Sponsor:	CureSPG50, a non-profit entity
IND #:	Not yet assigned
Product Name:	Melpida (scAAV9-UsP-hAP4M1opt)
Indication:	Treatment of patients with Spastic Paraplegia Type 50 (SPG50) caused by the AP4M1 gene mutation
Request Type:	**Request for Rare Pediatric Disease Designation (this is accompanied with a request for Orphan Drug Designation)**
Submission Date:	Tuesday 28 July 2020

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TABLE OF ABBREVIATIONS

AAV	adeno-associated virus
AP	Adaptor Protein
ATG9A	Autophagy Related 9A
BCH	Boston Children's Hospital
BGH	Bovine growth hormone
CNS	central nervous system
DAPI	4′,6-diamidino-2-phenylindole
DNA	deoxyribonucleic acid
FDA	Food and Drug Administration
GFP	green fluorescent protein
h	human
HSP	Hereditary spastic paraplegia
IND	Investigational New Drug
IRNHS	International registry Natural History Study
ITR	inverted terminal repeat(s)
kg	kilogram
MOI	Multiplicity of Infection
NIH	National Institutes of Health
opt	optimized
OOPD	Office of Orphan Products Development
sc	self-complimentary
SPG50	Spastic Paraplegia 50
RNA	ribonucleic acid
TGN	Trans-Golgi network
US	Unites States of America
UTSW	University of Texas Southwestern Medical Center
vg	vector genome(s)
WT	wild type

1. SPONSOR NAME AND ADDRESS

Sponsor	CureSPG50
Primary Contact	Terry Pirovolakis
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2. PRODUCT NAME

Name(s) of Drug (including all available names: Trade, Generic, Chemical, or Code):

Melpida (scAAV9-UsP-hAP4M1opt).

Generic and tradenames have not yet been requested for this product.

3. PROPOSED INIDICATION

The proposed indication is for the "Treatment of patients with Spastic Paraplegia Type 50 (SPG50) caused by the AP4M1 gene mutation".

4. APPLICATION AND DESIGNATIONS FOR RBIO1

There are no applications or designations currently for Melpida.

Orphan drug designation (ODD) is being requested alongside this request for Rare Pediatric Disease Designation (RPDD).

5. PRODUCT DESCRIPTION

The investigational agent, Melpida is a self-complimentary (sc) AAV9 gene therapy containing a mutant AAV2 inverted terminal repeat (ITR) with the D element deleted, the UsP promoter, codon-optimized human AP4M1 cDNA coding sequence, Bovine growth hormone (BGH) polyadenylation signal, and wild type (WT) AAV2 ITR (Figure 1; Table 1).

Figure 1 Schematic of Melpida

Promoter = USP; Poly A = BGHpA

Table 1 Product description for Melpida

Vector Genome Structure	AAV2 mutant ITR	UsP promoter	Optimized human AP4M1 transgene	BGH poly A sequence	AAV2 ITR
Size	100 bp	328 bp	1362 bp	250 bp	145 bp
Function	Encapsidation, self-priming, self-complementing	Recruitment of transcription factors for AP4M1 transcription	Encoding human AP4M1 protein more efficiently	Provides stability of AP4M1 transcript	Encapsidation, self-priming

6. PROPOSED DOSEAGE FORM AND ROUTE OF ADMINISTRATION

Melpida will be provided as a sterile clear colorless solution, vialed at a strength to be determined. Melpida will be administered as a single one-time administration by intrathecal injection at a dose and volume to be determined from ongoing nonclinical studies.

7. INFORMATION SUPPORING RARE PEDIATRIC DISEASE DESIGNATION

Per Section 529(a)(3), a rare pediatric disease is required to meet the following criteria:

(A) The disease is a serious or life-threatening disease in which the serious or life-threatening manifestations primarily affect individuals aged from birth to 18 years, including age groups often called neonates, infants, children and adolescents, AND

(B) The disease is a rare disease or condition, within the meaning of Section 526 of the FD&C act.

Additionally, the product application needs to be for a human drug for the prevention or treatment of a rare pediatric disease where the active moiety has not been previously approved, and provides data suggesting that the drug may be effective in the rare pediatric disease.

The following sections provide the information necessary for the determination of this designation; the below bullets summarize the more detailed content that follows:

- Spastic Paraplegia Type 50 (SPG50) is one of a group of 4 genetic disorders (SPG47, SPG50, SPG51 and SPG52) involving a mutation 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, Behne et al, 2018).

- SPG is an ultra-rare autosomal recessive disease with ~156 patients identified worldwide for all phenotypes and 59 identified with SPG50. There are ~9 patients with SPG50 in North America (AP4M1, OMIM #612936) (source: Ebrahimi-Fakhari et al, unpublished).

- Without new-born screening identification, patients are often 8 to 10 years of age before they are correctly diagnosed although symptoms start within infancy (Table 3). Patients are usually paralyzed by spasticity paraplegia within their first decade and tetraplegic and severely mentally delayed later on in life; epilepsy is also a key symptom (Ebrahimi-Fakhari, Behne et al, 2018). Only a few patients are identified over the age of 30 years (Figure 3) (Ebrahimi-Fakhari et al, under review);

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

- Melpida is a self-complimentary (sc) AAV9 gene therapy containing codon optimized human (h) AP4M1, which has been shown to rescue up to 77% of defective fibroblasts from two SPG 50 patients in vitro and transduce the brain of wildtype mice when administered by intrathecal injection, the intended clinical route of administration (Section 7.3).

- The therapeutic goal is to treat patients with a single one-time intrathecal administration of Melpida, which will provide the AP4M1 protein required to prevent further progression of the disease, improving functional outcomes and survival in these pediatric patients. The intrathecal route has been used with AAV9 as a way to deliver gene therapies to the central nervous system (CNS) (Bailey et al, 2018). As with similar pediatric diseases such as spinal muscular atrophy and aromatic l-amino acid decarboxylase, it is likely that earlier treatment could provide better outcomes for patients (Gray, 2016).

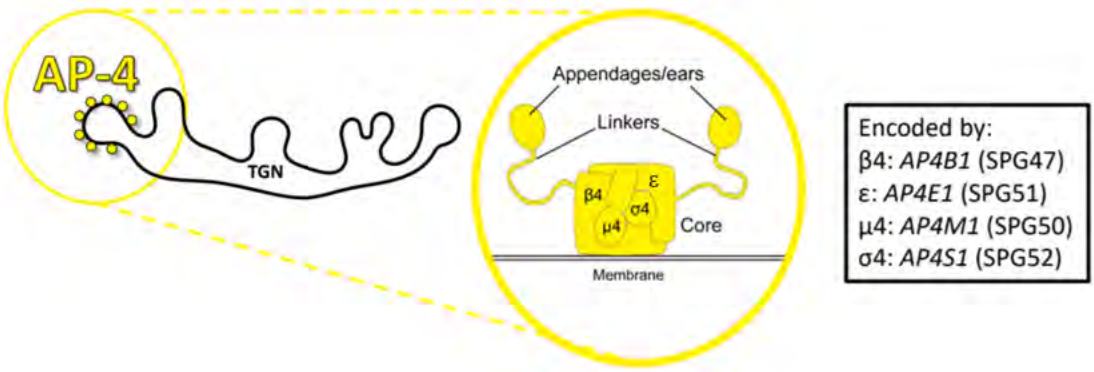
In summary, the data presented in this designation request support the granting of rare pediatric disease designation to Melpida.

7.1. Description of SPG50

7.1.1. Pathophysiology

SPG50 is one of a collection of adaptor protein complex 4 (AP-4) deficiencies (Figure 2). A homozygous loss-of-function mutation in any of the four AP-4 subunits causes a neurological disorder characterized by spastic paraplegia, developmental delay and intellectual disability (Ebrahimi-Fakhari et al, under review; Ebrahimi-Fakhari et al, 2018).

Figure 2 Schematic of Adaptor Protein complex 4



The four deficiencies are divided into SPG47, SPG50, SPG51 and SPG52 (Table 2).

Table 2 Subtypes of AP-4 deficiencies (Ebrahimi-Fakhari et al, under review)

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 demonstrated 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 early (Burgos et al, 2010; Toh et al, 2017) and late endosomes (Aguilar et al, 2001). 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 an AP4E1 knockout mouse has revealed widespread axonal pathology that includes reduced axonal development and prominent axonal swellings (De Pace et al, 2018; Ivankovic et al, 2020).

7.1.2. Natural History and Treatment 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, (under review) reports on 156 patients from 101 families that have registered. 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.

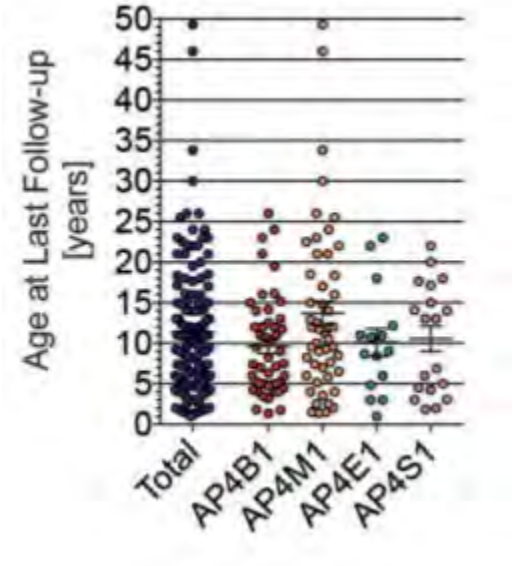
Onset of symptoms is typically prior to 1 year of age, with diagnosis often occurring 10 years later (Table 3). Often patients are initially misdiagnosed with cerebral palsy (Jameel et al, 2014).

Table 3 Key demographic data for 156 AP-4 deficient patients from Ebrahimi-Fakhari et al, (under review)

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)	47% (*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 3) (Ebrahimi-Fakhari et al, under review).

Figure 3 Age at last follow-up for 156 patients with AP-4 deficiency (from Ebrahimi-Fakhari et al, under review)



All phenotypes present with serious and quality of life limiting symptoms. From Ebrahimi-Fakhari, Behne 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 -2SD to -3SD 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 years: Delays become evident, low muscle tone is more evident and children are usually falling off WHO head growth charts
- 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 are now much more spastic and usually wheelchair-bound or soon to be. 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 all wheelchair bound and begin exhibiting spasticity in upper extremities.

- 15-20+ years: Children present with spasticity in all extremities and brain function appears to be degrading. Not much more is known beyond 15+ as there are very few patients in this category.

Unpublished data from the 156 patients with AP-4 deficiency described by Ebrahimi-Fakhari et al, (under review) support earlier findings that symptoms are severely debilitating and manifest prior to adulthood (Table 4). 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 4 Clinical and Radiographic features of AP-4 deficiency (from Ebrahimi-Fakhari et al, (under review)

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%

There are no approved treatments for SPG50.

7.1.3. Incidence and prevalence of SPG50

- SPG is an ultra-rare autosomal recessive disease with ~156 patients identified worldwide for all phenotypes and 59 identified with SPG50. There are ~9 patients with

SPG50 in North America (AP4M1, OMIM #612936) (source: Ebrahimi-Fakhari et al, unpublished).

CureSPG50 is developing Melpida to treat SPG50. The therapeutic goal of Melpida is to reverse and/or prevent progressive symptoms of SPG50 in pediatric patients with a single one-time intrathecal administration, resolving or preventing the serious manifestations, and providing patients the opportunity to experience an improved quality of life.

Melpida may be administered to patients only following diagnosis of SPG50, therefore, prevalence is the appropriate epidemiological parameter to report when describing the treatment or prevention of a disease or condition in this case.

The prevalence of a disease should be established using authoritative peer-reviewed references based on the U.S. population therefore a PubMed literature search was conducted (20 July 2020) without limit of publication date using the following search terms:

- "SPG50" (N=4),
- "AP-4 deficiency" (N=27),
- "hereditary spastic paraplegia", (N=1,382)
- "spastic paraplegia" (N=22,192)

Within each of these searches, "incidence," OR "prevalence" was added.

No publications were identified that spoke specifically to the incidence of SPG50 in the US. Most of the limited information available was related to hereditary spastic paraplegias, without necessary information specific to SPG50.

The most robust data to support prevalence comes from the work by Ebrahimi-Fakhari et al, (under review) which describes 156 patients worldwide with AP-4 deficiency and 59 (38%) with SPG50 (Table 3). North America accounted for 15% of all patients. Estimation for the number in North America is derived by:

59 * 0.15 = 8.85 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. These data support orphan designation for SPG50.

7.2. Mechanism of action of Melpida

Efficient transduction of 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 all 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 any other cell types within the body is also desired. The AAV9 vector utilized is expected to deliver the AP4M1 gene to these primary targets (Bailey et al, 2018). 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 should prevent/slow the onset of SPG50 if treated presymptomatic, or halt/reverse the progression of SPG50 if treated after symptom onset.

Nonclinical proof-of-concept has been established from in vitro patient-derived fibroblasts, and an in vivo mouse study supports brain targeting using the proposed clinical route of administration (intrathecal injection). These data are described in Section 7.3.

7.3. Non-clinical Proof of Concept studies supporting Melpida's usefulness to treat SPG50

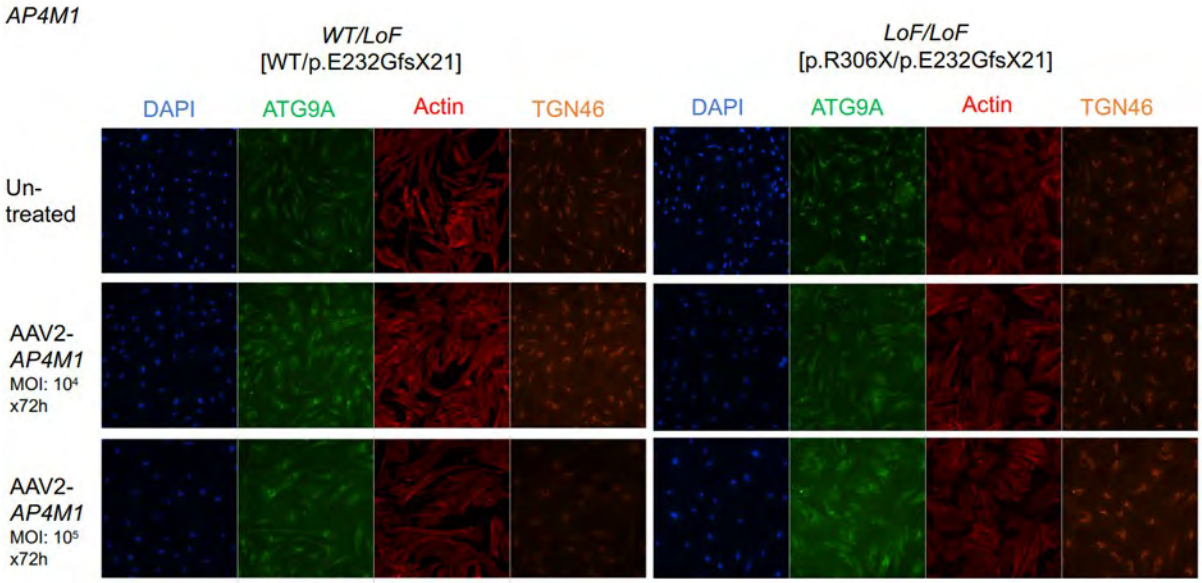
The following nonclinical studies provide proof of concept that Melpida provides promise for the clinical treatment of SPG50:

- **Study 1**: in vitro efficacy study using patient-derived fibroblasts in Dr. Darius Ebrahimi-Fakhari Lab at Boston Children's Hospital (BCH): Patient-derived fibroblast lines transduced with Melpida result in phenotype rescue with some toxicity in the higher MOI group (the capsid used here was AAV2 due to transfection issues in vitro, but the transgene and promoter were identical). These results provide strong proof-of-concept for benefit in SPG50 patient cells and will be repeated to confirm results.
- **Study 2**: in vitro efficacy study using patient-derived fibroblasts in Dr. Juan Bonifacino Lab at NIH: Patient-derived fibroblast lines transduced with Melpida result in phenotype rescue without any toxicity even with the higher MOI group (the capsid used here was AAV2 due to transfection issues in vitro, but the transgene and promoter were identical). These results further provide strong proof-of-concept for benefit in SPG50 patient cells. This study has been repeated twice with very similar conclusions.
- **Study 3**: in vivo safety study using WT C57BL/6J mice in Dr. Steven Gray's Lab at UTSW Medical Center. No toxicity has been observed in the in-life portion of the study up to 6 months post injection. The key information to support mechanism of action of Melpida from this study is that hAP4M1opt mRNA expression was found in all brain regions 4 weeks post AAV9/AP4M1 intrathecal (lumbar) injection.

7.3.1. Study 1: in vitro efficacy study using patient-derived fibroblasts in Dr. Darius Ebrahimi-Fakhari Lab at BCH (unpublished)

Fibroblasts from a single SPG50 patient were treated with Melpida for 72 hours at 104 and 105 multiplicity of infection (MOI) and then stained with 4′,6-diamidino-2-phenylindole (DAPI) for nuclear DNA and with antibodies against Autophagy Related 9A (ATG9A), Actin, or TGOLN2/trans-Golgi network protein 2 (TGN46). We found that treatment with Melpida restored ATG9A trafficking and hence AP-4 function, indicated by the ATG9A ratio going from 1.71 back to normal 1.25. The reduced cell account per well may suggest some possible toxicity with the higher MOI (Figure 4). Collectively, these results provide strong proof-of-concept for benefit in this particular patient's cells; repeat experiments with this patient and additional patients is planned.

Figure 4 Treatment with AAV2-AP4M1 restored ATG9A trafficking and levels in fibroblasts from SPG50 patient

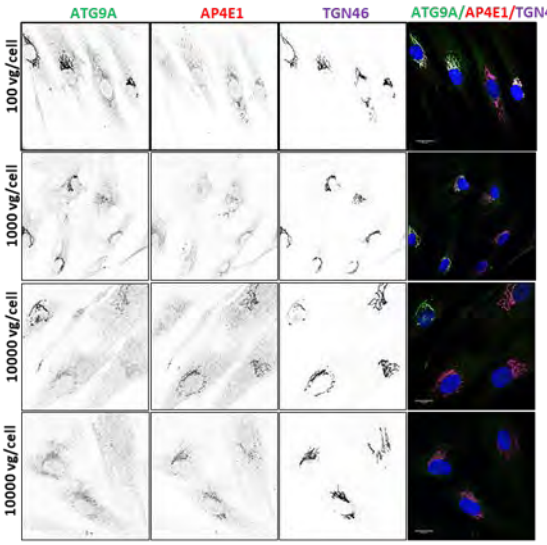


	WT/LoF [WT/p.E232GfsX21]			LoF/LoF [p.R306X/p.E232GfsX21]		
Mean	**Untreated**	**+AAV2-*AP4M1* (10^4)**	**+AAV2-*AP4M1* (10^5)**	**Untreated**	**+AAV2-*AP4M1* (10^4)**	**+AAV2-*AP4M1* (10^5)**
ATG9A Ratio	1.30	1.25	1.25	1.71	1.25	1.23
ATG9A inside TGN (A.U.)	1085.5	1086.2	1097.6	1625.1	1077.4	1048.4
ATG9A outside TGN (A.U.)	851.7	835.4	868.7	953.2	859.3	851.7
Cell count (per well)	482	454 (z=-0.3)	227 (z= -7.5)	713	415 (z=-3.1)	131 (z=-27.0)

7.3.2. Study 2: in vitro efficacy study using patient-derived fibroblasts in Dr. Juan Bonifacino Lab at NIH (unpublished)

Fibroblasts from two additional SPG50 patients were treated with Melpida for 72 hours at 102, 103, 104 or 105 MOI and then stained with DAPI for nuclear DNA and with antibodies against ATG9A, AP4E1 or TGN46. At the highest virus dose (100,000 vg) the rescue is 49% and 77% of the cells for patient #1 and #2 respectively (Figure 5). Unlike the cells from study 1, these cells did not show any sign of toxicity after infection, with nuclei, cell size and shape looking normal after staining. These results further provide strong proof-of-concept for benefit in SPG50 patient cells. This study has been repeated twice with very similar conclusion.

Figure 5 Treatment with AAV2-AP4M1 dose-dependently restored ATG9A and AP4M1 levels in up to 77% of fibroblasts from SPG50 patients



Patient	#1			#1			#1			#1		
# of viral particles	100			1000			10000			100000		
	imaged	rescued	% rescue	imaged	rescued	% rescue	imaged	rescued	% rescue	imaged	rescued	% rescue
	91	8	8.8	95	21	22.1	125	36	28.8	128	55	43.0
	106	7	6.6	111	32	28.8	181	58	32.0	116	64	55.2
Average	98.5	7.5	7.7	103.0	26.5	25.5	153.0	47.0	30.4	122.0	59.5	49.1

Patient	#2			#2			#2			#2		
# of viral particles	100			1000			10000			100000		
	imaged	rescued	% rescue	imaged	rescued	% rescue	imaged	rescued	% rescue	imaged	rescued	% rescue
	112	22	19.6	97	49	50.5	101	54	53.5	98	78	79.6
	117	32	27.4	107	63	58.9	103	65	63.1	112	83	74.1
Average	114.5	27.0	23.5	102.0	56.0	54.7	102.0	59.5	58.3	105.0	80.5	76.8

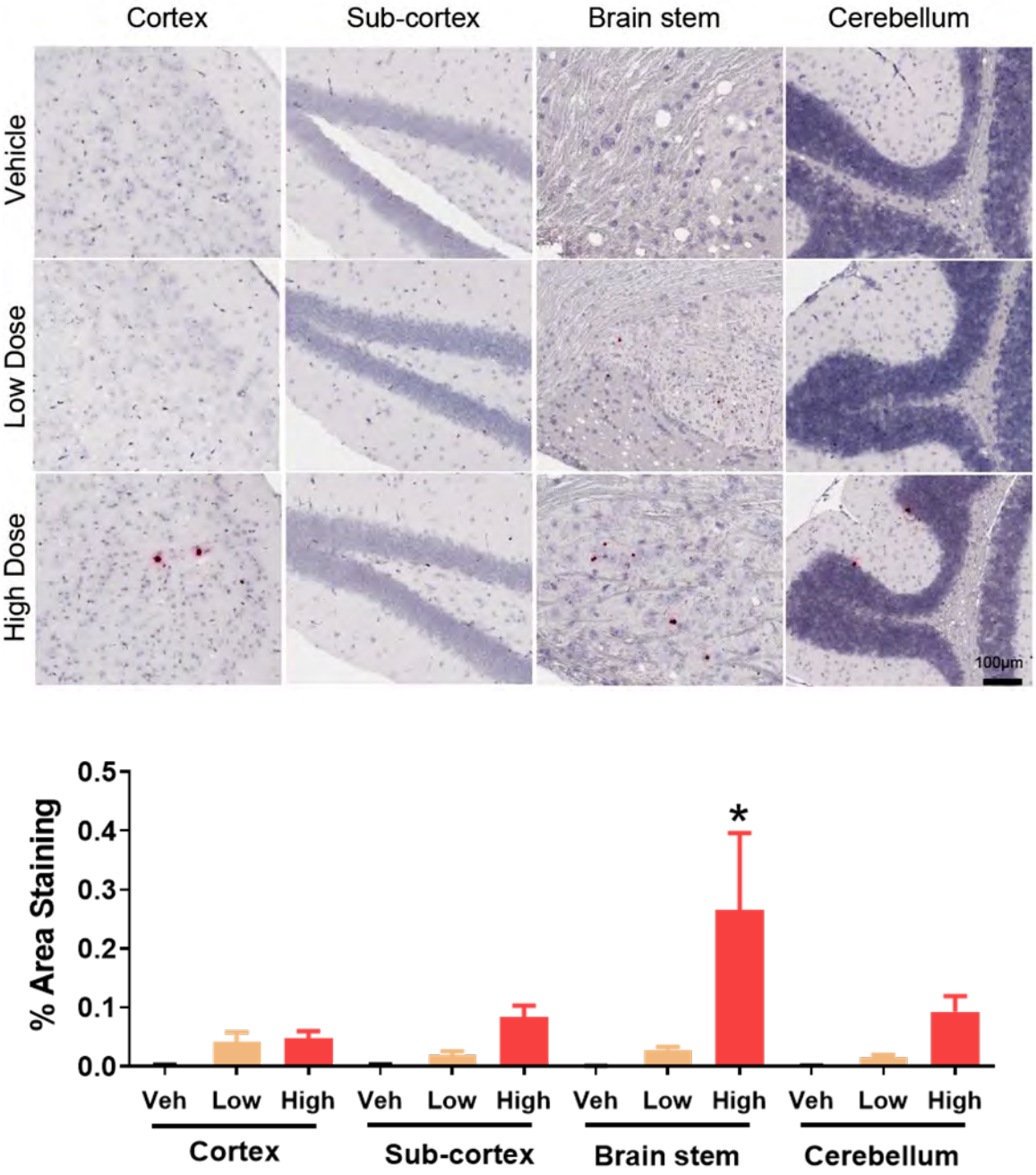
7.3.3. Study 3: in vivo safety study using WT C57BL/6J mice in Dr. Steven Gray Lab at UTSW Medical Center

Typically toxicology data are not provided to support orphan designation, however, this study supports the distribution of Melpida to the target tissues by the intrathecal route of administration.

Mice were randomized into 3 treatment groups for vehicle, low (1.25E11 vg) and high (5E11 vg) dose of Melpida (n=10 per group; 5 male, 5 female per group). Melpida or vehicle was administered via intrathecal lumbar injection in a volume of 5 µL, and monitored for 16 weeks. Three (3) mice per gender per group were taken down 4 weeks post injection for AP4M1 mRNA expression by RNAscope.

Dose-dependent hAP4M1opt mRNA expression was found in all brain regions at 4 weeks post AAV9/AP4M1 intrathecal injection (Figure 6), confirming Melpida reached the site of action.

Figure 6 AAV9/AP4M1 increases hAP4M1 mRNA expression in brain regions of WT BL/6J mice 4 weeks post injection



7.4. Basis for concluding product is for rare disease or condition

Section 7.1.3 provides the prevalence estimates for SPG50. The disease is ultra-rare with only 8 patients being identified in the US. No publications were found to support prevalence or incidence of SPG50 in any country; data were available to support prevalence of HSP, of which SPG50 is a subtype. Table 5 highlights the data used to substantiate prevalence estimates.

Table 5 Data supporting prevalence of SPG50

Data Source	Key data
Ebrahimi-Fakhari et al, under review	• 156 patients have been identified worldwide • Approximately 9 patients have been identified in North America, making a prevalence of 0.002:100,000 based on current US population size of 329,842,223 (source: U.S. Census Bureau and World Bank, June 25, 2020)
Fink et al (2013)	• Reported prevalence for 50 genetic subtypes of HSP as 1.2 to 9.6:100,000 across various countries (none were US specific); using the higher value of 9.6:100,000, estimates <30,000 patients based on current US population size of 329,842,223 (source: U.S. Census Bureau and World Bank, June 25, 2020)
McMonagle et al (2002)	• Reported autosomal dominant HSP prevalence for Ireland (North and South) as 1.27:100,000, and from around the world from 0.7 to 18.4:100,00. Using 18.4:100,000 estimates < 61,000 patients based on current US population size of 329,842,223 (source: U.S. Census Bureau and World Bank, June 25, 2020)

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

Data confirm that HSP qualifies as an orphan disease. SPG50 is one of many subgroups belonging under the umbrella of HSP and currently the estimated US prevalence is 0.002 per 100,000; significantly below the level required for orphan designation.

7.5. Basis for concluding product is for a rare and serious pediatric disease

Section 7.1 describes the seriousness of SPG50 and supports that these manifestations occur predominantly in pediatric patients. The following Table 6 highlights the key information from the publications supporting the pediatric nature and seriousness of SPG50.

Table 6 Key messages from publications supporting the pediatric nature and seriousness of SPG50

Reference	Key data
Ebrahimi-Fakhari et al, under review	• 156 patients with AP-4 deficiency are described, with ~9 from North America. • Symptom onset occurs during infancy (Table 3) with serious manifestations before adulthood (Table 4; Figure 3)
Ebrahimi-Fakhari et al (2018)	Of 68 patients identified in the International Registry with AP-4 deficiency (SPG47, 50, 51 and 52), natural history data indicated: • Early-onset developmental delay in all patients, with significant neonatal/infantile hypotonia, postnatal microcephaly, delayed myelination and loss of white matter in the majority of patients • At age 10 to 15 years, all patients are wheel-chair bound and exhibit spasticity in upper extremities
Bettencourt et al (2017)	• Describes 3 siblings from a Greek family with SPG50. • All 3 presented febrile tonic-clonic seizures during first year of life • Developmental delay since infancy

	• Serious deficiencies in language and social skills by puberty • 2 never were ambulatory and one was unable to walk independently by adolescence

8. SUMMARY AND CONCLUSIONS

In summary, the details presented in this submission support the following conclusions:

- Spastic Paraplegia Type 50 (SPG50) is one of a group of 4 genetic disorders (SPG47, SPG50, SPG51 and SPG52) involving a mutation 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, Behne et al, 2018).

- SPG is an ultra-rare autosomal recessive disease with ~156 patients identified worldwide for all phenotypes and 59 identified with SPG50. There are ~9 patients with SPG50 in North America (AP4M1, OMIM #612936) (source: Ebrahimi-Fakhari et al, unpublished).

- Without new-born screening identification, patients are often 8 to 10 years of age before they are correctly diagnosed although symptoms start within infancy (Table 3). Patients are usually paralyzed by spasticity paraplegia within their first decade and tetraplegic and severely mentally delayed later on in life; epilepsy is also a key symptom (Ebrahimi-Fakhari, Behne et al, 2018). Only a few patients are identified over the age of 30 years (Figure 3) (Ebrahimi-Fakhari et al, under review);

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

- Melpida is a self-complimentary (sc) AAV9 gene therapy containing codon optimized human (h) AP4M1, which has been shown to rescue up to 77% of defective fibroblasts from two SPG 50 patients in vitro and transduce the brain of wildtype mice when administered by intrathecal injection, the intended clinical route of administration (Section 7.3).

- The therapeutic goal is to treat patients with a single one-time intrathecal administration of Melpida, which will provide the AP4M1 protein required to prevent further progression of the disease, improving functional outcomes and survival in these pediatric patients. The intrathecal route has been used with AAV9 as a way to deliver gene therapies to the central nervous system (CNS) (Bailey et al, 2018). As with similar pediatric diseases such as spinal muscular atrophy and aromatic l-amino acid decarboxylase, it is likely that earlier treatment could provide better outcomes for patients (Gray, 2016).

The sponsor considers the data presented in this submission supports the granting of rare pediatric disease designation to Melpida.

9. REFERENCES

Abou Jamra R, Philippe O, Raas-Rothschild A, et al. Adaptor protein complex 4 deficiency causes severe autosomal-recessive intellectual disability, progressive spastic paraplegia, shy character, and short stature. Am J Hum Genet. 2011;88(6):788-795. doi:10.1016/j.ajhg.2011.04.019

Aguilar RC, Boehm M, Gorshkova I, et al. Signal-binding specificity of the mu4 subunit of the adaptor protein complex AP-4. J Biol Chem. 2001;276(16):13145-13152. doi:10.1074/jbc.M010591200

Bailey RM, Armao D, Nagabhushan Kalburgi S, Gray SJ. Development of Intrathecal AAV9 Gene Therapy for Giant Axonal Neuropathy. Mol Ther Methods Clin Dev. 2018;9:160-171. Published 2018 Feb 15. doi:10.1016/j.omtm.2018.02.005

Behne R, Teinert J, Wimmer M, et al. Adaptor protein complex 4 deficiency: a paradigm of childhood-onset hereditary spastic paraplegia caused by defective protein trafficking. Hum Mol Genet. 2020;29(2):320-334. doi:10.1093/hmg/ddz310

Bettencourt C, Salpietro V, Efthymiou S, et al. Genotype-phenotype correlations and expansion of the molecular spectrum of AP4M1-related hereditary spastic paraplegia. Orphanet J Rare Dis. 2017;12(1):172. Published 2017 Nov 2. doi:10.1186/s13023-017-0721-2

Burgos PV, Mardones GA, Rojas AL, et al. Sorting of the Alzheimer's disease amyloid precursor protein mediated by the AP-4 complex. Dev Cell. 2010;18(3):425-436. doi:10.1016/j.devcel.2010.01.015

Davies AK, Itzhak DN, Edgar JR, et al. AP-4 vesicles contribute to spatial control of autophagy via RUSC-dependent peripheral delivery of ATG9A. Nat Commun. 2018;9(1):3958. Published 2018 Sep 27. doi:10.1038/s41467-018-06172-7.

De Pace R, Skirzewski M, Damme M, et al. Altered distribution of ATG9A and accumulation of axonal aggregates in neurons from a mouse model of AP-4 deficiency syndrome. PLoS Genet. 2018;14(4):e1007363. Published 2018 Apr 26. doi:10.1371/journal.pgen.1007363.

Ebrahimi-Fakhari D, Behne R, Davies AK, Hirst J. AP-4-Associated Hereditary Spastic Paraplegia. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. GeneReviews®. Seattle (WA): University of Washington, Seattle; 1993.

Ebrahimi-Fakhari D, Teinert J, Behne R et al. Defning the clinical, molecular and imaging spectrum of adaptor protein complex 4-associated hereditary spastic paraplegia. Brain. Under Review/pending publication.

Fink JK. Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms. Acta Neuropathol. 2013;126(3):307-328. doi:10.1007/s00401-013-1115-8

Frazier MN, Davies AK, Voehler M, et al. Molecular Basis for the Interaction Between AP4 β4 and its Accessory Protein, Tepsin. Traffic. 2016;17(4):400-415. doi:10.1111/tra.12375

Gray SJ. Timing of Gene Therapy Interventions: The Earlier, the Better. Mol Ther. 2016;24(6):1017-1018. doi:10.1038/mt.2016.20

Hirst J, Irving C, Borner GH. Adaptor protein complexes AP-4 and AP-5: new players in endosomal trafficking and progressive spastic paraplegia. Traffic. 2013;14(2):153-164. doi:10.1111/tra.12028

Ivankovic D, Drew J, Lesept F, et al. Axonal autophagosome maturation defect through failure of ATG9A sorting underpins pathology in AP-4 deficiency syndrome. Autophagy. 2020;16(3):391-407. doi:10.1080/15548627.2019.1615302

Jameel M, Klar J, Tariq M, et al. A novel AP4M1 mutation in autosomal recessive cerebral palsy syndrome and clinical expansion of AP-4 deficiency. BMC Med Genet. 2014;15:133. Published 2014 Dec 14. doi:10.1186/s12881-014-0133-2

Langouët M, Siquier-Pernet K, Sanquer S, et al. Contiguous mutation syndrome in the era of high-throughput sequencing. Mol Genet Genomic Med. 2015;3(3):215-220. doi:10.1002/mgg3.134

Mattera R, Park SY, De Pace R, Guardia CM, Bonifacino JS. AP-4 mediates export of ATG9A from the trans-Golgi network to promote autophagosome formation. Proc Natl Acad Sci U S A. 2017;114(50):E10697-E10706. doi:10.1073/pnas.1717327114

McMonagle P, Webb S, Hutchinson M. The prevalence of "pure" autosomal dominant hereditary spastic paraparesis in the island of Ireland. J Neurol Neurosurg Psychiatry. 2002;72(1):43-46. doi:10.1136/jnnp.72.1.43

Moreno-De-Luca A, Helmers SL, Mao H, et al. Adaptor protein complex-4 (AP-4) deficiency causes a novel autosomal recessive cerebral palsy syndrome with microcephaly and intellectual disability. J Med Genet. 2011;48(2):141-144. doi:10.1136/jmg.2010.082263

Toh WH, Tan JZ, Zulkefli KL, Houghton FJ, Gleeson PA. Amyloid precursor protein traffics from the Golgi directly to early endosomes in an Arl5b- and AP4-dependent pathway. Traffic. 2017;18(3):159-175. doi:10.1111/tra.12465

Tüysüz B, Bilguvar K, Koçer N, et al. Autosomal recessive spastic tetraplegia caused by AP4M1 and AP4B1 gene mutation: expansion of the facial and neuroimaging features. Am J Med Genet A. 2014;164A(7):1677-1685. doi:10.1002/ajmg.a.36514.

Verkerk AJ, Schot R, Dumee B, et al. Mutation in the AP4M1 gene provides a model for neuroaxonal injury in cerebral palsy. Am J Hum Genet. 2009;85(1):40-52. doi:10.1016/j.ajhg.2009.06.004