INVESTIGATOR'S BROCHURE

Sponsor:	CureSPG50
Indication:	SPG50 / AP4M1

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REVISION HISTORY

Amendments: The following sections have been updated from November 2021 version:

Section/Appendix	Description	Date
Amendment 1		
1	Added information on subject dosed in March 2022	July 2022
1	Updated summary bullets for nonclinical with confirmed conclusions	
4	Updated nonclinical information based on completed studies and final study reports	
5/5.3	Added information on subject dose in March 2022	

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LIST OF ABBREVIATIONS

AAV	adeno-associated virus
ADME	Absorption, distribution, metabolism and elimination
AE	Adverse event
ALB	Albumin
AP-4	Adaptor Protein complex
AP4M1	Adaptor protein complex, μ4
AST	Aspartate transaminase
ATG9A	Autophagy Related 9A
BCH	Boston Children's Hospital
BD	biodistribution
BGH	Bovine growth hormone
BUN	Blood Urea Nitrogen
CK	Creatine Kinase
CNS	Central nervous system
CRF	Case report form
CRIM	cross-reactive immunologic material
CSF	Cerebrospinal fluid
CTA	Clinical trial application
CTL	cytotoxic lymphocyte
DAPI	4′,6-diamidino-2-phenylindole
ddPCR	Digital drop PCR
DNA	Deoxyribonucleic acid
DRG	Dorsal root ganglia
EEG	Electroencephalogram
FDA	Food and Drug Administration
GFP	Green fluorescent protein
GLP	Good laboratory practice
h	Human
hAP4M1opt	Human optimized AP4M1
HC	Health Canada
HSP	Hereditary spastic paraplegia
INFγ	Interferon gamma
IRB	Independent review committee
IRNHS	International registry Natural History Study
IT	Intrathecal
ITR	Inverted terminal repeat(s)

IV	Intravenous
kg	Kilogram
KO	Knock-out
LP	Lumber puncture
MAS	Modified Ashworth Scale
MOI	Multiplicity of Infection
MRI	Magnetic resonance imaging
NCV	Nerve conduction velocity
NHP	Non-human primate
NIH	National Institutes of Health
NOAEL	no-observed-adverse-effect level
NSAID	Nonsteroidal anti-inflammatory
opt	Optimized
PBS	phosphate buffered saline
PCR	Polymerase chain reaction
PICU	Pediatric intensive care unit
PND or p	Post-natal day
PRES	posterior reversible encephalopathy syndrome
qPCR	Quantitative PCR
SAE	Serious adverse event
sc	Self-complimentary
SD	Sprague Dawley (rat)
SEM	Standard error of the mean
SPG50	Spastic Paraplegia 50
SPRS	Spastic Paraplegia Rating Scale
StDev	Standard deviation
RNA	Ribonucleic acid
TBIL	Total bilirubin
TGN	Trans-Golgi network
US	Unites States of America
USP	Universal stress protein
UTSW	University of Texas Southwestern Medical Center
Veh	Vehicle
vg	Vector genome(s)
WoA	Weeks of age
WT	Wild type

1 Summary

MELPIDA is a gene therapy being progressed for the treatment of Spastic Paraplegia Type 50 (SPG50), a form of Spastic Paraplegia Disease (SPG), which is one of a group of 4 genetic disorders (SPG47, SPG50, SPG51 and SPG52) involving a pathologic variation that results in the production of an abnormal adaptor protein complex 4 (AP-4); all 4 subtypes present with the same clinical phenotype (Behne et al, 2020; Ebrahimi-Fakhari et al, 2020; Ebrahimi-Fakhari et al, 2018).

MELPIDA was administered to a single subject (male, 4y) at 1E15 vg in March 2022 under a CTA in Canada. As of 1 June 2022, almost 3 months post-dose, this subject has reported 4 adverse events; vomiting (mild) and upper respiratory tract infection (mild) were not considered related to product; excessive fatigue and low neutrophils were considered possible and probably related, both were mild and resolved within 2 months following treatment.

AP-4-associated hereditary spastic paraplegia (AP-4-HSP) is caused by bi-allelic loss-of-function variants in one of the 4 subunits that make up AP-4. AP-4-HSP is an ultra-rare autosomal recessive disease with ~156 patients identified worldwide and 59 identified with SPG50. There are ~9 patients with SPG50 in North America (AP4M1, OMIM #612936) (Ebrahimi-Fakhari et al, 2020, Registry and Natural History Study for AP-4 Associated Hereditary Spastic Paraplegia (AP-4-HSP; ClinicalTrials.gov Identifier: NCT04712812).

Symptoms of AP-4-HSP begin in infancy, though patients are often not correctly identified and diagnosed until age 5 to 10 years. Patients are usually paralyzed by spastic paraplegia within their first decade, become tetraplegic and severely mentally delayed by adolescence or adulthood; epilepsy is also a key symptom (Ebrahimi-Fakhari et al, 2018). Only a few patients are identified over the age of 30 years due in part to recent advances in genetic diagnostics and also to life limitation associated with the disease (Table 2) (Ebrahimi-Fakhari et al, 2020).

There are no approved treatments for SPG50, leaving an unmet medical need for this serious and quality-of-life limiting disease affecting pediatric patients.

The proposed clinical trial involves gene replacement via MELPIDA, a recombinant self-complementary (sc) adeno-associated virus (AAV) serotype 9 encoding a functional codon-optimized human AP4M1 transgene (hAP4M1opt). The final product is available as an injectable sterile solution developed for lumbar intrathecal (IT) administration. The study aims to deliver the functional *AP4M1* gene to SPG50 patients. AAV vectors are non-pathogenic, non-replicating, and transduce non-dividing cells (Goncalves, 2005). These recombinant vectors, however, are incapable of coding viral proteins or actively integrating with the host genome, thus making them useful for gene delivery (Duan et al, 1998; Choi et al, 2006).

Central nervous system (CNS) tissues generally are the gene therapy target for neurological diseases. AAV9 vectors in particular, demonstrate their suitability as vehicles for delivering the therapeutic products involving broad transgene distribution to CNS tissues (Snyder et al, 2011; Gray et al, 2011; Gray et al, 2013; Karumuthil-Melethil et al, 2016; Sinnett et al, 2017; Bailey et al, 2020; Chen et al, 2021). These vectors can be purified in large quantities at high concentrations, which makes them desirable for delivering a functional gene to target cells with aberrant disease mutations.

Some recent Phase I and II human clinical trials, such as Giant axonal neuropathy disease (NCT02362438), Gangliosidosis-1 disease (NCT03952637), Neuronal ceroid lipofuscinosis 7 Batten disease (NCT04737460), and Canavan Disease (NCT04998396), have successfully employed these vectors for gene replacement in treating neurological diseases, but definitive evidence of efficacy is still being gathered. Furthermore, safety data from both nonclinical and clinical studies are also being gathered. For example, an identified risk of inflammatory damage to dorsal root ganglia (DRG) or peripheral nerves has been reported from recent non-clinical studies (Hinderer et al, 2018; Hordeaux et al, 2020a and 2020b).

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 AP-4 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, AP4E1 localization to the TGN and unchanged staining for TGN46 with phenotypic rescue in up to 77% of fibroblasts and no associated toxicity (Study #01, Section 4.2.1).
- An in vivo efficacy study (Study 2020-06, Section 4.2.2) 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), mid (2.5E11) or high (5E11) doses of MELPIDA and assessed for potential phenotypic rescue. Interim results at 8 months post dose indicate the high dose of 5E11 vg provided maximal pharmacological effects on Hindlimb Clasping whether administered early on PND7-10 or later on PND90 in male mice. For the Maze total distance, female mice had a less robust response when dosed at PND90 compared to PND7-10. The mid dose of 2.5E11 vg provided some pharmacological effects on Hindlimb Clasping when administered PND90 in male mice, suggesting MELPIDA may also generate benefits when dosed at the early-symptomatic stage.
- An in vivo 12-month non-GLP toxicology study (Study #05, Section 4.8.1.1) 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, no deaths occurred. 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 [DHHS, 2019]).
- An in vivo 3-month GLP toxicology and biodistribution study in WT Sprague Dawley (SD) rats (Study CRL-5550008, Section 4.8.1.2) 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 ≥ 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. Biodistribution of MELPIDA in rats was consistent with the expected biodistribution pattern of AAV9 in previously published studies.

- 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. Biodistribution of MELPIDA in NHPs was consistent with the expected biodistribution pattern of AAV9 in previously published studies.

Published literature on animal studies and human trials point to minimal and manageable side effects caused by the AAV9 viral component (Gushchina et al, 2021; Meadows et al, 2015). Further, it is also realized that any primary anticipated complications arising from anti-capsid immune responses to AAV9 can often be countered with the use of transient immunosuppressive regimens. To avoid such potential undesirable immune responses, the clinical protocol incorporates immune management with T cell suppressive agents and a long-term monitoring plan.

Based on a natural history study being conducted at Boston Children's Hospital (BCH) it is evident that the disease severity ranges from child to child, but most children fall into the severely affected category. Those who are considered 'least severe', are able to speak in short sentences, walk with a significant gait and have few to no seizures early on in the disease progression when they are 6 to 10yrs of age. However, they ultimately end up in a wheelchair and become quadriplegic between the ages of 10 and 20 yrs of age. The majority of children with SPG50 are completely non-verbal, have microcephaly, never walk, have epilepsy and are severely mentally disabled 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. To date there has not been a single patient identified that is able to look after themselves without the need of continual support from family and/or caregivers. Simple day to day tasks such as changing, feeding, going to the bathroom or even getting into bed requires assistance. SPG50 is a deteriorating neurological disease both in intellectual and motor capabilities. Given the severity of the disease and lack of treatment options, gene replacement via MELPIDA offers the potential for benefit which could be life-changing for these children.

MELPIDA is proposed for the treatment of subjects with SPG50 and targets neuronal cells to deliver a fully functional human AP4M1 cDNA copy via intrathecal injection to counter the associated neuronal loss. Outcomes will evaluate the safety and tolerability of a single dose of MELPIDA, which will be measured by the treatment-associated adverse events (AEs) and serious adverse events (SAEs). Secondarily, the trial will explore efficacy in terms of disease burden assessments.

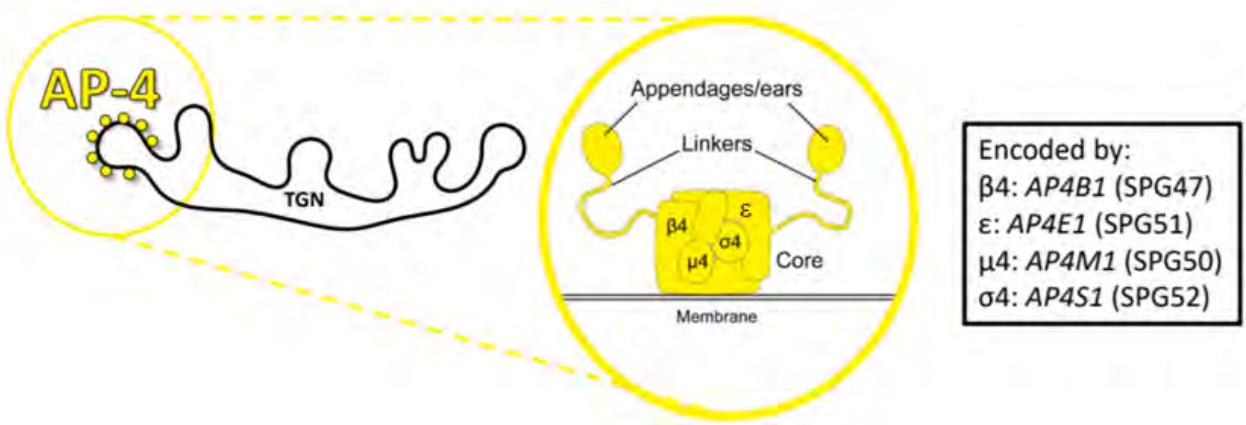
2 Introduction

2.1 Disease Background and Unmet Medical Need

2.1.1 Pathophysiology of SPG50

SPG50 is one of a group of adaptor protein complex 4 (AP-4) deficiencies (Figure 1). Homozygous (or compound heterozygous) loss-of-function variants in any of the four AP-4 subunits causes a neurological disorder characterized by spastic paraplegia, seizures, developmental delay and intellectual disability (Ebrahimi-Fakhari et al, 2020; Ebrahimi-Fakhari et al, 2018).

Figure 1 Schematic of Adaptor Protein Complex 4



The four subtypes are SPG47, SPG50, SPG51 and SPG52 (Table 1).

Table 1 Subtypes of AP-4 Deficiencies (Ebrahimi-Fakhari et al, 2018)

AP-4 subtype	OMIM	Mutation	Distribution	Male:Female
SPG47	#614066	AP4B1	34%	39% Female
SPG50	#612936	AP4M1	38%	43% Female
SPG51	#613744	AP4E1	13%	33% Female
SPG52	#614067	AP4S1	15%	52% Female

*From International Registry and Natural History Study (IRNHS) of Adaptor-Protein 4-Related Hereditary Spastic Paraplegia database (with 185 identified patients worldwide),

The AP-4 biology and molecular mechanisms of AP-4-hereditary spastic paraplegia (HSP) are not completely elucidated. Available data demonstrate that AP-4 complex consists of four subunits (β4, ε, μ4 and σ4) (Hirst et al, 2013) and has been implicated in trafficking of transmembrane proteins from the *trans*-Golgi network (TGN) to endosomes (Aguilar et al, 2001; Burgos et al, 2010; Toh et al, 2017). More recent cell culture studies have demonstrated that ATG9A, an autophagy protein, is a cargo of AP-4 and loss of AP-4 leads to a mislocalization of ATG9A (Mattera et al, 2017; Davies et al, 2018; De Pace et al, 2018; Ivankovic et al, 2020). Characterization of AP4B1- (Matsuda et al, 2008) and AP4E1-knockout mice has revealed widespread axonal pathology that includes reduced axonal development and prominent axonal swellings (De Pace et al, 2018; Ivankovic et al, 2020).

2.1.2 Natural History and Seriousness of SPG50

Due to the rarity of the condition, limited data are available on the natural history of AP-4 deficiency. Ebrahimi-Fakhari et al, (2020) reports on 156 patients from 101 families from an AP-4 HSP registry. Subsets of patients have provided information on The Spastic Paraplegia Rating Scale (SPRS) (N=37), The Modified Ashworth Scale (MAS) (N=28), A SPATAX-EUROSPA Disability score (N=39) and a Four Stage Functional Mobility Score (N=85). This is the most extensive data available on these patients.

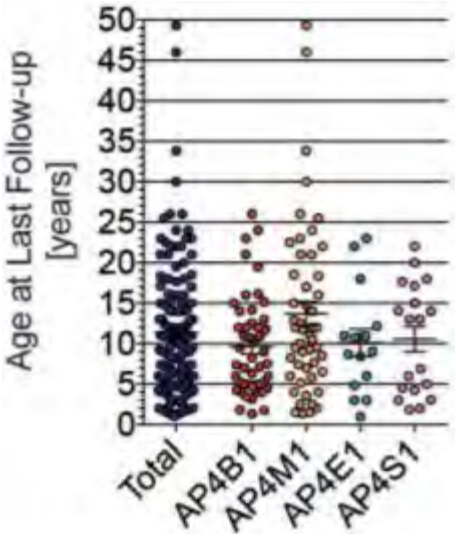
SPG50 is a serious and life-threatening disease with presentation and progression during childhood. Natural history data indicates age of symptom onset in infancy with diagnosis often delayed and occurring towards the end of the first decade (Ebrahimi-Fakhari et al, 2020) (Table 2). Of note, patients are often initially diagnosed with cerebral palsy (Jameel et al, 2014). Patients initially present with hypotonia, developmental delay, microcephaly, spasticity and paralysis in the lower limbs. As the disease progresses paralysis spreads to the hands and upper extremities and cognitive impairment worsens (Ebrahimi-Fakhari et al, 2018).

Table 2 Key Demographic Data for 156 AP-4 Deficient Patients from Ebrahimi-Fakhari et al, 2020

AP-4-HSP Subtype	Age at onset, years	Age at diagnosis, years	Age at last visit, years	Consanguinity	Familial case
AP4B1-HSP: *n* = 53	0.6 ± 0.4 (*n* = 34)	7.8 ± 6.9 (*n* = 31)	9.6 ± 6.1 (*n* = 48)	60% (*n* = 50)	55% (*n* = 53)
AP4M1-HSP: *n* = 59	0.9 ± 0.8 (*n* = 42)	11.7 ± 10.0 (*n* = 44)	13.7 ± 10.6 (*n* = 50)	77% (*n* = 52)	78% (*n* = 59)
AP4E1-HSP: *n* = 21	0.7 ± 0.3 (*n* = 6)	10.3 ± 6.7 (*n* = 14)	10.1 ± 6.6 (*n* = 15)	90% (*n* = 20)	86% (*n* = 21)
AP4S1-HSP: *n* = 23	0.8 ± 0.5 (*n* = 11)	10.4 ± 7.3 (*n* = 13)	10.6 ± 6.8 (*n* = 19)	17% (*n* = 19)	48% (*n* = 23)
Total: *n* = 156	0.8 ± 0.6 (*n* = 93)	10.2 ± 8.5 (*n* = 102)	11.4 ± 8.3 (*n* = 132)	69% (*n* = 141)	67% (*n* = 156)

There are relatively few adult patients for all phenotypes (Figure 2) (Ebrahimi-Fakhari et al, 2020). This is likely primarily due to the fact that the condition has only relatively recently been described, and that sequencing technologies such as whole exome sequencing are more frequently utilized in pediatric patients. It remains to be established whether the condition is life limiting.

Figure 2 Age at Last Follow-up for 156 Patients with AP-4 Deficiency (from Ebrahimi-Fakhari et al, 2020)



All phenotypes present with serious and quality of life limiting symptoms. From Ebrahimi-Fakhari et al, (2018) GeneReview using data from the International Registry and Natural History Study of Adaptor-Protein 4-Related Hereditary Spastic Paraplegia database (updated 5-20-18 with 68 identified patients worldwide), characteristic clinical findings include:

- Progressive spastic paraplegia with progression to tetraplegia in the later stages (94%, 58/62)
- Early-onset developmental delay (100%, 68/68)
  - Delayed motor milestones (100%, 54/54)
  - Failure to achieve or loss of independent ambulation (93%, 41/44)
  - Impaired or absent speech development (98%, 51/52)
- Neonatal/infantile hypotonia (usually mild) (100%, 41/41)
- Postnatal microcephaly (77%, 47/61) (usually in -2 StDev to -3 StDev range)
- Early-onset seizures including frequent febrile seizures (42%, 25/59)
- Thinning of the corpus callosum (with prominent thinning of the posterior parts) (88%, 37/42)
- Delayed myelination and nonspecific loss of the periventricular white matter (69%, 29/42)
- Ex-vacuo ventriculomegaly, often with prominent enlargement of the posterior horns of the lateral ventricles (60%, 24/40)

When categorized by age, the following is a typical progression of motor function:

- 6 months: Microcephaly and low muscle tone become pronounced; MRI results show thinning of the corpus callosum.
- 1 year: Delays become evident, low muscle tone is more evident and microencephaly progresses such that head circumference becomes <2 StDev from age related mean.
- 2 years: Children are usually placed in multiple forms of therapy yet are unable to walk independently or speak. Spasticity begins to present in the ankles, calves and in some cases thighs.
- 3-5 years: Children exhibit greater spasticity, their ability to walk is severely impaired and a walker along with braces is used to keep children mobile. Botox injections have been used by many families as an intervention for spasticity. Some children develop a limited ability to speak a few words.
- 5-10 years: Children experience progressive spasticity and usually wheelchair-dependent. Typically, disease has not progressed to upper extremities yet. Some children are able to speak sentences, but others remain non-verbal.
- 10-15 years: Children are mostly wheelchair-dependent and many begin exhibiting spasticity in upper extremities.
- 15-20+ years: Children present with spasticity in all extremities and associated complications. Not much more is known beyond 15+ as there are very few patients in this category.

Published data from the 156 patients with AP-4 deficiency described by Ebrahimi-Fakhari et al, (2020) support earlier findings that symptoms are severely debilitating and manifest prior to adulthood (Table 3). Motor milestones were often missed or delayed and although some patients were able to sit and walk independently, these abilities were lost as the patients aged; the mean age of wheelchair dependency was 13.4 ± 9.8 years. Loss of previously acquired skills or development regression and cognitive decline was reported in 41% of patients.

Table 3 Clinical and Radiographic Features of AP-4 Deficiency (from Ebrahimi-Fakhari et al, (2020)

Principal clinical features of AP-4-HSP	
Developmental delay / intellectual disability	100%
Motor delay	100%
Speech delay	99%
Mild neonatal or infantile hypotonia	89%
Spasticity	97%
Spastic diplegia	54%
Spastic tetraplegia	43%
Hyperreflexia	92%
Babinski sign	88%
Contractures	50%
Drooling	70%
Postnatal microcephaly	83%
Dysmorphic facial features	78%
Foot deformities	69%
Febrile seizures	62%
Epilepsy	66%
Episodes of stereotypic laughter	56%
Principal radiographic features of AP-4-HSP	
Thin corpus callosum	90%
Ventriculomegaly	65%
White matter loss / changes	68%

2.1.3 The Incidence and Prevalence of SPG50

AP-4-HSP caused by mutations in AP-4 subunits is an ultra-rare autosomal recessive disease with ~156 patients identified worldwide and 59 identified with SPG50. There are ~9 patients with SPG50 in North America (AP4M1, OMIM #612936) (source: Ebrahimi-Fakhari et al, 2020).

The most robust data to support prevalence comes from the work by Ebrahimi-Fakhari et al, (2020) which describes 156 patients worldwide with AP-4 deficiency and 59 (38%) with SPG50 (Table 2). North America accounts for 15% of all known patients. Estimation for the number in North America is derived by:

$$59 * 0.15 = 8.85 \text{ persons}$$

Based on N=9 and a current US population size of 329,842,223 (source: U.S. Census Bureau and World Bank, June 25, 2020), prevalence for SPG50 is ([9/329,842,223]*100,000) = 0.0027:100,000.

2.1.4 No Treatments are Available for Patients with SPG50

There are currently no commercial or investigational products available to treat SPG50.

2.2 Medical Plausibility of MELPIDA

AP4M1 gene transfer to the CNS is expected to positively impact the normal course of disease progression in SPG50 patients. The mode of action of MELPIDA is to target multiple types of cells including neurons and glia in the CNS (and to some extent peripheral organs) after a single one-time intrathecal injection. Primary cellular targets proposed for the treatment of SPG50 are neurons and glia throughout the CNS. AP4M1 is a ubiquitous intracellular housekeeping gene, so the targeting of other cell types within the body is also desired.

The AAV9 vector utilized is expected to deliver the AP4M1 gene by the IT route to the brain and spinal cord, as the primary targets (Bailey et al, 2020). Global transgene CNS delivery of AAV9 has been investigated in rodents, dogs, pigs and non-human primates (NHPs). Compared to other AAV vector serotypes and routes of administration, intrathecal (lumbar) administration of AAV9 vectors into the cerebrospinal fluid (CSF) achieves widespread distribution of the transgene to neurons and glia throughout the spinal cord and brain at a translationally-relevant dose. In pigs, a 50 to 100% transduction was achieved in spinal cord motor neurons along with transduction of neurons and glia in the brain at a dose of 1.7E11 vg/kg (Snyder et al, 2011). Similar results have been observed in cats, dogs, and non-human primates (Bradbury et al, 2020; Gray et al, 2013; Bucher et al, 2013; Haurigot et al, 2013; Samaranch et al, 2012; Samaranch et al, 2013).

Nonclinical proof-of-concept has been established with MELPIDA from various studies. These data are described in Section 4 of this document.

The introduced recombinant viral genome encoding *AP4M1* should exist primarily as an episome following transfer of MELPIDA, and express a normal version of functional human AP4M1 protein continuously, which is expected to prevent or slow the onset of SPG50 if treated pre-symptomatically, or slow/halt or reverse the progression of SPG50 if treated after symptom onset.

3 Physical, Chemical and Pharmaceutical Properties and Formulation

MELPIDA is a recombinant serotype 9 adeno-associated virus encoding a codon-optimized human *AP4M1* transgene (*hAP4M1opt*) (Figure 3). The final product consists of AAV9 capsids that are packaged with the self-complementary (sc) AAV genome comprising a mutant AAV2 inverted terminal repeat (ITR) with the D element deleted, the universal synthetic promoter (USP), codon optimized human AP4M1 DNA coding sequence, the bovine growth hormone (BGH) polyadenylation signal, and WT AAV2 ITR. In essence, the investigational product MELPIDA is the viral vector (scAAV9/USP-hAP4M1opt-BGHpA), which is composed of the AAV9 serotype capsid and the *AP4M1* transgene expression cassette insert.

Figure 3 MELPIDA (MELPIDA): Vector Construct Expressing hAP4M1opt

MELPIDA for intrathecal administration is supplied as a 2 mL Daikyo CZ® vials 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. The product is stored and shipped frozen (≤ -60°C).

4 Nonclinical Studies

4.1 Introduction

Several nonclinical studies have been conducted to date with MELPIDA or analogues of MELPIDA (Table 4).

Table 4 Status of Nonclinical Studies Conducted with MELPIDA/Other

Study # GLP Status	Description	Model Doses (total vg) Route	Source
Ex vivo Studies			
#01 non-GLP Complete	Ex-vivo efficacy study using patient-derived fibroblasts	AAV2/Fibroblasts (2x Patient Cells) MOI 1E2, 1E3, 1E4 or 1E5 vg/cell	NIH
In vivo: Efficacy			
2020-06 non-GLP Ongoing	In-Vivo efficacy study in *Ap4m1* KO mice dosed at PND 7 to 10 or 90 days; terminate at 21 months of age	Ap4m1 KO Mouse Model (+/- and -/-), and WT mice, (N=193). No Tmt, Veh, 1.25E11, 2.5E11 or 5E11 vg/mouse in 5uL IT	UTSW
In vivo Toxicology and Biodistribution			
#05 non-GLP Complete	A 12 month in-vivo toxicology and biodistribution study	C57BL/6J mice (N=60) Veh, 1.25E11 or 5E11 vg/mouse in 5uL IT	UTSW
CRL-5550008 GLP Complete	A 90 day in-vivo toxicology and biodistribution study; dosed 7 to 8 WoA;	Sprague Dawley Rat (N=132) Veh, 0.36E12, 1.1E12, or 3.3E12 vg/mouse in 20 or 60uL IT	CRL
CRL-5550014 GLP Complete	A 90 day in-vivo toxicology and biodistribution study; dosed 2 to 4 YoA;	Cynomolgus monkeys (N=6) Veh, 8.4E13, or 1.68E14 vg/animal in 1.55 or 3.1mL IT	CRL

Key: GLP, good laboratory practice; vg, vector genomes; KO, knock out; Veh, vehicle; WoA, weeks of age; YoA, year of age; IT, intrathecal; PND, post-natal day; MOI, Multiplicity of Infection.

The following nonclinical studies provide proof of concept that MELPIDA provides promise for the clinical treatment of SPG50:

- Fibroblasts from 2x patients with SPG50 transduced with MELPIDA (using an AAV2 capsid) restored autophagy related 9A (ATG9A) trafficking and hence AP-4 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, AP4E1 localization to the TGN and unchanged staining for TGN46 with phenotypic rescue in up to 77% of fibroblasts and no associated toxicity (Study #01, Section 4.2.1).
- An in vivo efficacy study (Study 2020-06, Section 4.2.2) 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), mid (2.5E11) or high (5E11) doses of MELPIDA and assessed for potential phenotypic rescue. Interim results at 8 months post dose indicate the high dose of 5E11 vg provided maximal pharmacological effects on Hindlimb Clasping whether administered early on PND7-10 or later on PND90 in male mice. For the Maze total distance, female mice had a less robust response when dosed at PND90 compared to PND7-10. The mid dose of 2.5E11 vg provided some pharmacological effects on Hindlimb Clasping when administered PND90 in male mice, suggesting MELPIDA may also generate benefits when dosed at the early-symptomatic stage.

- An in vivo 12-month non-GLP toxicology study (Study #05, Section 4.8.1.1) 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, no deaths occurred. 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 [DHHS, 2019]).
- An in vivo 3-month GLP toxicology and biodistribution study in WT Sprague Dawley (SD) rats (Study CRL-5550008, Section 4.8.1.2) 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 ≥ 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. Biodistribution of MELPIDA in rats was consistent with the expected biodistribution pattern of AAV9 in previously published studies.
- 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. Biodistribution of MELPIDA in NHPs was consistent with the expected biodistribution pattern of AAV9 in previously published studies.

4.2 Primary Pharmacology

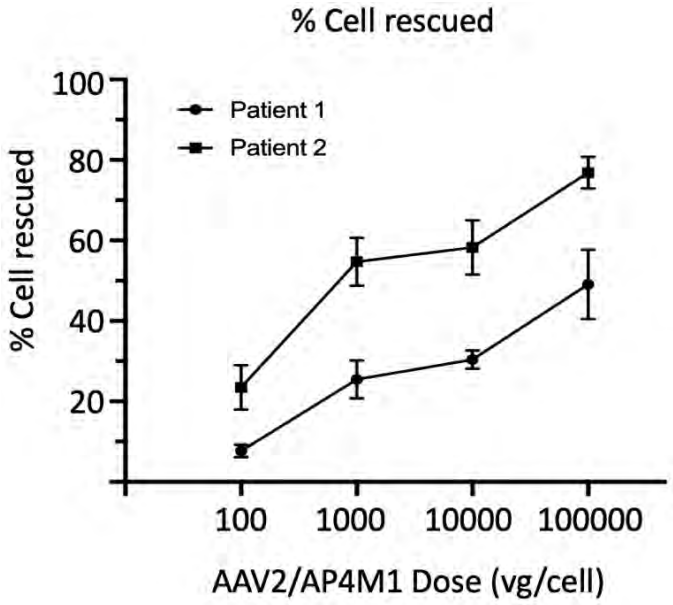
4.2.1 Study #01: In vitro Proof of Concept Study for MELPIDA Using SPG50 Patient Derived Fibroblasts

This in vitro study was conducted with an AAV2 capsid instead of AAV9 in fibroblasts derived from SPG50 patients. The objective of this study was to determine whether adeno-associated virus (AAV) -mediated expression of wild-type (WT) *AP4M1* could restore AP-4 level and correct ATG9A localization in SPG50 patient-derived fibroblast cultures expressing mutant *AP4M1*.

In two repeat *in vitro* efficacy experiments, fibroblast cell lines from two patients (#1 and #2) carrying *AP4M1* mutations were infected with the scAV2/*AP4M1* vector (similar to MELPIDA being progressed to the clinic, except for the use of an AAV2 serotype) for 72 hours (at multiplicity of infection [MOI] of 1E2, 1E3, 1E4 and 1E5 vg/cell). Twenty thousand (20,000) cells were plated in each well of a 24-well plate. The cells were then fixed and stained with 4′,6-diamidino-2-phenylindole (DAPI) and with antibodies against ATG9A, AP4E1, and TGN46. The total number of cells, and the number of cells exhibiting normal AP-4 and ATG9A staining (*i.e.*, "rescued" cells) were counted, and the percent rescue was calculated.

At the highest virus dose of 1E5 vg/cell, the rescue of AP-4 and ATG9A phenotypes was 49% and 77% of the cells for patients #1 and #2, respectively (Figure 4).

Figure 4 Dose-dependent Rescue of AP-4 and ATG9A Normal Localization by Expression of AAV2/AP4M1 in SPG50 Patient Cells



Total and rescued cells from two experiments were counted, and the percent rescue was calculated. Values are the mean ± SD from the two experiments

In summary, the vector scAAV2/*AP4M1* partially restored AP-4 levels and corrected ATG9A localization in fibroblasts from patients with SPG50.

4.2.2 Study 2020-06: An 8-month Interim Non-GLP Study to Determine the Efficacy of MELPIDA in a Mouse Knock-out (KO) Model of SPG50

The ability of MELPIDA to transduce multiple types of cells including neurons and glia in the CNS, which is the primary mechanism of action, was demonstrated in the Ap4m1 mouse through measurement of hAP4M1opt mRNA (Study 2020-06).

The design of this study is provided in Table 5. The in-life phase of this study is ongoing at University of Texas Southwestern (UTSW) Medical Center, Dallas, TX and assessments were as follows:

- Cage side observation to assess acute tolerability of MELPIDA following intrathecal administration.
- Monitoring for body weight, clinical signs including behavioral changes and alterations in neurological status, adverse events, and mortality.
- Three weeks post injection, 6 mice from each group were euthanized. Mouse brains were used for AP4M1 mRNA expression by RNAscope and mouse serum was used to check serum toxicity with a panel or markers including Aspartate transaminase (AST), Total bilirubin (TBIL), Albumin (ALB), Creatine Kinase (CK), and Blood Urea Nitrogen (BUN). Splenocytes from mouse spleen and lymph nodes were used in ELISpot assays to detect any immune responses to either AAV9 or transgene.
- Performance in a battery of behavioral tests is being assessed at 3, 5, 8, 12 and 18 months of age compared to homozygous (KO) control littermates.
- Blood and tissue samples are collected from mice that are euthanized for humane reasons. Where possible, a detailed necropsy is also performed to investigate or identify the reason for the ailment by a trained technician or veterinary staff.
- Terminal serum and tissue samples at 18 months (or 21 months) old will be collected for serum toxicity panel and histopathological assessment, respectively.

Two reports will be issued for this study, an interim from in-life for the initial 8 months which is included in this submission, and a final report with in-life and histopathology assessments after the 21 month assessments.

Table 5 21-month Efficacy Study in AP4M1 KO Mouse (ongoing)

***Ap4m1* Allele**	**AAV9/*AP4M1* Dose**	**Dose Group (Name)**	**Number of Animals (Male/Female)**	**Time of Dosing**
Ap4m1 (+/+)	na	A (WT)	12 (7/5)	na
Ap4m1 (+/-)	na	B (Het)	45 (23/22)	na
Ap4m1 (-/-)	Vehicle*	C	41 (19/22)	PND 7-10 or 90
Ap4m1 (-/-)	1.25E11 vg/animal*	D	16 (7/9)	PND 7-10
Ap4m1 (-/-)	5E11 vg/animal*	E	21 (8/13)	PND 7-10
Ap4m1 (-/-)	1.25E11 vg/animal*	F	17 (10/7)	PND 90
Ap4m1 (-/-)	2.5E11 vg/animal*	G	22 (9/13)	PND 90
Ap4m1 (-/-)	5E11 vg/animal*	H	19 (8/11)	PND 90

*Animals dosed via lumbar IT injection. WT, Wild type. Het, Heterozygous. KO, Knockout. PND, post-natal day.

Interim results for the initial 8 months demonstrated that MELPIDA dose dependently increases AP4M1 mRNA expression (Figure 5), induces minimal immune responses, causes minimal toxicity, generates minimal effects on body weight, creates minimal effects on survival, and improves abnormal behaviors (Figure 6).

Figure 5 MELPIDA Increased hAP4M1opt mRNA in All Brain Regions 3 Weeks Post Injection of PND 90 Mice

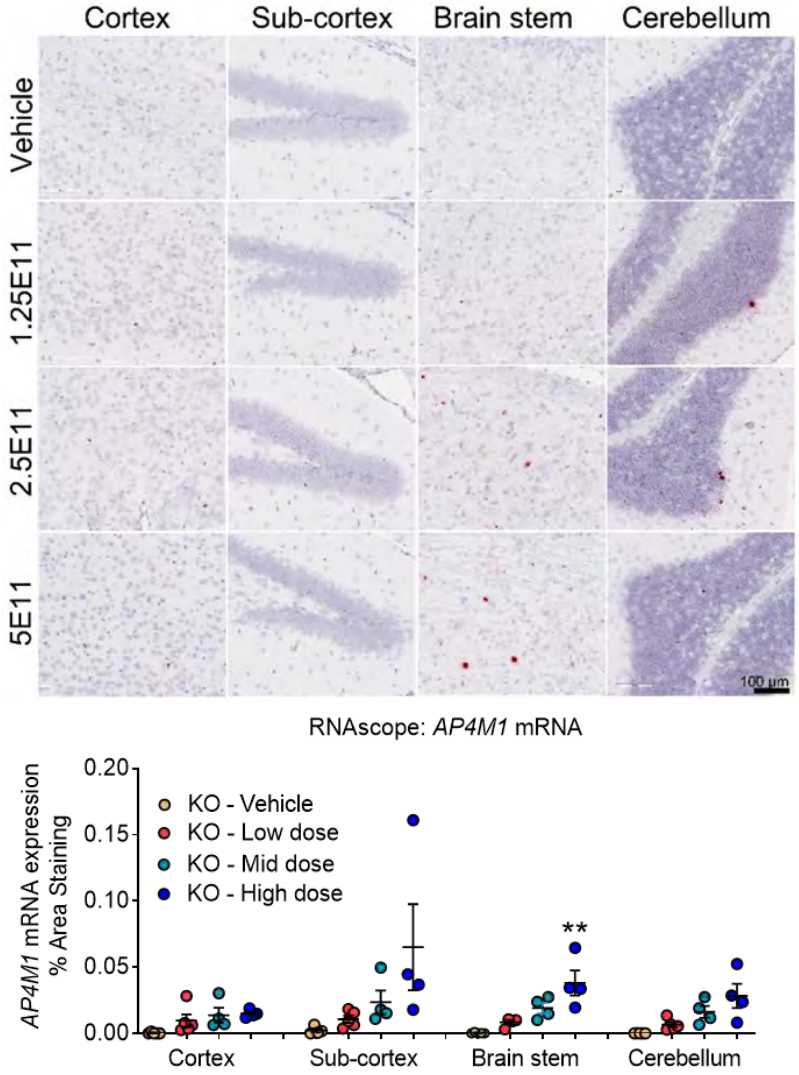
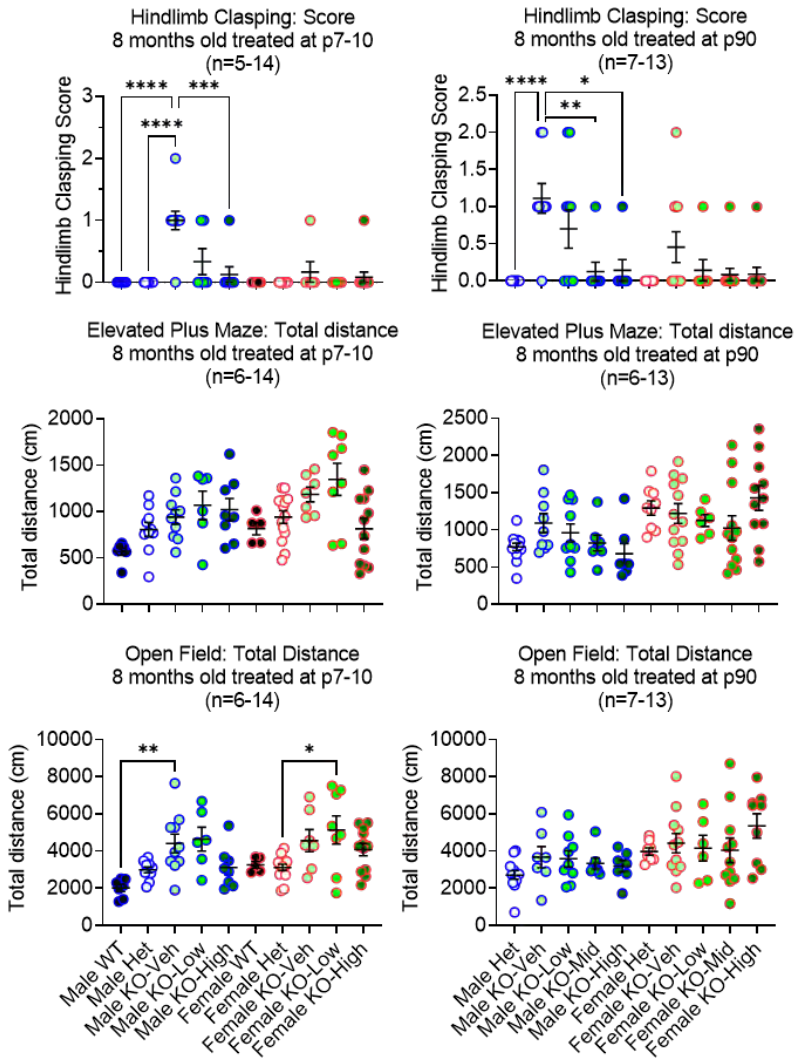


Figure 6 MELPIDA Improved Impaired Behaviors at 8 Months Post Dose



Hindlimb Clasping (upper panels), Elevated Plus Maze (middle panels), and Open Field (lower panels) performance of mice treated at p7-10 (left panels) and p90 (right panels). Each data point represents measurement from an individual animal, with lines representing the mean measurement ± SEM (n=5-14/group). *p<0.05, **p<0.001, ***p<0.001, and ****p<0.0001 compared to KO-Veh.

In summary, the high dose of 5E11 vg provided maximal pharmacological effects on Hindlimb Clasping whether administered early on PND7-10 or later on PND90 in male mice. For the Maze total distance, female mice had a less robust response when dosed at PND90 compared to PND7-10. The mid dose of 2.5E11 vg provided some pharmacological effects on Hindlimb Clasping when administered PND90 in male mice, suggesting MELPIDA may also generate benefits when dosed at the early-symptomatic stage.

4.3 Secondary Pharmacology

There were no studies conducted to evaluate secondary pharmacology for MELPIDA.

4.4 Safety Pharmacology

No stand-alone safety pharmacology studies were conducted with MELPIDA. Clinical evaluations were conducted as part of the GLP toxicity study.

4.5 Pharmacodynamic Drug Interactions

There were no studies conducted to evaluate secondary pharmacology for MELPIDA.

4.6 Pharmacokinetics, Drug Interactions and Product Metabolism in Animals

There were no studies conducted to evaluate adsorption, metabolism, excretion and drug interactions for MELPIDA.

Previous NHP data lends support for the inference that the unbound vector would be mostly (99%) cleared from circulation by 48 hours post-administration, but may persist at very low levels for weeks (Ramsingh et al, 2018). The delivered expression cassette (vector genome) will persist indefinitely in non-dividing cells. The USP promoter will permanently drive low level expression of normal AP4M1 protein, and the AP4M1 protein will remain intracellular in cells that were targeted by the vector.

Biodistribution was evaluated as part of the GLP toxicology study and is described in Section 4.7.

4.7 Biodistribution

Biodistribution of MELPIDA was evaluated in both non-GLP and GLP studies in WT mice, and AP4M1 KO mice, by visualizing mRNA expression by RNAscope. Vector genome biodistribution and AP4M1 mRNA expression was evaluated by qPCR and RT-qPCR (respectively) in GLP studies in both rats and NHPs.

Biodistribution of MELPIDA was evaluated in the 3-month GLP study in WT SD rats dosed intrathecally at the age of 7 weeks with 0 (vehicle), 3.6E11, 1.1E12, or 3.3E12 vg/rat. IT delivery of MELPIDA in rats resulted in a dose-dependent increase of *AP4M1* vector DNA across the CNS (brain and spinal cord) and peripheral organs (lung, dorsal root ganglia, sciatic nerve, heart, liver, thymus, spleen, kidney, and gonads) (Figure 7). The *AP4M1* vector DNA is concentrated closest to the injection site in the spinal cord and detected at lower levels in multiple brain regions. In the peripheral organs, similar high amounts of *AP4M1* DNA persist in heart, liver, and spleen and to a lesser extent in thymus, kidney, and gonads. The pattern of *AP4M1* biodistribution in this study is consistent with that expected from AAV9 and observed in previous studies (Table 6). Human *AP4M1* mRNA expression was detected across all tissues, with a pattern consistent with the vector DNA biodistribution (Figure 8). Collectively, IT delivery of MELPIDA resulted in broad AP4M1 vector DNA and expressed mRNA biodistribution across the rat body, which is considered to portray the normal biodistribution pattern expected for an AAV9 vector in rats with vector biodistribution increasing linearly with dose.

Figure 7 MELPIDA Dose-dependently Increases AP4M1 Tissue Biodistribution

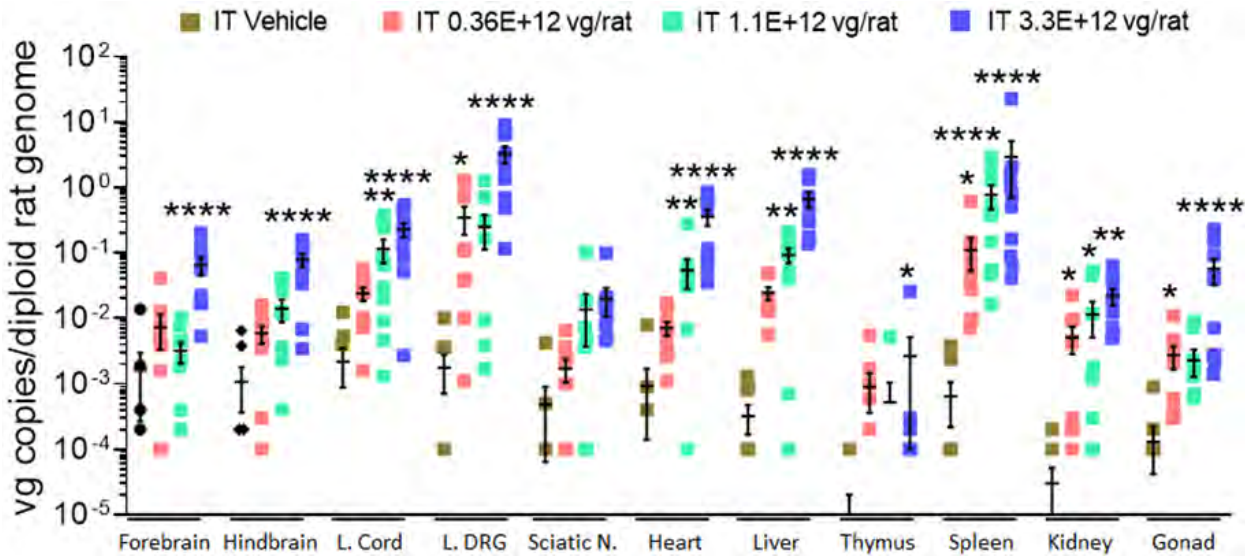
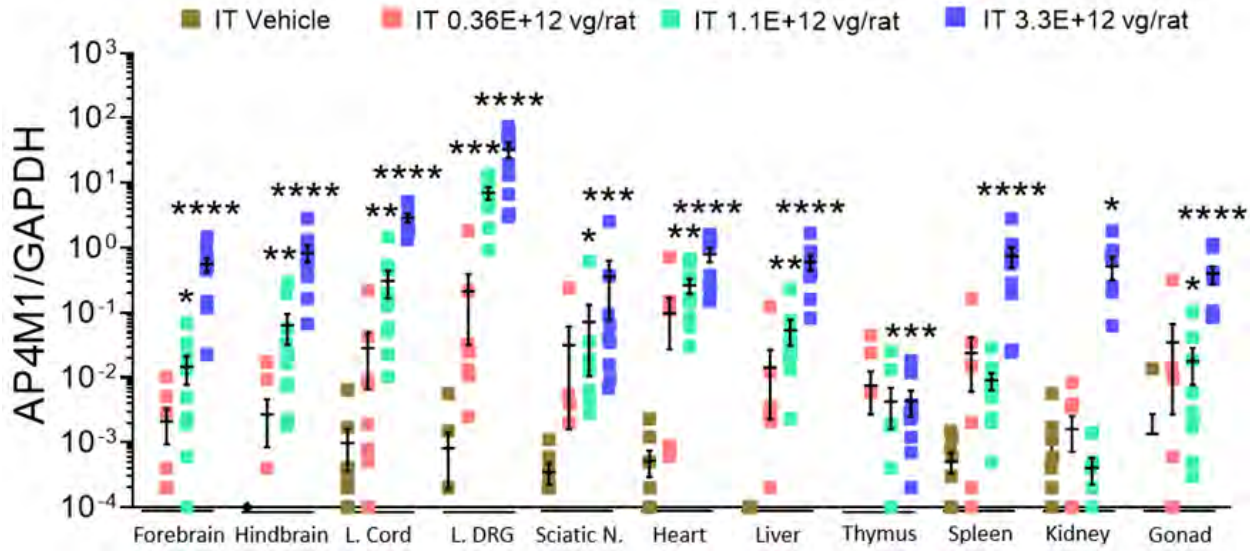
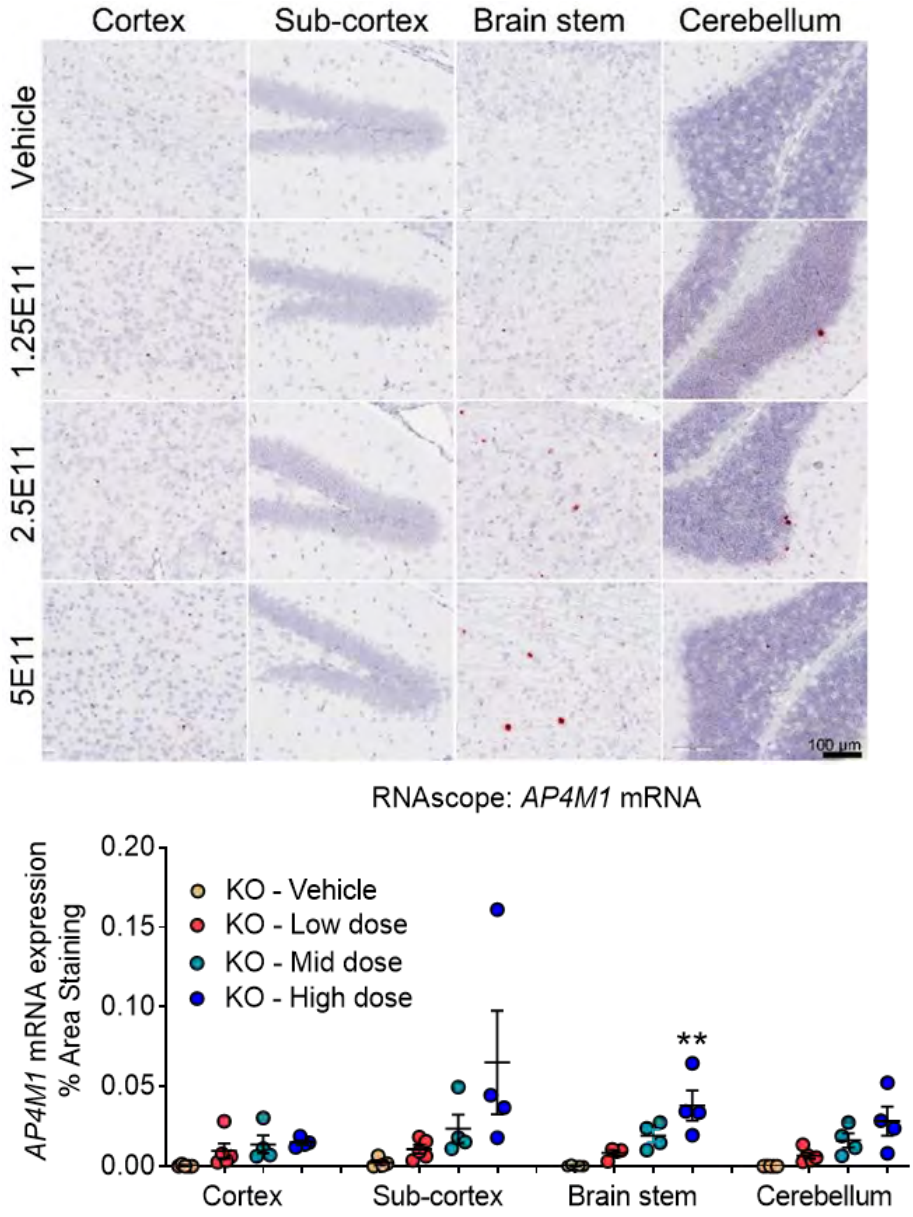


Figure 8 MELPIDA Dose-dependently Increases AP4M1 Tissue Expression



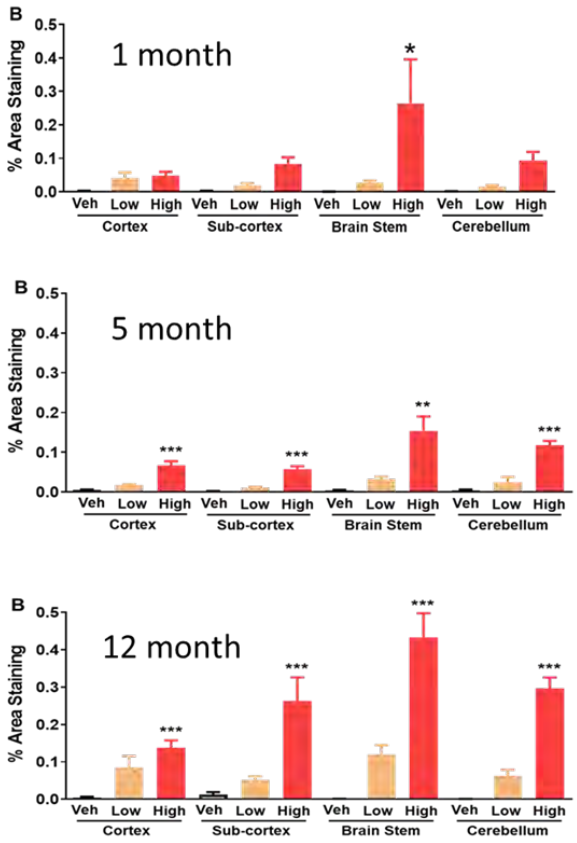
The data from the non-GLP efficacy study in AP4M1 KO mice (study 2020-06) indicated a dose-dependent increase in AP4M1 mRNA expression at 3 weeks post dosing in all areas of the brain (Figure 9), when administered at PND 90.

Figure 9 MELPIDA Increased hAP4M1opt mRNA in All Brain Regions



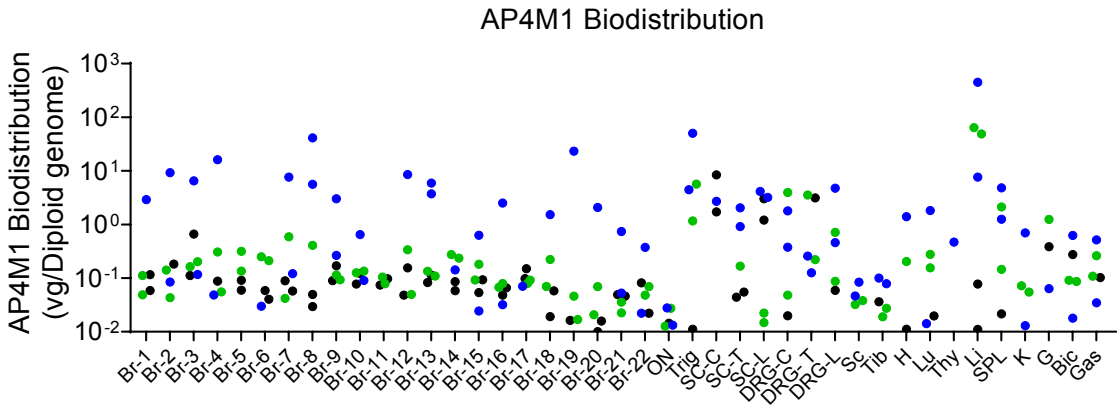
Study #05 in WT (C57BL/6J) mice provided hAP4M1 mRNA biodistribution data at 1, 5 and 12 months post dose at 1.25E11 and 5E11 vg/mouse and showed dose dependent increases in hAP4M1opt mRNA in all brain regions (Figure 10).

Figure 10 Distribution of hAP4M1opt mRNA in C57BL/6J Mice at 1, 5 and 12 Months After Vehicle, 1.25E11 and 5E11 vg Intrathecal Administration



Biodistribution of MELPIDA was evaluated in the 3-month GLP study in Cynomolgus monkeys doses of 0, 8.4E13 or 1.68E14 vg per animal. *AP4M1* vector biodistribution was quantified by qPCR and provided in Figure 11. IT delivery of MELPIDA results in delivery of *AP4M1* vector DNA across the central nervous system and peripheral organs. The *AP4M1* vector DNA is widely detected at high level in multiple brain regions. In the peripheral organs, even higher amounts of *AP4M1* DNA persist in liver and to a lesser extent in other organs tested. Consistent with this *AP4M1* DNA biodistribution data, *AP4M1* transgene expression is also widely detected at high level in multiple CNS and peripheral tissues (Figure 12). Collectively, IT delivery of AAV9/*AP4M1* results in broad *AP4M1* biodistribution and expression across the body of NHPs.

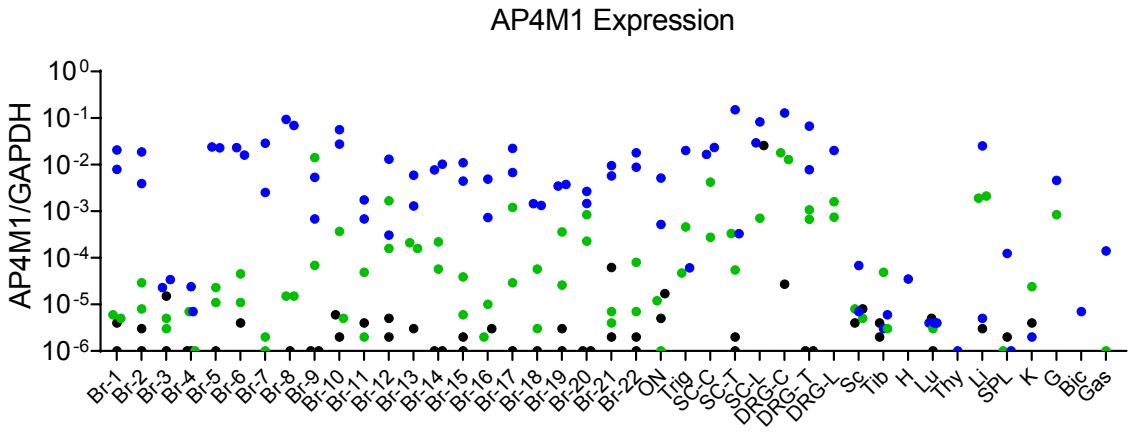
Figure 11 AP4M1 Tissue Biodistribution in NHPs Day 94



0 vg = black dots; 8.4E13 vg = green dots; 1.68E14 vg = blue dots;

Br-1, Br-2 = Frontal Cortex; Br-3, Br-4 = Striatum; Br-5, Br-6 = Parietal Cortex; Br-7, Br-8 = Temporal Cortex; Br-9, Br-10 = Hippocampus; Br-11, Br-12 = Thalamus; Br-13, Br-14 = Pons; Br-15, Br-16 = Midbrain; Br-17, Br-18 = Medulla; Br-19, Br-20 = Cerebellum; Br-21, Br-22 = Occipital Cortex; ON = Optic Nerve; Trig = Trigeminal Ganglion; SC-C = Cervical Spinal Cord; SC-T = Thoracic Spinal Cord; SC-L = Lumbar Spinal Cord; DRG-C = Cervical Dorsal Root Ganglion (DRG); DRG-T = Thoracic DRG; DRG-L = Lumbar DRG; Sc = Sciatic Nerve; Tib = Tibial Nerve; H = Heart; Lu = Lung; Thy = Thymus; Li = Liver; SPL = Spleen; K = Kidney; G = Gonad; Bic = Biceps Femoris; Gas = Gastrocnemius

Figure 12 AP4M1 Tissue Expression in NHPs Day 94



0 vg = black dots; 8.4×1013 vg = green dots; 1.68×1014 vg = blue dots;

Br-1, Br-2 = Frontal Cortex; Br-3, Br-4 = Striatum; Br-5, Br-6 = Parietal Cortex; Br-7, Br-8 = Temporal Cortex; Br-9, Br-10 = Hippocampus; Br-11, Br-12 = Thalamus; Br-13, Br-14 = Pons; Br-15, Br-16 = Midbrain; Br-17, Br-18 = Medulla; Br-19, Br-20 = Cerebellum; Br-21, Br-22 = Occipital Cortex; ON = Optic Nerve; Trig = Trigeminal Ganglion; SC-C = Cervical Spinal Cord; SC-T = Thoracic Spinal Cord; SC-L = Lumbar Spinal Cord; DRG-C = Cervical Dorsal Root Ganglion (DRG); DRG-T = Thoracic DRG; DRG-L = Lumbar DRG; Sc = Sciatic Nerve; Tib = Tibial Nerve; H = Heart; Lu = Lung; Thy = Thymus; Li = Liver; SPL = Spleen; K = Kidney; G = Gonad; Bic = Biceps Femoris; Gas = Gastrocnemius

The biodistribution of AAV9 following intra-CSF administration is well-established, based on numerous published studies across different species. Below is a non-comprehensive summary of published literature indicating wide-spread biodistribution of transgene delivered via AAV9 vectors across the CNS and to peripheral tissues, in small and large animal models including

non-human primates (Table 6). The overall biodistribution pattern of MELPIDA is consistent with these published studies.

Table 6 Summary of Published AAV9-GFP CSF Biodistribution Studies

Species	Reference	Promoter*	Total AAV9 dose (vg)	AAV9 dose** (vg/kg)	Vector dose per mL CSF*** (vg)
Rodent	Bailey et al, 2020	CBh	4.2E11	2.1E12	1.7E13
	Snyder et al, 2011	CBA	2.5E9	1.1E11	1E11
	Gray et al, 2013	CBh	1E10	5E11	4E11
	Meyer et al, 2015	CBA	7.3E11	3.3E13	2.9E13
	Bey et al, 2017	CAG	4E11	1.8E13	1.6E13
Cat	Bucher et al, 2013	CMV	1E12	1E12	2.3E11
Dog	Haurigot et al, 2013	CAG	2E13	2E12	1.6E12
	Hinderer et al, 2018	CBA	1.8E13	1.8E12	1.4E12
Pig	Federici et al, 2012	CBh	8.6E11	4.3E10	4.3E10
	Passini et al, 2014	CBA	3E12	1.5E11	1.5E11
NHP	Gray et al, 2013	CBh	5.5E12	1.45E12	4.6E11
	Samaranch et al, 2012	CBh	1.8E13	7E12	1.5E12
	Samaranch et al, 2013	CMV	1.8E13	7E12	1.5E12
	Passini et al, 2014	CBA	2.5E13	7E12	2.1E12
	Meyer et al, 2015	CBA	2E13	1E13	1.7E12
	Hinderer et al, 2018	CBA	2E13	3.3E12	1.7E12

* Promoters: CBA (chicken β-actin), CBh (chicken β-actin hybrid), CMV (cytomegalovirus), CAG (hybrid of the chicken β-actin promoter and CMV enhancer)

**CSF dosing was achieved via lumbar, intracisternal or intraventricular injection. Doses were normalized to body weights reported in the publications.

***CSF volumes in milliliters were: mouse – 0.035; cat – 4.4; dog – 12.5; pig – 20 and NHP – 12 (Morgan et al, 2004; Sullivan et al, 1979; Pardrigde et al, 2011). References. Note: Doses were per animal or by weight.

4.8 Toxicology

4.8.1 Single Dose

4.8.1.1 Study #05: 12m Non-GLP Toxicity Study in WT C57BL/6J Mice

The non-GLP 12-month Study #05 in WT C57BL/6J mice demonstrated that MELPIDA at doses of 0, 1.25E11 vg and 5E11 vg out to 12 months post administration was safe and well tolerated; no deaths occurred. Biodistribution data are presented in Section 4.7. There were no effects on body weight, hematology or clinical signs; minimal effects on clinical chemistry were noted. For example, 1 male mouse which received 1.25E11 vg/mouse dose had elevation of liver enzymes at 1-month post injection. One female mouse which received vehicle had elevation of liver enzyme at 5-months post injection. Two male mice which received 5E11 vg/mouse had liver and/or kidney toxicity at 12-months post injection (approximately 14 months old). The same 2 male mice plus 1 female mouse had granular appearance in the liver during necropsy, which was then diagnosed as hepatocellular adenomas by microscopic examination of major tissues/organs; up to 51% of male WT mice naturally develop these adenomas as they age (DHHS, 2019). The elevation in liver enzymes seen sporadically across the vehicle, low dose, and high dose groups,

without a clear dose-response, suggests that these may not be directly related to MELPIDA. However, there remains the possibility that high doses of MELPIDA may be associated with sporadic liver toxicity.

4.8.1.2 A 90-day GLP In-vivo Toxicology and Biodistribution Study in Sprague Dawley Rats (CRL-5550008)

This GLP 12-week toxicology and biodistribution study in SD rats with MELPIDA is described in Table 7.

Table 7 Single-dose GLP Toxicology and Biodistribution Study in Sprague Dawley Rats

Group No.	Test Material	Dose Level (vg)	Dose Volume (µL)	Dose Concentration (vg/µL)	No. of Animals					
					Main Study		Recovery Study			
					Day 8 Necropsy[a]		Day 29 Necropsy[b]		Day 91 Necropsy[c]	
					M	F	M	F	M	F
1	Reference Item	0	60	0	5	5	5	5	5	5
2	MELPIDA	0.36E12	20	0.18E11	5	5	5	5	5	5
3	MELPIDA	1.1E12	20	0.55E11	5	5	5	5	5	5
4	MELPIDA	3.3E12	60	0.55E11	5	5	5	5	5	5

M = Males; F = Females
[a]Animals scheduled for Necropsy on Day 8. Biodistribution will also be assessed.
[b]Animals scheduled for Necropsy on Day 29. Biodistribution will also be assessed.
[c]Animals scheduled for Necropsy on Day 91. Biodistribution will also be assessed.

The main study and associated endpoints were run by Charles River Laboratories under GLP. The biodistribution (BD) endpoints were run by the sponsor using qPCR, with a qualified assay that conforms to FDA guidelines but not in adherence with GLP.

The study demonstrated that MELPIDA at doses of 0, 3.6E11, 1.1E12 and 3.3E12 vg was well tolerated. Biodistribution data are provided in Section 4.7. 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 ≥ 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, although the 1.1E12 vg dose was also considered safe (even though adverse) based on the resulting findings of sporadic minimal to mild microscopic findings in the lumbar dorsal root nerve roots.

4.8.1.3 A 94-day GLP In-vivo Non-Human Primate Study (study # CRL-5550014)

A GLP 94-day NHP study is ongoing at doses of 0, 8.4E13 and 1.68E14 vg of MELPIDA to determine the effects on dorsal root ganglia. The study design is described in Table 8.

Table 8 Experimental Design for NHP Study with MELPIDA (CRL-5550014)

Group No.	Test Material	Dose Level (vg)	Dose Volume (mL)	Dose Concentration (vg/mL)	No. of Animals Main Study	
					Males	Females
1	Reference Item	0	1	0	1	1
2	MELPIDA	8.4E13	1.55	5.43E13	-	2
3	MELPIDA	1.68E14	3.10	5.43E13	1	1

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 and no mortality occurred during the course of the study. Biodistribution data are provided in Section 4.7. 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.

4.8.2 Repeated Dose

No repeat dose studies were conducted as this is a single, one-time administration.

4.8.3 Genotoxicity (mutagenicity)

No genotoxicity studies have been conducted with MELPIDA at this time.

4.8.4 Carcinogenicity

No carcinogenicity studies have been conducted with MELPIDA at this time.

4.8.5 Reproductive and Developmental Toxicity

No reproductive and developmental toxicity studies have been conducted with MELPIDA at this time.

4.8.6 Local Tolerance

No specific local tolerance studies have been conducted with MELPIDA at this time. The site of administration was examined as part of the GLP rat toxicity study and showed no evidence of irritation at the puncture site.

4.8.7 Other Toxicity Studies

No additional toxicity studies have been conducted with MELPIDA at this time.

4.8.8 Summary

Overall, the nonclinical data support the proposed mechanism of action, demonstrate efficacy in a disease model, and provide support for moving MELPIDA into the clinic.

A single patient (4y, male) was dosed with MELPIDA in March 2022 under a CTA in Canada at 1E15 vg, with only mild adverse events noted as of 1 June 2022 (see Investigator's Brochure Section 5.3).

Vector distribution of MELPIDA after a single intrathecal administration measured in two studies (rats and NHPs) was consistent with expected AAV9 biodistribution (Table 6).

Tissue transduction and expression of the biologically active gene product in vivo were demonstrated in WT NHPs, WT rats, 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 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.

Based on studies in rodents, there is some debated evidence of increased cancer risk associated with AAV vectors (Bolt et al, 2020; 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 and 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 (Chandler et al, 2015).

The device used in the rodent and NHPs studies differed from the clinical device and the specifics are outlined in Table 9. However, device compatibility was conducted for the pivotal nonclinical studies and the clinical device and indicated no loss of vector after hold-times and temperatures spanning those used in the nonclinical studies and proposed in the clinical study.

Table 9 Devices and Vector Compatibility for MELPIDA

Study #/Description	Devices Used for Administration	Concentrations and Hold Times Evaluated	Vector Recovery Within Acceptable Limits?
2020-06/Ap4m1 KO mouse efficacy study	Hamilton Syringe , 705RN (Hamilton Item # 7637-01) Hamilton needle 30GA RN 6PK 0.5" PT4 (Hamilton Item # 7803-07)	5.17E13 vg/mL No hold time	Yes
CRL-5550008/GLP SD rat, 90 day study	Insulin Syringe (EXEL Item # 26028)	5.17E13 vg/mL No hold time	Yes

Study #/Description	Devices Used for Administration	Concentrations and Hold Times Evaluated	Vector Recovery Within Acceptable Limits?
CRL-5550014/GLP NHP 94-day study	BD Platipak 3ml Syringe (BD Item # 309657) PENCAN Pencil Point Spinal Needle (B. Braunm Medical Inc., Item # 333878)	5.17E13 vg/mL 30 second hold time at room temperature.	Yes
Clinical study	Atraumatic Sprotte Needle (510K# K91126060; M: Pajunk; CN: 321151-31A), 60" Medical IV extension tubing (Baxter 2N3380) Discofix 4-way stopcock (B/Braun Medical Inc, 456020), 20 mL syringe (BD, CN 302830),	5.17E13 vg/mL 4 h hold time at 2 to 8°C	Yes

CN: catalogue number; M; manufacturer. For additional details see **Pharmacy Manual**

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 equivalent dose of 1.8E15 vg. Other toxicities of note included neuronal degeneration in the lumbar dorsal root ganglion at doses of ≥ 1.1E12 vg with no recovery, but the microscopic findings in the lumbar dorsal roots at 1.1E12 vg were sporadic and minimal to mild. The NOAEL was considered to be 3.6E11 vg corresponding to a human equivalent dose of 2.0E14 vg, but if the sporadic and minimal to mild histological findings are deemed acceptable the NOAEL in rats would be defined as 1.1E12 vg (human equivalent dose of 6.0E14 vg). In the NHP GLP study, microscopic findings were noted in the spinal cord with associated decreases in NCV and neurological effects at 1.68E14 vg and the NOAEL was considered to be 8.4E13 vg, corresponding to a 1.1-fold overage based on the proposed human dose of 1E15 vg.

The proposed dose extrapolation is based on age and brain size (Table 10).

Table 10 DoseExtrapolation 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

When considering the CSF volume in the various species, the target human dose of 1E15 vg corresponds to a dose equivalent of 6.6E12 vg per mL of CSF in humans, mice, rats and NHPs. The comparative absolute vg dose across species is 1E15 vg in humans, 8.0E13 vg in NHPs, 1.6E12 vg in rats, and 2.2E11 vg in mice (Table 11). 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

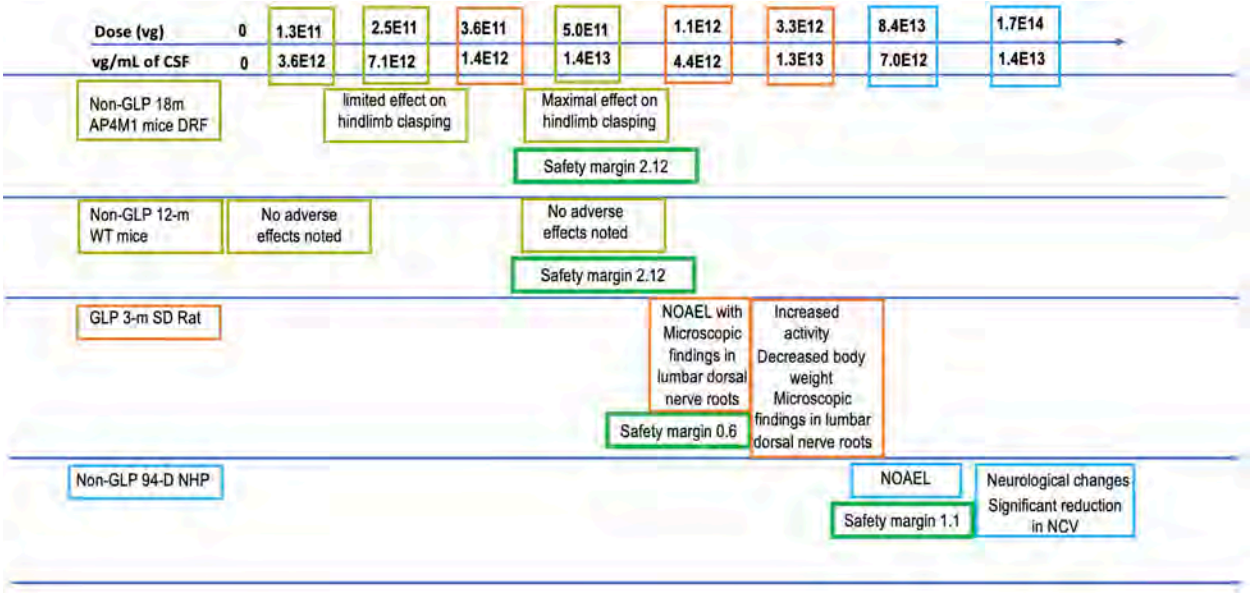
behavioral benefit with a 5E11 vg dose providing a greater benefit. Of note, 1.25E11 vg (equivalent to 5E14 vg in a human) did not provide a clear behavioral benefit, justifying 1E15 vg as the minimally effective human dose.

Table 11 Relationship Between Preclinical Study Doses and Proposed Human Intrathecal Dose

Species	Low Dose (vg)	Low Dose per CSF (vg/mL)	Mid Dose (vg)	Mid Dose per CSF (vg/mL)	Upper Dose (vg)	Upper Dose per CSF (vg/mL)	CSF Volume (mL)	HED of NOAEL (vg per mL CSF)	Intended Human Dose (vg)	Intended Human Dose (vg/mL)	Safety Margin (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*
NHP	8.4E13*	7.0E12*	-	-	1.7E14	1.4E13	12	7E12			1.1x*
Human ≥4 y							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 (Note that the middle dose in rats was also a NOAEL with the exception that sporadic minimal to mild microscopic findings in lumbar dorsal root nerve roots)
**based on upper mouse dose per CSF volume (vg/mL)

Figure 13 Summary of Nonclinical Findings to Support Clinical Dosing



In terms of safety, studies in mice up to 5E11 vg (equivalent to 2.0E15 vg human dose) were tolerated well 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) with associated microscopic changes, but otherwise showed minimal in-life adverse effects, and the lower dose of 8.4E13 vg (equivalent to 1.1E15 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 without an option to redose, a proposed dose of 1E15 vg (for patients 4yrs of age or older or based on Table 10 for patients younger than 4) should maximize benefit with acceptable risks. Preclinical pharmacology data in the SPG50 mouse model did not clearly support a benefit at any lower dose.

The totality of evidence and the seriousness of SPG50 suggests MELPIDA is able to provide benefit to patients at the planned dose of 1E15 vg in 10 mL (for patients 4yrs of age or older or based on Table 10 for patients younger than 4) and that there are sufficient safety data from the nonclinical studies to support this dose.

Additionally, a single patient (4y, male) has already been dosed with MELPIDA in March 2022 under a CTA in Canada at 1E15 vg, with only mild adverse events noted as of 1 June 2022.

5 Effects in Humans

5.1 Introduction

The objective of the clinical program is to evaluate the safety and tolerability of a single dose of MELPIDA at 1E15 vg in 10 mL (for patients 4yrs of age or older or based on Table 10 for patients younger than 4) administered intrathecally to patients affected by SPG50. Given the degenerative nature of the disorder, it is anticipated that younger children affected by SPG50 will have the highest benefit from MELPIDA. Supporting this notion, data in the treated AP4M1 KO mice at 8 months old indicates a stronger benefit in mice treated at 7-10 days old (equivalent to a human toddler) versus those treated at 90 days old (equivalent to a human adolescent).

Safety and tolerability will be measured by the incidence and severity of treatment-related adverse events and serious adverse events (AEs/SAEs). The secondary objective will be to explore efficacy in terms of disease burden assessments to be accomplished via a variety of assessments over an extended period, starting from screening to 24 months, with annual visits for an additional 3 years, for a total of 5 years follow-up post-dosing.

The dose selected for these subjects is justified from nonclinical studies and prior administration to a single subject in March 2022. Subjects will be selected based on a set of inclusion and exclusion criteria. Subjects will go through screening and a standard immunosuppressive strategy will be employed entailing prophylactic administration of different agents (details are included in the clinical protocol). The immune responses will be monitored out to 24 months, which will be based on the subject's CRIM (cross-reactive immune material) status relative to endogenous production of the AP4M1 protein at baseline. It is believed that some patients will not produce any AP4M1 protein at baseline (CRIM-negative), and will follow a more extensive immunomodulatory regimen (prednisone/methyl-prednisolone, sirolimus, and tacrolimus). The subjects will take sirolimus indefinitely based on their measure of tolerance and at the discretion of the PI. The dose levels and the delivery options for the immunosuppressive agents are described in detail in the clinical protocol.

For the intrathecal vector infusion, the subjects will have a spinal needle inserted percutaneously at the lumbar level into the intrathecal space of the spinal column. A volume of CSF approximately equal to the infusion volume is withdrawn from the lumbar thecal sac. Vector solution is infused at a rate of 1 mL per minute for a total of 10 mL for participants 4 years of age and older. Volume adjustments are made for younger participants. The subjects will remain side-lying in Trendelenburg position (head down) at 15-degrees for 1 hour following agent administration, during which time the subjects will be turned every 15 minutes. Dosing volumes will follow the calculated level to adjust to the final vector product concentration. The procedure will be performed in a procedure unit with an anesthesiologist or other qualified physician present to administer sedation as needed. Subjects will stay in the Pediatric Intensive Care Unit (PICU) overnight for intensive monitoring. Subjects monitoring will start from the initial screening/baseline testing (-28 to -7 days), out to 5 years post dose.

Exploring efficacy of MELPIDA constitutes the secondary endpoint of this study. It will involve clinical assessments to determine multiple aspects of SPG50 Disease, the primary endpoints will be reduction and or reversal related to spasticity. This will be achieved by leveraging the Modified Ashworth Scale (MAS) and Tardieu Scale.

Exploratory endpoints will be focused on disease burden as well as cognition and will be based on the standardized measurements below.

- Bayley 4 (Growth Scale Value) (Fine Motor & ADLS)
- Vineland (Inter-personal Domain, Fine Motor Domain, Personal Domain)
- Log Book Seizures
- Log Book Of Falls

In addition, surrogate assessments of efficacy using electroencephalogram (EEG) and brain MRI are expected to shed light on the actual status of gene replacement therapy, focusing on some critical functional aspects of the brain.

5.2 Pharmacokinetics and Product Metabolism in Humans

As a gene therapy product, standard ADME is not applicable. Shedding of the capsid will be evaluated out to 1 year by the collection of saliva, blood, stool and urine samples, which will be banked for future analysis.

A summary of information on the pharmacokinetics of the investigational product(s) should be presented, including the following, if available:

- Pharmacokinetics (including metabolism, as appropriate, and absorption, plasma protein binding, distribution, and elimination).
- Bioavailability of the investigational product (absolute, where possible, and/or relative) using a reference dosage form.
- Population subgroups (e.g., gender, age, and impaired organ function).
- Interactions (e.g., product-product interactions and effects of food).

- Other pharmacokinetic data (e.g., results of population studies performed within clinical trial(s).

5.3 Safety and Efficacy

MELPIDA was administered to a single subject (male, 4y) at 1E15 vg in March 2022 under a CTA in Canada. As of 1 June 2022, almost 3 months post-dose, this subject has reported 4 adverse events; vomiting (mild) and upper respiratory tract infection (mild) were not considered related to product; excessive fatigue and low neutrophils were considered possible and probably related, both were mild and resolved within 2 months following treatment.

A summary of information should be provided about the investigational product's/products' (including metabolites, where appropriate) safety, pharmacodynamics, efficacy, and dose response that were obtained from preceding trials in humans (healthy volunteers and/or patients). The implications of this information should be discussed. In cases where a number of clinical trials have been completed, the use of summaries of safety and efficacy across multiple trials by indications in subgroups may provide a clear presentation of the data. Tabular summaries of adverse drug reactions for all the clinical trials (including those for all the studied indications) would be useful. Important differences in adverse drug reaction patterns/incidences across indications or subgroups should be discussed.

The IB should provide a description of the possible risks and adverse drug reactions to be anticipated on the basis of prior experiences with the product under investigation and with related products. A description should also be provided of the precautions or special monitoring to be done as part of the investigational use of the product(s).

5.4 Marketing Experience

There is no marketing experience with MELPIDA.

6 Summary of Data and Guidance for the Investigator

The specific risks of MELPIDA are currently unknown. MELPIDA is designed to target and express in multiple types of cells in the CNS after a single one-time intrathecal injection. Primary cellular targets proposed for the treatment of SPG50 are neurons and glia throughout the CNS. AP4M1 is a ubiquitous intracellular housekeeping gene, so the targeting of other cell types within the body is also desired.

The introduced cDNA should exist primarily as an episome following transfer of MELPIDA, and express a normal version of functional human AP4M1 protein continuously, which is expected to prevent or slow the onset of SPG50 if treated pre-symptomatically, or slow/halt or reverse the progression of SPG50 if treated after symptom onset.

Nonclinical proof-of-concept has been established with MELPIDA from various studies. These data are described in Section 4 of this document.

These nonclinical results indicate a maximal efficacious dose of 2.5E11 to 5E11 vg in mice, which approximates to 1E15 to 2E15 vg in a human when scaled by CSF volume (Table 11). The suggested clinical dose is 1E15 vg in 10 mL (for patients 4yrs of age or older or based on

Table 10 for patients younger than 4). Overall, the toxicology studies across 3 species provide safety data up to an approximately 2-fold overdose in the equivalent human dose. While the preclinical toxicology studies predict the possibility of histological toxicity at the human 1E15 vg dose, this equivalent dose was not associated with adverse clinical findings in the animals. Considering MELPIDA as a one-time treatment for this degenerative condition for patients without an option to redose, we propose a dose of 1E15 vg in patients (for patients 4yrs of age or older or based on Table 10 for patients younger than 4y) to maximize benefit with acceptable risks. Preclinical pharmacology data in the SPG50 mouse model did not clearly support a benefit at any lower dose.

Overall, the nonclinical data support the proposed mechanism of action, demonstrate preliminary safety and efficacy in disease and non-diseased models, and provides support for this as a safe starting dose in this trial. Additionally, the information acquired from the dosing of a single male subject (4y old) in March 2022, support the safety of the 1E15 vg dose (Section 5.3).

There is no available information on the effects of MELPIDA on the developing human embryo or fetus. As the subjects are still considered a pre-pubescent child, and the disease course is severe, the benefits of treatment outweigh any unforeseeable future risks related to reproduction.

In any gene transfer study, regardless of the vector system employed (e.g., plasmid DNA or viral genomes), integration of the vector genetic material into the host chromosome poses a hypothetical risk for insertional mutagenesis. This event could result in oncogene activation or tumor suppressor gene inactivation and neoplastic transformation.

Recombinant AAV vectors (e.g., MELPIDA) lack the viral Rep protein and are unable to efficiently integrate in the host cell DNA. Numerous studies have clearly established that transduction with AAV vectors is mediated by the formation of episomal forms of the vector genome that persist within the transduced cell nucleus for extended periods of time (Nakai et al, 2001; Schnepp et al, 2003). AAV genome integration is as an extremely rare event and may preferentially occur in transcriptionally active genes (Nakai et al, 2003).

Rare chromosomal integration events of recombinant AAV vectors in vivo have been documented in mouse liver (Nakai et al, 2003; Nakai et al, 1999), and a potential link between AAV vector integration and tumor formation in mouse liver has been reported (Donsante et al, 2007). However, it appears that a particular combination of conditions and more specifically the high cycling rate of neonate hepatocytes and type of promoter could be responsible for tumor development noted by Donsante et al. Recently, it has been demonstrated that the risk for tumor formation appears to be limited to the newborn period in mice treated in newborn period with an AAV vector (Chandler et al, 2015). Based on the evidence to date, it appears that the oncogenic risk in current AAV trials is negligible (Nakai et al, 2003).

There are no approved and/or effective therapies for SPG50. MELPIDA may contribute to addressing this area of unmet medical need. The potential benefits based on the findings from nonclinical studies include histological evidence of AP4M1. Administration in the AP4M1 KO mouse model showed high dose treatment at post-natal day 7 to 10 normalized abnormal hindlimb clasping in male mice and hyperactivity assessed by the Elevated Plus Maze in female mice at the age of 5 and 8 months old. Potential benefits of the study intervention for patients

include preserved motor function and improvement on various other outcome measures designed to evaluate activities of daily living and quality of life. The sponsor believes that MELPIDA offers a favorable risk-benefit for the subjects to be enrolled.

As of September 2021, there have been several AAV9 vector-based products similar to MELPIDA administered by the IT route in the clinic which are presented in Table 12.

Table 12 Other Studies Conducted with AAV9 Products Administered by the IT Route

Name	Description NCT#	Route of Administration
CLN7	scAAV9 Gene therapy for CLN7 NCT04737460	Intrathecal (5E14 and 1E15 vg)
Zolgensma	scAAV9 gene therapy for Spinal Muscular Atrophy (SMA) NCT03381729	Intrathecal (6E13, 1.2E14 and 2.4E14 vg)
vLINCL6	scAAV9 gene therapy for variant late infantile neuronal ceroid lipofuscinosis NCT02725580	Intrathecal
GAN Gene Therapy	scAAV9 Gene Therapy for Giant Axonal Neuropathy NCT02362438	Intrathecal (3.5E13, 1.2E14, 1.8E14, and 3.5E14 vg)
CLN3 Gene Therapy	scAAV9 Gene Therapy for CLN3 Batten Disease NCT03770572	Intrathecal

Based on observations from previously evaluated AAV9-based gene therapies, liver enzyme elevations and immune reactions may occur with MELPIDA. The proposed protocol takes these risks into account and has included safety monitoring for liver- and immune-based adverse reactions, along with corticosteroid prophylaxis starting the day prior to dosing and extending at least 12 weeks post dosing.

6.1 Proposed Indication

The proposed indication is for the treatment of pediatric patients with Spastic Paraplegia Type 50 (SPG50) caused by biallelic pathogenic variation of the AP4M1 gene.

6.2 Administration of MELPIDA

MELPIDA will be administered via a single lumbar intrathecal (IT) injection to broadly treat the central nervous system as well as peripheral organs. Choice of IT dose administration is based on the following rationale. In comparison to the intravenous route, the IT route achieves the maximum possible transduction of the CNS, the organ system severely afflicted by the condition. Further, IT minimizes the exposure of the immune system to scAAV9 vector limiting the virus mostly to the CNS space. The proposed single dose of 1E15 vg (for patients 4yrs of age or older or based on Table 10 for patients younger than 4) is not expected to completely rescue the disease. This is because the AP4M1 protein is widely expressed and MELPIDA will not transduce 100% of cells across the CNS, and there is no known mechanism for non-cell-autonomous benefits of AP4M1 gene expression. Thus, the objective is to use the highest safe dose possible to achieve the maximum number of cells transduced.

If the subjects appear inadequately hydrated in the judgment of the Principal Investigator, bolus(es) of 10 to 20 mL/kg normal saline may be given during the time between subjects check-in and gene transfer. The lowest level of sedation required will be used, and sedation will occur at least 48 hours after Screening/Baseline MRI. The subjects will be continued on their usual diet until eight hours prior to gene transfer, after which they will have no solid food; clear liquids will be allowed up until NPO as per institutional guidelines for sedation, based on age. They will resume their usual diet after they have returned to pre-sedation baseline.

The intrathecal injection will be delivered in a volume of 10 mL, using an FDA approved Pajunk atraumatic Sprotte needle (part number 321151-31A; 510(k)#K911202), which 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 administration of MELPIDA. A volume of CSF approximately equal to the infusion volume will be withdrawn from the lumbar thecal sac and sent for standard evaluation (CSF cell count and differential, glucose, protein, culture and AAV9 neutralizing antibodies). The vector solution will then be infused at a rate of 1 mL per minute. Subject will remain 15-degree Trendelenburg (head down) for 1 hour following vector administration and while still sedated to promote distribution throughout the CSF space.

The subjects will be closely monitored for side effects during the infusion: Heart rate, respiratory rate, pulse oximetry, temperature, and blood pressure will be measured before the infusion, every 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. Neurology checks and continuous telemetry will be taken for at least 24 hours after dosing.

6.3 Contraindications, Warnings and Precautions

MELPIDA should not be administered to subjects with the following medical conditions:

- Inability to participate in the clinical evaluations necessary under the clinical protocol
- 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.

6.4 Adverse Events and Immune Modulation

6.4.1 MELPIDA Related Adverse Events

The most significant acute adverse effect of the experimental treatment that is reasonably anticipated would be a cytotoxic lymphocyte (CTL) response against the vector or against expressed wildtype AP4M1 protein. This could result in increased intracranial pressure, chemical meningitis, encephalitis, end organ damage, and death. Close serial examination and continuous monitoring in the PICU during the period of greatest threat of acute reaction to the investigational treatment, are expected to significantly reduce the risk of a poor outcome should this scenario present itself.

Patients who are presumed to have complete absence of the AP4M1 protein, CRIM-negative/'null' SPG50 patients, are at potentially higher risk of developing an immune response against the transgene. In order to minimize this risk to a foreign protein in the 'null' SPG50 patient, this proposed study will utilize the immune modulation regimen as outlined in Section 6.4.2.

6.4.2 Immunomodulation Protocol for MELPIDA

Crim –Negative Patients

In previous gene therapy studies, antigen specific T-cell responses to the AAV9 vector have been reported (Verdera et al, 2020). 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). This is summarized in the clinical protocol and consists of:

- 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: 4-8 ng/mL)
- 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)
- 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: 4-8 ng/mL)
  - Tacrolimus at 0.1 mg/kg/day divided into twice daily dosing (goal level: 4-8 ng/mL)
- 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: 4-8 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
- 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

Crim +Positive Patients

In previous gene therapy studies, antigen specific T-cell responses to the AAV9 vector have been reported (Verdera et al, 2020). 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). This is summarized in the clinical protocol and consists of:

- 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: 4-8 ng/mL)

- 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)
- 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: 4-8 ng/mL)
- 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 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.
- 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

6.4.3 Adverse Events Related to Procedure(s)

- Risks Related to Lumbar Puncture

Lumbar puncture (LP) is a routine procedure performed in childhood that is safe and well tolerated. Local site discomfort (from needle insertion) may occur, and can be prevented with EMLA cream or equivalent prior to injection. The most common side effect from LP is post procedure headache (for reference, approximately 7% of patients with SMA administered SPINRAZA via LP experience headache). This is usually transient and responds well to NSAIDs/acetaminophen. There is an extremely small risk of infection with LP, and a small risk of spinal fluid leak. The primary symptoms of leak are headache and backpain. It may persist for 1 to 3 days. Persistent symptoms may require spinal patching accomplished with a brief interventional procedure. There is a very small risk of spinal cord injury due to the LP needle contacting the spinal cord or emerging nerve roots.

- Risks Related to MRI and Electroencephalogram (EEG) Assessments

EEG is a well-tolerated procedure with minimal risk. There can be local irritation from the EEG leads, and the cleaning solution used to apply them.

MRI itself is a procedure with minimal risk. Gadolinium dye can be associated rarely with allergic reaction, requiring appropriate intervention (for example, anti-histamine therapy). If sedation is required for MRI, it can be associated with respiratory depression.

6.4.4 Concomitant Medication Adverse Events

Risks associated with Immunosuppression

- Methylprednisolone

Possible side effects from a single dose as used in this protocol include changes in mood such as depression; agitation; excitement; sleeplessness; Increased blood sugar levels, especially if the patient also has diabetes; and fluid retention.

- Prednisone

Use of prednisone in this patient population has not been studied and specific effects of this drug in SPG50 are unknown. Similar patient populations with limited mobility who require prednisone treatment tolerate similar doses of prednisone relatively well (0.75 mg /kg /day in Duchenne muscular dystrophy).

In addition, based on experience with use of prednisone in children and adults for different indications, we suspect that therapy at the proposed doses for the indicated amount of time are less likely to lead to long-term sequelae associated with prolonged glucocorticoid use such as adrenal insufficiency, cataracts, cushingnoid changes, or osteoporosis to name a few.

However, several risks exist and will be discussed with the patients and reflected in the consent forms. The following are thought to be more likely to occur given the relatively short-term use of steroids proposed above.

- Insomnia: this is a well-known effect of prednisone therapy in adults and children. We will try to minimize this by administering the medication in the morning.
- Neuropsychiatric symptoms: Increased energy, mania, or suicidal ideation have been rarely reported. The patients will be monitored for such side effects and their caregivers will be specifically educated about this risk at the time of consent and/or at the time of hospital discharge after the first week of hospitalization.
- Glucose intolerance: Elevated blood glucose levels will be monitored by serial blood testing.
- Blood pressure: Elevated blood pressures will be monitored by vital signs measurements at each clinic visit.
- Increased appetite and weight gain: This potential side effect will be monitored by vital sign and weight measurements though we anticipate this to be less of an issue given the relatively short period of prednisone therapy at the proposed doses.

- Avascular necrosis of the bones: This is an idiosyncratic adverse event associated with steroid use in rare patients that may not be dose dependent. We will screen the patients at follow up visits for joint pain and have a low threshold for x ray evaluation should they develop persistent hip or knee pain.
- Infections: Given the relative level of immunosuppression, the patients will be at higher risk of developing infections. SPG50 is not known to further reduce the patients' immunocompetency; however, we will monitor for any signs of infection especially during the period of steroid therapy and its taper both based on clinical symptoms and blood testing.

The patient will also be monitored for symptoms and signs of adrenal insufficiency during investigator follow up visits especially if any signs of superimposed infections are present. In the unlikely event that adrenal insufficiency develops, the patients will be treated with stress dose steroids per standards of care with consultation with endocrinology.

It may be difficult to conclusively ascertain whether a given side effects may be related to the prednisone therapy vs the vector administration in the first few weeks. However, prednisone has a known expected group of adverse events that can help in distinguishing between these to some extent.

- Tacrolimus

Tacrolimus is an immunosuppressive calcineurin inhibitor that interferes with several calcium-dependent processes in immune cells. The most common adverse reactions to tacrolimus include abnormal renal function, hypertension, diabetes mellitus, fever, tremor, paresthesias, hyperglycemia, cytopenia (leukopenia, anemia), abdominal complaints, electrolyte abnormalities (hyperkalemia, hypomagnesemia) and hyperlipemia. More severe neurotoxicity is described at higher whole blood trough concentrations and therapeutic drug monitoring is required during the trial. As with other immune suppressive agents, chronic use is associated with increased risk of infection and malignancy, in particular, lymphoma and skin cancer. Tacrolimus should be used with supervision by a physician with experience in immunosuppressive therapy. Tacrolimus may induce changes in blood concentrations of concomitant medications and may also be affected by their use. Potential drug-drug interactions with tacrolimus must be considered during therapy (Manns et al, 2010).

Participants will be monitored closely and serum concentration of tacrolimus will be collected and dose adjusted if either greater than or below the target therapeutic range of 5-10 ng/mL.

Acute tacrolimus nephrotoxicity is usually manifested by a moderate decline in renal excretory function, which is readily reversible by a decrease in drug dosage. Although some degree of transient renal dysfunction may occur in patients with therapeutic levels of tacrolimus, significant renal toxicity is associated with elevated trough or steady state levels. In addition to an increase in BUN and creatinine, hyperkalemic hyperchloremic acidosis, low fractional excretion of sodium and the onset of hypertension with hypomagnesemia are seen with tacrolimus nephrotoxicity. Hypertension occurs in up to 60% of patients. Hypomagnesemia can be associated with neurologic symptoms, including seizures, cerebellar ataxia and depression.

Dose-related hepatotoxicity, manifested by elevation of serum transaminases and bilirubin, has been reported.

- Rapamycin

Use of sirolimus is associated with a wide variety of well-described side effects (Wyeth Pharmaceuticals, Inc., 2010; Pfizer Europe MA EEIG, 2011). Warnings and precautions for sirolimus use include but are not limited to increased susceptibility to infection and the possible development of lymphoma, hypersensitivity reactions, angioedema, fluid accumulation and impairment of wound healing, hyperlipidemia, proteinuria, latent viral infections, and embryo-fetal toxicity. Exposure to sunlight and ultraviolet (UV) light should be limited by wearing protective clothing and using a sunscreen with a high protection factor. Most common adverse reactions (≥30% of participants) associated with sirolimus use for prophylaxis of organ rejection in clinical studies are peripheral edema, hypertriglyceridemia, hypertension, hypercholesterolemia, creatinine increased, constipation, abdominal pain, diarrhea, headache, fever, urinary tract infection, infection of the ear, nose, throat or sinus, anemia, nausea, arthralgia, pain and thrombocytopenia.

Participants will be monitored closely to minimize the risk of side effects related to use of sirolimus in the proposed study. Serum concentration of sirolimus will be collected and dose adjusted if either greater than or below the target therapeutic range of 4 to 8 ng/ml.

The anticipated toxicities of sirolimus in this trial are those related to its immune-suppressive properties, such as an increased likelihood of infection, and mucosal (including mouth, gastric, small bowel, or large bowel) ulcers, which may bleed. Other possible toxicities are listed here and include those reported with > 3% and <20% incidence in patients in any Sirolimus treatment group in the two controlled clinical trials for the prevention of acute organ graft rejection:

- Body as a Whole: abdomen enlarged, abscess, ascites, cellulitis, chills, face edema, flu syndrome, generalized edema, hernia, Herpes zoster infection, lymphocele, malaise, pelvic pain, peritonitis, sepsis;
- Cardiovascular System: atrial fibrillation, congestive heart failure, hemorrhage, hypervolemia, hypotension, palpitation, peripheral vascular disorder, postural hypotension, syncope, tachycardia, thrombophlebitis, thrombosis, vasodilatation;
- Digestive System: anorexia, dysphagia, eructation, esophagitis, flatulence, gastritis, gastroenteritis, gingivitis, gum hyperplasia, ileus, liver function tests abnormal, mouth ulceration, oral moniliasis, stomatitis;
- Endocrine System: Cushing's syndrome, diabetes mellitus, glycosuria, hypercholesterolemia, hyperlipidemia;
- Hematologic and Lymphatic System: ecchymosis, leukocytosis, lymphadenopathy, polycythemia, thrombotic thrombocytopenic purpura / hemolytic-uremic syndrome;
- Metabolic and Nutritional: acidosis, alkaline phosphatase increased, BUN increased, creatine phosphokinase increased, dehydration, healing abnormal, hypercalcemia, hyperglycemia, hyperphosphatemia, hypocalcemia, hypoglycemia, hypomagnesemia, hyponatremia, lactic dehydrogenase increased, SGOT increased, SGPT increased, weight loss;

- Musculoskeletal System: arthrosis, bone necrosis, leg cramps, myalgia, osteoporosis, tetany:
- Nervous System: anxiety, confusion, depression, dizziness, emotional lability, hypertonia, hypesthesia, hypotonia, insomnia, neuropathy, paresthesia, somnolence;
- Respiratory System: dyspnea, changes in PFTs, asthma, atelectasis, bronchitis, cough increased, epistaxis, hypoxia, lung edema, pleural effusion, pneumonia, rhinitis, sinusitis, diffuse alveolar hemorrhage;
- Skin and Appendages: fungal dermatitis, hirsutism, pruritus, skin hypertrophy, skin ulcer, sweating;
- Special Senses: abnormal vision, cataract, conjunctivitis, deafness, ear pain, otitis media, tinnitus;
- Urogenital System: albuminuria, bladder pain, dysuria, hematuria, hydronephrosis, impotence, kidney pain, kidney tubular necrosis, nocturia, oliguria, pyelonephritis, pyuria, scrotal edema, testis disorder, toxic nephropathy, urinary frequency, urinary incontinence, urinary retention.

Less frequently occurring adverse events included: mycobacterial infections, Epstein-Barr virus infections, BK virus-associated nephropathy, skin cancer, lymphoma, pericardial effusion, posterior reversible encephalopathy syndrome (PRES), and pancreatitis.

- Risks Related to Other Concurrent Drug Administration

Subjects may have concurrent medications that will require dose adjustment, or will necessitate adjustment of rapamycin and/or tacrolimus dosing. These may include anti-seizure medicine, and medicines for spasticity (such as baclofen). Consultation with a study site pharmacologist will occur prior to initiation of immunosuppression, in order to adjust medication dosing as necessary.

6.5 Overdose

MELPIDA will be prepared by a pharmacist, and the volume calculations will be checked by another pharmacist unconnected to the study, therefore the risk of overdose is low. For these subjects, any dose of MELPIDA greater than the assigned dose will be considered an overdose.

The sponsor does not recommend specific treatment for an overdose. In the event of an overdose, the investigator should:

- Contact the Medical Monitor immediately.
- Closely monitor the participant for any AE/SAE and laboratory abnormalities (at least 12 weeks).
- Document the quantity of the excess dose in the Case Report form (CRF).

6.6 Administration to Pregnant or Breastfeeding Patients

Not applicable.

6.7 Administration to patients able to reproduce

Not applicable.

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