CLINICAL PROTOCOL

A SINGLE-STAGE, ADAPTIVE, OPEN-LABEL, DOSE ESCALATION SAFETY STUDY OF ADENO-ASSOCIATED VIRUS ENCODING HUMAN AROMATIC L-AMINO ACID DECARBOXYLASE (AAV2-hAADC) ADMINISTERED BY MR-GUIDED CONVECTIVE INFUSION INTO THE MIDBRAIN IN PEDIATRIC PATIENTS WITH AADC DEFICIENCY

IND Number: 16127

Clinical Sites:

University of California San Francisco

The Ohio State University / Nationwide Children's Hospital

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

APPENDICES

LIST OF ABBREVIATIONS

Abbreviation	Definition
3-OMD	3-O-methyldopa
5-HIAA	5-Hydroxyindoleactic acid
5-MTHF	5-methyltetrahydrofolate
AADC	Aromatic L-amino acid decarboxylase
AAV2	Adeno-associated virus Type 2
AC	Advisory Committee
AE	Adverse event
CED	Convection-enhanced delivery
CNS	Central nervous system
CRF	Case report form
CSF	Cerebrospinal fluid
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CUP	Compassionate Use Program
DAT	Dopamine transporter
DDC	Dopa decarboxylase
DLT	Dose-limiting toxicity
DSMB	Data safety monitoring board
DTPA	Diethylenetriaminepentaacetate
FDA	Food and Drug Administration
FLAIR	Fluid attenuation inversion recovery
GMFM	Gross Motor Function Measure
hAADC	Human aromatic L-amino acid decarboxylase
HVA	Homovanillic acid
ICF	Informed consent form
ICH	International Conference on Harmonisation
ICU	Intensive care unit
IND	Investigational New Drug Application
INC	Interventional Neurotherapy Center
IRB	Institutional Review Board
IV	Intravenous
LP	Lumbar puncture

Abbreviation	Definition
MAO	Monoamine oxide
MR	Magnetic resonance
MRI	Magnetic resonance imaging
nAb	Neutralizing antibody(ies)
NCI	National Cancer Institute
NHP	Nonhuman primate
NIH	National Institutes of Health
NORD	National Organization for Rare Diseases
PCR	Polymerase chain reaction
PD	Parkinson's disease
PDMS-2	Peabody Developmental Motor Scales 2nd edition
PEDI-CAT	Pediatric Evaluation of Disability Inventory-Computer Adaptive Test
PedsQL	Pediatric Quality of Life Questionnaire
PET	Positron emission tomography
PICU	Pediatric Intensive Care Unit
PLP	Pyridoxal phosphate
PND	Pediatric Neurotransmitter Diseases
SAEs	Serious adverse events
SCD	Sequential compression device
SNc	Substantia nigra pars compacta
SPECT	Single-photon emission computed tomography
UK	United Kingdom
USA	United States of America
vg	Vector genomes
VTA	Ventral tegmental area

1 PROTOCOL SYNOPSIS

STUDY TITLE

A Single-Stage, Adaptive, Open-Label Dose Escalation Safety Study of Adeno-Associated Virus Encoding Human Aromatic L-Amino Acid Decarboxylase (AAV2-hAADC) Administered by MR-guided Infusion into the Midbrain in Pediatric Patients with AADC Deficiency

INVESTIGATIONAL PRODUCT

The AAV2-hAADC vector consists of an adeno-associated virus, serotype 2 (AAV2) containing human AADC complementary DNA (cDNA), human cytomegalovirus (CMV) promoter and 3'UTR sequences. Study drug is provided in a sterile formulation of phosphate buffered saline with 0.001% Pluronic acid (F-68). AAV2-hAADC is supplied in 0.5 mL aliquots as a suspension at a target concentration of 4.9×10^{12} vector genomes per mL.

The vector and excipient for dilution will be supplied to the clinical site by the Clinical Vector Core, Children's Hospital of Philadelphia.

The parenchymal use of gadoteridol is off-label, and also part of this study.

CLINICAL PHASE

Phase 1. Given the extremely rare nature of the disease and limited number of subjects, this study will generate data for dose selection, evaluation of safety, and confirmatory evidence of efficacy. A single stage study will address dose selection and a future study will further evaluate safety and efficacy of the selected dose.

STUDY OBJECTIVES

The overall objective of this study is to determine the safety and efficacy of AAV2-hAADC delivered to the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) in children with aromatic L-amino acid decarboxylase (AADC) deficiency. Specifically, the study will assess:

- Safety, as measured by adverse events (AEs), safety laboratory tests, brain imaging, and the relationship of AEs to study/surgical procedures or to AAV2-hAADC.
- Clinical responses to treatment with AAV2-hAADC. The primary clinical outcomes will reflect the predominant motor deficits of loss of motor function and dystonic movements.

STUDY RATIONALE

Aromatic L-amino acid decarboxylase (AADC) deficiency is a rare recessive genetic disorder in which mutations in the gene encoding the AADC enzyme lead to deficient synthesis of catecholamines (dopamine, norepinephrine, epinephrine) and serotonin. Over 100 cases have been identified worldwide since the original description of the disorder in 1990. Affected children suffer chronic and severe motor, cognitive, and behavioral disability. The most prominent neurological symptoms are motor: hypokinesia, hypotonia, oculogyric crises, involuntary movements and motor developmental delay. Additional symptoms include emotional lability, sleep disturbance, and hypotension. Because of the specific location of the metabolic block in this disorder (the conversion of levodopa to dopamine), motor symptoms do not respond to therapy with levodopa, in contrast

to the positive response observed in Parkinson's disease and in other inborn errors of dopamine metabolism. Most patients with AADC deficiency derive little or no benefit from currently available medical therapies.

In recent years, use of a viral vector, adeno-associated virus type 2 (AAV2), encoding the human AADC gene (hAADC), has been developed for the treatment of Parkinson's disease. Early studies have demonstrated that the gene can be safely delivered to the striatum in human subjects via targeted infusion. The current protocol proposes to adopt a similar strategy to deliver AAV2-AADC to select midbrain regions to treat AADC deficiency in pediatric patients.

STUDY POPULATION

Male and female patients aged 4 and up years with a confirmed diagnosis of AADC deficiency who, in the Investigator's opinion, are candidates for surgical therapy and who meet all inclusion/exclusion criteria, will be enrolled.

The initial patients screened for this study will be those with severe motor impairment and motor developmental delay despite treatment with currently available medications. The selection of patients who have failed to benefit from existing treatments will exclude newly diagnosed patients, as it would take at least 12 months to evaluate response to medications and determine lack of motor benefit.

For the first group of 3 subjects (Group 1), the primary goal is to assess procedural safety. The minimum age for the first group of patients will be 5 years. The selection of this lower age limit is intended to minimize the risks because response to standard therapy is less certain in younger children, and there are increased risks associated with skull fixation in very young children. Based on previous clinical experience in the treatment of neurotransmitter disorders, younger patients may have the potential to benefit the most from AADC gene transfer. Thus, the Investigator contacted the Agency after treating Cohort 1 to amend the clinical protocol and enroll subjects as young as 4 years old. Although it is anticipated that younger patients may have greater potential than older patients to benefit from AADC gene transfer, there is no specific evidence that older patients would not also benefit.

INCLUSION/EXCLUSION CRITERIA

Inclusion Criteria

1. Definite diagnosis of AADC deficiency, confirmed by at least two of the following three criteria: (1) CSF neurotransmitter profile demonstrating reduced HVA and 5-HIAA, and elevated 3-OMD concentrations, (2) plasma AADC activity less than or equal to 5 pmol/min/mL, (3) molecular genetic confirmation of homozygous or compound heterozygous mutations in DDC.
2. Age 4 years and up.
3. Failed to derive adequate benefit from standard medical therapy (dopamine agonists, monoamine oxidase inhibitor, pyridoxine or related form of Vitamin B6).
4. Unable to ambulate independently (with or without assistive device)
5. Cranium sufficiently developed, with sutures closed, to enable surgical placement of SmartFrame® system on the skull for MRI-guided stereotactic targeting.

6. Brain MRI does not show any conditions or malformations that are clinically significant with respect to risks for stereotactic brain surgery.
7. Parent(s)/guardian(s) of the study subject must agree to comply with the requirements of the study, including the need for frequent and prolonged follow-up.
8. Both parents (or legal guardians) must give their consent for their child's participation in the study.
9. Stable medication regimen for treatment of AADC deficiency: no new medications introduced for at least 6 months, and no existing medication dose changes for at least 3 months prior to Baseline.
10. Baseline hematology, chemistry, and coagulation values within the normal pediatric laboratory value ranges, unless in the Investigator's judgment, the out of range values are not clinically significant with respect to subject suitability for surgery.

Exclusion Criteria

1. Intracranial neoplasm or any significant structural brain abnormality or lesion (e.g., severe brain atrophy, white matter degenerative changes), which, in the opinion of the clinical investigators, would confer excessive risk and/or inadequate potential for benefit.
2. Presence of other significant medical or neurological conditions that would create an unacceptable operative or anesthetic risk (including congenital heart disease, respiratory disease with daytime home oxygen requirement, prior history of serious anesthesia complications during elective procedures, history of cardiorespiratory arrest), liver or renal failure, malignancy, or HIV positive.
3. Previous stereotactic neurosurgery.
4. Coagulopathy, or need for ongoing anticoagulant therapy.
5. Contraindication to sedation during surgery or imaging studies (FDOPA PET DAT SPECT, or MRI scans).
6. Receipt of any investigational agent within 60 days prior to the Baseline and during study participation.
7. Evidence of clinically active infection with adenovirus or herpes virus on physical examination.

PRIMARY ENDPOINTS

Safety: Assessment of AE or SAE and its relationship to study surgery, infusion, or treatment effect (graded as definite, probable, possible, unlikely or unrelated).

- Adverse Events and Serious Adverse Events
- Post-operative MRI and/or CT (with contrast if clinically indicated)
- Clinical laboratory assessments (hematology, chemistry, immunology)

Biological Activity: Demonstration of effective restoration of AADC function by assays of CSF neurotransmitter metabolites and F-DOPA PET imaging, as allowed by subject's ability to travel

to undergo follow-up visits.

SECONDARY & EXPLORATORY ENDPOINTS

To obtain preliminary data regarding the potential for clinical responses by assessing the magnitude and variability of changes in clinical outcome parameters.

The principal clinical outcome measures are:

- Motor function, as assessed by the Gross Motor Function Measure (GMFM-88)
- Frequency of oculogyric episodes, as measured by a Behavior Diary Secondary clinical outcome measures include:
- Assessment of subject disability, as assessed using the Pediatric Evaluation of Disability Inventory (PEDI); adaptive behavior, as assessed using Vineland Adaptive Behavior Scale; Patient's Global Impression of Change (PGI-C); and quality of life, as determined using the Pediatric Quality of Life Inventory (PedsQL).

It is anticipated that the utility of established developmental and cognitive assessments may be limited because of the study population's severe physical disability. Such developmental assessments include:

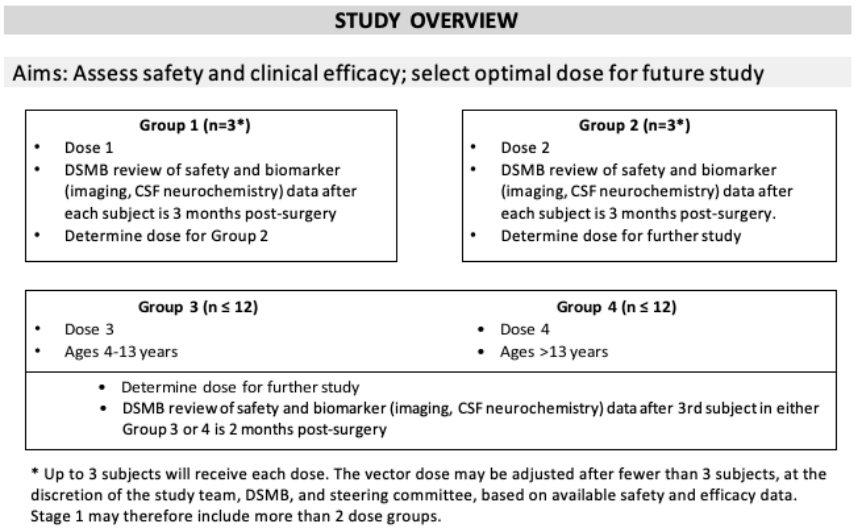
- Peabody Developmental Motor Scales 2nd edition (PDMS-2)

STUDY DESIGN

This study is an adaptive, single-stage dose-escalation, open-label safety study of AAV2-hAADC delivered by real-time image-guided convection-enhanced delivery bilaterally into the substantia nigra pars compacta and the ventral tegmental area of pediatric patients with AADC deficiency.

There will be 31 subjects divided into 4 groups of up to 12 subjects each. The primary aim of this study is to determine the dose for future studies based on safety, biomarkers of pharmacological activity of AADC and clinical outcomes.

Subjects will be enrolled sequentially into the first 2 dose groups, Group 1 followed by Group 2. Groups 3 and 4 will be enrolled in parallel after Group 2.



Group 1 will consist of three (3) subjects that meet the inclusion criteria will receive a single low dose of AAV2 hAADC (1.3×10^{11} vector genomes (vg), delivered as an infusate volume of up to 160 µL of vector at concentration of 8.3×10^{11} vg/mL). The total AAV2-hAADC dose will be infused bilaterally via magnetic resonance (MR)-guided convective infusion into a total of up to 4 sites per subject in both the SNc and VTA (left and right). The infusate will be divided between the VTA and SNc (e.g., 30 µL into the VTA and 50 µL into the SNc). The inter-subject dosing interval will be no shorter than 90 days for the first 3 subjects.

A Data and Safety Monitoring Board (DSMB) will be established to review safety data after each subject has been treated and will review the accumulated safety and efficacy data of Group 1. The DSMB report will be submitted to the FDA for review before Group 2 may commence. The DSMB will be notified of dose limiting toxicities (DLT) (see ***Dose-limiting Toxicity*** for definition) within 5 business days of the initial report to the PI. If none of the first 3 subjects in Group 1 experiences a DLT that results in hospitalization beyond 90 days post-surgery, enrollment in Group 2 may commence. If 1 subject experiences a DLT, treatment of additional subjects will not proceed until review and approval of the DSMB. If 2 or more subjects experience a DLT, both treatment and enrollment will be stopped, pending DSMB review. The occurrence of serious safety problems may lead to discontinuation of further treatments, based on pre-determined Stopping Rules.

The vector dose (volume and/or concentration) may be adjusted after the treatment of <3 subjects in Group 1 if the study team, DSMB, and study Advisory Committee determine, based on review of available data, that dose escalation or reduction is warranted. If dose escalation is warranted, total vg in Group 2 will be increased by factor of 3.1 (Table 2). Volume of infusion will remain constant between Group 1 and Group 2. Group 3 and 4 will receive the same vector concentration as Group 2 in 80-300 µL per hemisphere for coverage optimization of the target structures.

The final analysis of the safety and clinical outcome assessments will be performed 1 year post-surgery. A follow-up analysis will be performed 2 years post-surgery. Thereafter, subjects will be followed long-term to assess safety and clinical status updates annually for up to 5 years following gene transfer.

RECRUITMENT

Subject recruitment will be conducted via 3 main sources: (1) collaboration with two foundations, the Pediatric Neurotransmitter Diseases (PND) Association (USA), and the AADC Research Trust (UK), which both maintain registries of children with AADC deficiency; (2) the International Working Group on Neurotransmitter Related Disorders (iNTD), a group of physicians who treat patients with AADC deficiency, and (3) notification about the trial through the following organizations and societies: the National Organization for Rare Diseases (NORD), Genetic Alliance, Child Neurology Society mailing list, ChildNeuro Listserve. Study information will be available on Clinicaltrials.gov and a study website.

SUBJECT NUMBER

A total of up to thirty-one (31) subjects are planned to be enrolled and treated in this study

TREATMENT DURATION

Eligible subjects who have parental consent to be part of the clinical study must have screening evaluations and baseline functional testing over a period of up to three months prior to surgery.

Subjects who at the time of surgery still meet all Eligibility Criteria will undergo surgery to deliver the gene vector in a single surgery. Given the nature of gene transfer, the duration of the treatment is expected to be life-long, hence we anticipate the need for a separate long-term follow-up study.

DURATION OF FOLLOW-UP

Systematic clinical assessments will occur over a 24-month period postoperatively. Patients will be monitored for at least 5 years in a Long-Term Follow-up Study to the protocol. Permission for autopsy studies will be requested for all patients who die during the study.

DOSE LEVEL (S) AND DOSE JUSTIFICATION

The dose levels chosen for this study are based on results obtained from analysis of a safety study in non-human primates, in which the clinical cannula and surgical procedure were used, as well as GMP-process-comparable AAV2-hAADC vector. Relevant volumes of anatomical target structures were used to provide planned dose levels in this study. Recent experience from an ethics committee (EC)-approved compassionate use of AAV2-AADC in AADC deficient children was started in Europe. This program has shown a good safety profile of higher doses and volume of infusion of AAV2-AADC in the AADC deficient population.

DATA ANALYSIS PLAN

All subjects who received study medication will be included in the analysis of the safety and efficacy data.

Safety data will be summarized for the total study population and will be further broken down by the dose level. Treatment-emergent adverse events will be coded with the Medical Dictionary for Regulatory Activities (MedDRA) and summarized by body system and preferred term. In addition, separate summaries will be provided for treatment-emergent adverse events by severity and relationship to study drug, as well as adverse events leading to study drug discontinuation and serious adverse events. The tolerability will be assessed by number of patients having DLTs and with premature discontinuations of study treatment.

For the evaluation of efficacy, there will be two primary clinical outcome measures:

- Motor Function, as assessed by the Gross Motor Function Measure (GMFM-88). The GMFM-88 total score will be used as the endpoint. The total score will be derived as an unweighted average of the 5-dimension scores: lying and rolling (17 items); sitting (20 items); crawling and kneeling (14 items); standing (13 items) and walking, running, and jumping (24 items). Each dimension score will be defined as % of maximum score for the dimension in question.
- Frequency of oculogyric episodes, as measured by a Behavior Diary: each recorded episode will be classified as mild, moderate or severe. The area under the curve (AUC) of the diary symptoms will be used as the endpoint. The AUC will be calculated for each study month as a sum of duration of symptoms (hours) multiplied by the severity (0-3) of the symptom in question. The endpoint is the change from baseline (average of 3 months preceding the baseline) to 1 year (average of 3 months preceding the visit at Month 12.

All data collected during the study will be used for the evaluation of the primary clinical outcome measures. The evaluation will be focused on the following comparisons:

- Change from baseline to one year in all patients treated with the selected dose level (primary comparison). However, if no dose-response is seen at Stage 1 of the study, both dose levels will be included in the primary comparison.
- Difference in change from baseline to one year between the patients treated with the selected dose level and the other dose level used.

In addition to the comparisons defined above, efforts will be made to compare the changes seen in the present study to natural progression of the disease as assessed in The Natural History of Symptoms and Motor Function in the AADC Deficiency Study, a related external study at Washington University in St. Louis, led by Dr. Toni Pearson,. These comparisons may involve comparison of the changes between the treated patients from the present study and untreated patients, using endpoints derived from the elements of the primary outcome measures. The definition and analysis of these endpoints will depend on the availability of the data collected from The Natural History Study.

MEDICAL MONITOR AND DATA SAFETY MONITORING BOARD

A Medical Monitor with clinical expertise in pediatric movement disorders will be responsible for the review of all safety and adverse experience data.

A Data Safety Monitoring Board, consisting of a minimum of 3 individuals with broad expertise in pediatrics, neurology, and gene transfer will be appointed. Dose-limiting toxicity will be defined as any Grade 3 or 4 toxicity (NCI CTCAE v.4) that is thought to be possibly or probably related to AAV2-hAADC.

If none of the first 3 subjects experiences a Dose Limiting Toxicity (DLT) that results in hospitalization beyond 90 days post-surgery, more subjects may be enrolled. Should 2 or more subjects in either cohort experience DLT as defined above, the study will be stopped pending review and discussion with FDA.

STOPPING RULES

The occurrence of any of the following events(regardless of suspected causal associations) during or after the administration of AAV2-hAADC would result in the halting of study enrollment and notification of the DSMB, FDA, and IRB:

- Any significant procedural deviation or violations, e.g. dosing error, equipment failure
- Any symptomatic intra-cerebral hemorrhage or stroke that results in a significant new neurologic deficit that persists one month following surgery
- Any central nervous system infections related to study interventions
- Dyskinesias severe enough to require ICU admission for greater than 30 days
- Any death

Once a stopping rule is reached, the trial would be suspended (i.e., cessation of AAV2-hAADC administration to any subjects) pending a comprehensive safety review by the DSMB. Triggering of any Stopping Rule will prompt notification to FDA, IRB, NIH Office of Biotechnology, and the Institutional Biosafety Committee (IBC).

Additional Stopping Rules may be developed if unexpected serious adverse events (SAE) with

likely related to AAV2-hAADC delivery, including delivery procedure, appear during the study.

Events that do not clearly meet the above criteria may be submitted to the DSMB, at the discretion of the study investigators, for independent review.

2 INTRODUCTION

Aromatic L-Amino Acid Decarboxylase (AADC) deficiency is an inborn error of neurotransmitter biosynthesis with an autosomal recessive inheritance. Affected children typically present in infancy with hypotonia, oculogyric episodes, dystonia and developmental delay, and suffer chronic and severe neurological dysfunction. Mutations in the gene encoding for the enzyme AADC lead to a combined deficiency of serotonin and catecholamines (dopamine, norepinephrine, and epinephrine). Since the initial description of the condition, over 100 patients with this disorder have been identified worldwide (Hyland and Clayton 1990) (Manegold et al. 2009; Brun et al. 2010). Despite the severity of the disease, many patients do survive to adolescence and young adulthood. Because the disease is caused by a profound loss of functional AADC, gene replacement is seen as one of the most desirable and potentially transformative candidate therapies.

AADC deficiency causes motor, cognitive, and behavioral symptoms. The most prominent neurological symptoms are motor: hypokinesia, hypotonia, oculogyric crises, involuntary movements and motor developmental delay. Additional symptoms include emotional lability, sleep disturbance, and hypotension (Hyland and Clayton 1990; Hyland and Clayton 1992; Hyland et al. 1992; Swoboda et al. 1999; Swoboda et al. 2003) (Lee et al. 2009) (Manegold et al. 2009); Table 1).

Table 1: Common signs and symptoms in patients with AADC deficiency

Symptom	%	All Patients	Infancy ≤18 mo	Childhood ≤18 mo	Adoles-cence ≥11 years	Adulthood
Hypotonia	95	74/78	35/38	33/33	3/4	3/3
Oculogyric crises	86	67/78	33/38	28/33	3/4	3/3
Sweating	65	51/78	20/38	26/33	2/4	3/3
Developmental retardation	63	49/78	22/38	24/33	1/4	2/3
Dystonia	53	41/78	21/38	16/33	1/4	2/3
Hypertonia	44	35/78	14/38	18/33	1/4	2/3
Feeding/swallowing difficulties	42	33/78	17/38	16/33	0/4	0/3
Dysarthria/speech difficulties	41	32/78	9/38	20/33	1/4	2/3
Hypersalivation	41	32/78	12/38	17/33	1/4	2/3

Ptosis	39	30/78	18/38	10/33	2/4	0/3
Insomnia	37	29/78	11/38	17/33	1/4	0/3
Irritability	35	27/78	12/38	12/33	1/4	2/3
Hypokinesia	32	25/78	8/38	14/33	1/4	2/3
Nasal congestion	31	24/78	10/38	12/33	2/4	0/3
Temperature instability	29	23/78	12/38	9/33	1/4	1/3
Poor head control	28	22/78	10/38	9/33	2/4	1/3
Athetosis	27	20/78	8/38	11/33	0/4	1/3
Poor eye fixation	26	19/78	10/38	9/33	0/4	0/3
Chorea	22	17/78	7/38	9/33	1/4	0/3
Brain Imaging:						
Abnormal MRI	24	19/78	-	-	-	-
Abnormal EEG	13	10/78	-	-	-	-
Abnormal CT	6	5/78	-	-	-	-

A description of the clinical and biochemical features of AADC deficiency in 78 patients was published in 2010 (Brun et al. 2010) and features are tabulated in a public database (JAKE). (available at: www.biopku.org/BioPKU_DatabasesJAKE.asp).

Current treatment strategies are limited, and in many cases not beneficial. Administration of the neurotransmitter precursors L-dopa and 5-hydrotryptophan is ineffective, as these precursors cannot be further metabolized to dopamine and serotonin without functional AADC. Therefore current therapies aim to correct neurotransmitter abnormalities by: 1) augmenting residual AADC activity with the cofactor of AADC, pyridoxine or pyridoxal phosphate (PLP); and 2) potentiating dopaminergic neurotransmission through the use of monoamine oxide (MAO) inhibitors and dopamine agonists. Of the 78 patients currently in the JAKE database, a positive response to therapy was observed in 15 patients, with unsatisfactory or no response in the other 63 patients (Brun et al. 2010).

In recent years, use of a gene therapy vector, adeno-associated virus Type 2 (AAV2), encoding the human AADC (hAADC) gene has been developed for the treatment of Parkinson's disease. Early studies have demonstrated that the gene can be safely be delivered to the striatum via targeted infusion in adults with Parkinson's Disease (Christine et al. 2009) and in four children with AADC deficiency (Hwu et al. 2012).

The current protocol proposes to deliver the AAV2-hAADC vector to two specific regions of the

midbrain, substantia nigra pars compacta (SNc) and ventral tegmental area (VTA), taking advantage of anterograde transport of AAV2 from these regions to deliver AAV2-hAADC to other brain regions important in the pathophysiology of the disease. By delivering AAV2-hAADC to the VTA and SNc of affected children, we postulate that we will substantially rescue dopamine biosynthesis. If successful, this therapy thus has the potential to improve motor function and the quality of life of patients with AADC deficiency.

2.1 Supporting Information and Prior Clinical Experience

2.1.1 AADC Deficiency

AAV2-hAADC gene therapy for patients with AADC deficiency is being investigated clinically at National Taiwan University Hospital, Taipei, Taiwan. In a compassionate treatment study, four (4) patients with AADC deficiency aged 4 to 6 years were treated with AAV2-hAADC that was delivered bilaterally to the putamen ($1.5x10^{11}$vg) (Hwu et al. 2012). The treatment protocol was based on the AAV2-hAADC studies in Parkinson's disease (PD). All 4 patients in the published study were reported to tolerate the procedure without significant peri-operative complications. However, patients experienced transient dyskinesias (involuntary movements) as a result of putaminal AADC transduction, beginning approximately one (1) month after surgery. An additional 4 subjects, aged 2-9 years old, have been treated, with mean follow up for these 8 patients of 3.8 years (Hwu, Press Release, 2015).

2.1.2 Rationale for the Study

In the current study, AAV2-hAADC will be administered into the SNc and VTA of subjects with AADC deficiency. In contrast to PD, in which there is extensive degeneration of dopaminergic nigrostriatal neurons, DAT imaging reveals that the nigrostriatal pathway is largely intact in AADC deficiency (Dr. Wang-Tso Lee, Taiwan National Hospital, personal communication). By directly targeting the dopaminergic neurons that normally express AADC, we anticipate widespread distribution of AADC to their axon terminals within the caudate nucleus, putamen and nucleus accumbens. In addition, we have demonstrated in non-human primates that AAV2 vectors undergo anterograde transport. AAV2-hAADC delivered to the midbrain is expected to transport AADC via the nigrostriatal projection to axon terminals in the caudate nucleus and putamen (Johnston et al. 2009; Kells et al. 2012; Salegio et al. 2014).

Although the SN/VTA is a smaller target as compared to the putamen for neurosurgical intervention, it is anticipated that broader distribution of AADC, achieved in a smaller delivery volume, has a greater

likelihood of achieving a better clinical outcome. In particular, it is our strong belief that restoring AADC in the dopaminergic axonal terminals results in a more physiologic endpoint than does expression of AADC in the post-synaptic striatal neurons, as is the case after putaminal delivery. Studies in transgenic dopamine-deficient mice with tyrosine hydroxylase deficiency demonstrate better motor outcomes when restoring tyrosine hydroxylase expression in midbrain dopaminergic neurons (Hnasko et al. 2006), than when tyrosine hydroxylase expression was induced in striatal neurons (Szczypka et al. 2001).

In both mouse studies, hyperkinetic activity was observed after restoration of tyrosine hydroxylase expression. Hypersensitivity of postsynaptic dopamine receptors is postulated to be the mechanism underlying this hyperkinetic activity. A separate study in the same population of dopamine-deficient mice demonstrated reversibility of striatal dopamine receptor hypersensitivity after chronic L-dopa treatment (Kim et al. 2006), suggesting that receptor hypersensitivity responsible for hyperactivity would be corrected with the chronic restoration of dopamine production.

The post-treatment hyperkinetic activity observed in mice is analogous to the involuntary movements (*dyskinesias*) observed in patients with congenital dopamine deficiency when levodopa treatment is initiated (Pons et al. 2013). In the proposed trial in patients with AADC deficiency, our aim is to restore dopamine production through gene transfer. We anticipate that treated subjects will develop dyskinesia after AAV2-hAADC delivery. Dyskinesia may emerge approximately one month after surgery, and eventually resolve as dopamine production stabilizes at the new, increased level leading to reduction of dopamine receptor hypersensitivity. If post-therapy dyskinesia does occur, we have a protocol for their assessment and management (see 7.4.6, Management of post-therapy dyskinesia).

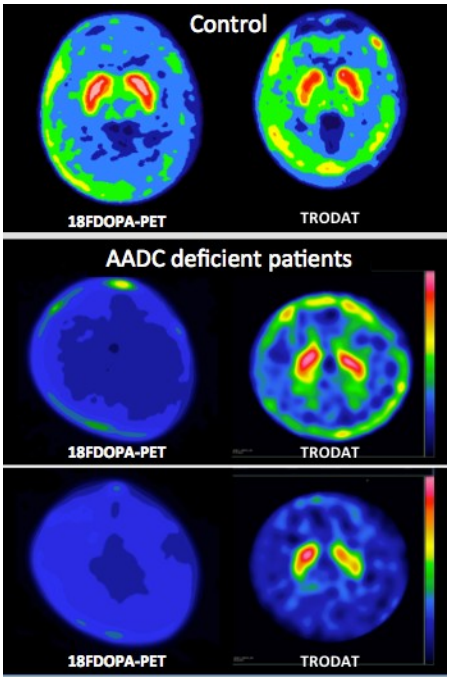


Figure 1: Imaging results from healthy children and children with AADC deficiency

All subjects will receive bilateral delivery of AAV2-hAADC. AADC-deficient patients do not display symptomatic asymmetry and no asymmetry is observed on structural or functional (PET) imaging (see Figure 1 provided by Dr. Lee, Taiwan National Hospital).

Unilateral treatment carries the additional risk of causing motor dysfunction through asymmetry in the dopaminergic system that would be impossible to control with medications. A second negative feature of unilateral treatment would be the risk of inducing antibody formation to AAV2, precluding the effective treatment of the second side in a patient who obtains benefit from the unilateral infusion. Furthermore, there would be additional risks and challenges for the patients if a second neurosurgical procedure were required to treat the other side at a later time.

2.1.3 Rationale for the Starting Dose and Infusion Volume

The selection of the infusion volume and vector dose levels for the intended clinical study is based upon extensive non-clinical data, as well as prior and ongoing clinical studies.

Overall brain volume reaches its maximum by the end of the first decade of life with gray matter structures developing in advance of that of the cerebral white matter. The brain of a child aged 7-11 years has completed 95% of its anticipated growth (Caviness et al. 1996). Cortical gray matter reaches its

maximum volume by 4 years of age whereas white matter volume continues to increase through the second decade of life (Pfefferbaum et al. 1994). The ratio of gray to white matter declines over time, reflecting both a reduction in gray matter and an increase of white matter volume beyond the first decade of life (Jernigan and Tallal 1990; Jernigan et al. 1991; Pfefferbaum et al. 1994).

The volumes of the human ventral tegmental area (VTA), and SNc are 150 mm^3 and 700 mm^3 respectively (Eapen et al. 2011), with the volume of the SNc being 140 mm^3 (Ziegler et al. 2013). In order to adequately cover the VTA, we will deliver 30 µL and 50 µL of AAV2-hAADC to the SNc. This calculation is based on data showing a ratio of 3:1 between the volume of vector infused into the NHP brain and the volume of AAV2-hAADC gene transfer (Su et al. 2010). Because the VTA is located adjacent to the midline, we reduced the volume of infusion to 30 µL in order to avoid leakage into the ventricular system.

In a nonclinical safety study (**S12-10383**) in nonhuman primates (NHP), doses of 60 µL (30 µL/hemisphere) of 5×10^{10} vg (8.3×10^{11} vg/mL) or 5×10^{11} vg (8.3×10^{12} vg/mL) were administered into the VTA/SN region without any adverse events. Because larger volumes of AAV2-hAADC vector could not be infused in this study (the vector would leak outside of the target area), a 60-µL infusion (30 µL per hemisphere) is the optimal volume. The corresponding structures in human children are 10-fold greater in volume. However, allowing for structural differences as well as clinical experience from our AAV2-hAADC studies in Parkinson's disease and the Taiwanese AADC deficiency study where a dose of 1.6 x 10^{11} vg of AAV2hAADC was delivered (Hwu et al. 2012), the planned doses were adjusted to 1.3 x 10^{11} vg (low dose) and 4.2 x 10^{11} vg (mid dose) in 160 µL (30 µL in the VTA and 50 µL in the SN on each side). The long-term safety study in NHP and results of a Phase 1 AAV2-hAADC clinical study in subjects with PD support the dose selection in the current clinical study.

More recently, further delivery experience has been gained as AADC deficiency patients (n=11) have been treated in Europe (Poland) under an ethics committee (EC)-approved compassionate use program (CUP). In order to increase safety of the delivery, subjects treated under CUP received AAV2-AADC in a single-cannula insertion and using a higher volume (150 µL/side) and vector concentration (up to $4.2\text{x}10^{12}$ vg/mL). The optimized procedure reduced was safe and well-tolerated by all subjects. Based on this evidence and in order to minimize the surgical risk, the vector for Groups 3 and 4 (ages 4-13 y and >13 y, respectively; n≤12 subjects per group) will be delivered at the same concentration used in Group 2

($2.6x10^{12}$ vg/mL) in a larger volume of up to 300 µL per side (for up to a total of 600 µL). The increased total volume of infusion is intended as a maximum volume, and termination of delivery will remain at the discretion of the operating neurosurgeon who will monitor distribution in real-time by intraoperative MRI. This vector dose is 2.8-fold lower than estimated human equivalent dose calculated for the highest dose tested in the GLP toxicology study in NHP (Table 2).

Table 2: Dose Justification and Scaling Factor

Study	Group	Volume (µL)	Vector conc. (vg/mL)	Dose (vg)	HED (vg)*
Non-Clinical Studies					
NHP GLP tox	Low High	60 60	8.3×10^{11} 8.3×10^{12}	5.0×10^{10} 5.0×10^{11}	4.5×10^{11} 4.5×10^{12}
Clinical Studies					
First PD study	Low Mid	200 200	1.1×10^{12} 3.7×10^{12}	2.3×10^{11} 7.5×10^{11}	NA
Current PD study	Mid High	900 900	8.3×10^{11} 2.6×10^{12}	7.5×10^{11} 2.3×10^{12}	NA
Current AADC Deficiency CUP		300	4.2×10^{12}	1.3×10^{12}	NA
Proposed Clinical Study					
AADC Deficiency	Low Mid High	160 160 600	8.3×10^{11} 2.6×10^{12} 2.6×10^{12}	1.3×10^{11} 4.2×10^{11} 1.6×10^{12}	NA NA NA

* HED: Human Equivalent Dose is based on a 10-fold difference in brain volume between Cynomolgus monkey and a human child.

3 STUDY OBJECTIVES

The overall objective of this study is to determine the safety and efficacy of AAV2-hAADC delivered to the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) in children with aromatic L-amino acid decarboxylase (AADC) deficiency. Specifically, the study will assess:

- Safety, as measured by adverse events (AEs), safety laboratory tests, brain imaging, and the relationship of AEs to study/surgical procedures or to AAV2-hAADC.
- Biological activity of AADC, measured by assays of neurotransmitter metabolites and FDOPA PET imaging.
- Clinical responses to treatment with AAV2-hAADC. The primary clinical outcomes will reflect the predominant motor deficits of loss of motor function and dystonic movements.

3.1 Primary Endpoints

Safety: Assessment of AE or SAE and its relationship to study surgery, infusion, or treatment effect (graded as definite, probable, possible, unlikely or unrelated).

- Adverse Events and Serious Adverse Events
- Post-operative MRI and/or CT (with contrast if clinically indicated)
- Clinical laboratory assessments (hematology, chemistry, immunology)

Biological Activity: Demonstration of effective restoration of AADC function by assays of CSF neurotransmitter metabolites and F-DOPA PET imaging.

3.2 Secondary and Exploratory Endpoints

To obtain preliminary data for clinical response by assessing the magnitude and variability of changes in specific outcomes.

The principal clinical outcome measures are:

- Motor function, as assessed by the Gross Motor Function Measure (GMFM-88)
- Frequency of oculogyric episodes, as measured by a Behavior Diary

Secondary clinical outcome measures include:

- Assessment of subject disability, as assessed using the Pediatric Evaluation of Disability Inventory (PEDI); adaptive behavior, as assessed using Vineland Adaptive Behavior Scale; Patient's Global Impression of Change (PGI-C); and quality of life, as determined using the Pediatric Quality of Life Inventory (PedsQL) and caregiver's priorities with the Caregiver Priorities and Child Health Index of Life with Disabilities (CP Child).

Although we recognize that the utility of established developmental and cognitive assessments may be limited because of the study population's severe physical disability, we will use the following:

- Peabody Developmental Motor Scales 2nd edition (PDMS-2)

4 STUDY DESIGN

This study is an adaptive, single-stage dose-escalation, open-label safety and efficacy study of AAV2-hAADC delivered by real-time image-guided convection-enhanced delivery bilaterally into the SNc and

VTA of pediatric patients with AADC deficiency.

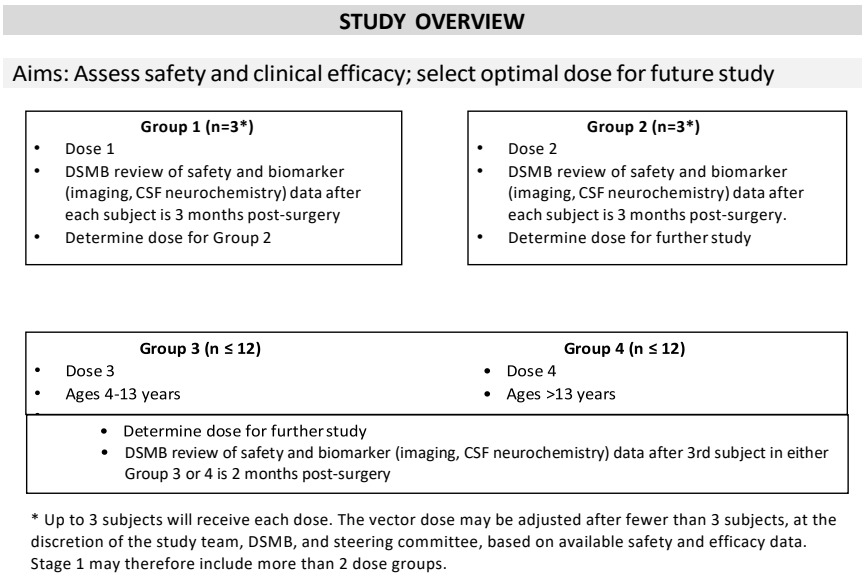


Figure 2: Flowchart for Study Treatment and Dose Escalation

There will be 31 subjects in this study. The primary aim is to determine the dose for future clinical trials based on safety, biomarkers, of pharmacological activity of AADC and clinical outcomes.

The first 6 subjects will be enrolled sequentially into 2 dose groups, Group 1 followed by Group 2. Initially, up to 3 subjects initially will be enrolled in Group 1 and treated with a single dose of AAV2 hAADC (1.3×10^{11} vg, delivered as an infusate volume of up to 160 µL of vector at concentration of 8.3×10^{11} vg/mL) on Day 0. Enrollment in Group 2 may commence after the last subject in Group 1 is treated and followed through Month 3 post-surgery, with the approval of the data safety monitoring board (DSMB) and the FDA. If dose escalation is warranted, total vg in Group 2 will be increased by factor of 3.1 (Table 2). Volume of infusion will remain constant between Group 1 and Group 2.

The final safety and clinical outcome assessments will be performed 1 year post-surgery. A follow-up analysis will be performed for 5 years post-surgery to assess safety and clinical status updates. An overview of the study schedule on a per-subject basis is presented in Figure 3.

Up to 24 additional subjects will receive a larger volume of infusion (up to a maximum of 300 µL) in each hemisphere to cover both VTA and SNc with a single cannula insertion (Table 3). Groups 3 and 4 will be enrolled in parallel, will each include up to 12 subjects ages 4-13 years and >13 years,

respectively, and will receive the same vector concentration as Group 2. DSMB will review safety data after the 3rd subject (in either cohort) reaches 2 months post-surgery. By that time, it is likely the report will include safety data for 3 subjects followed for at least 2-3 months, which is the most meaningful period for detecting surgery and dose-related complications.

Table 3: Dose Groups

	Cohort 1	**Cohort 2**	**Cohort 3**	**Cohort 4**
No. of Subjects	N=3	N=4	N≤12	N≤12
Age (years)	5-9	4-6	4-13	>13
Vector Concentration (vg/mL)	8.3×10^{11}	2.6×10^{12}	2.6×10^{12}	2.6×10^{12}
Total Vg	1.3×10^{11}	4.2×10^{11}	1.6×10^{12}	1.6×10^{12}
Total Volume of Infusion	160 µL	160 µL	600 µL	600 µL
Cannula Insertions	2 per side	2 per side	1-2 per side	1-2 per side

5 SELECTION AND ENROLLMENT OF SUBJECTS

We anticipate that the initial patients screened for this study will have severe motor impairment and motor developmental delay. Patients must also have tried currently available treatments without any obvious benefit. Because it would take at least 12 months to evaluate response to medications and determine lack of motor benefit, newly diagnosed patients will be excluded.

For the first group of 3 patients, the primary goal is to assess procedural safety. The minimum age for the first group of patients will be 5 years. The selection of this lower age limit is intended to minimize the risks because response to standard therapy is less certain in younger children, and there are increased risks associated with skull fixation in very young children. Based on previous clinical experience in the treatment of neurotransmitter disorders, younger patients may ultimately have the potential to benefit the most from AADC gene transfer. Thus, the Investigator will contact the Agency after the first 3 subjects have been treated to discuss modifying the age requirement to enroll younger patients. Although it is anticipated that younger patients may have greater potential than older patients to benefit from AADC gene transfer, there is no evidence that older patients would not also benefit.

5.1 Inclusion Criteria

1. Definite diagnosis of AADC deficiency, confirmed by at least two of the following three criteria: (1) CSF neurotransmitter profile demonstrating reduced HVA and 5-HIAA, and elevated 3-OMD concentrations; (2) Plasma AADC activity less than or equal to 5 pmol/min/mL; (3) Molecular genetic confirmation of homozygous or compound heterozygous mutations in *DDC*.
2. Age 4 years and up.
3. Failed to derive adequate benefit from standard medical therapy (dopamine agonists, monoamine oxidase inhibitor, pyridoxine or related form of Vitamin B6).
4. Unable to ambulate independently (with or without assistive device).
5. Cranium sufficiently developed, with sutures closed, to enable surgical placement of SmartFrame® system on the skull for MRI-guided stereotactic targeting.
6. Brain MRI does not show any conditions or malformations that are clinically significant with respect to risks for stereotactic brain surgery.
7. Parent(s)/legal guardian(s) of the subject must agree to comply with the requirements of the study, including the need for frequent and prolonged follow-up.
8. Parent(s)/legal guardian(s) with custody of subject must give their consent for subject to enroll in the study.
9. Stable medication regimen for treatment of AADC deficiency: (i.e. no new medications introduced for at least 6 months, and no existing medication dose changes for at least 3 months prior to Baseline).
10. Baseline hematology, chemistry, and coagulation values within the normal pediatric laboratory value ranges, unless in the Investigator's judgment, the out of range values are not clinically significant with respect to subject's suitability for surgery.

5.2 Exclusion Criteria

11. Intracranial neoplasm or any structural brain abnormality or lesion (e.g., severe brain atrophy,

white matter degenerative changes), which, in the opinion of the study investigators, would confer excessive risk and/or inadequate potential for benefit.

12. Presence of other significant medical or neurological conditions that would create an unacceptable operative or anesthetic risk (including congenital heart disease, respiratory disease with home oxygen requirement, history of serious anesthesia complications during previous elective procedures, history of cardiorespiratory arrest), liver or renal failure, malignancy, or HIV positive.

13. Previous stereotactic neurosurgery.

14. Coagulopathy, or need for ongoing anticoagulant therapy.

15. Contraindication to sedation during surgery or imaging studies (SPECT, PET or MRI).

16. Receipt of any investigational agent within 60 days prior to Baseline and during study participation.

17. Evidence of clinically active infection with adenovirus or herpes virus on physical examination.

6 STUDY METHODS

Subjects will be followed by a series of clinical assessments for a period of 2 years and undergo a long-term follow-up of annual visits up to 5 years from AAV2-hAADC administration (surgery date). Although not analyzed as part of the safety assessment portion of this study, patients will be screened yearly for up to five years after surgery. Scheduled visits are summarized in Schedule of Events (Appendix 1). An overview of the study schedule on a per-subject basis is presented in Figure 3.

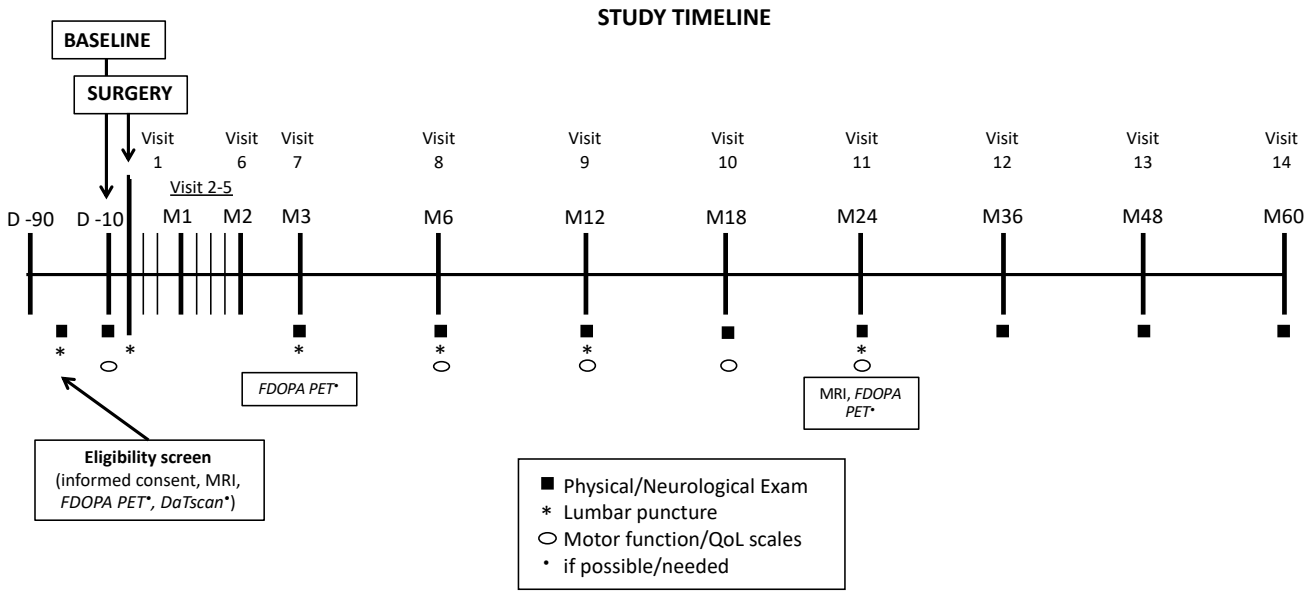


Figure 3: Study Schedule

6.1 Recruitment

Subject recruitment will be conducted through 3 main sources: (1) in collaboration with two foundations, the Pediatric Neurotransmitter Diseases (PND) Association (USA), and the AADC Research Trust (UK), which both maintain registries of children with AADC deficiency; (2) via the International Working Group on Neurotransmitter Related Disorders (iNTD), a group of physicians who treat patients with AADC deficiency; and (3) notification about the trial through the following organizations and societies: the National Organization for Rare Diseases (NORD), Genetic Alliance, Child Neurology Society mailing list, ChildNeuro Listserve. Details of the clinical protocol will be listed on the ClinicalTrials.gov website and on a study-specific website.

6.2 Informed Consent Procedures

The proposed clinical research study is complex and requires a substantial time commitment from the subject and family. For this reason, the informed consent process will occur as a series of steps, through which families will learn about the rationale, objectives, and logistics of the trial. This will take place in a series of meetings with members of the study team over a number of weeks. The final study consent will be signed prior to the first trial-related procedure. The study investigators will ensure the subject's parents/guardians:

- understand the experimental nature of the intervention,

- have a thorough understanding of the procedures and evaluations involved in the trial over a period of several years,
- are aware of the potential risks, and
- have realistic expectations, including the possibility that there will be no benefit.

Anticipated steps in the process:

1. Parent(s)/guardian(s) will acquire general information about the trial from a written summary made available through the recruitment channels outlined above. Or, if group information sessions are organized by parent-run foundations, study investigators will discuss the trial in more detail and be available to answer questions.

2. During the pre-screening process, study investigators will meet with parent(s)/guardian(s) in person, or by telephone, for individual discussion. A preliminary discussion of risks and benefits will occur, and any specific concerns will be addressed. The time commitment required will be explicitly described.

3. If the parent(s)/guardian(s) of the subject wish to proceed to the in-person screening stage, they will travel to meet in person with one of the study investigators at the beginning of the visit. The screening process and the study will be reviewed. Potential risks of the screening tests, operative procedure, and subsequent follow-up procedures will be described and reviewed in detail. The discussion will be documented by a written summary within 48 hours.

4. Parent(s)/guardian(s) assessment. At this point, the parent(s)/guardian(s) understanding and expectations of study participation will be assessed, primarily to determine if they have realistic expectations of the potential risks and benefits to the subject.

5. Following the screening procedures, if the subject is eligible for ongoing participation in the study, the parent(s)/guardian(s) will have a final discussion with a study investigator to determine their commitment to the next phase of the trial which includes the baseline assessments, the surgical procedure, and long-term follow-up. If the parent(s)/guardian(s) agree, they will confirm their willingness to proceed by signing the informed consent document on the final page. The two-step process will assure they have an improved understanding of the risks and commitment to return for all follow-up visits over 60 months. A copy of the signed consent will be provided to the parent(s)/guardian(s) at each stage of the process and the original will be kept with study records.

7 STUDY RELATED TREATMENT AND PROCEDURES

7.1 Screening Procedures

7.1.1 Pre-screening questionnaire

In the first stage of screening, the following information will be forwarded to the study investigators for review:

- Documentation of the subject's diagnosis of AADC deficiency with the presence of *at least two* of the following three criteria:

    1. CSF neurotransmitter metabolites demonstrating reduced HVA and 5-HIAA and elevated 3-OMD concentrations
    2. Plasma AADC activity less than or equal to 5 pmol/min/mL
    3. Molecular genetic confirmation of two homozygous or compound heterozygous mutations in *DDC*.

- A detailed medical questionnaire regarding the subject's course of symptoms directly related to AADC deficiency, current and past medications, other past medical and surgical history (especially as relevant to assessment of anesthetic risk), developmental history, and current level of function.
- The subject's medical records, and discharge summaries from all previous relevant hospital admissions.
- Brain MRI images if available. A brain MRI will be performed for all subjects that progress to the in-person screening stage.
- If eligibility criteria are met, a **medication review** will be performed to determine if any medications need to be tapered and/or discontinued prior to the in-person screening visit

    a. Medications that may interfere with DaTscan (refer to Appendix 8). DaTscan may be performed if, after reviewing the subject's medical history and screening materials, the study clinical team deems necessary to assess the integrity of the subject's nigrostriatal pathway.

b. Levodopa. (Exogenous levodopa would cause elevation of CSF 3-OMD beyond the level due to the primary disease, and confound post-operative measurements that will be relied upon to assess the effect of gene transfer)

The study investigators will review the pre-screening information. If the subject is a candidate for advancement to the in-person eligibility screen, the study team will then arrange for subject travel to the center.

7.1.2 In-person Eligibility Screen (Day -90 to D-11)

Screening tests and procedures will be conducted at the study center over the course of 1-2 weeks:

- The parent(s)/legal guardian(s) of the subject will meet with one of the study investigators to review the **consent** form in detail. A visit with a social worker will be scheduled as part of the informed consent process. The consent form must be signed prior to any study procedures being performed.
- Neurological and anesthesiology evaluation: The subject will be evaluated by the study neurologist. In addition, **anesthesiology evaluation** will be performed. Patients with AADC deficiency have an increased risk of hypoglycemia, hypotension, and bradycardia during periods of physiological stress due to peripheral catecholamine deficiency. Particular factors to be assessed include: 1) the appropriate protocol for pre-procedure fasting if the subject has any history of fasting hypoglycemia; 2) any history of symptomatic hypotension or bradycardia; 3) history of upper airway or respiratory complications.
- A **contrast brain MRI** will be performed to examine brain anatomy, exclude any structural abnormalities that would make the subject ineligible, and for the purposes of neurosurgical planning.
- An **[^{18}F]-FDOPA PET** may be performed as a marker of baseline AADC activity if study team determines that additional data are required to confirm the diagnosis. In cases when it is not in the subject's best interest to travel and undergo examination under anesthesia the PET scan might be not performed.
- **DaTscan™ Ioflupane I-123 SPECT** imaging may be performed if after reviewing the subject's medical history and screening materials the study clinical team deems necessary assess the integrity of the nigrostriatal pathway.

- A **lumbar puncture** will be performed to obtain a sample of CSF for neurotransmitter analysis (baseline sample #1). The LP will be done with sedation if necessary and may be scheduled at the same time as the MRI.
- **Blood** will be collected for plasma AADC activity, 3-O-Methyldopa, routine hematology and clinical chemistry, coagulation, and AAV nAb and AAV PCR analysis.
- **Urinalysis** will be performed, and female subjects with reproductive capacity will have a urine pregnancy test.

Data obtained during the Screening period will be reviewed by the study investigators. If at the end of the Screening period, the subject is eligible for inclusion in the study and his/her parent(s)/guardian(s) have provided consent, the caregivers will be instructed how to complete a behavior diary. The diary of motor and behavioral symptoms should be completed twice between the Screening and Baseline visits, each over a consecutive 7-day period at least 1 month apart. A log of the timing, duration, and severity of oculogyric crises will be maintained on an ongoing basis.

Participation in the treatment component of the study will be confirmed with the parents/guardians by the study investigators prior to scheduling of the actual surgical procedure. The parents/guardians will confirm their consent to proceed by completing the final signature page of the informed consent document.

7.2 Baseline Assessments (D -10 to D -1)

The subject and parent(s)/legal guardian(s) will meet with members of the study team for Baseline clinical evaluations within 10 days to 24 hours before study drug administration. The following tests and procedures will be performed:

- The Investigator will review medications and the symptom diaries and perform a physical and neurological examination.
- Motor function (Gross Motor Function Measure [GMFM]-88), cognitive, and developmental assessments will be performed.
- Parent(s)/legal guardian(s) will complete the Pediatric Quality of Life Questionnaire (PedsQL), Pediatric Evaluation of Disability Inventory-Computer Adaptive Test (PEDI-CAT) and CP Child questionnaires and Vineland-II survey.
- A second set of baseline blood, CSF, and urine samples will be collected for neurotransmitter

metabolite, AADC enzyme analysis and 3-O-Methyldopa on the day of surgery, following induction of anesthesia and prior to the start of the surgical procedure. A type andscreen will be obtained at the discretion of the neurosurgeon.

7.3 Study Treatment and Dosing

The surgical procedure to deliver the AAV2-hAADC vector will be performed in a magnetic resonance (MR) suite with the skull-mounted SmartFrame® and ClearPoint® targeting system (or similar FDA-approved system that allows for MR-controlled delivery of AAV2-hAADC vector). The procedure will be performed by a neurosurgeon experienced with MR-based stereotactic placement of MR-based delivery of therapeutics and/or DBS leads into the brain. Subjects will be secured in a head-frame and four target sites (bilateral SNc and VTA) will be located by MR images of the brain.

For Groups 1 and 2, 80 µL of AAV2-hAADC will be delivered: 50 µL in SNc and 30 µL in VTA in 2 separate cannula passes. Recently, 11 AADC deficient patients have been treated with the same vector in the Mazovian Bródnowski Hospital in Warsaw Poland under an EC-approved CUP. Under this program the first 5 subjects received the same amount of total vg as Group 2 (4.2×10^{11} vg), administered in a larger volume of infusion (150 µL) with a single-cannula insertion strategically positioned to cover both SNc and VTA in the same infusion. Subsequent patients (n=6) received the same volume of a 1.6-fold higher concentration than Group 2 (4.2×10^{12} vg/mL). The modification of the surgical delivery reduced the surgical risks (lower number of cannula insertions and associated risks, shorter surgical and anesthesia times) and demonstrated a favorable safety profile (no AE/SAE related to the study drug or surgical procedure) and was well-tolerated by all subjects.

Based on this experience, and in order to improve the safety and reduce the procedure time, for Groups 3 and 4 the operating neurosurgeon will plan to deliver the vector with the same cannula-insertion in each hemisphere aiming to cover both SNc and VTA in the same infusion. The cannula will be positioned in between both structures and a volume of up to 300 µL per side will be administered at a flow rate of 1-15 µL/min. The use of 1 or more cannula insertions to cover the SNc and VTA on each hemisphere (i.e., 1 cannula insertion for SNc/VTA or 1 cannula insertion for SNc plus 1 cannula insertion for VTA) will remain at the discretion of the operating neurosurgeon on the day of surgery based on pre-surgery planning that considers individual subject variations in the anatomy of target sites and planned trajectory. To accommodate possible anatomical and trajectory needs, the Vi delivered in a single infusion will be

based on MR imaging of the infusate for up to 300 µL per side to cover SNc/VTA combined, or separately as in Groups 1 and 2. Near real-time monitoring of the infusion by MRI-contrast admix to the vector solution will allow the neurosurgeon to monitor the infusion, adjust the volume as needed and determine the final volume that is infused at each site based on best judgment for delivery to each SNc/VTA while minimizing off-target distribution.

If significant backflow or distribution into non-targeted areas is observed at a particular cannula placement, the infusion at that site will be stopped and consideration will be given to moving the cannula to a new position within the SNc/VTA region of the midbrain. Likewise, if continued infusion at a particular cannula placement is not observed to be increasing distribution within the SNc/VTA region, infusion at that site will be stopped. The larger volume gives the operating neurosurgeon greater discretion to help match the volume of infusion with the size and shape of the SNc/VTA, which may vary from side-to-side and between patients. The number of planned cannula placements will remain at two sites per target as specified in the current protocol. If distribution of the vector is sub-optimal, the cannula may be repositioned to allow remaining vector to be infused in the SNc. The majority of the surgical time is required to 1) place burr holes in the skull at the entry points, 2) position the targeting system, 3) align the cannula guide with each target site by intraoperative MRI, and 4) place the MRI-compatible infusion cannula. With the single cannula pass delivery, the infusion time should be 30-60 minutes for the SNc/VTA per hemisphere, reducing the total infusion time for both structures bilaterally less than 2 hours. This represents a reduction of up to 50% of the infusion time form the way the vector was originally delivered in Groups 1 and 2 (SNc infusion (50 µL) time - 30-60 minutes; VTA infusion (30 µL) time - 20-60 minutes for the VTA; total infusion time for both structures bilaterally - up to 4 hours). Shortening the infusion time will reduce anesthesia time, which is important since AADC deficiency subjects present severe autonomic and hemodynamic regulatory dysfunctions and are at risk of anesthesia complications for several reasons (i.e., nasal congestion, temperature instability, hypotension, and hypoglycemia; Swoboda et al. 1999; Swoboda et al. 2003)..

7.3.1 Investigational Drug

The AAV2-hAADC vector consists of an adeno-associated virus, serotype 2 (AAV2) containing human AADC complementary DNA (cDNA), human cytomegalovirus (CMV) promoter and 3'UTR sequences. AAV2-hAADC is supplied in 0.5 mL aliquots as a suspension at a target concentration of 4.9×10^{12} vector genomes per mL. The vector and excipient for dilution will be supplied to the clinical site by the Clinical

Vector Core, Children's Hospital of Philadelphia.

7.3.2 Handling, Storage and Disposal of Drug

The vector will be stored in an Investigational Drug Pharmacy at <-60˚C. Just prior to use, the material will be thawed by allowing it to come to ambient temperature.

Any manipulation of the vials or the contents in preparation for clinical use will be performed in the Investigational Drug Pharmacy with standard safety measures.

Any remaining material and supplies coming into contact with the vector will be disposed of following the same procedures as for handling biohazardous human clinical samples.

7.3.3 Dosing and Dose Escalation

AAV2-hAADC vector will be delivered bilaterally into the SNc and VTA of the midbrain in a single surgical setting via SmartFlow® cannulae (MRI Interventions Inc.; Product code NGS-NC-02) connected to Medfusion infusion pumps (Smiths Medical Inc.). Convection-enhanced delivery (CED) will be used, with an increasing infusion rate (1-15 µL/min) to maximize distribution of the vector within the target. Both the SmartFlow® cannula and Medfusion pumps will be used "off-label" in this study and are considered experimental for this application.

The flowchart for staged dosing is shown in Figure 2. Three subjects meeting the entry criteria will be enrolled sequentially in Group 1; a subject must be followed through Month 3 (90 days) post-surgery before the next subject in the group may be treated.

A Data and Safety Monitoring Board (DSMB) established by the NIH will review safety data after each subject has been treated and will review the accumulated safety and efficacy data. The DSMB will be notified of the occurrence of dose limiting toxicities (DLT) (see Section 22.2 for definition) within 5 business days of the initial report to the Sponsor. If 1 subject experiences a DLT, treatment of additional subjects will not proceed until review and approval of the DSMB. If 2 or more subjects experience a DLT, both treatment and enrollment will be stopped, pending DSMB review. The occurrence of serious safety problems may lead to discontinuation of further treatments, based on pre- determined Stopping Rules.

The vector dose (volume and/or concentration) may be adjusted after the treatment of <3 subjects in

Group 1 if the study team, DSMB, and study Advisory Committee determine, based on review of available data, that dose escalation or reduction is warranted.

The DSMB will review study data once the third subject in Group 1 has reached 3 months post-surgery (Fig. 2) and the DSMB report will be shared with FDA. With the approval of the DSMB and FDA three subjects also will be enrolled in Group 2. Subjects in Group 2 also will be enrolled sequentially, with a minimum of 45 days between dosing of each subject. The DSMB review process for Group 2 will be the same as that for Group 1. After the last subject in Group 2 reaches Month 6 post-surgery, the DSMB will review safety and clinical outcome data for all subjects in Groups 1 and 2. Clinical outcome endpoints include improvement of motor symptoms and gains in motor function. If DSMB review is positive and no safety concerns are raised by the experience in Groups 1 and 2, the following groups (Groups 3 and 4) will be dosed with no time limitations other than those inherent to the scheduling availability for the Screening, Baseline and Surgery visits. For Groups 3 and 4 DSMB will review safety data after the 3rd subject (in either cohort) reaches 2 months post-surgery. By that time, it is likely the report will include safety data for 3 subjects followed for at least 2-3 months, which is the most meaningful period for detecting surgery and dose-related complications. After that, the DSMB will review safety data every 6 months.

7.3.4 Definition of Dose-limiting Toxicity

DLT is defined as any ≥Grade 3 toxicity, based on the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.03, that is determined by the Investigator to be at least possibly related to AAV2-hAADC.

7.4 Procedures for Delivery of AAV2-hAADC

7.4.1 Preoperative Procedures

Subjects will undergo standard preoperative evaluations in preparation for surgery. This will include pre-operative visits with the study neurosurgeon and anesthesiologist. The medical history will be taken with regards to any recent changes in medical status and a review of current prescribed and over-the-counter medications. Routine clinical laboratory analyses, including CBC with platelets, blood chemistries, PT/PTT and urinalysis, will be reviewed (from screening visit), as well as electrocardiogram. At the discretion of the clinical team, additional clinical assessments specific to concurrent medical conditions

to ensure subject safety may also be obtained.

Patients with AADC deficiency have an increased risk of hypoglycemia, hypotension, and bradycardia during periods of physiological stress due to their peripheral catecholamine deficiency. Treating physicians will be made alert to these risks to ensure appropriate preparation and close monitoring during all procedures requiring anesthesia, and post-operative care in the intensive care unit.

7.4.2 CED and Imaging of Distribution

To overcome the problems associated with current delivery methods, we and others have investigated the use of convection, or pressurized infusion, to deliver and distribute molecules and genetic vectors to targeted sites within the central nervous system (CNS). Previous methods of delivering AAV into the parenchyma by simple injection have not been effective at distributing vector over significant volumes of target tissue. In contrast, a technique known as CED (convection-enhanced delivery) uses a specially designed reflux-resistant cannula to permit high infusion pressures to be exerted at the cannula tip, thereby overcoming the interstitial pressure in the brain and allowing the infusate to permeate large volumes of targeted areas in a homogeneous manner. Moreover, by co-infusing a chelated Gadolinium contrast reagent (gadoteridol), the distribution of the infusate can be accurately measured by MR imaging. The distribution of the gadoteridol closely matches distribution of the infused AAV2 vector with no evidence of toxicity (Fiandaca et al. 2009) (Su et al. 2010; Richardson et al. 2011c). Pre-clinical and clinical studies have shown that co-delivery of gadoteridol, a widely used contrast agent for MR-imaging marketed for intravenous administration and used in this protocol off-label, and real-time MR-imaging helps to ensure that the target region is exposed to the investigational agent while minimizing exposure of non-targeted CNS tissues (Voges et al. 2003; Lonser et al. 2007b).

The MR images acquired during the CED procedure will be analyzed after surgery to measure the volume of gadoteridol contrast enhancement. Calculations will be made of the total volume of gadoteridol distribution and percentage of VTA and SN coverage by gadoteridol. Correlations between coverage and other outcome measures, such as PET signal may be assessed.

7.4.3 Infusion Procedure

7.4.3.1 Stereotactic Placement of Infusion Cannula

Surgery will be performed in a 1.5T or 3T interventional MRI suite. The team at UCSF has used such

suites to implant more than 330 deep brain stimulation electrodes since 2004, as well as 7 laser ablations (2 Parkinson's disease, 2 pediatric hypothalamic hematomas and 4 seizure disorders) and 12 CED infusions for gene therapy (8 Parkinson's disease and 4 brain tumors). In addition, many standard neurosurgical procedures, including craniotomies and needle biopsies have been performed in the interventional MRI suites at UCSF.

For the procedure, the subject will be placed under general anesthesia and the subject's head secured to an MRI-compatible head-holder attached to the MRI gantry. A combination of volumetric T1, slab T2, and slab inversion recovery sequences will be acquired and bilateral VTA and SNc infusion sites and cannula trajectories will be identified. Trajectories will be trans-frontal and planned to avoid cortical veins and sulci. The surgical sites will be prepared and draped in the usual neurosurgical fashion and locally anesthetized. One or two transverse linear incisions will be made in the region of the coronal suture. A burr-hole in the skull will be placed, and the dura will be coagulated and opened. Bilateral skull-mounted aiming devices (SmartFrame®) will be mounted over each burr-hole. End-lumen cannula with a progressive bullet-shaped step design connected to an infusion pump with a syringe containing a mixture of AAV2-hAADC and gadoteridol (2 mM) will be placed with its tip in the SNc initially, and 50 µL of vector will be infused per side with continuous MR imaging to monitor progress of the infusion. The cannula will then be repositioned into the ventral tegmental area followed by infusion of 30 µL of vector per side. For Groups 3 and 4, the operating neurosurgeon will plan to position the cannula with its tip in the space between SNc and VTA initially, and up to 300 µL of vector will be infused per side with while MR imaging to monitor progress of the infusion. Depending on the shape of the infusion cannula may be inserted deeper along the same trajectory to allow for more complete coverage of VTA and SN regions. Infusion may be terminated before reaching full infusion volume once planned coverage is achieved. The use of 1 or more cannula insertions to cover the SNc and VTA on each hemisphere (i.e., 1 cannula insertion for SNc/VTA or 1 cannula insertion for SNc plus 1 cannula insertion for VTA) will remain at the discretion of the operating neurosurgeon on the day of surgery based on pre-surgery planning that considers individual subject variations in the anatomy of target sites and planned trajectory.

7.4.3.2 AAV2-hAADC Infusion

Convection-enhanced delivery will be used, with a stepped infusion rate to maximize distribution of the vector within the target. In order to monitor the pattern of infusion, serial intraoperative T1-weighted images will be obtained until its completion (images will be acquired every 4 minutes consecutively). If

significant backflow along the cannula is detected, the infusion rate may be decreased or the cannula may be advanced slightly. If further backflow is still detected or leakage into non-targeted areas is observed, and less than half of the intended volume is infused, the infusion will be stopped and consideration will be given to moving the cannula to a new infusion site. If backflow or leakage into non-targeted areas is seen, and more than half of the intended volume has been infused, the infusion will be stopped and no further infusion will be performed in that region. During SNc/VTA, SNc or VTA infusion, if infusate is seen distributing along the white matter tracts (descending tracts) in the tegmentum, suggesting off target infusion into cranial nerves III - IV, the infusion will be immediately halted. All subjects will remain on study regardless of total volume infused. Data will be analyzed with and without subjects that receive less than the intended dose of AAV2-hAADC.

Once the full AAV2-hAADC dose has been delivered, the infusion will be terminated. The cannula will be removed after terminating the infusion and the incision will be closed with absorbable plates over the burr-holes, along with absorbable sutures in the galea and skin. The subject will then undergo final imaging to rule out hemorrhage after cannula removal and will then be removed from the scanner.

7.4.4 Postoperative Procedures

After leaving the MRI suite, the subject will be extubated and taken to the Pediatric Intensive Care Unit (PICU) for observation for the ensuing 48-72 hours. If the subject is stable on postoperative day 3, he/she will then be transferred to the children's floor for the remainder of the hospital stay.

7.4.5 Discharge Criteria

The duration of the subject's hospitalization is expected to be 2-3 days, excluding any complications. After hospital discharge, subjects will attend study center visits on an outpatient basis. Discharge criteria are as follows:

For transfer from PICU to inpatient floor:

4. Stable cardiorespiratory status off mechanical ventilation and pressor support for at least 24 hours
5. Awake and alert
6. Stable neurological examination for 24 hours
7. No evidence of hemorrhage on brain MRI 48 hours after surgery
8. If post-surgery brain MRI shows hemorrhage, then PICU discharge will be subject to

neurosurgical clearance following further clinical monitoring and follow-up brain imaging.

From hospital:

1. Baseline level of alertness
2. No requirement for respiratory support or oxygen
3. Able to tolerate usual diet or enteral feeds
4. Stable neurological examination for 48 hours

From clinical site:

1. Able to comfortably tolerate all routine daily activities including mobility, sleep, and feeding
2. Stable medication regimen for 3 days.

7.4.6 Management of Post-therapy Dyskinesia

Gene transfer is expected to result in an increase in brain dopamine production within approximately 1 month after surgery. One anticipated effect of the increase in dopamine is the occurrence ofinvoluntary jerky or writhing movements (dyskinesia). Based on the prior experience with putaminal transfer of hAADC in a total of 8 patients with AADC deficiency (Hwu, 2012), and the experience of others in treating congenital dopamine deficiency with levodopa (Pons, 2013), dyskinesia is expected to subside over weeks to months, as compensatory changes in postsynaptic dopamine receptors occur in response to the increase in nigrostriatal dopamine.

Subjects will be monitored frequently for dyskinesia in the 3 months after gene transfer by the study team via teleconference, and the clinical course will be followed closely and documented via serial video examinations at specific visits. The severity of dyskinesia is to be assessed by the Investigator using the guidelines in Table 3.

Suggested guidelines for medical management of dyskinesia can be found in Appendix 9.

Table 3: Grading of Post-therapy Dyskinesia

Grade 1	Mild, low amplitude movements which cause no functional impairment. Child is able to sit (supported or unsupported) comfortably and has regular periods of uninterrupted sleep.
Grade 2	Mild-moderate movements, associated with minor disruption to routine care or interference with sitting. Child may have some difficulty settling to sleep.

Grade 3	Moderate-severe movements causing significant discomfort and/or self-injury. Unable to get to sleep. No evidence of metabolic compromise (i.e. normal hydration status, electrolytes, renal function, with CK <1,000 IU/L)
Grade 4	Severe movements causing significant discomfort and/or self-injury. Unable to get to sleep. Evidence of metabolic decompensation (e.g. elevated BUN or creatinine, CK >1,000 IU/L

Modified from Dystonia Severity Action Plan (Lumsden, 2013, Dev Med Child Neurol).

8 SAFETY AND RESEARCH ASSESSMENTS

Safety assessments include:

- co-infusion of gadoteridol with AAV2-hAADC to confirm dosing to appropriate region
- intraoperative and post-operative MRI
- grading of adverse events
- physical examinations
- routine clinical laboratory analysis (hematology, clinical chemistries, immunologic assessments)

8.1 Clinical Assessments

8.1.1 Adverse Event (AE) Log

A systematic assessment of adverse events and side effects will be performed at all study visits based on standardized discussions with the subject's caregiver using open-ended questions. Information will be systematically recorded on an Adverse Events (AE) Log which includes type of AE, dates of onset and resolution, severity, perceived relationship to experimental therapy. The severity of each AE will be rated based on the NCI Common Terminology Criteria for Adverse Events v4.0 (CTCAE; Appendix 2).

8.1.2 Medication Log

The use and dosages of medications will be systematically recorded. A substantial decline in medication requirement may provide evidence of a beneficial effect of our experimental therapy.

8.1.3 Behavior Diary

A standardized Behavior Diary (Appendix 7) will be used to capture the frequency and duration of clinical symptoms caused by brain dopamine dysregulation.

- One category of symptoms is attributable to dopamine deficiency, and includes oculogyric

crises and dystonic episodes.

- The second category is treatment-related involuntary movements, or dyskinesia, which is attributed to dopamine receptor hypersensitivity induced by chronic dopamine deficiency. Patients may experience dyskinesia pre-treatment, due to their oral medications, and/or post-treatment, due to increased endogenous production of brain dopamine as a result of the gene transfer.
- The third category of symptoms is pre- or post-treatment psychiatric and behavioral symptoms, which may manifest as changes in behavior and/or sleep.

Subject's caregivers will be trained in the completion of a behavior diary by study center personnel during Screening. The behavior diary then is to be completed twice during the period between the Screening and Baseline visits, each over a consecutive 7-day period at least 1 month apart. The diary will be reviewed by study center personnel for legibility and completeness. If there are any difficulties in interpreting the diary, the caregiver will be retrained. If the caregiver reports difficulty in recalling how to record the subject diary for subsequent visits, he or she will be retrained by study center personnel. In addition to the intermittent 7-day Behavior Diary, caregivers will be asked to keep a separate log of oculogyric crises in real time during study participation.

8.1.4 Medical and Neurologic Examinations

A general medical and a full neurologic examination will be conducted and any abnormality will be recorded.

Some neurological examinations will be recorded on video, with the aim of documenting changes in motor function from baseline over time, and documenting the evolution of involuntary movements that may emerge after surgery. Video examinations may also be viewed and scored by independent, blinded raters in the process of data analysis. All subjects will have a brief examination recorded on video at Baseline, 1 month, weekly between 1 and 2 months, 3 months, 6 months, 12 months, 24 months, 36 months, 48 months and 60 months (See Video Protocol, Appendix 10). If motor symptoms emerge at other points in time, additional recordings may be made at the discretion of one of the study neurologists.

Video recordings will be transferred immediately from the camera to an encrypted desktop or laptop computer (endpoint device), and deleted from the camera storage card. The files will be stored indefinitely.

Steps will be taken to protect subject identity and privacy as much as possible. Subjects will not be identified by name on the video. Each video filename will contain only the subject's numerical identification code and date of recording. Recording of the face will be limited to only what is necessary to adequately demonstrate the relevant clinical features. If the study investigators show brief video excerpts at any future scientific presentations, the identity of subjects will be protected by masking the face unless it is not possible to do so in the event that a facial symptom is being illustrated.

8.2 CSF, Blood and Urine Neurotransmitter Analytes

8.2.1 CSF Neurotransmitter Analytes

CSF will be collected by lumbar puncture, to be performed with sedation if necessary. (See Appendix 3 for CSF collection protocol)

Metabolites to be assayed:

- Homovanillic acid (HVA)
- 5-Hydroxyindoleacetic acid (5-HIAA)
- 3-O-methyldopa (3-OMD)
- 5-methyltetrahydrofolate (5-MTHF)

8.2.2 Plasma AADC and 3-O-Methyldopa Activity

Plasma AADC enzyme activity and 3-OMD levels will be determined by quantitative methods (Hyland and Clayton 1992). It will be performed by Medical Neurogenetics, Atlanta GA (Appendix 3).

8.2.3 Urine vanillactate

Vanillactate is a metabolite of 3-OMD, and may be assayed in urine, together with other urine organic acids, using tandem mass spectrometry. A quantitative assay is under development (Dr. Simon Heales, Great Ormond Street Hospital, London, UK). Urine samples will be collected so that we may correlate urine vanillactate with CSF 3-OMD levels to explore its future potential as a biomarker that may be performed non-invasively.

Sample requirements: 5 mL random fresh urine, frozen immediately at -20 C for storage and transport.

8.3 Clinical Measures

The following measures will assess motor and cognitive function, adaptive behavior, and patient/family

quality of life. They are collectively referred to in the Schedule of Events table as "Motor /QoL Measures." These measures will be obtained at Baseline, 3 months, 6 months, 12 months, 18 months, 24 months, 36 months, 48 months and 60 months.

8.3.1 GMFM-88

The GMFM is a standardized instrument to evaluate changes in gross motor function over time in children (Russell et al. 2002). It has been validated in patients with cerebral palsy. It is designed to assess how much of an activity a child can accomplish, rather than the quality of the motor performance, across five dimensions: lying/rolling, sitting, crawling/kneeling, standing, and walking/running/jumping. Each dimension contributes 20 percentage points towards a total possible score of 100. We expect that, at baseline, most subjects will have very limited voluntary movement and will lack the ability to sit without support (baseline score <30). If gene transfer successfully restores dopamine production, we anticipate that subjects will experience gradual improvement in trunk control, mobility, and voluntary reaching and grasping movements. We estimate that this could correspond with an approximate 10 point increase in GMFM score at 12 months. (Appendix 4).

8.3.2 Pediatric Evaluation of Disability Inventory (PEDI)

The PEDI, originally published in 1992 (Haley et al. 1992), was revised as a computer adaptive test, the PEDI-CAT, in 2012 (Dumas and Fragala-Pinkham 2012; Dumas et al. 2012). The PEDI-CAT is designed for use with children and youth (birth through 20 years of age) with a variety of physical and/or behavioral conditions. It measures abilities in 3 functional domains: Daily Activities, Mobility, Social/Cognitive. (A fourth domain, Responsibility, assesses abilities in more complex tasks that enable independent living, and will not be applicable to our study population). The PEDI-CAT takes 10-15 minutes for parents to complete. The software utilizes Item Response Theory statistical models to estimate a child's abilities from a minimal number of the most relevant items or from a set number of items within each domain. All respondents begin with the same item in each domain in the middle of the range of difficulty or responsibility and the response to that item then dictates which item will appear next (a harder or easier item), thus tailoring the items to the child and avoiding irrelevant items. For each item, the child's ability is rated on a 4-point difficulty scale with responses ranging from 'Unable' to 'Easy'. The CAT generates scaled scores, which can be used to follow changes in a child's functional skills over time (Appendix 5).

8.3.3 Vineland Adaptive Behavior Scales-2nd edition

The Vineland-II is designed to measure the adaptive behavior of individuals across the domains of communication, daily living skills, and socialization. The questions are administered in the form of an interview with the child's caregivers and will be administered at Baseline, and 12 and 24 months.

8.3.4 Pediatric Quality of Life Inventory (PedsQL™)

The PedsQL is a modular instrument for measuring health-related quality of life in children and adolescents ages 2 to 18 years. The PedsQL 4.0 Generic Core Scales questionnaire (Varni et al. 2001; (Appendix 6)) contains 23 items and takes approximately 5-10 minutes for parents to complete. Children aged 5 and over may complete a corresponding self-report questionnaire if able. (We anticipate that most if not all of the subjects in this study will not be able to complete it themselves). The questionnaire yields information on the physical, emotional, social and school functioning of the child during the previous 4 weeks. Each item on the scale is reverse-scored on a 0-4 scale, and raw scores are transformed to a 0-100 scale and averaged, so that higher scores indicate better quality of life.

8.3.5 Patient/Caregiver Global Impression of Change (PGI-C)

Caregivers will be asked to provide a global impression of change in the subject's overall functional level and quality of life on a 7-point scale ranging from 1 (much worse) to 7 (much better). The PGI-C will be completed 3, 6, 12, 18, and 24months after gene transfer.

8.3.6 Caregiver Priorities and child health Index of Life with Disabilities Questionnaire (CP Child)

Caregivers will be asked to assess the health status, comfort, wellbeing and ease of caregiving of children with severe cerebral palsy. We are using cerebral palsy as the closest disability comparison to AADC deficiency.

8.3.7 Cognitive and Developmental Assessments

We will explore the potential utility of established developmental and cognitive assessments in our study population. Their use may be limited as a result of our subject's severe physical disability. Developmental assessments, including the Peabody Developmental Motor Scales 2nd edition (PDMS- will be attempted. The study neuropsychologist will try to administer cognitive assessments in which responses may be given by pointing rather than speaking (e.g., Peabody Picture Vocabulary test, Raven's Progressive Matrices).

8.4 Neuroimaging

8.4.1 MR-imaging

MR-scans will be performed pre-operatively as part of the screening process, during the infusion procedure, and then at 48 hours and 24 months postoperatively.

Scans may be obtained at other time-points if deemed necessary by the investigators. Sequences will be obtained on a 3T field strength MRI and will include full head 3D high resolution T1 with contrast, 3D T2, Diffusion Tensor Imaging (DTI), and susceptibility weighted imaging (SWAN). The projected duration of the imaging study will be less than 1 hour. The 48 hour MR scan will use a 'rapid' protocol and will consist of a 3D T2 FSE sequence, and axial diffusion; the anticipated duration of this study is 15 minutes. Sedation is typically not required for the 'rapid' protocol. Any abnormalities, seen as a change from the screening MR imaging and not anticipated from the surgical procedure, will be reviewed by the study neurosurgeon and neuroradiologist and reported as an AE.

8.4.2 [^{18}F]-FDOPA Positron Emission Tomography (PET)

[^{18}F]-FDOPA is a PET radiopharmaceutical that has been used for approximately 30 years to image dopamine synthesis capacity. It has been used for clinical research purposes in thousands of patients to study neuropsychiatric disorders, patients with neuroendocrine disorders, as well as in healthy controls. It has been widely used in both patients and normal control subjects. Details of the production have been provided in IND #15660.

Brain [^{18}F]-FDOPA PET scans may be performed at screening, 3 months and 24 months. In cases when it is not in the subject's best interest to undergo procedure under prolonged anesthesia and/or subject presents travel limitations, the PET scan might be not performed. The dose of 0.043mCi/kg for F-DOPA is a weight-based dose calculation that will allow a safe dose yet produce a more detailed scan and optimal visualization of the brain (Barthlen et al. 2008; Liu et al. 2016). Weight based dosing will not exceed 3 mCi per injection (RUA approval: RU133031-01A). We anticipate that subjects will require sedation to remain still during the scan. Sedation will be administered by a pediatric anesthesiologist. PET scans will be analyzed for quantification of AADC activity and differences in the distribution of AADC activity between baseline and post-surgery scans.

Fluorinated radiopharmaceutical emits a little amount of radiation. They have been used in the research and clinical arenas for more than 30 years with no measurable adverse effects.

8.4.3 DaTscan™ Ioflupane I-123 SPECT

DaTscan imaging may be performed as part of subject screening if, after reviewing the subject's medical history and screening materials, the study clinical team deems necessary will be performed to assess the integrity of the nigrostriatal pathway. Ioflupane I-123 selectively binds to presynaptic dopamine transporters and provides a method for imaging nigrostriatal terminals in the striatum. DaTscan™ is an FDA-approved radiopharmaceutical used in conjunction with single photon emission computed tomography (SPECT) scan for use in adults. DaTscan will be performed at the screening visit to document nigrostriatal pathway integrity.

Iodine-123 is a cyclotron-produced radionuclide that decays to ^{123}Te by electron capture and has a physical half-life of 13.2 hours. The recommended dose is 111 to 185 MBq (3 to 5 mCi) administered intravenously in adults. The estimated radiation absorbed doses to an average adult from intravenous injection of DaTscan are shown in Table 1. The values are calculated assuming urinary bladder emptying at 4.8-hour intervals and appropriate thyroid blocking (iodine-123 is a known Auger electron emitter).

Table 4: Estimated Radiation Absorbed Doses from DaTscan

ORGAN / TISSUE		ABSORBED DOSE PER UNIT ADMINISTERED ACTIVITY (μGy / MBq)
*The absorbed dose to the colon wall is the mass-weighted sum of the absorbed doses to the upper and lower large intestine walls, D Colon = 0.57 DULI + 0.43 DLLI [Publication 80 of the ICRP (International Commission on Radiological Protection); Annals of the ICRP 28 (3). Oxford: Pergamon Press; 1998]		
	Adrenals	12.9
	Brain	17.8
	Striata	230.0
	Breasts	7.8
	Esophagus	10.0
	Gallbladder Wall	26.4
	Stomach Wall	11.2
	Small Intestine Wall	21.2
GI Tract	Colon Wall *	39.8
	Upper Large Intestine Wall	38.1
	Lower Large Intestine Wall	42.0
	Heart Wall	12.9
	Kidneys	10.9
	Liver	27.9
	Lungs	41.2
	Muscle	9.4
	Osteogenic Cells	28.2

ORGAN / TISSUE	ABSORBED DOSE PER UNIT ADMINISTERED ACTIVITY (μGy / MBq)
Ovaries	16.8
Pancreas	13.0
Red Marrow	9.2
Skin	6.0
Spleen	10.4
Testes	8.5
Thymus	10.0
Thyroid	9.0
Urinary Bladder Wall	53.1
Uterus	16.1
Total Body	11.3
EFFECTIVE DOSE PER UNIT ADMINISTERED ACTIVITY (μSv / MBq)	21.3

The Effective Dose resulting from a DaTscan administration with an administered activity of 185 MBq (5 mCi) is 3.94 mSv in an adult.

The DaTscan injection may contain up to 6% of free iodide (iodine 123). To decrease thyroid accumulation of iodine-123, a dose up to 100 mg of Potassium Iodide Oral Solution or Lugol's Solution will be administered.

8.5 Hematology, Clinical Chemistry and Immunological Assessments

Routine hematology and clinical chemistry assessments will be performed on blood samples collected at Screening, 1 week after surgery, and as clinically indicated in the post-operative period. Blood coagulation parameters will be assessed 1-10 days prior to surgery.

Blood samples will be collected at Screening, and Months 1, 3, and 6 to measure levels of neutralizing anti-AAV2 antibodies (nAb): Samples will be stored at <-60°C and analyzed in groups after completion of each dose group. Samples will be analyzed in conjunction with any observed clinical events. These assays are being obtained for research purposes, and safety information cannot be obtained from them alone.

Hematology: Hemoglobin (Hgb), Hematocrit (Hct), Red Blood Cells (RBC), Mean Corpuscular Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), White Blood Cells (WBC), Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, Platelets

Clinical Chemistry: Blood Urea Nitrogen (BUN), Creatinine, Sodium, Potassium, Chloride, Bicarbonate, Magnesium, Phosphorus, Calcium, Glucose, Total Protein, Albumin, Total Bilirubin, Alkaline Phosphatase (ALP), Serum Glutamic Aminotransferase/Aspartate Transaminase (SGOT/AST), Serum Pyruvate Aminotransferase/ Alanine Aminotransferase (SGPT/ALT), Gamma-glutamyl transpeptidase (GGT)

Coagulation studies: PT, aPTT, INR, Thrombin Time, and Fibrinogen

Immunological Assessment: Determination of anti-AAV2 Capsid Neutralizing Serum Antibodies. Samples will be collected at Screening and Month 3 and banked until further analysis.

9 SCHEDULE OF STUDY ASSESSMENTS

Patients with AADC deficiency are a fragile population, thus, when possible, the option will be open for the subject to have a scheduled visit at the clinical site or, if it is not in the subject's best interest to travel, the study team will visit her/him at their home place.

The Schedule of Assessments is detailed in Appendix 1; assessments by Visit are listed below.

9.1 Pre-Screen

- Medical History
- Prior Medications
- Current Medications (screen for medications that need to be discontinued prior to LP; see section 10, "Medications for AADC Deficiency," below)
- Review previous results of CSF neurotransmitter analysis, molecular genetic testing, and plasma AADC enzyme assay

9.2 Screening (Day -56 to Day -11)

- Informed Consent
- Medical History
- Physical Exam/Vitals
- Neurological Exam
- Anesthesia Consult

- Social Worker Visit
- 12 lead ECG
- Brain MRI
- FDOPA PET Scan (AADC activity), if possible
- Baseline Neurotransmitter Analytes (CSF, Blood, Urine) - #1 of 2
- Prior Medications
- Current Medications
- Clinical Chemistry
- Hematology
- Coagulation studies
- Immunology (AAV-nAb)
- Blood Sample (PCR)
- If needed, DaTscan™ Ioflupane I-123 SPECT

9.3 Baseline (Day -10 to D -1)

- Physical Exam/Vitals
- Neurological Exam - videotaped
- Type and Screen (may be done on day of surgery – day 0, at discretion of surgeon)
- Motor/Quality of Life Measures
- Cognitive Assessment
- Behavior Diary
- Oculogyric Crisis Log
- Adverse events
- Current medications

9.4 Surgical Delivery of AAV2-hAADC – Pre Surgery & Surgery (Day 0)

- Physical Exam/Vitals
- Neurosurgical Exam
- Baseline Neurotransmitter Analytes (CSF, Blood, Urine) - #2 of 2
- MRI-Guided surgery
- Adverse events
- Current Medications

9.5 Hospital Stay (Day 2)

- Physical Exam/Vitals
- Brain MRI (rapid scan protocol) at ~ 48 hours
- Neurosurgical Exam
- Current Medications
- Adverse Events

9.6 Treatment Follow-up Phase

9.6.1 Visit 1 (day 10 +/- 4 days; at clinical site or clinic close to home)

- Physical Exam/Vitals
- Neurological Exam
- Neurosurgical Exam (includes suture removal)
- Current Medications
- Adverse Events

9.6.2 Visit 2-5 (day 28, 35, 42, 49 +/- 3 days - videoconference)

- Behavior Diary
- Oculogyric Crisis Log
- Current Medications
- Adverse Events

Between Visit 2 and Visit 5 subjects will be contacted by videoconference at least once per week (Days 28, 35, 42 and 49). This is the period when post-surgery dyskinesia may emerge and require active management.

9.6.3 Visit 6 (day 56 +/- 3 days - videoconference)

- Current Medications
- Adverse Events

9.6.4 Visit 7 (Month 3 +/- 14 days)

- Physical Exam/Vitals
- Neurological Exam - videotaped
- Social Worker Visit as indicated/requested

- Clinical Chemistry
- Hematology
- Immunology (AAV nAb)
- Neurotransmitter metabolites (CSF, Blood, Urine)
- Motor/QoL Measures
- PGI-C
- Behavior Diary
- Oculogyric Crisis Log
- FDOPA PET Scan (AADC), if possible
- Blood sample (PCR)
- Current Medications
- Adverse Events

9.6.5 Visit 8 (Month 6 +/- 14 days)

- Physical Exam/Vitals
- Neurological Exam - videotaped
- Social Worker Visit as indicated/requested
- Neurotransmitter metabolites (CSF, Blood, Urine)
- Motor/QoL Measures
- Behavior Diary
- Oculogyric Crisis Log
- Current Medications
- Adverse Events

9.6.6 Visit 9 (Month 12+/- 14 days)

- Physical Exam/Vitals
- Neurological Exam - videotaped
- Neurotransmitter metabolites (CSF, Blood, Urine)
- Motor/QoL Measures
- PGI-C
- Cognitive Assessment

- Behavior Diary
- Oculogyric Crisis Log
- Current Medications
- Adverse Events

9.6.7 Visit 10 (Month 18+/- 14 days)

- Physical Exam/Vitals
- Neurological Exam - videotaped
- Motor/QoL Measures
- PGI-C
- Behavior Diary
- Oculogyric Crisis Log
- Current Medications
- Adverse Events

9.6.8 Visit 11 (Month 24+/- 14 days)

- Physical Exam/Vitals
- Neurological Exam - videotaped
- Brain MRI
- Neurotransmitter metabolites (CSF, Blood, Urine)
- Motor/QoL Measures
- PGI-C
- Cognitive Assessment
- Behavior Diary
- Oculogyric Crisis Log
- FDOPA PET Scan (AADC), if possible
- Current Medications
- Adverse Events

9.6.9 Visit 12 (Month 36 +/- 14 days)

- Physical Exam/Vitals
- Neurological Exam - videotaped

- Motor/QoL Measures (only GMFM)
- Oculogyric Crisis Log
- Current Medications
- Adverse Events

9.6.10 Visit 13 (Month 48 +/- 14 days)

- Physical Exam/Vitals
- Neurological Exam - videotaped
- Motor/QoL Measures (only GMFM)
- Oculogyric Crisis Log
- Current Medications
- Adverse Events

9.6.11 Visit 14 (Month 60 +/- 14 days)

- Physical Exam/Vitals
- Neurological Exam - videotaped
- Motor/QoL Measures (only GMFM)
- Oculogyric Crisis Log
- Current Medications
- Adverse Events

10 MEDICATIONS FOR AADC DEFICIENCY

We anticipate that most subjects will be taking multiple medications for management of AADC deficiency at the time of entry into the trial. Medications commonly used to treat AADC deficiency, such as dopamine agonists, pyridoxine, pyridoxal phosphate, selegiline (or other MAO inhibitor), and trihexyphenidyl, are permitted. We require that no new medication have been started in the 6 months prior to Baseline, and that doses have been stable for 3 months prior to Baseline.

If a DaTscan is deemed necessary, it needs to be taken into account that some medications interfere with DAT SPECT (Appendix 8). The medications listed above do not, but some patients may be taking other medications. For example, selective serotonin reuptake inhibitors (SSRI's) interfere with DAT SPECT and would need to be discontinued before the scan (recommended stopping time prior to scan is ≤8 days

for all SSRI's except fluoxetine, which needs to be stopped 45 days prior).

Levodopa would cause elevation of CSF 3-OMD beyond the level due to the primary disease and confound post-operative measurements that will be relied upon to assess the effect of gene transfer. Levodopa must be discontinued at least 7 days prior to lumbar puncture.

At the time of pre-screening, all of the subject's medications will be reviewed. In the event that any subject needs to undergo a DaTscan and is taking levodopa or a medication that would interfere with DAT SPECT, a recommendation will be made to taper the medication off 3 months prior to the Screening visit.

Any patient treated with a rotigotine patch should have the patch removed prior to MRI scan (the patch contains aluminium).

11 POST-MORTEM BRAIN EXAMINATION

Each subject's parent(s)/guardian(s) will be informed that, in the event of death, even after completing the formal protocol, careful neuropathological examination of the brain is desirable and encouraged. Information will be provided to families regarding the process of brain donation. Gross and microscopic examinations will seek the location of needle tracks and the localization of the infusion sites. Post-mortem analysis may include assessment of frozen or fixed brain sections. Immunohistochemistry for AADC will be performed to assess gene transfer.

12 SUBJECT WITHDRAWAL / DISCONTINUATION OF STUDY

12.1 Voluntary Participation

Participation in this study is voluntary. All subjects have the right to withdraw at any point during the study with no penalty or loss of benefits to which the subject is otherwise entitled. Every attempt will be made to provide safety follow-up for the subject. Subjects who withdraw for reasons other than safety/tolerability will be replaced.

The Principal Investigator may withdraw a subject from the protocol if the subject is unwilling or unable to comply with the requirements of the protocol.

12.2 Discontinuation of the Study

In the course of the study, if the Investigator, the DSMB, or the Food and Drug Administration discover

conditions including, but not limited to, the discovery of an unexpected, serious, or unacceptable risk to subjects, the study will be discontinued.

13 RISKS/DISCOMFORTS

13.1 Neurosurgical Complications

Known complications of real time image guided brain infusion fall into four groups: 1) risks associated with intra-cerebral infusion, 2) risks associated with infusion of MRI contrast agent, 3) immediate complications that occur during the actual procedure, and 4) delayed complications that occur in the postoperative period.

13.1.1 Risks of Intra-cerebral Infusion

Direct delivery within the interstitial space of the brain has been safely performed in numerous clinical studies. Previous gene transfer studies for Parkinson's disease include direct infusion into the putamen, substantia nigra and subthalamic nucleus. Approximately 250 subjects have been treated in these previous studies.

No SAEs related to the AAV2-hAADC vector were reported in the two clinical studies, at UCSF and Jichi Medical University, completed to date (Christine et al. 2009; Muramatsu et al. 2010). In both open-label studies the same AAV2-hAADC vector was directly delivered to the putamen. However, out of the 16 subjects treated, four subjects had cerebral hemorrhages (two asymptomatic and two symptomatic with almost complete recovery). In the UCSF trial (10 subjects) two intracranial hemorrhages (one subject in each dose cohort) and a cerebral venous infarction (subject in low dose cohort) were early events that occurred on the day of surgery (temporally related; Christine et al. 2009). Of the 6 subjects enrolled in the Jichi Medical University study, one subject experienced a venous hemorrhage in the right frontal lobe beneath and anterior to the burr hole, which resulted in symptoms related to hemorrhage and surrounding edema. All hemorrhages occurred along the trajectory of the catheters but far from the infusion site and were unrelated to the infusion of AAV2-hAADC. The subjects in these original two studies were treated a number of years ago, and with improved targeting techniques being used in current trials, we believe the likelihood of significant hemorrhage is reduced. An additional 18 patients have been treated in ongoing trials without the occurrence of any cerebral hemorrhage (personal communication from PI's).

A critical aspect of the infusion procedure for this study is the use of convection-enhanced delivery (CED, allowing for small and large molecules to be distributed within deep brain structures in a highly reliable, homogenous, and targeted manner (Laske et al. 1997a; Lonser et al. 2002) (Gill et al. 2003). This technique has been used clinically to deliver immunotoxins over large volumes of brain parenchyma (several magnitudes larger than what would be used in this study) in patients with brain tumors. In a Phase 1 study conducted at the NIH by the Surgical Neurology Branch, there were reported 4 instances (in 2 of 28 patients) of cerebral hemorrhage occurring at 79 days (median) after the start of drug infusion, and 1 instance of hydrocephalus occurring 125 days after infusion. These complications were attributed to the tumor biopsy obtained before beginning the infusion or to the immunotoxin therapy itself, rather than catheter placement or this method of delivery. Another Phase 1 study of CED of immunotoxins in 15 adult tumor patients reported no complications from catheter placement or CED (Laske et al. 1997b). A Phase 2 study of CED of the Tf-CRM107 immunotoxin in patients with glioblastoma was recently completed at the NIH and cooperating centers, and again the infusion was not linked to any complications. More recently, there have been 11 AADC deficiency patients that received a CED infusion of AAV2-AADC into the midbrain under an EC-approved CUP at the Mazovian Bródnowski Hospital in Warsaw, Poland. No SAEs related to the investigational product or the surgical procedure were reported.

13.1.2 Risks of Gadoteridol Co-Infusion

Co-infusion of a gadolinium-based imaging agent allows MRI detection of vector distribution within the brain. No histopathologic evidence of gadolinium-related changes or toxicity have been observed in non-human primate studies when infused in the brain via CED. Gadoteridol (ProHance) has proved to be an excellent imaging marker for gene expression after intra-cerebral CED of AAV2 vectors.

Several clinical studies have been performed that included the direct infusion of gadoteridol in the human CNS with no evidence of toxicity related to gadolinium. A Phase 1/2 clinical trial for glioblastoma multiforme involved cerebral infusion of gadolinium-DTPA (1 mM) before delivery of a liposomal vector bearing the HSV-1-*tk* gene (Voges et al. 2003). The NIH has treated an 8-month-old male with neuronopathic (type 2) Gaucher's disease with glucocerebrosidase administered via CED to the brainstem co-infused with gadolinium-DTPA to monitor infusate distribution and a 3-year-old female with diffuse brainstem glioma with interleukin 13-*Pseudomonas exotoxin* administered via CED to the

brainstem co-infused with gadolinium-DTPA to monitor infusate distribution (Lonser et al. 2007a; Lonser et al. 2007b). Similar CED parameters (as outlined in this protocol) were used in these patients and they tolerated infusions without clinical or radiographic evidence of toxicity. More recently, a study was conducted at UCSF involving CED of gadoteridol (ProHance) and a retroviral replicating vector encoding cytosine deaminase into glioblastoma multiforme tumors (ClinicalTrials.gov identifier: NCT01156584).

Gadolinium-based contrast agents are marketed for intravenous administration to visualize lesions in the central nervous system with MRI. In a typical gadolinium-contrast enhanced MRI scan with ProHance, a 70-kg man would receive approximately 3,900 mg of gadolinium intravenously. In the study proposed here, subjects will receive gadoteridol at a maximum concentration of 2.8 mg/mL in a volume of 0.9 mL delivered intra-cerebrally, i.e. 2.5 mg gadoteridol, more then 1,500-fold less than the amount delivered during a typical intravenous MRI study. It should be noted that all subjects will also have intravenous gadoteridol administered for a routine contrast-enhanced MRI on the day of surgery to assist with surgical planning. Intra-cerebrally administered gadoteridol is used “off label” in this study and is considered experimental.

The most common side effect associated with systemic administration of gadolinium-based contrast agents is headache with an incidence of 4.8%. The majority of headaches are transient and of mild-to-moderate severity. Nausea is the second most common side effect at 2.7%. Injection site coldness/localized coldness is the third most common side effect at 2.3%. Dizziness occurs in 1% of patients. People with kidney disease are at risk for a serious reaction to gadolinium contrast called nephrogenic systemic fibrosis, which has resulted in a very small number of deaths.

13.1.3 Immediate Surgical Complications

Misalignment or slippage of the neurosurgical guidance system during surgery would result in loss of accurate target localization and any surgical infusion not completed by that point will be aborted if the error cannot be corrected. Air embolism, or entry of air into the venous circulation through the vascular spaces in the bone of the skull or by cerebral veins, can cause oxygen desaturation and hypotension if significant. The risk of this is much lower in this procedure than in many other stereotactic procedures as the subject is supine in the MRI. Air embolisms have not been observed in any of our MRI guided DBS implantations. Should an air embolism occur, the surgical field will be flooded with irrigation

solution immediately. If the surgeon and anesthesiologist deem that the embolism is causing persistent and/or significant oxygen desaturation or hypotension, the surgical procedure will be stopped. Subjects will undergo frequent MR imaging during surgery, which will allow the detection of any of intracranial hemorrhage that may occur. The procedure will be stopped if any evidence of intracranial hemorrhage is detected on imaging.

13.1.4 Delayed Surgical Complications

Cerebral hemorrhage or infarction has been observed with comparable brain surgery procedures (e.g., 1-3% of patients undergoing deep brain stimulation experience cerebral hemorrhage resulting in stroke). Hemorrhage could result from an arterial vessel puncture as a result of introducing the device into the brain, which would result in bleeding immediately or within 24 hours. Arterial bleeding events are more likely to be symptomatic with parenchymal involvement manifesting as acute neurological symptoms. A hemorrhage resulting from a venous vessel puncture could be slower to appear and more likely to be asymptomatic as the blood takes longer to accumulate. Delayed, asymptomatic hemorrhages or venous infarctions can be detected on postoperative MR or CT imaging; if found, they will be reported as an SAE but will not alter the evaluation schedule. Symptomatic hemorrhages or infarcts will be managed with supportive care and intervention appropriate to the degree and location of the lesion.

Infections can involve the surgical wounds superficially, or may be deep infections. Any evidence of infection will be reported immediately and will result in immediate evaluation by the neurosurgeon. Superficial wound infections may be treated aggressively with antibiotics. Deep infection may be explored surgically. No more than 1 subject will be expected to have a central nervous system infection based on a 9.0% rate (Weaver et al. 2009).

13.2 Immune Response, Vector and Gene Therapy Risks

Preclinical studies show that peripherally circulating neutralizing antibodies to AAV can affect the efficiency of transduction in gene transfer experiments (Peden et al. 2004; Sanftner et al. 2004; Peden et al. 2009). No adverse effects attributed to the presence of neutralizing AAV antibodies have been clinically observed, even in human subjects with high antibody titers prior to AAV delivery. Systemic levels of AAV2 antibodies will be assessed in this study and may be analyzed in conjunction with adverse events or other outcome measures.

Data from a hemophilia clinical trial involving the intrahepatic administration of AAV transducing factor IX revealed a transient transaminitis followed by decline in the patient's expression of factor IX (Mingozzi & High 2007; Li et al. 2007). The apparent immunological response has been attributed to immunodominant epitopes on the AAV capsid although the mechanism of antigen presentation is still obscure (Mingozzi et al. 2007). Although the CNS is relatively immunologically privileged, we will employ an ELISpot assay to assess T-cell immune responses in the study subjects**.**

Based on multiple clinical trials using the AAV2 viral vector, no adverse events have been noted, and are anticipated. Over 250 patients have received AAV2 for a variety of brain conditions without any direct adverse events associated with the vector. Overexpression of the AADC gene, a normal human gene, in the context of AADC deficiency may result in dyskinesias (see section below). However, this is an anticipated, and transient, event which would be consistent with a functional gene product in the SN, and would represent a proof of biological activity. We would treat patients with dyskinesia as needed and specified in the clinical protocol.

13.3 Risks of Imaging (MR imaging, PET)

13.3.1 Risks of MR imaging

People are at risk for injury from the MR imaging magnet if they have pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, older generation aneurysm clips (metal clips on the wall of a large artery), some prostheses (such as heart valves and cochlear implants) or shrapnel fragments. Subjects will be screened for these conditions before having any MRI scans. In addition, all magnetic objects (such as watches, coins, jewelry, and credit cards) will be removed before entering the MRI suite. Any patient treated with a rotigotine patch should have the patch removed prior to MRI (patch contains aluminium). Operating room personnel involved in the surgical procedure are also screened prior to entering the MRI suite as part of routine safety procedures.

It is not known if MRI is completely safe for a developing fetus. Therefore, all adolescents of childbearing potential will have a pregnancy test performed if there is a reasonable chance that they could be pregnant at screening. No scanning will be done if the pregnancy test is positive, and subjects who are discovered to be pregnant will be excluded from the study.

People with fear of confined spaces may become anxious during MR imaging. As study subjects will be

under anesthesia for their imaging procedures, we do not anticipate this problem occurring. Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss. Everyone having a MRI scan, either awake or under anesthesia, will be fitted with hearing protection.

The risks of an IV catheter for administration of contrast (Gadolinium) include bleeding, infection, or inflammation of the skin and vein with pain and swelling. Symptoms from the contrast infusion are usually mild and may include coldness in the arm during the injection, a metallic taste, headache, and nausea. In an extremely small number of patients, more severe symptoms have been reported including shortness of breath, wheezing, hives, and lowering of blood pressure. People with kidney disease are at risk for a serious reaction to gadolinium contrast called "nephrogenic systemic fibrosis" which has resulted in a very small number of deaths. Blood test results will be reviewed for kidney function prior to MRI scanning. Subjects will not receive Gadolinium for an MR scan if their kidney function is abnormal and it is felt that contrast would pose significant risk to them.

13.3.2 Risks of PET

This research study involves exposure to radiation from PET scans for all study subjects. The total amount of radiation received in this study is from three injections of [^{18}F] fluoro-L-Dopa (FDOPA) (0.043 mCi/kg per injection) and from the associated transmission scans with a Cs-137 external source. The dose of 0.043mCi/kg for F-DOPA is weight based and will not exceed the dose of 3.0 mCi per injection. The UCSF and OSU Radiation Safety Committees have reviewed the use of radiation in this research study and has approved this use as involving slightly greater than minimal risk (RU133031-01A), but is regarded as necessary to obtain the research information desired.

Subjects in this study will receive 3 scans over about 24 months. Although each organ will receive a different dose, the total amount of radiation exposure received from these procedures is equal to a uniform whole-body exposure of 12 mSv. This is equivalent to 4 times the yearly natural background of radiation in the US (3 mSv).

If any of the subjects undergoing PET scanning is a female of child-bearing potential, pregnancy testing will be performed (and must be negative) prior to each PET scan.

13.3.3 Risks of DAT SPECT

This research study involves exposure to radiation. DaTscan™ Ioflupane I-123 is a radiopharmaceutical agent that emits a small amount of radiation and is used to image the neurons that transport dopamine within the striatum. The UCSF and OSU Radiation Safety Committees have reviewed the use of radiation in this research study and has approved this use as involving slightly greater than minimal risk, but is regarded as necessary to obtain the research information desired.

Subjects in this study will receive DaTscan™ Ioflupane I-123 on SPECT scan during the baseline period. The dose of radioactive ligand will be calculated based on patient weight. Assuming a total dose of 185 mBq, individual organs will receive a different dose of radiation: 53 μGy to the urinary bladder wall, 9.2 μGy to blood-forming organs (e.g. marrow), 16.8 μGy to the ovaries, 8.5 μGy to the testes, and 17.8 μGy to the brain. The total amount of radiation exposure received from these procedures is equal to a uniform whole-body exposure of 3.9 mSv. This is equivalent to 1.3 times the yearly natural background of radiation in the US (3 mSv). As noted above in section 8.4.3, the DaTscan injection may contain up to 6% of free iodide (iodine-123). To decrease thyroid accumulation of iodine-123, a dose up to 100 mg of Potassium Iodide Oral Solution or Lugol's Solution will be administered. If any of the subjects undergoing DAT SPECT scanning is a female of child-bearing potential, pregnancy testing will be performed (and must be negative) prior to each scan.

13.4 Risks of Blood Samples

The risk of blood drawing includes a bruise at the needle insertion site and possible fainting.

13.5 Risks of Sedation

The risks associated with having sedation include: dizziness, drowsiness, allergic reaction, tiredness, weakness and the rare possibility of dry mouth.

Patients with AADC deficiency have an increased risk of hypoglycemia, hypotension, and bradycardia during periods of physiological stress due to their peripheral catecholamine deficiency.

13.6 Post-therapy Dyskinesia

Gene transfer is expected to result in an increase in brain dopamine production within approximately one month after surgery. One anticipated effect of the increase in dopamine is the occurrence of involuntary jerky or writhing movements (*dyskinesia*). Subjects will be monitored frequently for the emergence of

dyskinesia in the 2 months after gene transfer, and the clinical course will be followed closely and documented via serial video examinations. Refer to section 7.4.6 for further information. Guidelines for the management of post-treatment dyskinesia are in Appendix 9.

13.7 Behavioral Adverse Effects

Increased dopamine in mesocortical and mesolimbic circuits has the potential to cause behavioral effects such as agitation, vivid dreams, hallucinations, and compulsive behaviors. We will monitor subjects closely at each visit for changes in behavior or sleep that may be subtle indicators of such effects. If behavioral adverse effects occur, treatment should be promptly initiated. If the subject has co-existing dyskinesias requiring treatment with tetrabenazine, then this should be the first step (following protocol above) as behavioral symptoms may respond to the same treatment. If tetrabenazine is not required for treatment of dyskinesias, then treatment with quetiapine should be started, beginning at a low dose (6.25-12.5 mg daily) and increasing gradually while monitoring for adverse effects including hypotension.

14 SUBJECT SAFETY MONITORING

There will be ongoing extensive caregiver education before and after surgery to ensure that the caregiver reports all adverse events at the time they occur. All personnel involved in this study are provided with training and written guidelines concerning the definitions of adverse events (AE) and serious adverse events (SAE) and their responsibilities if they believe they have identified an AE/SAE, including reporting any SAE within 24 hours and the completion of the AE/SAE Form.

Monitoring of safety of individual subjects during study procedures will be performed by the medical staff and the research nurses. Safety assessments include: co-infusion of gadoteridol with AAV2-hAADC to confirm dosing to appropriate region, intra-operative and post-operative MRI, grading of adverse events, physical examinations, clinical laboratory analysis (hematology, clinical chemistries, immunologic assessments).

Adverse effects will be monitored systematically throughout the study with formal assessments at each evaluation visit. In addition, subjects will be encouraged to contact the investigators by telephone any time there is concern about side effects. If necessary, an unscheduled clinical visit with the investigator will be arranged. Subjects will have access to clinicians day and night for the duration of the study. The Principal Investigator, in collaboration with the Data and Safety Monitoring Board, will be responsible

for monitoring data collected to ensure the safety of subjects.

14.1 Toxicity Criteria

Toxicities and adverse events will be recorded and categorized according to severity, relationship to procedure, and relationship to the study procedure or study drug. Toxicity criteria are based on the NCI CTCAE version 4.0. The scale can be accessed at:

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf.

14.2 Criteria for Individual Subject Withdrawal

The subject may be withdrawn from the study at any time for the following reasons:

1. The subject's parents/Durable Power of Attorney desires to discontinue participation in this study.
2. Investigator's prerogative. The subject is unwilling or unable to comply with the protocol.

15 OUTCOME MEASURES

Initial outcome measures will be determined after all subjects in each cohort have reached one year after surgery. Follow-up analysis will be performed after all subjects have reached 2 years after surgery. A second longer-term clinical follow-up study of all subjects will occur for 5 years to obtain additional longitudinal outcome data for safety and efficacy measures.

15.1 Primary Outcome Measures

Safety:

- Assessment of AE or SAE and its relationship to study surgery, infusion, or treatment effect (graded as definite, probable, possible, unlikely or unrelated)

Biological Activity:

- Effective improvement of AADC function by assays of CSF neurotransmitter metabolites and PET imaging.
- Improvements in motor function and reduction of neurological symptoms as

measures of clinical efficacy resulting from AAV2-hAADC treatment.

15.2 Secondary Outcome Measures

- GMFM-88
- Oculogyric Crisis Log
- PEDI-CAT
- PedsQL
- Behavior Diary

16 SAMPLE SIZE CALCULATIONS AND DATA ANALYSIS

This is an open-label safety study that has not been designed as a powered efficacy study. Statistical analysis will be performed on quantifiable measures of clinical responses to treatment by comparing pre- and post-operative assessments, and analyzing any differences between the dose cohorts.

16.1 Sample Size

The number of subjects is based on the desire to gain adequate safety and preliminary clinical outcome information to support future work. Inferential statistical hypothesis testing is not the primary intent of the study. It is judged that the selected sample size, based on previous animal experience and empiric criteria, will provide acceptable clinical validity for the study objectives.

Previous Phase 1 studies involving innovative surgical and medical interventions for rare CNS disorders have historically enrolled similar numbers of subjects.

16.2 Data Analysis

Analysis of the study data will primarily be tabular, graphical and descriptive, with a view to elucidating the time course and pattern of responses to AAV2-hAADC treatment and thoroughly exploring the antecedents of any observed adverse effects of therapy. Particular attention will be paid to verifying that all subjects enrolled satisfy the stated inclusion criteria, and on checking for any protocol violations. Statistical analysis will be performed on quantifiable measures of clinical responses to treatment by comparing pre- and post-operative assessments, and analyzing any differences between the two dose cohorts.

Characteristics of the surgical procedure such as accuracy of cannula placement, number of cannula

passes, any events during infusion such as reflux or non-targeted delivery will be analyzed. Coverage of target structures will be measured on the MR images acquired during the CED procedure. Calculations will be made of the total volume of gadoteridol distribution, percentage covered by gadoteridol, and percentage of gadoteridol distribution contained within the target structures.

16.3 Safety Analysis

Safety data will be summarized for the total study population and the pooled safety data will be further broken down by the dose level. The disposition of the patients will be summarized by tabulating the number of screened, completed, and discontinued patients. The reasons for premature discontinuations will be tabulated. The extent of exposure to the study treatment will be summarized by tabulating the number of patients being exposed to each dose level.

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary. The treatment-emergent AEs (TEAEs), i.e., events which start or worsen during the study treatment will be summarized by body system and preferred term. In addition, separate summaries will be provided for TEAEs by severity and relationship to study drug, as well as adverse events leading to study drug discontinuation and serious adverse events. The tolerability will be assessed by number of patients having DLTs and with premature discontinuations of study treatment.

The other safety analyses include the evaluation of safety laboratory tests, vital signs and brain imaging. The safety laboratory tests and vital signs will be evaluated by summarizing the changes from baseline with descriptive statistics and by tabulating the number of patients with abnormal values. The evaluation of the brain imaging is based on post-operative MRI and/or computed tomography (CT) (with contrast if clinically indicated) findings.

16.4 Efficacy Analysis

For the evaluation of the efficacy, there will be two primary clinical outcome measures:

- Motor Function, as assessed by the Gross Motor Function Measure (GMFM-88). The total score will be derived as an unweighted average of the 5 dimension scores: lying and rolling (17 items); sitting (20 items); crawling and kneeling (14 items); standing (13 items) and walking, running, and jumping (24 items). Each dimension score will be defined as % of maximum score for the dimension in question.
- Frequency of motor behaviors, as measured by a Oculogyric Crisis Log: each recorded episode

will be classified as mild, moderate or severe. The area under the curve (AUC) of the diary symptoms will be used as the endpoint. The AUC will be calculated for each study month as a sum of duration of symptoms (hours) multiplied by the severity (0-3) of the symptom in question. The endpoint is the change from baseline (average of 3 months preceding the baseline) to 1 year (average of 3 months preceding the visit at Month 12).

The secondary analyses of the primary endpoints include the evaluation of the data at time points other than 1 year. In addition, the following endpoints will be evaluated as secondary analyses:

- Evaluation of each of the 5 GMFM-88 dimension scores separately.
- Evaluation of the OGC Log as total number of episodes, total duration of episodes and distribution of the severity scores.

All data collected during the study will be used for the evaluation of the clinical outcome measures. The evaluation will be focused on the following comparisons:

- Change from baseline to one year in all patients treated with the selected dose level (primary comparison). However, if no dose-response is seen, both dose levels will be included in the primary comparison.
- Difference in change from baseline to one year between the patients treated with the selected dose level and the other dose level used.

In addition to the comparisons defined above, efforts will be made to compare the changes seen in the present study to natural progression of the disease as assessed in The Natural History of Symptoms and Motor Function in the AADC Deficiency Study, a related external study at Washington University in St. Louis, led by Dr. Toni Pearson,. These comparisons may involve comparison of the changes between the treated patients from the present study and untreated patients, using endpoints derived from the elements of the primary outcome measures. The definition and analysis of these endpoints will depend on the availability of the data collected from The Natural History Study.

17 HUMAN SUBJECTS PROTECTION

17.1 Human Subjects Involvement and Characteristics

This protocol, advertisements (if applicable) and/or web-based information, written information to be provided to subjects' families, and stipends will be reviewed and approved by the study site Human Subjects Committee/IRB prior to enrollment of subjects in the study. A signed consent form will be obtained from parents/guardians of each subject. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the parents, or legal guardian, and this fact will be documented in the subject's record. Any revisions to the above-mentioned documents will also be submitted for review.

17.2 Adequacy of Protection Against Risks

There will be ongoing extensive caregiver education before and after surgery to ensure that the caregivers report all adverse events at the time they occur. All personnel involved in this study are provided with training and written guidelines concerning the definitions of adverse events (AE) and serious adverse events (SAE) and their responsibilities if they believe they have identified an AE/SAE, including reporting any SAE within 24 hours and the completion of the AE/SAE form.

Adverse events will be monitored systematically throughout the study with formal assessments at each evaluation visit. In addition, subjects will be encouraged to contact the investigators by telephone any time there is concern about side effects. If necessary, an unscheduled clinical visit with the investigator will be arranged. Subjects will have access to clinicians day and night for the duration of the study. The Investigators, in collaboration with the Data and Safety Monitoring Board, will be responsible for monitoring data collected to ensure the safety of subjects. Confidential identification codes will be used on all study data forms. Should results of the study be published or reported, individual names or other identifying information will not be used.

Treatment intervals between subjects have been set at a minimum observation period of 90 days between each treated subject and the next subject within the first group of 3 subjects. This will provide time to identify serious safety or tolerability problems that might arise.

All reasonable and appropriate activities will be employed to try to prevent adverse events and to minimize the consequences of any that do occur.

17.3 Safeguards for Vulnerable Populations

Children with AADC have limited capacity to understand the nature of their condition or the proposed treatment. Therefore, both parent(s)/guardian(s) will be asked to give their permission for their child to participate in the clinical study. We will request that the IRB waive the requirement for assent in this situation where the child is not capable of assent given his cognitive and emotional maturity.

Gaining informed consent from parent(s)/guardian(s) will be an ongoing/iterative process. Parent(s)/guardian(s) will have ample time to review the study information and consent form on the website should they have interest in participation in the study. Initial consent will be obtained prior to screening procedures. After screening, should the subject be eligible, parent(s)/guardian(s) will be asked to sign the consent form indicating their desire to move forward to the treatment phase.

Should any new information become available during the study that might affects the decision to continue participation, parent(s)/guardian(s) will be contacted to discuss that information and further provide consent should they wish to continue.

Investigators and study personnel are sensitive of the influence of therapeutic misconception about participation in the study. The informed consent has been designed to appropriately indicate that the treatment is experimental, and that no direct benefit is expected.

Parents will be provided with additional resources available on NIH and FDA Website to help them through the decision making process.

- NIH Website — Children and Clinical Studies: http://www.nhlbi.nih.gov/childrenandclinicalstudies/index.phphttp://www.nhlbi.nih.gov/childrenandclinicalstudies/index.php
- FDA Website — Should your child be in a clinical trial? http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm048699.htm

Families will also have visits with a Social Worker as part of the informed consent process and as needed or requested throughout the study duration. During these visits, families can discuss any issues they might not feel comfortable with discussing with the study personnel. The Social Worker will also make recommendations for further evaluation if any of the family members need additional support.

18 ANTICIPATED BENEFITS

18.1 Potential Benefits to Subjects and Others

Subjects in this study will have disabling symptoms that remain refractory to currently available medication therapies. Subjects may experience an improvement in symptoms after experimental therapy. Complications caused by their primary condition may progress more slowly than the natural course. We feel that the potential risks to our disabled and treatment-refractory subjects are reasonable in relation to the possible benefits of experimental therapy.

18.2 Importance of the Knowledge to be Gained

A careful, systematic scientific evaluation of the safety, tolerability and eventually the clinical effects of AADC gene transfer in AADC deficiency are scientifically rational and could lead to a breakthrough in disease-modifying therapy.

19 CLASSIFICATION OF RISK

Subjects may receive a direct benefit from participation in this study. There is greater than minimalrisk for all subjects in the study, but because of the possible benefit, the risks are reasonable.

20 CONSENT DOCUMENTS AND PROCESS

A copy of the informed consent form will be provided to the parent(s)/guardian(s) of potential study subjects. Parent(s)/guardian(s)will be encouraged to review it with relatives, friends or their primary care physician and be prepared for the face-to-face meetings with the study team.

As part of the informed consent process, parent(s)/guardian(s) will be informed about the most likely possible adverse effects of the operation to infuse the viral vectors. These include stroke, bleeding or infection. Parent(s)/guardian(s) will be informed about the potential side effects of viral vector delivery such as immunological reactions. They will be informed about the most common side effects reported in prior studies of AAV2-hAADC. Parent(s)/guardian(s) will be informed that AADC gene expression cannot be regulated and potential consequences of excessive dopaminergic function will be described, including dyskinesias, confusion, psychosis and impulse control disorders. As with other stereotactic brain procedures, there could be alterations in mood or cognitive functions. More information about potential adverse experiences is provided above in the section on Safety Monitoring.

Informed consent will be obtained after meetings involving parent(s)/guardian(s), the study coordinator and the study neurologist and neurosurgeon. The informed consent document will be reviewed in detail and there will be an opportunity to answer any questions or clarify any issues. The parent(s)/guardian(s) of the subject will provide consent by signing the informed consent document. One signed copy of the consent form will be provided to the parent(s)/guardian(s) and the original will be kept on file. A standard operative consent will also be obtained by the study neurosurgeon prior to actual procedure.

A copy of the blank Informed Consent document is provided separately.

21 DATA AND SAFETY MONITORING

The Principal Investigator will provide overall supervision of the study and review the data on a monthly basis. The data will be presented and reviewed by the Data and Safety Monitoring Board (DSMB) approximately 90 days after completion of treatment of every 3rd subject and as needed. Data will be presented and reviewed by the study site IRB annually in conjunction with the Continuing Review.

21.1 Data and Safety Monitoring Board, Medical Monitor and Advisory Committee

The study will incorporate oversight by a Medical Monitor, a Data Safety Monitoring Board, and an Advisory Committee to assess the safety data for each subject before proceeding with the next subject, as well as prior to dose escalation.

The roles and responsibilities of the DSMB will be described in detail in the DSMB charter. Briefly, the primary charge of the DSMB will be to monitor safety and the occurrence of the following:

1. Serious adverse events (SAEs)
2. Deaths (also reported as SAEs)
3. All other adverse events (AEs)
4. Premature withdrawal of research participants from the study
5. Dose Limiting Toxicity.
6. Stopping Rules (see below).

The DSMB may meet in person or through a teleconference. Reports summarizing study progress, SAEs

and enrollment will be sent to the DSMB by the study coordinator every 6 months, at least one week prior to the scheduled DSMB meeting. Information in the reports should be current as of one month prior to the date of the DSMB meeting. The DSMB will also be available to discuss ad hoc (at the request of the Sponsor, Principal Investigator or Medical Monitor) any concerns or events during the course of the study. Unscheduled DSMB meetings may be called as necessary by the DSMB.

The Medical Monitor, who is not on the study team, is Dr. Erika Augustine (University of Rochester), a pediatric neurologist who is a movement disorders specialist. Her role will be to regularly review all safety data and adverse events, and provide an objective opinion regarding clinical outcomes and adverse events, which she will communicate to the study PI and DSMB.

The Advisory Committee (AC) will consist of 6 individuals who will directly with the PI and study team to provide overall scientific oversight for the study. The role of the AC will be to provide overall oversight regarding the interpretation of the results, review possible changes in the protocol resulting from obtained data, and to assess safety data. The AC will provide scientific guidance and advice to the PI and study team as required. The AC will meet either in person or by teleconference ad hoc as data becomes available for review. . The members of the AC are:

Ron Crystal - Professor and Chairman, Department of Genetic Medicine at Weill Cornell Medical College, New York, NY

Darryl De Vivo - Sidney Carter Professor of Neurology, Professor of Pediatrics, and Director Emeritus (1979-2000) of the Pediatric Neurology Service at Columbia University Medical Center, New York, NY

Jonathan Mink - Frederick A. Horner, MD Endowed Professor in Pediatric Neurology, Professor of Neurology, Neuroscience, and Pediatrics, Chief, Division of Child Neurology, Vice Chair, Department of Neurology, University of Rochester Medical Center, Rochester, NY

Un Kang - H. Houston Merritt Professor of Neurology and Chief of the Division of Movement Disorders at Columbia University, New York, NY

Roser Pons - Assistant Professor, First Pediatric Clinic, Medical School, National and Kapodistrian University of Athens, Agia Sofia Hospital, Athens, Greece

Manju Kurian - Professor of Neurogenetics, Developmental Neurosciences Department, University College London, Great Ormond Street Institute of Child Health, London, England

21.2 Dose Limiting Toxicity

Dose-limiting toxicity will be defined as any Grade 3 or 4 toxicity (NCI CTCAE v.4) that is thought to be possibly or probably related to AAV2-hAADC.

If none of the subjects in the first group experiences a Dose Limiting Toxicity (DLT), dose escalation may proceed and subjects may be enrolled in the higher dose level group. Should 2 or more subjects in any group experience DLT as defined above, the study will be stopped pending review and discussion with FDA.

21.3 Stopping Rules

The occurrence of any of the following events (regardless of suspected causal associations) during or after the administration of AAV2-hAADC would result in the halting of study enrollment and notification of the DSMB, FDA, and IRB:

- Any significant procedural deviation or violations, e.g. dosing error, equipment failure
- Any symptomatic intra-cerebral hemorrhage or stroke
- Any central nervous system infections
- Dyskinesia severe enough to require ICU admission for greater than 30 days
- Any death

Once a stopping rule is reached, the trial would be suspended (i.e., cessation of AAV2-hAADC administration to any subjects) pending a comprehensive safety review by the DSMB. Triggering of any Stopping Rule will prompt notification to FDA, IRB, NIH Office of Biotechnology, and the Institutional Biosafety Committee (IBC).

Additional Stopping Rules may be developed if unexpected serious adverse events (SAE) with causal relationship to AAV2-hAADC, including delivery procedure, appear during the study.

22 QUALITY ASSURANCE

A Study Management Service (SMS) will provide monitoring for this study. The expected level of monitoring will be 2 subjects longitudinally, i.e., monitored throughout the study, plus random monitoring of 1 or 2 randomly selected subjects, depending on whether 6 or less, or 7 or more subjects have been enrolled, respectively. Subject monitoring will be by comparison of individual subject records and other source documents with case report forms (CRFs). In addition, critical information will be monitored 100%. Critical information includes but is not limited to IRB approvals and correspondence, subject tracking and eligibility, informed consent, drug accountability and storage, and adverse event reporting.

23 ADVERSE EVENT AND UNANTICIPATED PROBLEM REPORTING

Adverse events (AEs) will be collected from the establishment of baseline conditions (admission) through 36 months following study drug administration. “Adverse Events” and “Serious Adverse Events”, the determination of the relatedness to the investigational treatment, and reporting will follow the definitions and reporting requirements mandated in 21CFR 312.32.

Serious or unexpected adverse events and other unanticipated problems related to the research aspects of the protocol will be submitted to the study site IRB/Committee on Human Research (CHR) within 10 working days of PI awareness. Expected or non-serious adverse events will be reported at the time of continuing review.

24 ALTERNATIVES TO PARTICIPATION

In addition to participation in this clinical study, there may be other investigational studies for which these patients may qualify.

25 CONFIDENTIALITY

Information about study subjects will be handled as confidentially as possible to the extent permitted by applicable laws and/or regulations. Data and blood samples will be coded and stored in cabinets. Blood samples sent to outside labs will be labeled with a code number assigned by the Principal Investigator or clinical study coordinator.

26 CONFLICT OF INTEREST

The Sponsor, Krystof Bankiewicz MD, PhD, is an inventor on patents held by the Regents of the University of California covering AAV delivery to the brain.

27 TECHNOLOGY TRANSFER

1. MRI Interventions Inc.

   SmartFlow® Cannula and ClearPoint® neuro-navigational devices will be provided by MRI Interventions Inc. under a collaborative research agreement.

2. Children's Hospital of Philadelphia

   AAV2-hAADC was manufactured and provided by Children's Hospital of Philadelphia.

28 RESEARCH AND TRAVEL COMPENSATION

Subjects will not be paid for participation in this protocol. However, meals, parking, and lodging costs will be paid for . All airfare for subjects and caregivers for visits will be booked in advance by the study coordinator. The study site will also coordinate and pay for in-town transport.

29 REFERENCES

Brun, L., L. H. Ngu, W. T. Keng, G. S. Ch'ng, Y. S. Choy, W. L. Hwu, W. T. Lee, M. A. Willemsen, M. M. Verbeek, T. Wassenberg, L. Regal, S. Orcesi, D. Tonduti, P. Accorsi, H. Testard, J. E. Abdenur, S. Tay, G. F. Allen, S. Heales, I. Kern, M. Kato, A. Burlina, C. Manegold, G. F. Hoffmann and N. Blau (2010). "Clinical and biochemical features of aromatic L-amino acid decarboxylase deficiency." Neurology **75**(1): 64-71 PMCID:

Caviness, V. S., Jr., D. N. Kennedy, C. Richelme, J. Rademacher and P. A. Filipek (1996). "The human brain age 7-11 years: a volumetric analysis based on magnetic resonance images." Cereb Cortex **6**(5): 726-736 PMCID:

Christine, C. W., P. A. Starr, P. S. Larson, J. L. Eberling, W. J. Jagust, R. A. Hawkins, H. F. VanBrocklin, J. F. Wright, K. S. Bankiewicz and M. J. Aminoff (2009). "Safety and tolerability of putaminal AADC gene therapy for Parkinson disease." Neurology **73**(20): 1662-1669 PMCID:

Dumas, H. M. and M. A. Fragala-Pinkham (2012). "Concurrent validity and reliability of the pediatric evaluation of disability inventory-computer adaptive test mobility domain." Pediatr Phys Ther **24**(2): 171-176; discussion 176 PMCID:

Dumas, H. M., M. A. Fragala-Pinkham, S. M. Haley, P. Ni, W. Coster, J. M. Kramer, Y. C. Kao, R. Moed and L. H. Ludlow (2012). "Computer adaptive test performance in children with and without disabilities: prospective field study of the PEDI-CAT." Disabil Rehabil **34**(5): 393-401 PMCID: 3668545

Eapen, M., D. H. Zald, J. C. Gatenby, Z. Ding and J. C. Gore (2011). "Using high-resolution MR imaging at 7T to evaluate the anatomy of the midbrain dopaminergic system." AJNR Am J Neuroradiol **32**(4): 688-694 PMCID: 3076524

Fiandaca, M. S., V. Varenika, J. Eberling, T. McKnight, J. Bringas, P. Pivirotto, J. Beyer, P. Hadaczek, W. Bowers, J. Park, H. Federoff, J. Forsayeth and K. S. Bankiewicz (2009). "Real-time MR imaging of adeno-associated viral vector delivery to the primate brain." Neuroimage **47 Suppl 2**: T27-35 PMCID: PMC2719019

Gill, S. S., N. K. Patel, G. R. Hotton, K. O'Sullivan, R. McCarter, M. Bunnage, D. J. Brooks, C. N. Svendsen and P. Heywood (2003). "Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease." Nat Med **9**(5): 589-595 PMCID:

Haley, S., W. Coster, L. Ludlow, J. Haltiwanger and A. PA (1992). Pediatric Evaluation of Disability Inventory: Development, Standardization and Administration Manual. Boston, MA, Trustees of Boston University.

Hnasko, T. S., F. A. Perez, A. D. Scouras, E. A. Stoll, S. D. Gale, S. Luquet, P. E. Phillips, E. J. Kremer and R. D. Palmiter (2006). "Cre recombinase-mediated restoration of nigrostriatal dopamine in dopamine-deficient mice reverses hypophagia and bradykinesia." Proc Natl Acad Sci U S A **103**(23): 8858-8863 PMCID: 1466546

Hwu, W. L., S. Muramatsu, S. H. Tseng, K. Y. Tzen, N. C. Lee, Y. H. Chien, R. O. Snyder, B. J. Byrne, C. H. Tai and R. M. Wu (2012). "Gene therapy for aromatic L-amino acid decarboxylase deficiency." Sci Transl Med **4**(134): 134ra161 PMCID:

Hyland, K. and P. T. Clayton (1990). "Aromatic amino acid decarboxylase deficiency in twins." J Inherit Metab Dis **13**(3): 301-304 PMCID:

Hyland, K. and P. T. Clayton (1992). "Aromatic L-amino acid decarboxylase deficiency: diagnostic methodology." Clin Chem **38**(12): 2405-2410 PMCID:

Hyland, K., R. A. Surtees, C. Rodeck and P. T. Clayton (1992). "Aromatic L-amino acid decarboxylase deficiency: clinical features, diagnosis, and treatment of a new inborn error of neurotransmitter

amine synthesis." Neurology **42**(10): 1980-1988 PMCID:

Jernigan, T. L., S. L. Archibald, M. T. Berhow, E. R. Sowell, D. S. Foster and J. R. Hesselink (1991). "Cerebral structure on MRI, Part I: Localization of age-related changes." Biol Psychiatry **29**(1): 55-67 PMCID:

Jernigan, T. L. and P. Tallal (1990). "Late childhood changes in brain morphology observable with MRI." Dev Med Child Neurol **32**(5): 379-385 PMCID:

Johnston, L. C., J. Eberling, P. Pivirotto, P. Hadaczek, H. J. Federoff, J. Forsayeth and K. S. Bankiewicz (2009). "Clinically relevant effects of convection-enhanced delivery of AAV2-GDNF on the dopaminergic nigrostriatal pathway in aged rhesus monkeys." Hum Gene Ther **20**(5): 497-510 PMCID: 19203243

Kells, A. P., J. Forsayeth and K. S. Bankiewicz (2012). "Glial-derived neurotrophic factor gene transfer for Parkinson's disease: anterograde distribution of AAV2 vectors in the primate brain." Neurobiol Dis **48**(2): 228-235 PMCID: 3289735

Kim, D. S., R. D. Palmiter, A. Cummins and C. R. Gerfen (2006). "Reversal of supersensitive striatal dopamine D1 receptor signaling and extracellular signal-regulated kinase activity in dopamine-deficient mice." Neuroscience **137**(4): 1381-1388 PMCID:

Laske, D. W., P. F. Morrison, D. M. Lieberman, M. E. Corthesy, J. C. Reynolds, P. A. Stewart-Henney, S. S. Koong, A. Cummins, C. H. Paik and E. H. Oldfield (1997a). "Chronic interstitial infusion of protein to primate brain: determination of drug distribution and clearance with single-photon emission computerized tomography imaging." J Neurosurg **87**(4): 586-594 PMCID:

Laske, D. W., R. J. Youle and E. H. Oldfield (1997b). "Tumor regression with regional distribution of the targeted toxin TF-CRM107 in patients with malignant brain tumors." Nat Med **3**(12): 1362-1368 PMCID:

Lee, H. F., C. R. Tsai, C. S. Chi, T. M. Chang and H. J. Lee (2009). "Aromatic L-amino acid decarboxylase deficiency in Taiwan." Eur J Paediatr Neurol **13**(2): 135-140 PMCID:

Lonser, R. R., R. Schiffman, R. A. Robison, J. A. Butman, Z. Quezado, M. L. Walker, P. F. Morrison, S. Walbridge, G. J. Murray, D. M. Park, R. O. Brady and E. H. Oldfield (2007a). "Image-guided, direct convective delivery of glucocerebrosidase for neuronopathic Gaucher disease." Neurology **68**(4): 254-261 PMCID:

Lonser, R. R., S. Walbridge, K. Garmestani, J. A. Butman, H. A. Walters, A. O. Vortmeyer, P. F. Morrison, M. W. Brechbiel and E. H. Oldfield (2002). "Successful and safe perfusion of the primate brainstem: in vivo magnetic resonance imaging of macromolecular distribution during infusion." J Neurosurg **97**(4): 905-913 PMCID:

Lonser, R. R., K. E. Warren, J. A. Butman, Z. Quezado, R. A. Robison, S. Walbridge, R. Schiffman, M. Merrill, M. L. Walker, D. M. Park, D. Croteau, R. O. Brady and E. H. Oldfield (2007b). "Real-time image-guided direct convective perfusion of intrinsic brainstem lesions. Technical note." J Neurosurg **107**(1): 190-197 PMCID:

Lumsden, D. E., C. Lundy, C. Fairhurst and J. P. Lin (2013). "Dystonia Severity Action Plan: a simple grading system for medical severity of status dystonicus and life-threatening dystonia." Dev Med Child Neurol **55**(7): 671-672 PMCID:

Manegold, C., G. F. Hoffmann, I. Degen, H. Ikonomidou, A. Knust, M. W. Laass, M. Pritsch, E. Wilichowski and F. Horster (2009). "Aromatic L-amino acid decarboxylase deficiency: clinical features, drug therapy and follow-up." J Inherit Metab Dis **32**(3): 371-380 PMCID:

Mingozzi, F., N. C. Hasbrouck, E. Basner-Tschakarjan, S. A. Edmonson, D. J. Hui, D. E. Sabatino, S. Zhou, J. F. Wright, H. Jiang, G. F. Pierce, V. R. Arruda and K. A. High (2007). "Modulation of tolerance to the transgene product in a nonhuman primate model of AAV-mediated gene transfer

to liver." Blood **110**(7): 2334-2341 PMCID: 1988950

Muramatsu, S., K. Fujimoto, S. Kato, H. Mizukami, S. Asari, K. Ikeguchi, T. Kawakami, M. Urabe, A. Kume, T. Sato, E. Watanabe, K. Ozawa and I. Nakano (2010). "A phase I study of aromatic L-amino acid decarboxylase gene therapy for Parkinson's disease." Mol Ther **18**(9): 1731-1735 PMCID: 2956925

Peden, C. S., C. Burger, N. Muzyczka and R. J. Mandel (2004). "Circulating anti-wild-type adeno-associated virus type 2 (AAV2) antibodies inhibit recombinant AAV2 (rAAV2)-mediated, but not rAAV5-mediated, gene transfer in the brain." J Virol **78**(12): 6344-6359 PMCID:

Peden, C. S., F. P. Manfredsson, S. K. Reimsnider, A. E. Poirier, C. Burger, N. Muzyczka and R. J. Mandel (2009). "Striatal readministration of rAAV vectors reveals an immune response against AAV2 capsids that can be circumvented." Mol Ther **17**(3): 524-537 PMCID:

Pfefferbaum, A., D. H. Mathalon, E. V. Sullivan, J. M. Rawles, R. B. Zipursky and K. O. Lim (1994). "A quantitative magnetic resonance imaging study of changes in brain morphology from infancy to late adulthood." Arch Neurol **51**(9): 874-887 PMCID:

Pons, R., D. Syrengelas, S. Youroukos, I. Orfanou, A. Dinopoulos, B. Cormand, A. Ormazabal, A. Garzia-Cazorla, M. Serrano and R. Artuch (2013). "Levodopa-induced dyskinesias in tyrosine hydroxylase deficiency." Mov Disord **28**(8): 1058-1063 PMCID:

Richardson, R. M., F. Gimenez, E. A. Salegio, X. Su, J. Bringas, M. S. Berger and K. S. Bankiewicz (2011a). "T2 imaging in monitoring of intraparenchymal real-time convection-enhanced delivery." Neurosurgery **69**(1): 154-163; discussion 163 PMCID:

Richardson, R. M., A. P. Kells, A. J. Martin, P. S. Larson, P. A. Starr, P. G. Piferi, G. Bates, L. Tansey, K. H. Rosenbluth, J. R. Bringas, M. S. Berger and K. S. Bankiewicz (2011b). "Novel platform for MRI-guided convection-enhanced delivery of therapeutics: preclinical validation in nonhuman primate brain." Stereotact Funct Neurosurg **89**(3): 141-151 PMCID: 3085040

Richardson, R. M., A. P. Kells, K. H. Rosenbluth, E. A. Salegio, M. S. Fiandaca, P. S. Larson, P. A. Starr, A. J. Martin, R. R. Lonser, H. J. Federoff, J. R. Forsayeth and K. S. Bankiewicz (2011c). "Interventional MRI-guided putaminal delivery of AAV2-GDNF for a planned clinical trial in Parkinson's disease." Mol Ther **19**(6): 1048-1057 PMCID: 3129792

Russell, D. J., P. L. Rosenbaum, L. M. Avery and M. Lane (2002). The Gross Motor Function Measure (GMFM-66 & GMFM-88) User's Manual. London, Mac Keith Press.

Salegio, E. A., H. Streeter, N. Dube, P. Hadaczek, L. Samaranch, A. P. Kells, W. San Sebastian, Y. Zhai, J. Bringas, T. Xu, J. Forsayeth and K. S. Bankiewicz (2014). "Distribution of nanoparticles throughout the cerebral cortex of rodents and non-human primates: Implications for gene and drug therapy." Front Neuroanat **8**: 9 PMCID: 3956368

Sanftner, L. M., B. M. Suzuki, M. M. Doroudchi, L. Feng, A. McClelland, J. R. Forsayeth and J. Cunningham (2004). "Striatal delivery of rAAV-hAADC to rats with preexisting immunity to AAV." Mol Ther **9**(3): 403-409 PMCID:

Su, X., A. P. Kells, E. A. Aguilar Salegio, R. M. Richardson, P. Hadaczek, J. Beyer, J. Bringas, P. Pivirotto, J. Forsayeth and K. S. Bankiewicz (2010). "Real-time MR imaging with Gadoteridol predicts distribution of transgenes after convection-enhanced delivery of AAV2 vectors." Mol Ther **18**(8): 1490-1495 PMCID: 2927064

Swoboda, K. J., K. Hyland, D. S. Goldstein, K. C. Kuban, L. A. Arnold, C. S. Holmes and H. L. Levy (1999). "Clinical and therapeutic observations in aromatic L-amino acid decarboxylase deficiency." Neurology **53**(6): 1205-1211 PMCID:

Swoboda, K. J., J. P. Saul, C. E. McKenna, N. B. Speller and K. Hyland (2003). "Aromatic L-amino acid decarboxylase deficiency: overview of clinical features and outcomes." Ann Neurol **54 Suppl 6**:

S49-55 PMCID:

Szczypka, M. S., K. Kwok, M. D. Brot, B. T. Marck, A. M. Matsumoto, B. A. Donahue and R. D. Palmiter (2001). "Dopamine production in the caudate putamen restores feeding in dopamine-deficient mice." Neuron **30**(3): 819-828 PMCID:

Varni, J. W., M. Seid and P. S. Kurtin (2001). "PedsQL 4.0: reliability and validity of the Pediatric Quality of Life Inventory version 4.0 generic core scales in healthy and patient populations." Med Care **39**(8): 800-812 PMCID:

Voges, J., R. Reszka, A. Gossmann, C. Dittmar, R. Richter, G. Garlip, L. Kracht, H. H. Coenen, V. Sturm, K. Wienhard, W. D. Heiss and A. H. Jacobs (2003). "Imaging-guided convection-enhanced delivery and gene therapy of glioblastoma." Ann Neurol **54**(4): 479-487 PMCID:

Weaver, F. M., K. Follett, M. Stern, K. Hur, C. Harris, W. J. Marks, Jr., J. Rothlind, O. Sagher, D. Reda, C. S. Moy, R. Pahwa, K. Burchiel, P. Hogarth, E. C. Lai, J. E. Duda, K. Holloway, A. Samii, S. Horn, J. Bronstein, G. Stoner, J. Heemskerk, G. D. Huang and C. S. P. S. Group (2009). "Bilateral deep brain stimulation vs best medical therapy for patients with advanced Parkinson disease: a randomized controlled trial." JAMA **301**(1): 63-73 PMCID: 2814800

Ziegler, D. A., J. S. Wonderlick, P. Ashourian, L. A. Hansen, J. C. Young, A. J. Murphy, C. K. Koppuzha, J. H. Growdon and S. Corkin (2013). "Substantia nigra volume loss before basal forebrain degeneration in early Parkinson disease." JAMA Neurol **70**(2): 241-247 PMCID: