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2.4 Nonclinical Overview

2.4.1. Overview of the Nonclinical Testing Strategy

[Note to Reviewers: For this submission, only module 2.4 will be provided and therefore additional details on the study design of the nonclinical studies is added here in lieu of not providing module 2.6 information].

CureSPG50 is a non-profit entity developing MELPIDA (a self-complementary [sc] recombinant adeno-associated virus [serotype 9] encoding a codon-optimized human AP4M1 transgene [hAP4M1opt]) gene therapy administered as a single one-time intrathecal infusion for targeted expression of AP4M1, an adaptor protein complex 4 protein, deficient in patients with Spastic Paraplegia Disease 50 (SPG50). The final drug product is a clear and sterile aqueous solution stored in AT-1 cryovials. The final fill material contains the viral vector MELPIDA (AAV9/AP4M1) in a phosphate-buffered sterile saline (PBS) solution formulated with 5% D-sorbitol and 0.001% pluronic F-68 stored at ≤60°C. The target concentration of MELPIDA is 1E14 vg/mL with a volume of 1 mL per vial.

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).

The clinical protocol submitted with this original IND is a single dose intrathecal administration for 2 patients at a dose of 1E15 vg in a volume of 10 mL based on CSF volume (for patients 4y of age or older or based on Table 6 for patients younger than 4y) with a final concentration of approximately 7E12 vg per mL of CSF to establish the safety, tolerability and preliminary efficacy of MELPIDA. Measurable clinical benefits of improving spasticity and muscle tone are expected by 6 months and beyond.

The scAAV9 vector that comprises MELPIDA encodes human AP4M1. Thus, the primary mechanism of action of MELPIDA is the expression of AP4M1 in multiple types of cells including neurons and glia in the CNS cells (both brain and spinal cord). The resulting biological activity of MELPIDA, therefore, is essentially that of the AP4M1 gene product. AP4M1 expression in the CNS 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.

The nonclinical program for MELPIDA included an in vitro proof of concept (POC) study and then focused on the pharmacology and safety of the product after intrathecal administration to mice, rats, and non-human primates (NHPs). The overall objectives of the nonclinical program were to:

- Demonstrate POC in SPG50 patient-derived fibroblasts
- Demonstrate the mechanism of action through detection of hAP4M1 messenger RNA in all brain regions from animals receiving an intrathecal dose of MELPIDA

- Demonstrate the pharmacology of MELPIDA and the biological effects of the gene product in a mouse model of SPG50 (Ap4m1 knock out [KO])
- Define the dose levels that achieve efficacy in mice
- Assess the biodistribution of MELPIDA vector DNA after a single administration
- Evaluate the safety of MELPIDA at several doses in wild-type (WT) mice, rats, and NHPs

One in vitro and 4 in vivo nonclinical studies in mice, rats and NHPs were performed to meet the objectives listed above (Table 1). Since the product is intended to produce a functional protein in patients with a dysfunctional protein caused by a genetic mutation, the nonclinical studies used genetically engineered mice with mutations that model SPG50 disease (Ap4m1 KO) and WT mice, WT rats, and NHPs.

Table 1 Nonclinical Studies for IND Submission

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 Lots used for the nonclinical in vivo studies are described in **Table 3.2.P.5.4-1 of Module 3** and are summarized in Table 2 for ease. The lot used for the in vitro study (#01) was an AAV2 product from lot 8829 as AAV9 will not infect fibroblasts in vitro.

Table 2 Lots Used for the Nonclinical In vivo Studies

Study	Lots Used	Grade/Size/Manufacturer	Titer*
2020-06	LAV112	Research/10L/UNC VC	9.9E13 vg/mL (1.03E14 vg/mL UNC VC)
#05	LAV112	Research/10L/UNC VC	9.9E13 vg/mL (1.03E14 vg/mL UNC VC)
CRL-5550008	T-GEMINIS-033	GMP/50L/VVC	5.43E13 vg/mL
CRL-5550014	T-GEMINIS-033	GMP/50L/VVC	5.43E13 vg/mL
Clinical			
CT-MEL-01	G-GEMINIS-029	GMP/250L/VVC	1.0E14 vg/mL
*Titer = ddPCR reported from VVC; VVC, Viralgen vector core; UNC VC, University of North Carolina Vector Core			

In brief, the in vitro study (#01/SPG50_NC_01) indicated that scAAV2/AP4M1 partially restored AP-4 levels and corrected ATG9A localization in fibroblasts from patients with SPG50. For in vivo studies, WT, heterozygous and homozygous Ap4m1 KO mice, which exhibit a mild phenotype of SPG50, were used to establish the efficacy of MELPIDA (study 2020-06). This is the first time this Ap4m1 KO mouse has been developed and studied. It appears that phenotypically the homozygous KO mice have abnormal hindlimb clasping and altered motility in a slowly progressive fashion, which corresponds to patients with mild to moderate SPG50. The homozygous KO mice have a comparable lifespan without any other abnormal clinical signs compared to WT or heterozygous littermates, up to 8 months of age. A efficacy study to test MELPIDA in the mouse model is ongoing with a 21-month endpoint (currently at 8-month timepoint) where these mice were dosed intrathecally at post-natal day (PND) 7 to 10 or PND 90 with no treatment (WT+/+ [N=12] and heterozygous+/- [N=45]), vehicle (-/-, N=41), low (1.25E11 vg, N=33), mid (2.5E11 vg, N=22) or high (5E11 vg, N=40) doses of MELPIDA and assessed for potential phenotypic rescue. Body weights and clinical signs and assessments are monitored over the entire span of the study. Some mice were euthanized 3 weeks post treatment to evaluate mRNA expression, clinical chemistry and immune responses. Behavioral testing is assessed at 3, 5, 8, 12 and 18 months post dosing of MELPIDA. At the conclusion of the study, serum and tissues will be collected for clinical chemistry and histopathology.

For the evaluation of safety, two in vivo studies were conducted in rodents, (i) Study #05 was a 12-month non-GLP toxicology study in WT C57BL/6J mice dosed intrathecally at the age of 7 weeks with vehicle, low (1.25E11 vg), or high (5E11 vg) doses of MELPIDA, and (ii) Study CRL-5550008 was an in vivo 3-month GLP toxicology and biodistribution study in WT Sprague Dawley (SD) rats dosed intrathecally at the age of 7 weeks with 0 (vehicle), 0.36E12, 1.1E12, or 3.3E12 vg/rat of MELPIDA. In addition, an in vivo 3-month GLP toxicology and biodistribution study was conducted in WT Cynomolgus Monkeys (Study CRL-5550014) dosed intrathecally at the age of 2 to 4 years with 0 (vehicle), 8.4E13, or 1.68E14 vg/monkey of MELPIDA. The NHP study used a PENCAN® Pencil Point Spinal Needle (B. Braun Medical Inc., Item # 333878) for administration whereas the clinic will use a PAJUNK® SPROTTE® lumbar needle with introducer 30mm (Item # 321151-31A; 21G x 90mm with wings; 510(k) #K911202). Both are approved devices for the intended route of administration, with the PENCAN being 25G and 25 mm in length compared to 21G and 90 mm for the SPROTTE. The difference in gauge and

length is not expected to have any impact on the safety profile when considering the nonclinical safety supporting the clinical intended route of administration. The compatibility of MELPIDA was assessed for all injection devices used across the studies in mice, rats, and NHPs, and found compatible in all cases with minimal loss of MELPIDA, similar to that seen with the clinical device (see **Device Compatibility Results in Module 3.2.P.2.6**).

The conclusions from these studies are discussed below in the pharmacology, biodistribution, and toxicology summary sections. An integrated discussion on how the nonclinical data supports the overall safety and proposed clinical dosing plan is also provided in Section 2.4.5.

2.4.2. Pharmacology

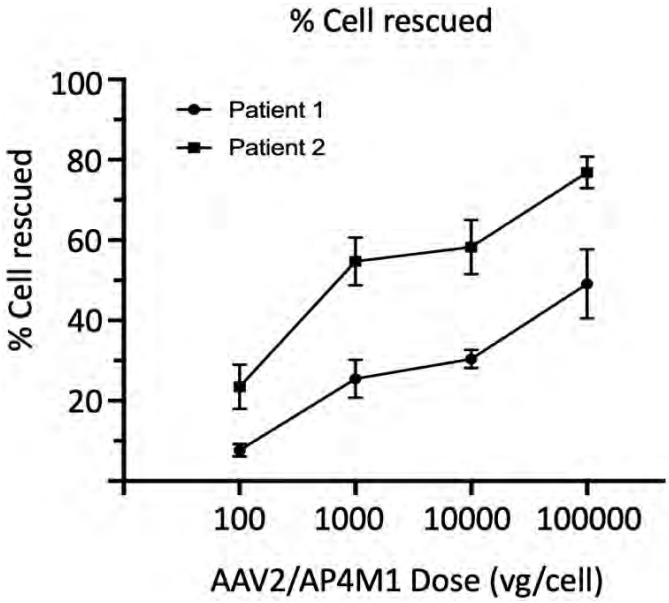
2.4.2.1. Study #01; Report SPG50_NC_1

An in vitro study (#01, Report SPG50_NC_01) 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 scAAV2/*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 1).

Figure 1 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.

2.4.2.2. Study 2020-06; Ongoing Study of MELPIDA in the Ap4m1 Mouse

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 3. 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 21 months old will be collected for serum toxicity panel and histopathological assessment, respectively.

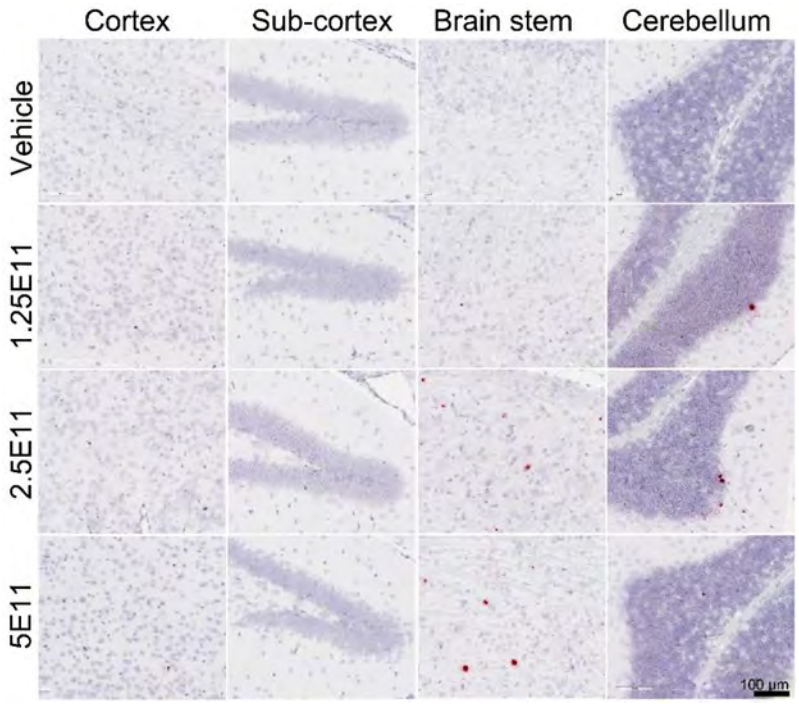
Two reports will be issued for this study, an interim from in-life for the initial 8 months which is included in this submission, and a final report with in-life and histopathology assessments after the 21 month assessments.

Table 3 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 2), induces minimal immune responses, causes minimal toxicity, generates minimal effects on body weight, creates minimal effects on survival, and improves abnormal behaviors (Figure 3) (see **Investigator's Brochure** for additional information, or 8-month interim **Study Report 2020-06 in Module 4**).

Figure 2 MELPIDA Increased hAP4M1opt mRNA in All Brain Regions 3 Weeks Post Injection of PND 90 Mice



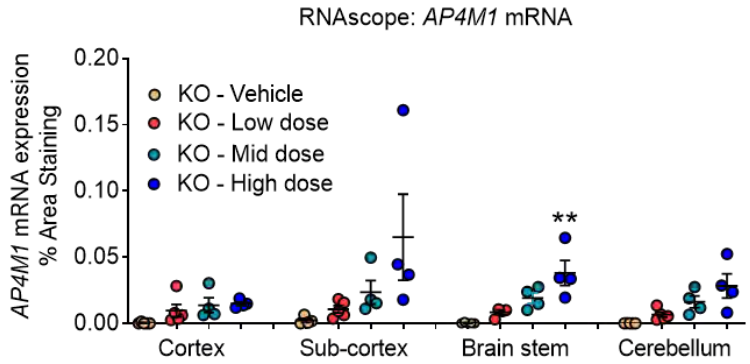
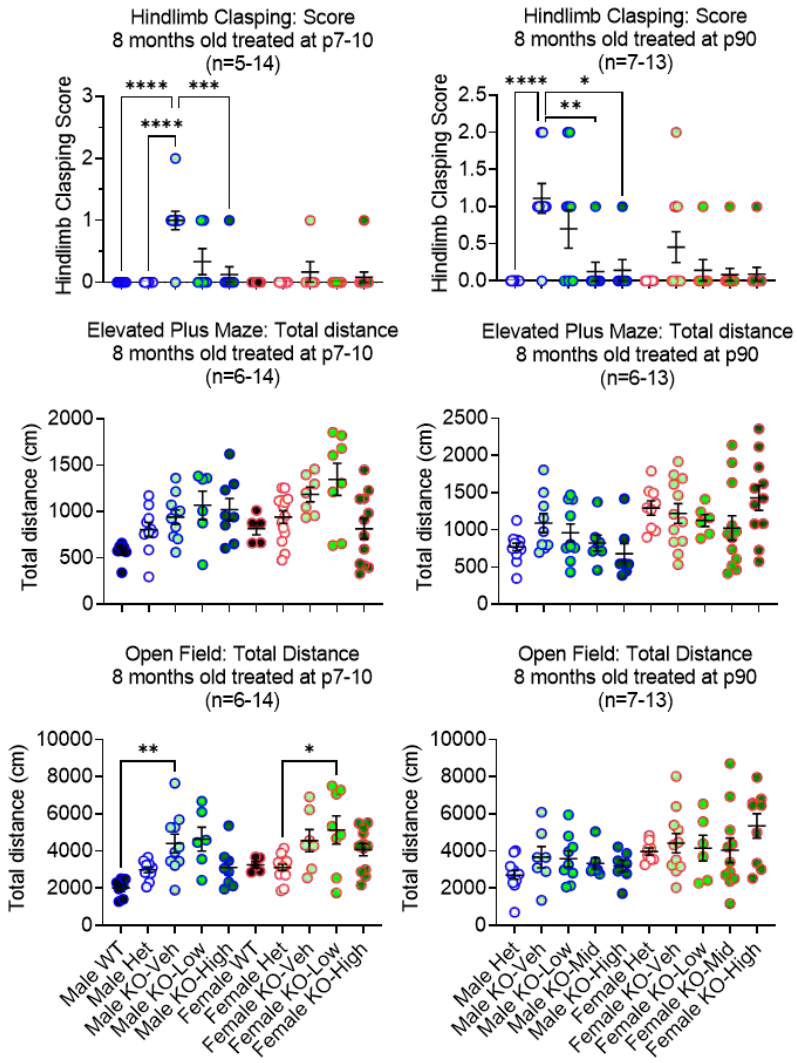


Figure 3 MELPIDA Improved Impaired Behaviors at 8 Months Post Dose



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.

2.4.3. 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 (**Study CRL-5550008, Module 4**) 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 4). 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 4). Human *AP4M1* mRNA expression was detected across all tissues, with a pattern consistent with the vector DNA biodistribution (Figure 5). 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 4 MELPIDA Dose-dependently Increases AP4M1 Tissue Biodistribution

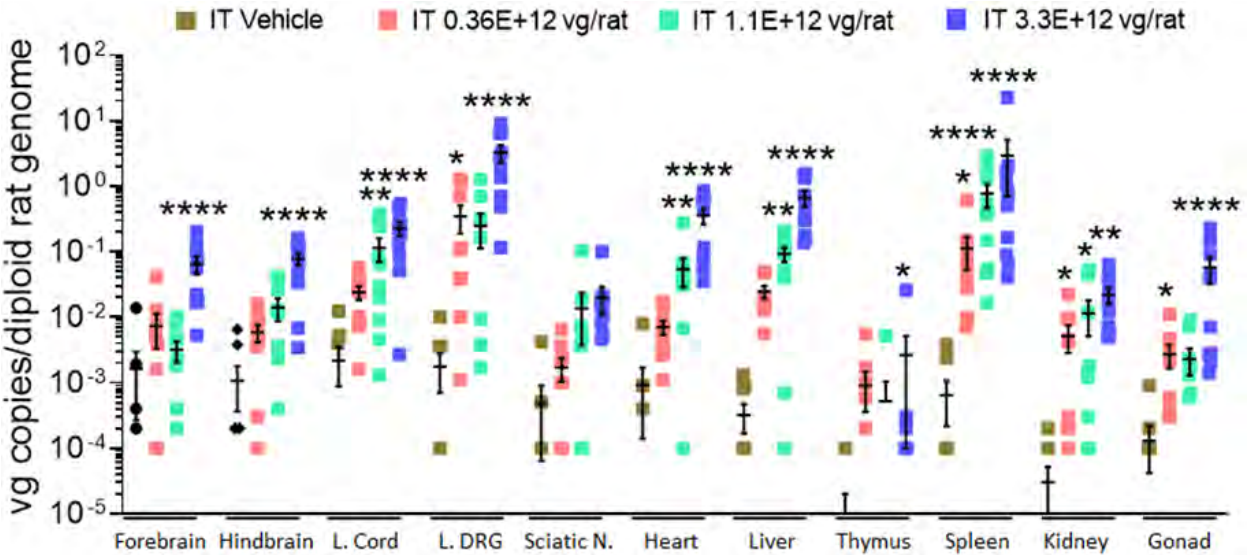
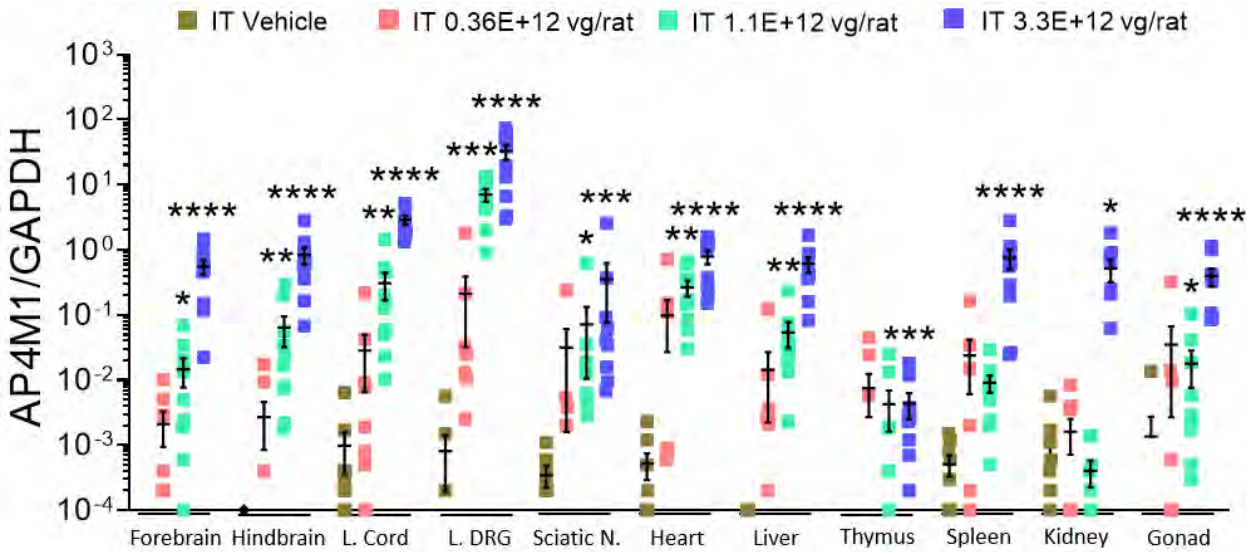
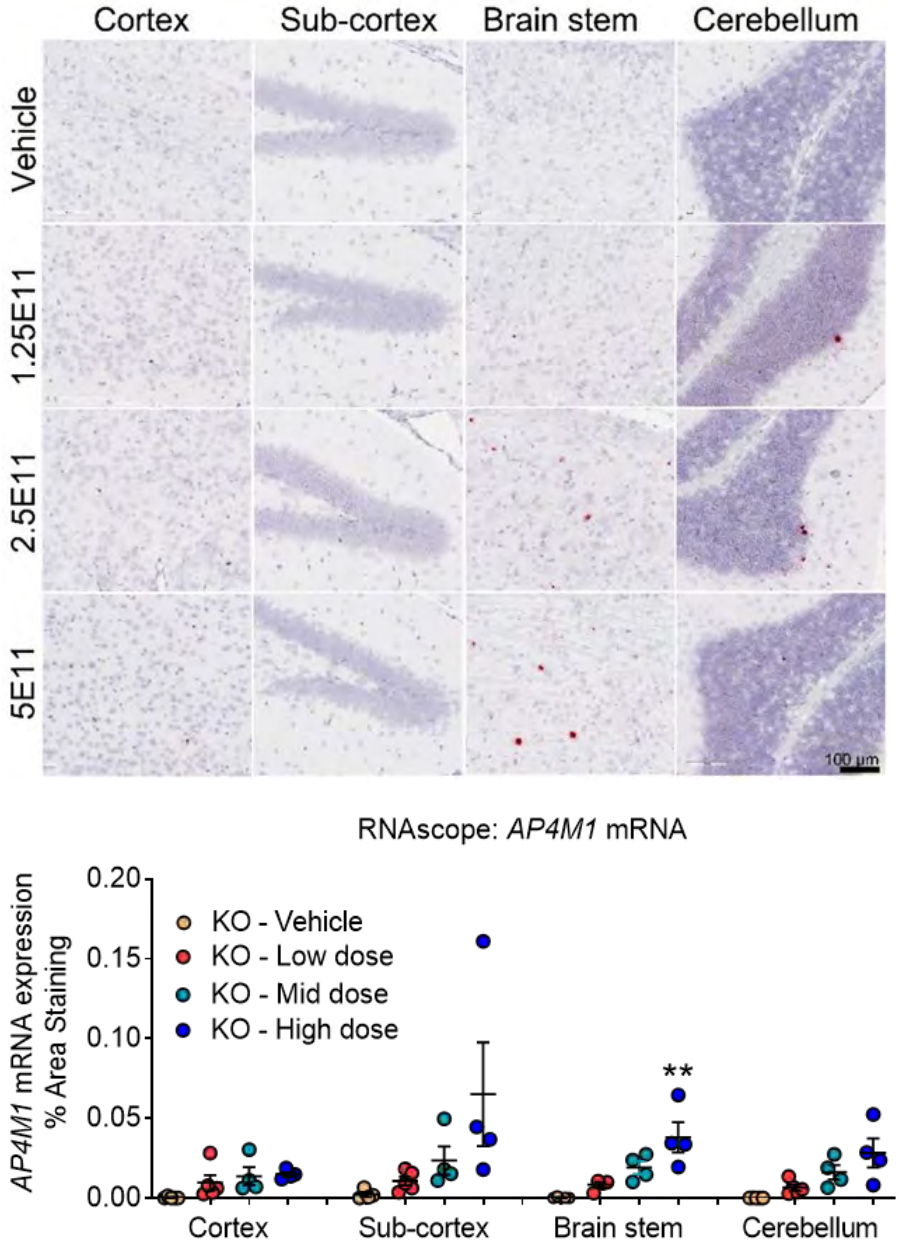


Figure 5 MELPIDA Dose-dependently Increases AP4M1 Tissue Expression



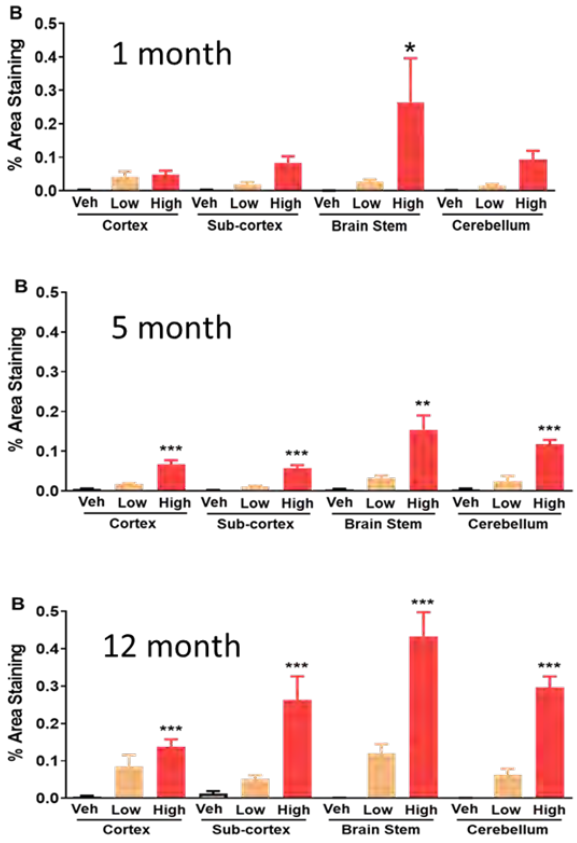
The data from the non-GLP efficacy study in AP4M1 KO mice (Study 2020-06) indicated a dose-dependent increase in AP4M1 mRNA expression at 3 weeks post dosing in all areas of the brain (Figure 6), when administered at PND 90.

Figure 6 MELPIDA Increased hAP4M1opt mRNA in All Brain Regions



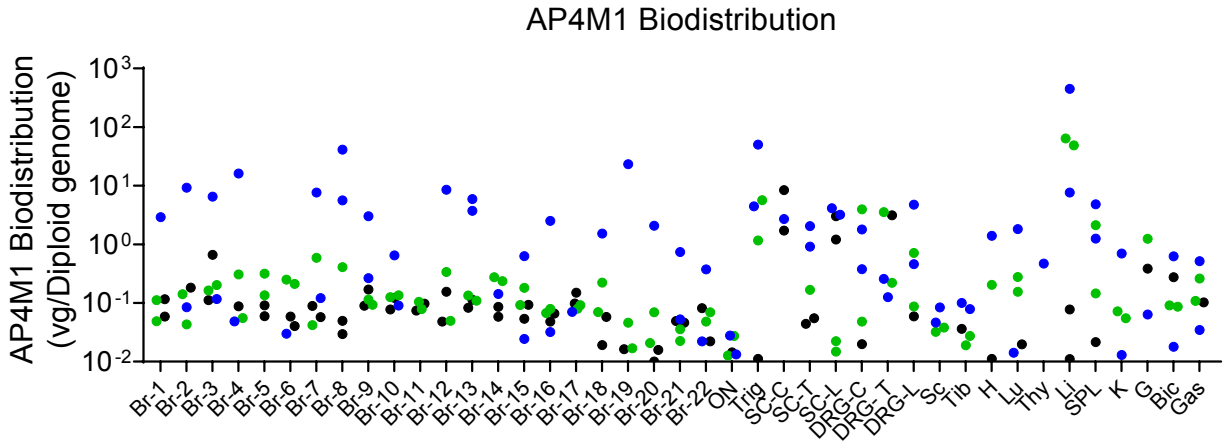
Study #05 in WT (C57BL/6J) mice provided hAP4M1 mRNA biodistribution data at 1, 5 and 12 months post dose at 1.25E11 and 5E11 vg/mouse and showed dose dependent increases in hAP4M1opt mRNA in all brain regions (Figure 7).

Figure 7 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 (**Study CRL-5550014, Module 4**) at doses of 0, 8.4E13 or 1.68E14 vg per animal. *AP4M1* vector biodistribution was quantified by qPCR and provided in Figure 8. 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 9). Collectively, IT delivery of AAV9/*AP4M1* results in broad *AP4M1* biodistribution and expression across the body of NHPs.

Figure 8 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;

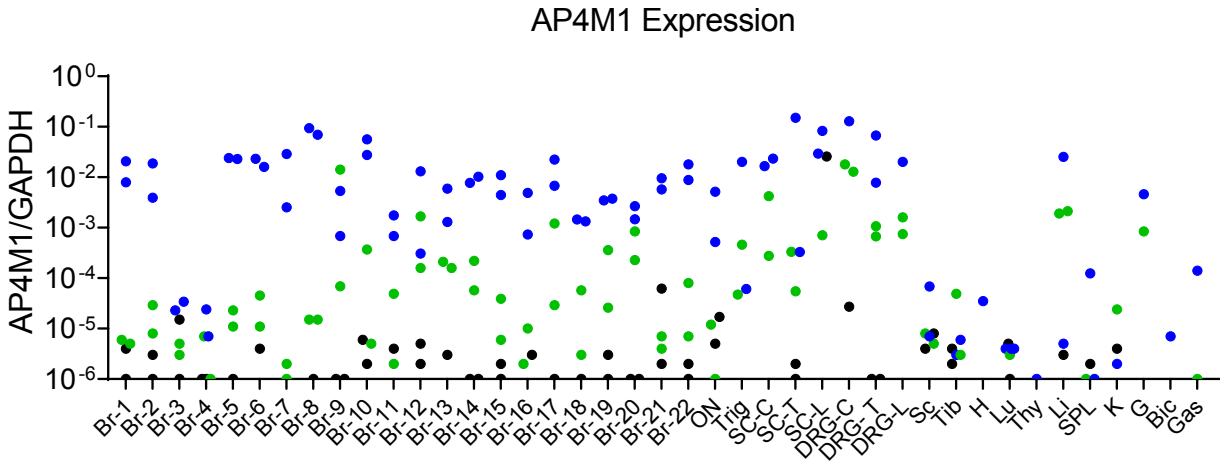
Br-19, Br-20 = Cerebellum; Br-21, Br-22 = Occipital Cortex; ON = Optic Nerve; Trig = Trigeminal Ganglion; SC-C = Cervical

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 9 AP4M1 Tissue Expression 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

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 4). The overall biodistribution pattern of MELPIDA is consistent with these published studies.

Table 4 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; Pardridge et al, 2011). References. Note: Doses were per animal or by weight.

2.4.4. Toxicology

2.4.4.1. 3 Month GLP SD Rat Study (CRL5550008)

The 3-month GLP study in WT SD rats (**CRL5550008, Module 4**) demonstrated that MELPIDA at doses of 0, 3.6E11, 1.1E12 and 3.3E12 vg 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, 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.

2.4.4.2. 12m Study (#05) in WT C57BL/6J Mice (Module 4)

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. 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.

2.4.4.3. 94-day GLP Study in NHP (CRL5550014, Module 4)

A GLP 94-day NHP study was conducted with intrathecal doses of 0 (N=2), 8.4E13 (N=2) and 1.68E14 vg (N=2) of MELPIDA to determine the effects on dorsal root ganglia. 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. 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.

2.4.5. Integrated Overview and Conclusions

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).

Vector distribution of MELPIDA after a single intrathecal administration measured in two studies (rats and NHPs) was consistent with expected AAV9 biodistribution (Table 4).

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, 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 5. 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 5 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
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 in Module 5**

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 6).

Table 6 Dose Extrapolation Based on Age and Brain Size

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

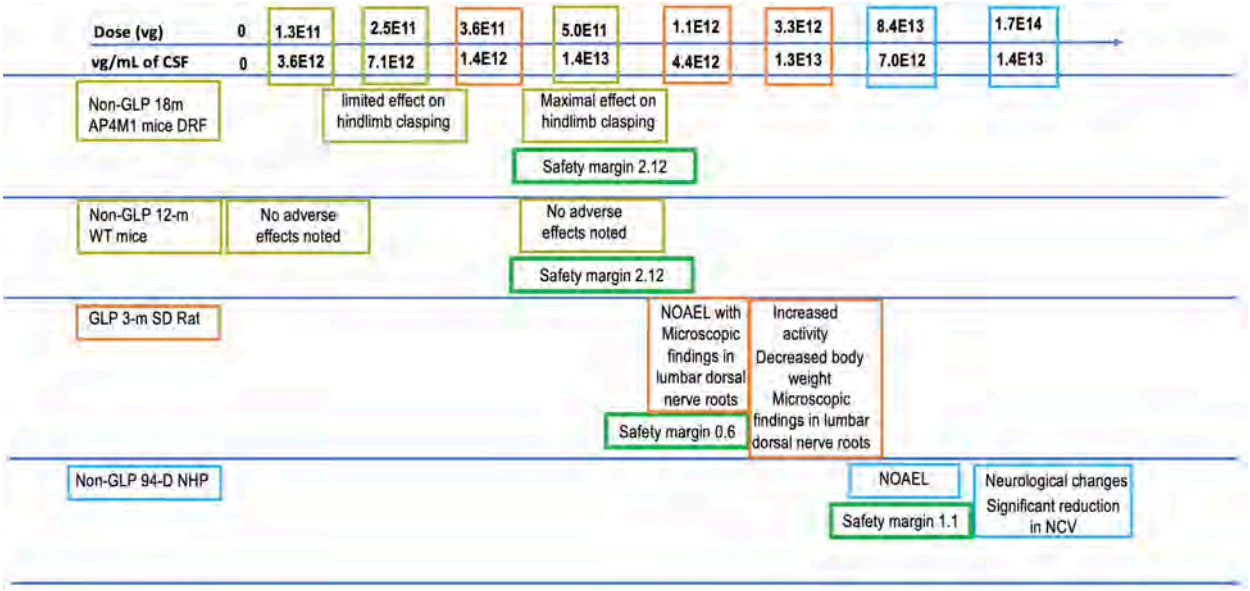
When considering the CSF volume in the various species, the target human dose of 1E15 vg corresponds to a dose equivalent of 6.6E12 vg per mL of CSF in humans, mice, rats and NHPs. The comparative absolute vg dose across species is 1E15 vg in humans, 8.0E13 vg in NHPs, 1.6E12 vg in rats, and 2.2E11 vg in mice (Table 7). 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 (Figure 10).

Table 7 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 10 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 6 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 6 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 (see **Investigator's Brochure**).

2.4.6. References

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