridge, 2011; Pardridge, 1991.
*NOAEL (no observed adverse effect level)
**based on upper mouse dose per CSF volume (vg/mL)

Figure 2 Summary of Nonclinical Findings to Support Clinical Dosing



A feature of some SPG50 patients is microcephaly. Since dosing extrapolation across species is based on CSF or brain volume, this raises a possible concern that patients with microcephaly may receive a higher relative dose, since dosing extrapolations assume a patient with normal CSF and brain volume. According to Peterson et al. (2021) and Centers for Disease Control growth charts, a patient with microcephaly (3rd percentile) would have a brain volume approximately 6% lower than an average person. A <10% correction factor for dosing would not be expected to have a strong impact on either safety or efficacy. Conversely, the added trial complexity and increased sedation risk to do a detailed volumetric MRI does not seem justified to impose such a corrective dosing factor individualized for each pediatric patient, especial1y those younger than 4 years. Considering this, the proposed plan is for any patient > 4 years old to receive the same 1E15 vg dose in 10 mL. Intrathecal AAV9 gene therapy was previously administered in an infant of 7 months old enrolled in the STRONG trial (NCT03381729). This was well tolerated at a dose of 1.2E14 vg with no treatment emergent adverse events.

In terms of safety, studies in mice up to 5E11 vg (equivalent to 2.0E15 vg human dose) were well-tolerated to one-year post-injection, with no significant drug-related effects on survival, body weight, body condition, blood chemistry, or histopathology. Studies in rats found increased activity, reduced weight, and histopathological findings at 3.3E12 vg (equivalent to 1.8E15 vg in humans), whereas lower doses were better tolerated with only sporadic minimal to mild histopathological findings. Studies in NHPs found reduced sensory nerve conduction at 1.7E14 vg (equivalent to 2E15 vg in humans), but otherwise showed minimal in-life adverse effects, and the lower dose of 8.4E13 vg (equivalent to 9.7E14 vg in humans) was tolerated well. Overall, the toxicology studies across 3 species provide safety data up to an approximately 2-fold overdose in the human equivalent dose (HED). While the preclinical toxicology studies predict the possibility of dorsal root ganglion specific pathology at the human 1E15 vg dose, this HED was not associated with adverse clinical findings in the animals. Considering MELPIDA as a one-time treatment for this severe neurodegenerative condition for patients without an option to redose, a proposed dose of 1E15 vg (for patients 4 years of age or older or based on Table

1 for patients younger than 4 years of age) in these patients should maximize benefit with acceptable risks. Preclinical pharmacology data in the SPG50 mouse model did not clearly support a benefit at any lower dose.

Based on studies in rodents, there is some debated evidence of increased cancer risk associated with AAV vectors (Bolt et al., 2021; Bell et al., 2006; Rosas et al., 2012; Donsante et al., 2007). One large-scale study in mice found no evidence for tumorigenesis following AAV administration (Bell et al., 2005), despite other studies having found evidence for limited AAV integration (Chandler et al., 2015, 2016). While clonal integration of wildtype AAV2 has been detected in patient hepatocellular carcinomas (Nault et al., 2015), integration and increased cancer risk from rAAVs has not been identified in a clinical setting, and it is considered a very low risk for the proposed clinical trial. Moreover, our vector utilizes a relatively weak promoter, minimizing the risk of transactivation (overexpression) of oncogenes if the vector genome integrates nearby.

In summary, AAV9-based AP4M1 gene therapy has promise to treat SPG50 disease, but as with any other new modality, it has some development challenges. Preclinical safety studies in mice, rats, and NHPs demonstrate a favorable safety profile of MELPIDA, at up to twice the proposed human dose of 1E15 vg (doses extrapolated across species by CSF volume, Table 2).

Pharmacology studies in the AP4M1 KO mouse model demonstrated a dose-dependent benefit of MELPIDA, with the greatest benefit seen at a dose of 5E11 vg in mice (scaled to approximately 1E15 to 2E15 vg in humans by CSF volume). Importantly, the safety concerns noted in the toxicology studies are balanced against the severe unmet medical need of this patient population and the potential benefit of intrathecal administration of MELPIDA.

4.5.2. Dosing schedule

The Data Safety Monitoring Board (DSMB) appointed will review all relevant safety data from the first participant at Day 60, before a second participant is dosed. If there are no safety concerns, the second patient will be dosed not less than 90 days apart from the first patient to allow for any clinical evidence and safety signals due to toxicities in dorsal root ganglia (DRG) and peripheral nerves (PN) to be detected. See Appendix 1 (Section 11.1).

5. INCLUSION/EXCLUSION CRITERIA

5.1. Inclusion Criteria

- Age 4 months to10 years old
- Confirmed diagnosis of SPG50 disease by:
    - Genomic DNA mutation analysis demonstrating homozygous or compound heterozygous, pathogenic and/or confirmed pathogenic variants in the *AP4M1* gene

- Parent/legal guardian willing to accompany the participant to all study visits and who will provide permission for their child's participation.

For subjects less than 1 year:

- Body weight at or above 3rd percentile for age.

For subjects 1 year or more:

- Modified Ashworth Score of 2 or below (Ankles).
- or stand independently for 5 seconds
- or walk 5 steps in a walker.

5.2. Exclusion Criteria

- Inability to participate in the clinical evaluation
- Presence of a concomitant medical condition that precludes lumbar puncture or use of anesthetics
- Bleeding disorder or any other medical condition or circumstance in which a lumbar puncture is contraindicated according to local institutional policy
- Inability to be safely sedated in the opinion of the clinical anesthesiologist
- Active infection based on clinical observations
- Concomitant illness or requirement for chronic drug treatment that in the opinion of the PI creates unnecessary risks for gene transfer
- Any item which would exclude the patient from being able to undergo MRI according to local institutional policy
- Any other situation that would exclude the patient from undergoing any other procedure required in this study
- The presence of significant non-SPG50 related CNS impairment or behavioral disturbances that would confound the scientific rigor or interpretation of results of the study
- Have received an investigational drug within 30 days prior to screening or plan to receive an investigational drug (other than gene therapy) during the study.
- Enrollment and participation in another interventional clinical trial
- Contraindication to MELPIDA or any of its ingredients
- Contraindication to any of the immune suppression medications used in this study
- Clinically significant abnormal laboratory values (GGT, ALT, and AST, or total bilirubin > 3 x ULN, creatinine $\geq$ 1.5 mg/dL, hemoglobin [Hgb] < 6 or > 20 g/dL; white blood cell [WBC] > 20,000 per mm3) prior to gene replacement therapy.

5.3. Participant Withdrawal

The participant/parents/guardians will be consented prior to their enrollment in the study. They will be made aware that participation in the study is voluntary and they can withdraw at any time. Participants are free to withdraw from participation in the study at any time upon request.

If possible, the study site should attempt to have the participant return for one last study visit (for the 36 month study visit procedures).

Early withdrawal may also occur for any of the following reasons:

1. Protocol deviation (at the Investigator's discretion)
2. Investigator discretion
3. Study termination by Investigator
4. Lost to follow up
5. Participant enrollment in a different interventional study for SPG50

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

Follow-up Procedures following Withdrawal

For the safety of the participant, every effort will be made for a final evaluation (per t = 36 month study visit). This includes follow-up for any unresolved adverse events.

6. SCHEDULE OF EVENTS

6.1. Screening

The participant's parents/legal guardians will provide written informed consent for their child. The informed consent process can take place remotely via video call; the study and the risks/ benefits to study participation will be discussed in detail. If consent is obtained virtually via video conference, the family will be emailed a copy of the consent form to sign and return electronically. No study procedures will occur prior to consent/assent.

Between 28 and 7 days before potential dosing, the participant will be screened in-person at the UT Southwestern Medical Center and inclusion/exclusion criteria assessed. If the requirements (all of the inclusion criteria and none of the exclusion criteria) are met, the

participant will be enrolled. The PI will also confirm that local care can supply adequate support to the subject in between visits.

At the screening visit, confirmation of genetic diagnosis, medical history, a review of concomitant medications, and the following clinical evaluations will be performed:

- A complete physical exam
- Vital signs - including heart rate, respiration rate, blood pressure, temperature, and oxygen saturation
- Height and weight
- EKG - a 12 lead electrocardiogram will record the participant's electrical heart rhythm
- A neurologic exam to include (but not be limited to) the testing of cranial nerves, muscle bulk/tone and strength, sensation, cerebellar function, involuntary movements, myotatic reflexes, toe sign, gait, and stance.
- Blood and urinalysis
    - safety labs - Complete blood count (CBC) with differential, coagulation (INR, PT, PTT), ESR, CRP, Complete metabolic panel (CMP) GGT, cardiac safety panel (Tn, ProBNP, CK-MB), lipid panel and urinalysis
    - Screening labs - Specifically for screening, the following will be assessed: HIV Ab, Hepatitis A Ab, Hepatitis B Surface Ab, Hepatitis Surface Ag, Hepatitis C Core Ab, PPD skin test for TB, HTLVl

The following exploratory assessments will also be performed at screening for all subjects unless otherwise stated:

- AAV9 Antibody titers
- ELISpot on whole blood for T cell response to AAV9 and AP4M1
- Bayley Scales of Infant and Toddler Development, 3rd edition (Bayley-III)
- Vineland Adaptive Behavior Scale, 3rd edition (Vineland-3)
- 10-meter walk test (item 3 on the Spastic Paraplegia Rating Scale) for subjects 1 year and above.
- Improvement of gait (three-minute walk test) for subjects 1 year and above.
- Spastic Paraplegia Rating Scale (SPRS) for subjects 1 year and above.
- Modified Ashworth Scale (MAS) for subjects 1 year and above.
- Tardieu Scale for subjects 1 year and above.
- Gross Motor Function Measure (GMFM)-88
- SPATAX-EUROSPA Disability Score for subjects 1 year and above.
- CPCHILD (Caregiver Priorities and Child Health Index of Life with Disabilities) for health related quality of life

- Clinical Global Impression (CGI) of Overall Change by Physician
- Nerve conduction studies (NCS)
- Parent-assessed Global Impression (PGI)
- Developmental Quotients (DQs)

6.2. Enrollment and Pre-Dosing Schedule

Following the Screening study visit, if the participant is eligible for enrollment, they will begin the immunosuppression regimen. Starting 7 days before dosing, the participant will begin Sirolimus loading at l mg/m2 every 4 hours on days -7, -6, and -5. On days -4, -3, -2, and -1, the sirolimus dose will be lowered to 0.5 mg/m2 divided into twice a day dosing, with the goal level being 4 to 8 ng/ml.

The day before dosing, the following procedures will be done:

- Physical exam
- Vital signs
- EKG (telemetry overnight)
- Blood gases (capillary or venous)
- Recording of adverse events and concomitant medications

6.3. Dosing Day

The following will be done on the day of dosing:

1. Sirolimus dosing will be 0.5 mg/m2 divided in twice a day dosing
2. Administration of additional immunosuppressants and analgesic:
   - Methyl Prednisolone (IV) - 10 mg/kg to a maximum single dose 500 mg, infused over 30 minutes
   - Acetaminophen - 15 mg/kg dose (maximum 650 mg/ dose)
   - Diphenhydramine - 0.5 mg/kg/dose (maximum 50 mg/dose)
3. Lumbar puncture/Intrathecal administration of MELPIDA under anesthesia
   - Removal of spinal fluid to equal volume of MELPIDA dose
   - Cerebral-spinal fluid analysis, including cell count, differential, protein, glucose, gram stain, culture.
   - Administration of MELPIDA (see below)

6.3.1. Administration of Study Intervention (MELPIDA)

A Pajunk atraumatic Sprotte needle (part number 321151-31A) will be inserted percutaneously at the lumbar level into the intrathecal space of the spinal column. Spinal

needle placement will be confirmed using fluoroscopic intraoperative imaging (CArm) scanner at the chosen injection site prior to and after vector administration.

A volume of CSF approximately equal to the infusion volume (up to 10 mL depending on age) will be withdrawn from the lumbar thecal sac. The MELPIDA vector solution will be loaded into a 20 mL BD syringe, connected to the needle with 60 inch mini volume IV extension tubing and a Braun 4-way stopcock. The vector solution is then infused at a rate of 1 mL per minute, using a CareFusion Alaris 8110 syringe pump or similar pump able to deliver at a rate of 1 mL/min.

6.3.2. Anesthesia Safety

The participant will undergo a comprehensive pre-anesthesia evaluation prior to dosing per hospital policy. Physiologic monitoring in accordance with the standards set by the American Society of Anesthesiologists will be utilized while they are receiving analgesia/anesthesia and until they have fully recovered from its effects. Active warming devices will be used during anesthesia as needed since patients are prone to hypothermia during the anesthesia.

6.3.3. Post-Procedure Recovery

After the procedure, the participant will be transported to a Post Anesthesia Care Unit (PACU) or (PICU) with continuous pulse oximetry monitoring and oxygen if needed by bag/mask/nasal canula or blow-by. Vital signs including heart rate, respiratory rate, blood pressure, and pulse oximetry will be monitored every 15 minutes for the first 2 hours post infusion, every 30 minutes during the third and fourth hour post infusion, then hourly for 4 hours, and finally every 4 hours until discharge from PICU. In the event that abnormalities are detected, appropriate medical intervention will occur, including the possibility of extending the hospitalization and/or subsequent testing.

6.4. Day 2

Vital signs, physical exam, neurologic exam, safety labs and a review of concomitant medications and any adverse events will be assessed prior to discharge. The first dose of tacrolimus at 0.1 mg/kg/day divided into twice daily dosing (goal level: 4 to 8 ng/mL) The first dose of prednisolone at 1 mg/kg/day will also be given. When the attending physician determines that the participant is stable, then they may be discharged. Immunosuppressive medications (prednisolone, tacrolimus [CRIM-Negative patients only] and sirolimus) will be continued daily starting from Day 2.

6.5. Days 7, 14, 21, 30 (+/- 2 days)

The participant will return to Childrens Health on Day 7, 14, 21, and 30. Vitals, safety labs, brief physical exam, viral shedding samples, concomitant medications and adverse events will be collected. On Day 7 and 21 exploratory labs will also be done. ELISpot for T-Cell response to AAV9 and AP4M1 samples will be collected on Days 7 and 30.

Immunosuppression with prednisolone, tacrolimus (CRIM-Negative patients only) and sirolimus will continue daily. Nerve conduction studies will be performed at 28 days.

6.6. Months 2, 6, 9, and 12 (+/- 14 days)

The participant will return to Childrens Health for safety evaluation as outlined in the Schedule of Events (SoE) in Table 3 for months 3, 6, and 12. Month 2 will be a remote standard of care visit. Brain MRI and lumbar punch1re with CSF analysis will be performed at 3, 6, and 12 months.

Nerve conduction studies will be performed at 3, 6, and 12 months. If at month 3, there is no evidence of inflammation, prednisolone tapering may begin at month 3 (see Immune Modulation Protocol Section 6.10). Tacrolimus (CRIM-Negative patients only) and sirolimus dosing will continue with tapering, detailed in (Immune Modulation Protocol Section 6.10). If there is evidence of inflammation at Month 3, prednisolone tapering will not commence until after the next examination at Month 6. When there is no evidence of inflammation, then the schedule for tapering may commence. If signs of inflammation continue to be present at 6 months, immunosuppressive medicines will be continued, and MRI and LP will be repeated at 9 months to again assess.

6.7. Months 18, 24, 36, 48, 60 (+/- 14 days)

Follow up safety evaluation will consist of physical exam, vitals, height and weight and safety labs. If possible, further exploratory labs will be collected and efficacy assessments out lined in SoE. Month 18 will be a remote standard of care visits.

The participant/parents/guardians will be encouraged to contact the investigator for any suspected adverse event reporting between visits. Unscheduled visits may occur if the PI determines that they are necessary to assess safety, repeat labs, etc.

A complete schedule of events is found in Table 3.

Table 3 Schedule of Events

	Screening		Inpatient stay			Period A monitoring											Period B monitoring		
Visit	(0)	(1)	(2)	(2)	(2)	(3)	(4)	(5)	(6)	(7)[12]	(8)	(9)	(10)[12]	(11)	(12)[12]	(13)	(14)	(15)	(16)
Screen Day	-28 to -7	-7 to-1	-1	1	2[13]	7	14	21	30	2M	3M	6M	9M	12M	18M	24M	36M	48M	60M
Informed Consent	X																		
Genetic Confirmation	X																		
Medical History	X																		
Vitals	X	x	x	x	x	x	x	x	x	x	x	x	x	x		x			
Height And Weight	X		x								x	x	x	x		x			
Physical Exam	X		x	x	x	x			x		x	x	x	x		x	x	x	x
Concomitant Medications	X	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Adverse Events		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Screen Labs*	X																		
Exploratory Labs****	X					x		x			x	x		x		x			
Safety Labs**	X	x		x	x	x	x	x	x	x	x	x	x	x		x	x	x	x
MELPIDA Dosing				x															
PICU Admit for Dosing				x	x														
EKG	X		x								x	x		x		x			
Neurologic Exam	X		x		x	x			x		x	x	x	x		x	x	x	x
Lumbar Puncture				x							x	x		x		x			
CSF Analysis***				x							x	x		x		x			
MRI[11]	x+										x	x		x		x	x	x	x
NCS	X								x		x	x		x		x	x	x	x
Anti-AAV9 Antibody Titers	X					x			x		x	x	x	x		x			
ELISpot For T-Cell Response to AAV9 & AP4M1 Protein	X					x			x		x	x	x	x		x			
Viral Shedding	X			x		x	x		x		x	x		x		x			

	Screening		Inpatient stay			Period A monitoring											Period B monitoring		
Visit	**(0)**	**(1)**	**(2)**	**(2)**	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**	**(7)[12]**	**(8)**	**(9)**	**(10)[12]**	**(11)**	**(12)[12]**	**(13)**	**(14)**	**(15)**	**(16)**
Screen Day	**-28 to -7**	**-7 to-1**	**-1**	**1**	**2[13]**	**7**	**14**	**21**	**30**	**2M**	**3M**	**6M**	**9M**	**12M**	**18M**	**24M**	**36M**	**48M**	**60M**
Prednisolone[9,10]				x	x	x	x	x	x	x	x								
Acetaminophen[1]				x															
Diphenhydramine[2]				x															
Methylprednisolone (Iv)[3]				x															
Tacrolimus[4,5]					x	x	x	x	x	x	x	x							
Sirolimus[6,7,8]		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
Spasticity Assessments																			
Bayley Scales of Infant and Toddler Development, 3rd Edition	x											x		x		x			
Clinical Global Impression of Overall Change by Physician (CGI)	x								x		x	x		x		x			
Vineland Adaptive Behavior Scale	x											x		x		x	x	x	x
10-Meter Walk Test (Item 3 on the Spastic Paraplegia Rating Scale)	x											x		x		x			
Improvement Of Gait (Three-Minute Walk Test)	x											x		x		x			
Spastic Paraplegia Rating Scale (SPRS)	x											x		x		x			

	Screening		Inpatient stay			Period A monitoring											Period B monitoring		
Visit	**(0)**	**(1)**	**(2)**	**(2)**	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**	**(7)[12]**	**(8)**	**(9)**	**(10)[12]**	**(11)**	**(12)[12]**	**(13)**	**(14)**	**(15)**	**(16)**
Screen Day	**-28 to -7**	**-7 to-1**	**-1**	**1**	**2[13]**	**7**	**14**	**21**	**30**	**2M**	**3M**	**6M**	**9M**	**12M**	**18M**	**24M**	**36M**	**48M**	**60M**
CPCHILD (Caregiver Priorities and Child Health Index of Life with Disabilities) for health-related quality of life	x											x		x		x	x	x	x
Modified Ashworth Scale	x											x		x		x			
Tardieu Scale	x											x		x		x			
Gross Motor Function Measure (GMFM)-88	x											x		x		x			
SPATAX-EUROSPA Disability Score (Similar To GMFM)	x											x		x		x			
Parent-Assessed Global Impression (PGI)	x											x		x	x	x	x	x	x
Developmental Quotients (DQs)	x											x		x		x			
~~Logbook of Seizures[12]~~	~~x~~		~~x~~			~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~
~~Logbook of Falls[12]~~	~~x~~		~~x~~			~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~	~~x~~

*Screening labs include HIV Ab, Hepatitis A Ab, Hepatitis B Surface Ab, Hepatitis Surface Ag, Hepatitis C Core Ab, PPD skin test for TB, HTLVI
**Safety labs include CBC With Differential, ESR, CRP, CMP GGT, INR, PT, PTT, Urinalysis, Cardiac Safety (Tn, ProBNP, CK-MB), Sirolimus Levels, Tacrolimus levels, Lipid Profile
***CSF Analysis includes cell count, differential, protein, glucose, gram stain, culture, oligoclonal bands, cytokine analysis.
****Exploratory labs include: PAXgene DNA, PAXgene RNA, serum (red top) or plasma (green top).
X+ = MRI Brain & Spine With contrasts. Screening MRI required if not performed within 2 years.
1- Acetaminophen 15 mg/kg dose (max 650 mg/ dose)

2- Diphenhydramine 0.5 mg/kg/dose (max 50 mg/dose)
3- Methylprednisolone (IV) 10 mg/kg to a max single dose 500 mg, infused over 30 minutes.
4- Tacrolimus 0.1 mg/kg divided in to twice DAILY (goal level 4-8 ng/ml) from day 2 to month 6 (Crim-Negative patients only)
5- Tacrolimus - Based on clinical results of LP, MR is at Month 6, tacrolimus taper may begin at Month 6 (Week 24). If any evidence of ongoing inflammation in any exam at Month 6, continue with 0.1 mg/kg/day DAILY; do not begin taper until results of next clinical evaluation inflammation and reassess (Crim -Negative patients only)
6- Sirolimus load 1 mg/m2 every 4h for 3 doses (days -7), then 0.5 mg/m2/day divided in twice a day dosing (goal level 4-8 ng/ml) (days -6, -5 -4, -3, -2, -1)
7- Sirolimus maintenance dose 0.5 mg/m2 divided in twice a day dosing on day 1
8- Sirolimus maintenance dose 0.5 mg/m2 divided in twice a day dosing DAILY from day 2 to Month 60
9- Prednisone 1 mg/kg/day DAILY until month 4
10- Prednisone - Based on clinical results of LP and MR is at Month 3, prednisone taper may begin at Month 4 (Week 16). If any evidence of ongoing inflammation in any exam at Month 3, continue with 1 mg/kg/day DAILY; do not begin taper until results of next clinical evaluation of inflammation and reassess.
11- If inflammation evident at month 6, repeat MRI, LP and CSF analysis at month 9
12- Visit 7, 11, 13 are SOC remote visits if feasible
13- Additional inpatient stay (PICU or other units) may be warrented for safety evaluations per PIs discretion.

6.8. Specific Study Procedures

6.8.1. Nerve Conduction Study (NCS)

The participant will undergo nerve conduction studies to evaluate for dorsal root ganglion injury. A baseline study will be performed within 28 days of dosing. Standard methodology, as used in routine clinical care, will be used. At least one sensory and one motor nerve in the upper and lower extremity will be sampled. Sensory action potentials (SAP) and Compound muscle action potential (CMAP) of Sural and Radial nerves will be measured and studies should be always done on the same side each time.

NCVs will be done at baseline, Day 30, 3, 6, 12 months and years 2 to 5 after dosing.

6.8.2. Magnetic Resonance Imaging of Brain and Spine (MRI)

Brain MRI (with and without contrast) will be done at baseline and at 3-month intervals. Standard imaging sequences will be obtained (Tl, T2, FLAIR, etc.) MRI studies will be done at University of Texas Southwestern Medical Center. The primary role for brain MRI in the study is to evaluate for signs of inflammation and inflammatory change associated with the study drug. Of note, patients with SPG50 typically have structural brain abnormalities. On MRI, the following changes have been observed in SPG50 patients: (1) thin splenium of the corpus callosum, (2) absent or thin anterior commissure, (3) characteristic signal abnormalities of the forceps minor ("ears of the grizzly sign"), and (4) periventricular white matter.

All MR imaging will be supervised by an attending pediatric neuroradiologist to ensure acquisition of complete, high-quality scans. MR imaging will be acquired at baseline and at multiple time points after administration of the viral vector using the same 3T MR Skyra scanner (Siemens, Erlangen, Germany) using a 64-channel head coil. Sequences will include whole brain sagittal 3D-T1 SPGR with isotropic voxels, axial T2-FLAIR, axial T2 FSE, axial susceptibility- weighted images, and axial Diffusion Tensor Imaging (DTI). The latter sequence uses 2 x 2 mm isotropic voxels, 32 directions, b =1000 s/mm2. Post-contrast imaging includes axial T1 FLAIR and axial T2 FLAIR for identification of parenchymal and leptomeningeal injury respectively will be acquired. Intravenous gadolinium will be administered in a dose of 0.2 mL/kg (0.1 mmol/kg) body weight administered as an intravenous bolus injection through a peripheral IV, after confirming adequate renal function (GFR > 30 mL/min/1.73 m2 and the patient is not on any type of dialysis). The gadolinium agent gadoterate meglumine (Dotarem) will be used.

6.8.2.1. Adverse Reactions to Dotarem in Pediatric Patients

The safety and efficacy of Dotarem at a single dose of 0.1 mmol/kg have been established in pediatric patients from birth (term neonates ≥ 37 weeks gestational age) to 17 years of age based on clinical data. Adverse reactions in pediatric patients were similar to those reported in adults. No dose adjustment according to age is necessary in pediatric patients. During clinical trials, 185 pediatric patients (52 aged < 24 months, 33 aged 2 to 5 years, 57 aged 6 to 11 years, and 43 aged 12 to 17 years) received Dotarem. Overall, 7 pediatric

patients (3.8%) reported at least one adverse reaction following Dotarem administration. The most frequently reported adverse reaction was headache (1.1%). Most adverse events were mild in intensity and transient in nature. Patients on dialysis will not receive gadolinium. We will assess patients for prior allergies to gadolinium prior to administration. Trained personnel are present in the hospital to treat hypersensitivity reactions.

6.8.2.2. Visual Analysis of Conventional MR Images

MR images will be reviewed by an experienced pediatric neuroradiologist who will assess overall image quality on the baseline and post-treatment MR images and reach a consensus on the findings. A standardized report template will be used.

The size of the cerebral and cerebellar sulci and ventricles will be subjectively classified as normal, moderately, or severely enlarged on the baselines and follow-up scans.

White matter signal abnormalities will be classified as absent, focally abnormal, or diffusely abnormal. White matter signal abnormalities will be compared between baseline and follow-up.

Susceptibility weighted images will be evaluated for thalamic T2 shortening and hemosiderin deposition.

Post-contrast images will be assessed for pathologic parenchymal and/or leptomeningeal enhancement potentially indicative of treatment-induced brain irritation or inflammation.

ADC and trace images from DTI will be assessed for areas of restricted diffusion and compared to areas of abnormal T2 signal and/or enhancement.

Transverse dimensions of frontal horns will be measured in the axial plane and transverse cerebellar dimension measured in axial plane will be reported.

6.8.2.3. DTI Analysis

DTI provides information about local microstructure. And may be more sensitive to conventional MR imaging at demonstrating toxicity in the form of white matter injury or therapeutic efficacy. Diminished fractional anisotropy and/or increased diffusivities would be presumptive markers of white matter injury while increases in FA and decreased mean diffusivity could indicate regrowth of axonal membranes or remyelination. Diffusion images will be corrected for subject motion and eddy current and EPI-induced distortions. Tensor data will be sent to an offline workstation for analysis using Tract-based Spatial Statistics. FA skeletons will be created for each of the tensor for comparison purposes. The mean values for fractional anisotropy (FA), mean diffusivity, and axial and radial diffusivity will be calculated across the FA skeleton.

Voxelwise microstructural analysis will be performed using TBSS. Unlike settings wherein the most "typical" subject is used as a mean FA image, each patient will provide their own baseline FA skeleton against which subsequent FA skeletons will be compared.

Randomise (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/ Randomise), a permutation program, will be used for statistical testing of the voxelwise differences between baseline and follow-up with 5000 permutations. Cluster like structures in the data will be enhanced with threshold-free cluster enhancement, and multiple correction for the family-wise error rate with a significance threshold of $P < .05$ will be performed using permutation-based nonparametric testing.

6.8.3. Lumbar Puncture

The participant will undergo one lumbar puncture via interventional radiology guidance. The participant will undergo additional post-dosing lumbar punctures as performed without IR guidance by the study PI. For all LPs, a 21 gauge standard LP needle will be used. The area around lumbar 4/5 will be sterilely addressed. EMLA will be applied for local anesthesia. The needle will be inserted into the inter thecal space between L4/L5 or L3/L4. An appropriate quantity of CSF will be removed for relevant laboratory studies. The needle will be removed, the site cleaned, and a sterile dressing will be applied.

6.9. Adverse Event and Concomitant Medication Monitoring

The participant's caregivers are encouraged to contact the study team whenever an adverse event or change in medication occurs in a timely manner. Long term adverse event monitoring will include annual study visits.

6.10. Immune Modulation Protocol

6.10.1. CRIM-Negative Patients

In previous gene therapy studies, antigen specific T-cell responses to the AAV9 vector have been reported (Harrison et al., 1977). This is an expected response between 2- and 12-weeks following gene transfer, even when administered IT. One possible consequence to such antigen specific T-cell responses is clearance of the transduced cells and loss of transgene expression.

To reduce the risk of the host immune response to the AAV9-based MELPIDA, an initial proposal for an immunosuppression regimen has been designed based on advice from investigators in the ongoing trial of AAV9 gene transfer to CSF for giant axonal neuropathy (Clinicaltrials.gov # NCT02362438).

6.10.1.1. 1 Week Prior to Vector Administration

- Sirolimus load: 1 mg/m2 every 4 hours x 3 doses (load only given on one day)
- Starting the day after the sirolimus load, begin enteral daily dosing at 0.5 mg/m2/day, divided in twice per day dosing (goal level: 4 to 8 ng/mL)

6.10.1.2. Day of Vector Administration (Day 1)

- Acetaminophen (15 mg/kg/dose enteral; maximum 650 mg per dose)
- Diphenhydramine (0.5 mg/kg/dose enteral; maximum 50 mg/dose)
- IV methylprednisolone (10 mg/kg to a maximum single dose of 500 milligrams, infused over 30 minutes)

6.10.1.3. Day after Vector Administration (Day 2)

- Begin daily enteral prednisone/prednisolone at 1 mg/kg/day x 3 months
- Continue enteral daily sirolimus dosing at 0.5 mg/m2/day, divided in twice per day dosing (goal level: 4 to 8 ng/mL)
- Tacrolimus at 0.1 mg/kg/day divided into twice daily dosing (goal level: 4 to 8 ng/mL)

6.10.1.4. Maintenance

- Enteral prednisone/prednisolone at 1 mg/kg/day x 3 months, then taper according to schedule
- Sirolimus 0.5 mg/m2/day, divided in twice per day dosing. If there are signs or symptoms of transgene mediated CNS inflammation by examination, brain imaging, and/or laboratory testing, longer administration of immunomodulatory medications and possibly addition of other immunomodulatory agents may be required
- Tacrolimus at 0.1 mg/kg/day divided into twice daily dosing (goal level: 4 to 8 ng/mL); tacrolimus will be continued for 6 months and will begin taper by 7 months after gene transfer. The taper will be started if there are no signs or symptoms of transgene mediated CNS inflammation by examination, brain imaging, and/or laboratory testing, which if present may require longer administration of immunomodulatory medications

6.10.1.5. Monitoring

- Weekly BP checks x 4 weeks
- Sirolimus troughs every 1 week x 4, then every 2 weeks x 4, then monthly once levels are stable within the desired range
- Tacrolimus troughs every 1 week x 4, then every 2 weeks x 4, then monthly once levels are stable within the desired range
- CBC with differential testing at every blood draw
- 8 AM cortisol level when participants are on 5 mg dose prednisone/prednisolone for 1 week
- Monthly fasting lipid profile while on immunomodulation and at PI's discretion

6.10.2. CRIM Positive Patients

In previous gene therapy studies, antigen specific T-cell responses to the AAV9 vector have been reported (Harrison et al., 1977). This is an expected response between 2- and 12-weeks following gene transfer, even when administered IT. One possible consequence to such antigen specific T- cell responses is clearance of the transduced cells and loss of transgene expression.

To reduce the risk of the host immune response to the AAV9-based MELPIDA, an initial proposal for an immunosuppression regimen has been designed based on advice from investigators in the ongoing trial of AAV9 gene transfer to CSF for giant axonal neuropathy (Clinicaltrials.gov # NCT02362438).

In addition, as participants are immunocompromised, particapants will be placed on Bactrim (trimethoprim/sulfamethoxazole) for prophylaxis against opportunistic infections such as documented Pneumocystis jirovecii pneumonia (PCP). Dosing will be 150 mg TMP/m2/day enteral divided q12 hours for 3 days/week on consecutive or alternate days. If a participant has an allergy to trimethoprim/sulfamethoxazole, Dapsone will be used at 4 mg/kg/dose enteral qWeek (not to exceed 200 mg/week).

Preventative gastroesophageal/Famotidine/modulation can be prescribed per PI's discretion, to prevent gastric complications secondary to immune modulation medication.

6.10.2.1. 1 Week Prior to Vector Administration

- Sirolimus load: 1 mg/m2 every 4 hours x 3 doses (load only given on one day)
- Starting the day after the sirolimus load, begin enteral daily dosing at 0.5 mg/m2/day, divided in twice per day dosing (goal level: 4 to 8 ng/mL)

6.10.2.2. Day of Vector Administration (Day 1)

- Acetaminophen (15 mg/kg/dose enteral; maximum 650 mg per dose)
- Diphenhydramine (0.5 mg/kg/dose enteral; maximum 50 mg/dose)
- IV methylprednisolone (10 mg/kg to a maximum single dose of 500 milligrams, infused over 30 minutes)

6.10.2.3. Day after Vector Administration (Day 2)

- Begin daily enteral prednisone/prednisolone at 1 mg/kg/day x 3 months
- Continue enteral daily sirolimus dosing at 0.5mg/m2/day, divided in twice per day dosing (goal level: 4 to 8 ng/mL)

6.10.2.4. Maintenance

- Enteral Prednisone/Prednisolone 1 mg/kg/day x 3 months, then taper according to schedule

- Sirolimus 0.5 mg/m2/day, divided in twice per day dosing until tapering at approximately 10 months. Tapering at 10 months (following vector administration) will be completed by 12 month post-gene-transfer. If there are signs or symptoms of transgene mediated CNS inflammation by examination, brain imaging, and/or laboratory testing, longer administration of immunomodulatory medications and possibly addition of other immunomodulatory agents (such as tacrolimus) may be required.

6.10.2.5. Monitoring

- Weekly BP checks x 4 weeks
- Sirolimus troughs every 1 week x 4, then every 2 weeks x 4, then monthly once levels are stable within the desired range
- CBC with differential testing at every blood draw
- 8 AM cortisol level when participants are on 5 mg dose prednisone/prednisolone for 1 week
- Monthly fasting lipid profile while on immunomodulation and at PI's discretion

6.11. Immunomodulation Taper Plan

6.11.1. Steroid Taper

Participants will be on maintenance prednisone/prednisolone for 90 days. The steroid taper will be started after the Day 90 labs are completed and are deemed acceptable by the PI.

The weeks numbered below are weeks in taper schedule:

6.11.2. For Steroid Dose Below 60 mg

At the Day 90 visit, the dose will be incrementally decreased by 15% of the total dose each week. The numbers will be rounded in 2.5 mg increments.

At the Day 90 visit, safety labs and ELISpot will be checked. Once lab results have been reviewed and if normal, the steroid taper will begin as indicated below.

- Week 1: Prednisone/Prednisolone will be reduced by 15% of the baseline dose.
- Week 2: Prednisone/Prednisolone will be reduced by another 15% of the baseline dose.
- Week 3: Prednisone/Prednisolone will be reduced by another 15% of the baseline dose.
- Week 4: Prednisone/Prednisolone will be reduced by another 15% of the baseline dose.

- Week 5: Prednisone/Prednisolone will be reduced by another 15% of the baseline dose.
- Week 6: Prednisone/Prednisolone will be reduced by another 15% of the baseline dose.

The taper will continue until the participant reaches 5 mg, at which time tapering will be stopped temporarily. If the last dose is above 5 mg, it will be rounded down to 5 mg between Days 120 to 134. After participants are on a 5 mg dose for 1 week, an 8 AM cortisol level would be checked. If the 8 AM cortisol level is normal, prednisone/prednisolone will be tapered off in 1 mg increments every 4 to 7 days. All participants will be off steroids by Day 150. At Day 180, scheduled safety and immunomodulation labs will be checked.

6.11.3. For Steroid Dose at or Above 60 mg

At the Day 90 visit, safety labs and ELISpot will be checked. Once lab results have been reviewed and if normal, the steroid taper will begin as indicated below.

Week 1: Prednisone/ Prednisolone will be reduced to 40 mg/day. Week 2: Prednisone/ Prednisolone will be reduced to 30 mg/day. Week 3: Prednisone/ Prednisolone will be reduced to 20 mg/day. Week 4: Prednisone/ Prednisolone will be reduced to 10 mg/day. Week 5: Prednisone/ Prednisolone will be reduced to 5 mg/day.

All participants will continue at a 5 mg dose for one week. At this point, an 8 AM cortisol level will be checked. If the 8 AM cortisol level is normal, prednisone/prednisolone will be tapered in 1 mg increments every 4 to 7 days. All participants be off steroids by Day 150. At Day 180, scheduled safety and immunomodulation labs will be checked.

If safety lab results are not normal at any point during the time participants are on maintenance steroids, prednisone/prednisolone dose may be increased to 2 mg/kg/day (max daily dose 60mg) at the PI's discretion, depending on the T-cell response measured by ELISpot assay specifically to the AP4M1 protein. The PI might also decide to prolong the tapering protocol based on the individual participant's immune response to the gene transfer, again assessed by ELISpot assay specifically for the AP4M1 protein.

6.11.4. Sirolimus Taper

The sirolimus taper will be started after the Day 270 labs are normal. The taper will start no later than 10 months. The taper will complete over the following 4 to 6 weeks. On Day 360 visit, safety labs will be checked.

6.11.5. Tacrolimus Taper

The tacrolimus taper will be started after Day 180 if labs are normal. Taper will complete over next 4 to 6 weeks. On Day 270, safety labs will be checked.

7. STATISTICAL ANALYSIS

7.1. Data Monitoring

7.1.1. General Plan

For this Phase I study of Gene Therapy the safety oversight will be focused not only on the initial treatment but also on an extended observation.

7.1.2. Monitoring Entity

Dr. Susan Iannaccone (University of Texas Southwestern Medical Centre is the lead investigator and will be responsible for assuring ongoing safety monitoring of the trial. All medical decisions will be made in the best interest of the participant. Management of toxicities will be at the discretion of the lead investigator in consultation with experts in the field.

7.2. Plans for Assuring Participant Safety, Adverse Event Collection, and Reporting

Expected adverse events could be due to the infusion and are listed in Section 6.3. Other adverse events could be due to immunomodulatory drugs.

Maximum efforts will be undertaken to ensure the safety of all study participants.

The primary and secondary endpoint assessment is identifying the safety and tolerability of intrathecal administration of MELPIDA. Monitoring for safety will be performed by recording and evaluating type and occurrences of Adverse Events (AEs), concomitant medication usage, and by conducting physical examinations, vital sign assessments, cardiovascular evaluations, and laboratory evaluations (chemistry, hematology, coagulation, immunology).

7.3. Definitions

7.3.1. Adverse Event

Adverse events (AE) are defined as any untoward occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21CFR 312.32.a). These events will be reviewed by the PT and determined if they are clinically significant requiring adjustments to medications or interventions.

Clinically significant signs and symptoms or lab abnormalities will be recorded as an AE. This could include a laboratory result for which there is no intervention, but the abnormal value suggests a disease or organ toxicity. The PI will evaluate all AEs with respect to Seriousness, Severity (intensity or grade), and Causality (relationship to study agent and relationship to research) according to the following guidelines. All AEs will be classified

in accordance with the CTCAE v.5. AEs will be coded in accordance with the most current version of the MedDRA coding dictionary.

All events will then be reviewed by the DSMB and an evaluation will be made as to whether the study should be terminated early following the discontinuation rules.

7.3.2. Classification of Adverse Events

Monitoring AEs requires that they be classified as to seriousness, expectedness, and potential relationship to the investigational product, all of which drive the reporting process.

7.3.2.1. Seriousness

A serious adverse event (SAE) is one that:

- Results in death,
- Is life-threatening (the participant was in immediate danger of death from the event as it occurred),
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect in the offspring of a participant.

All SAEs that occur after any patient has been enrolled, before vector dosing, during vector dosing, or up through the last study visit, whether or not they are related to the study, must be recorded on case report forms. All SAEs will be required to be reported to the REB within 24 hours of it being brought to the attention of the PI.

CTCAE v.5 provides a grading system that is used to categorize the severity of adverse events, as follows:

- Grade 1 Mild: transient, requires no special treatment or intervention, does not interfere with daily activities
- Grade 2 Moderate: alleviated with simple treatments, may limit daily activities
- Grade 3 Severe: requires therapeutic intervention and interrupts daily activities
- Grade 4 Life-threatening or disabling
- Grade 5 Death

An SAE, as defined above, encompasses CTCAE grades 4 and 5, and any Grade 3 event that requires or prolongs hospitalization, or that is disabling. Other SAEs that are considered Important Medical Events (IME) requiring medical judgement that need reporting is when the event does not fit the outcomes listed above, but the event may jeopardize the patient and may require medical or surgical intervention (treatment) to prevent one of the other outcomes.

7.3.2.2. Expectedness

The purpose of reporting is to provide new, important information on serious reactions or events previously unobserved or undocumented. Therefore, all AEs will be evaluated as to the expectedness of its occurrence as follows:

- Unexpected: An unexpected AE or adverse drug reaction is one for which the nature or severity is not consistent with information in the protocol, consent form, or Investigator's brochure.
- Expected: While there remains limited data on the safety of AAV9 and AAV based gene therapy programs in general, some AEs have emerged in across different programs. Ones that have been noted in multiple individuals and in more than one patient include:
- Transient thrombocytopenia
- Transient transaminitis
- An AE is considered expected if it is known to be associated with any of the study procedures (i.e., blood draw sticks, LPs, etc.)
- Expected adverse events due to underlying disease are listed below:
- Worsening of spasticity
- Worsening of ataxia
- Worsening seizures
- Progressive atrophy in brain noted in follow-up MRIs

7.3.2.3. Causality

Causality assessment is required in clinical investigations to help determine which events require expedited reporting. The PI must make the determination of relationship to the investigational product for each AE (Unrelated, Possibly Related, Probably Related, or Definitely Related). The PI should decide whether, in his/her medical judgment, there is a reasonable possibility that the event may have been caused by the investigational product. If no valid reason exists for suggesting a relationship, then the AE should be classified as "unrelated." If there is any valid reason, even if undetermined, for suspecting a possible causative relationship between the investigational product and the occurrence of the AE, then the AE should be considered "related." If the relationship between the AE/SAE and the investigational product is determined to be "possible" or "probable", the event will be considered to be related to the investigational product for the purposes of expedited regulatory reporting.

The following criteria will be used to determine causality:

- Unrelated: The event is clearly related to other factors, such as the participant's clinical state or non-study drugs or interventions.

- Possibly Related: The event follows a compatible temporal sequence from the time of administration of the study agent but could have been produced by other factors such as the participant's clinical state or non-study drugs or interventions.
- Probably Related: The event follows a reasonable temporal sequence from the time of study agent administration and cannot be reasonably explained by other factors such as the participant's clinical state or non-study drugs or interventions.

7.4. Dose Limiting Toxicity

Dose limiting toxicity (DLT) is defined as any SAE or AE that is possibly, probably, or definitely related to the investigational product. This would include any AE Grade 3 or greater event, according to the CTCAE v.5; these classifications are outlined below:

- Grade 1 Mild: transient, requires no special treatment or intervention, does not interfere with daily activities
- Grade 2 Moderate: alleviated with simple treatments, may limit daily activities
- Grade 3 Severe: requires therapeutic intervention and interrupts daily activities
- Grade 4 Life-threatening or disabling
- Grade 5 Death

Study enrollment will be halted by the investigators when any subject experiences a Grade 3 or higher AE or SAE that is unanticipated and possibly, probably, or definitely related to the investigational product. The event will then be reviewed by the DSMB and an evaluation will be made as to whether the trial should be terminated early following the discontinuation rules, or if a protocol modification should be considered.

7.4.1. Discontinuation Rules:

Study enrollment will be halted by the investigators when any subject experiences a Grade 3 or higher AE or SAE that is unanticipated and possibly, probably, or definitely related to the investigational product that presents with clinical symptoms and requires medical treatment. This will include any patient death, important clinical laboratory finding, or any severe local complication in the injected area related to administration of the study agent. If after review by the DSMB, IRB and FDA, the decision is made to continue, the study will proceed with a lower dose per the dose de-escalation plan.

7.4.2. Other Adverse Events

Other adverse events (OAEs) may be identified by the PT and the DSMB. Significant AEs of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the participant from the study, will be classified as OAEs.

7.5. Sponsor Reporting Procedures to the DSMB

The DSMB will have access to real-time review of participant data during the course of the study through access to the participants electronic medical record. In addition, following the dosing of the first participant, a detailed review of safety will be conducted by the DSMB prior to dosing subsequent participants. After 90 days following infusion of each participant, the PI will submit a safety report to the DSMB for review. Requests for additional data by the DSMB can be made by communicating the request to the PI.

Other reports submitted will include:

- Immediate and interim subject data reviews. Data for individual subject reviews or for SAEs will be made available as soon as possible
- Summaries of adverse events (SAEs and AEs)
- Information necessary to review the conduct of the trial, including recruitment, enrollment, and unexpected problems
- Information from nonclinical findings that may impact the safety assessment of the trial will be provided by the PI or his designee
- The reports to the DSMB are considered privileged and not subject to disclosure except as required by law
- Ad hoc data summaries may be prepared upon written request by the DSMB to address a specific safety concern (email is an acceptable method of communication)

7.6. Sponsor Reporting Procedures to the IRB

Unanticipated problems involving risks to subjects or others (UPIRSO) will be reported to the IRB within 5 working days of discovery if they follow the following definition:

An event that meets ALL three (3) of the following criteria: 1. Unexpected (in nature, severity, or frequency), AND 2. Probably or definitely related to the research, AND 3. Suggests the research places subjects or others at a greater risk of harm than previously known or recognized.

All other research-related events and reports will be summarized at annual continuing review (CR) or notice of study closure, whichever comes first. That includes, but is not limited to:

- Noncompliance events (e.g., deviations) that do not meet the UTSW HRPP definition of either serious or continuing noncompliance
- AEs/SAEs that do not meet ALL 3 UPIRSO criteria
- Events/reports the sponsor wants submitted to the UTSW IRB/HRPP
- Data safety monitoring (DSMB) reports
- Other safety reports (e.g., IND)

- Monitoring/audit reports
- Any other new information since the last IRB/HRPP review

A Summary Report of AEs will be prepared by the PT annually and will be sent to the TRB at continuing review. The Summary Report will contain the following information:

- A statement that for DSMB review of outcome data, AEs, and information relating to study performance took place on a given date.
- A statement as to whether or not the frequency of AEs exceeded what was expected and indicated in the informed consent.
- The DSMB recommendation to either proceed with the study or modify the protocol or informed consent document. If the DSMB recommends changes to the protocols or informed consent document, the rationale for such changes and any relevant data will be provided.
- A statement that if safety concerns are identified, they will be communicated promptly to the investigators.

7.7. Sponsor Reporting Procedures to the FDA

The sponsor CureSPG50 will notify the FDA of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the PI determines that the information qualifies for reporting (see below) per 21CFR312.32. In each IND safety report, the sponsor will identify all IND safety reports previously submitted to FDA concerning a similar suspected adverse reaction and must analyze the significance of the suspected adverse reaction in light of previous, similar reports or any other relevant information.

Information that qualifies reporting:

- Serious and unexpected suspected adverse reaction. The PI will report any suspected adverse reaction that is both serious and unexpected. The PI will report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as o single occurrence of an event that is uncommon and known to be strongly associated with drug exposure.
- One or more occurrences of an event that is not commonly associated with drug exposure but is otherwise uncommon in the population exposed to the drug.
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently following treatment.
- Findings from other studies. The PI will report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies that suggest a significant risk in humans exposed to the drug.

- Findings from animal or in vitro testing. The PI will report any findings from animal or in vitro testing, whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug.
- Increased rate of occurrence of serious suspected adverse reactions. The sponsor must report any clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol or investigator brochure.
- Submission of IND safety reports. The PT will submit each IND safety report in a narrative fo1mat or on FDA Form 3500A or in an electronic format that FDA can process, review, and archive. Reports of overall findings or pooled analyses from published and unpublished in vitro, animal, epidemiological, or clinical studies must be submitted in a narrative format.
- Unexpected fatal or life-threatening suspected adverse reaction reports. The PI will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the PI's initial receipt of the inflammation.

7.8. Protocol Deviations and Continuing Review

Deviations to the protocol will not be reported to the IRB unless they occur at a rate greater than anticipated by the study team. If the rate of these events exceeds the rate expected by the study team, the events will be classified and reported as though they are unanticipated problems.

The following items will be reported to the UTSW IRB in summary at the time of Continuing Review:

- Serious and non-serious unanticipated problems,
- Expected serious adverse events that are possibly, probably, or definitely related to the investigational product,
- Serious adverse events that are not related to the investigational product
- All adverse events, except expected AEs and death granted a waiver of reporting,
- Any trends or events which in the opinion of the investigator should be reported, and
- Any protocol-specific reporting requirements (as applicable).

7.9. Stopping Rules

Our experience with managing adverse events is based on other clinical trials our Investigators are involved in using the same administration, delivery system, viral load and dosing method (clinical trial.gov identifiers NCT02122952, NCT02362438, NCT02716246, NCT04737460, NCT04798235).

The DSMB will have the responsibility and authority to stop or suspend the trial based on their review of the data and the pre-determined stopping rules. If DSMB determines that the trial should be stopped or suspended, they will notify Dr Iannaccone as soon as

feasible. Dr. Iannaccone will be responsible for notifying the IRB within three days. The DSMB will review the study data that the PI will submit listed below:

- A narrative summary of trial activity to date,
- A line listing of all AEs reportable per protocol,
- A narrative summary assessment of any safety concerns including:
- AE and SAE trends,
- Unanticipated Problems relating directly to protocol-driven activities,
- Participants withdrawn for safety reasons,
- Trial halting or pausing activity, and
- Other events relating to the overall safety of the trial.

After each DSMB review, a recommendation as to whether the study is to continue, be modified, or be terminated will be provided in a summary report. All SAEs, all unanticipated problems, and all IND Safety Reports will be reported by the PI to the DSMB at the same time they are submitted to the IRB. The DSMB will be notified immediately if pausing or halting rules are met and the DSMB will provide a recommendation for continuation, modification, or termination of the study. The PI will submit the written DSMB summary reports with recommendations to the IRB.

Halting the study requires immediate discontinuation of study agent administered for all participants and suspension of enrollment until a decision is made whether or not to continue study agent administration.

7.10. Halting Criteria for the Protocol:

The DSMB will review all safety data from the first subject in order to permit the second subject to be dosed with the study drug. The halting criteria will be if the first subject has had a grade 3 or above AE and an unexpected and possibly, probably, or definitely related to the study agent.

The study will be halted to allow for investigation of a safety signal if one subject has a serious adverse event > Grade 3-regardless of relatedness.

The study will also pause if any subject has an adverse event related to new or worsening DRG or PN signs or symptoms.

Any safety issue that the site investigators determine should halt the study.

We will monitor participants closely for any idiosyncratic allergic reaction related to the infusion of the gene product under investigation.

The procedure will be performed in a procedure unit with a board-certified anesthesiologist present to administer sedation. Physiologic monitoring in accordance with the standards set by the American Society of Anesthesiologists will be utilized for

all participants while they are receiving analgesia/anesthesia during the infusion and until they have fully recovered from its effects. Vital signs including blood pressure (BP), heart rate (HR), respiration rate (RR), temperature (T), oxygen saturation (02 sat), and heart rhythm (via telemetry) will be monitored.

If any adverse reaction is noted during the infusion, infusion will be stopped immediately. Participants will undergo a thorough clinical assessment to assure medical safety. Participants will be treated for any adverse event as set by clinical standards and guidelines of care and based upon the clinical findings (e.g., if low oxygen saturation is measured, the patient will be evaluated and treated as clinically indicated). This may include, but is not limited to, assessments on physical examination, initiating of oxygen therapy, measurement of blood oxygen levels by ABG (a1terial blood gas), Chest X-ray, CT imaging of the chest, PFTs (pulmonary function tests), or aerosol therapy. In the unlikely event that a severe allergic reaction should occur, the medical and nursing staff will follow the anaphylaxis guidelines.

Subjects who receive a partial infusion will continue to be monitored for period A and period B per protocol.

Since drug reactions can be idiosyncratic and not necessarily dose dependent, we will proceed with the same dose in the next subject once cleared to move forward with the study. We will not, however, increase the dose if a reaction occurs in the first patient.

7.11. Reporting of Study Halting:

If a halting requirement is met, a description of the event(s) or safety issue must be reported by the PI within one business day by fax or email AND the PI must inform the IRB that a halting role has been met.

Resumption of a Halted Study will be dependent on the DSMB and IRB feedback, although the sponsor maintains overall decision-making authority for resuming the study.

8. DATA COLLECTION

Data will be collected at specified time intervals as outlined in the protocol. Once the participant and/or parent/guardian has signed the informed consent/assent form, data can then be collected, including pertinent retrospective medical records per PI discretion.

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, pathology reports, laboratory notes, memoranda, participants' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, digitized imaging data, x-rays, participant files,

and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

The study electronic case report forms (eCRF) are where all data collection will be inputted for the study. All data requested on the eCRF will be recorded by the clinical operations team consisting of the clinical research coordinators/managers and research nurses. The electronic data capture (EDC) platform used in this clinical trial will be REDCap. REDCap is a secure web-based application that is FDA compliant per 21 CFR11 regulation. It supports regulatory trials to ensure Good Clinical Practice (GCP). All missing data must be explained, and it will have automatic data verification in place to ensure complete and accurate data is entered. REDCap will be accessed via a secure personalized login, thus allowing for role-appropriate access, and providing audit trails for data entry, exports, and reports. Trial data will be entered using participant identification numbers. PHI will not be shared outside of IRB approved entities.

8.1. Database Locks

For key deliverables requiring analysis of the trial data, an export of the entire database from REDCap will be performed at such periodic intervals in order to have a locked dataset from which all results will be generated. At trial end, a final lock and export will occur after all data queries are resolved and statistical analysis will be performed. The final trial results, FDA summary report, and publications will be prepared from this locked dataset. The study database will be retained at the site 5 years after trial end.

8.2. Study Monitoring Plan

Dr. Susan Iannaccone (UT Southwestern) is the lead investigator and will be responsible for assuring ongoing safety monitoring of the trial.

Members on the DSMB will serve as independent safety reviewers. The DSMB charter defines the role and responsibilities of the members of the group and contains assurances of freedom from conflicts of interest.

UTSW Human Research Protection Program Office (HRPPO) will provide data monitoring. The ongoing data monitoring responsibilities are contracted by the UTSW PI, with the UTSW HRPPO on a fee-for-service basis to monitor the study progress and will function independently from the study team. The UTSW PI is responsible for providing copies of the HRPPO monitoring reports and disclosing any reportable events (REs) submitted to the UTSW HRPPO/IRB to the FDA in accordance with federal requirements.

8.3. Quality Assurance of Data

Quality assurance (QA) processes are in place to ensure the data will be collected and entered into the EDC accurately and consistently. QA of trial data will employ several approaches, including auditing of CRFs to identify data fields that require adaptation for clinical relevance and accessibility, a Manual of Operations (MOO) prepared by the

clinical operations team with detailed instructions for data collection and entry into EDC, and real-time validations of submitted data.

9. INSTRUMENTS FOR THE ASSESSMENT OF DISEASE

9.1. Modified Ashworth Scale

Modified Ashworth Scale Instructions

General information (derived Bohannon and Smith, 1987):

- Place the patient in a supine position
- If testing a muscle that primarily flexes a joint, place the joint in a maximally flexed position and move to a position of maximal extension over one second (count "one thousand one")
- If testing a muscle that primarily extends a joint, place the joint in a maximally extended position and move to a position of maximal extension over one second (count "one thousand one")
- Score basic on the classification below

Scoring (taken from Bohannon and Smith, 1987):

- 0 No increase in muscle tone
- 1 Slight increase in muscle tone, manifested by a catch and release or by minimal resistance at the end of the range of motion when the affected part(s) is moved in flexion or extension
- 1+ Slight increase in muscle tone, manifested by a catch, followed by minimal resistance throughout the remainder (less than half) of the ROM
- 2 More marked increase in muscle tone through most of the ROM, but affected part(s) easily moved
- 3 Considerable increase in muscle tone, passive movement difficult
- 4 Affected part(s) rigid inflexion or extension

9.2. Tardieu Scale

Patient must remain in a constant position throughout the test. Head should be in midline or in a constant position each time tested.

X: SPASTICITY ANGLE (THRESHOLD):

The difference $X_{V1} - X_{V3}$ is the Spasticity Angle (X), which reflects the velocity-dependence of the stretch reflex. The larger the spasticity angle the more spastic the muscle.

Velocity of Stretch is indicated for each muscle and remains the same from one test to another, as follows:

X_{V1} = As *slow* as possible

X_{V3} = As *fast* as possible

Y: SPASTICITY GRADE: Quality of the muscle reaction (GAIN):

- **0** = No resistance throughout passive movement
- 1. = Slight resistance throughout passive movement
- 2. = Clear catch at precise angle, interrupting passive movement, followed by release
- **3** = Fatigable clonus (*less* than 10 seconds when maintaining pressure) occurring at a precise angle, followed by release
- **4** = Unfatigable clonus (*more* than 10 seconds when maintaining pressure) occurring at a precise angle

Catch without release:
graded 0 if $X_{V1} = X_{V3}$

Catch without release:
graded 'unratable' if $X_{V1} \neq X_{V3}$

Catch with "minimal" release:
graded 2 if X_{V3} is consistent and consistently $< X_{V1}$

Catch with "minimal" release:
graded 'unratable' if X_{V3} is variable / inconsistent

For grades 0 and 1, the Spasticity Angle = 0 by definition

TARDIEU SPASTICITY SCALE

Date:_____

Patient:_____

Investigator:_____

X = Degree Y = 0 - 4

***Indicate 'NR' if not ratable**

		LEFT	RIGHT
Muscle Group:	X_{V1}		
	X_{V3}		
	X (V1-V3)		
	Y		
Muscle Group:	X_{V1}		
	X_{V3}		
	X (V1-V3)		
	Y		
Muscle Group:	X_{V1}		
	X_{V3}		
	X (V1-V3)		
	Y		

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11. APPENDICES

11.1. Appendix 1: Dosing Schedule