19 May 2022
EMA/CHMP/571076/2022
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Upstaza

International non-proprietary name: eladocagene exuparvovec

Procedure No. EMEA/H/C/005352/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

18F-DOPA	L-6-[18F] fluoro-3,4-dihydroxyphenylalnine
3-OMD	3-O-methyldopa
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptophan
5-HTP	5- hydroxy-tryptophan
AADC	Aromatic L-amino acid decarboxylase
AADC	Aromatic L-amino acid decarboxylase
AAV	Adeno-associated virus
AAV2	Adeno-associated virus serotype 2
Ad	Adenovirus
AE	Adverse event
AET	Analytical evaluation threshold
AIMS	Alberta Infant Motor Scale
Alb	Albumin blood test
ALT	Alanine aminotransferase
APQR	Annual product quality report
AQL	Acceptable quality limit
AS-DA	Rabbit anti-dopamine anti-serum
AST	Aspartate aminotransferase
ATMP	Advanced therapy medicinal product
AUC	Analytical ultracentrifugation
Bayley-III	Bayley Scales of Infant Development – Third Edition
BLOD	Below limit of detection
BSA	Bovine serum albumin
BSC	Biosafety cabinet
BSE	Bovine spongiform encephalopathy
BUN	Blood urea nitrogen
CA	Cancer antigen
CAT	Committee for Advanced Therapies
CBC	Complete blood count
CCS	Container closure system

cDNA	Coding DNA
CEX	Cation exchange chromatography
CFB	Change from baseline
CFU	Colony forming units
CI	Confidence interval
CMV	Cytomegalovirus
CNS	Central nervous system
CO2	Carbon dioxide
CP	Capsid particle
CPE	Cytopathic effects
CPP	Critical process parameter
CPV	Continuous process verification
CQA	Critical quality attribute
Cre	Creatinine
CRF	Case Report Form
CS	Cell stacks
CSF	Cerebrospinal fluid
Ct	Cycle threshold
CT	Computed tomography
CV	Column volumes
DAD	Diode array detector
DBS	Deep brain stimulation
DC	Differential count
DF	Degrees of freedom
DIA	Dopamine Induction Assay
DLS	Dynamic light scattering
DMEM	Dulbecco’s modified Eagle Media
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DNA	Deoxyribonucleic acid
DP	Drug Product
DPBS	Dulbecco phosphate buffered saline
DS	Drug Substance

DTR	Deep tendon reflex
EBV	Epstein Barr virus
EC50	Concentration of a drug that gives half-maximal response
ECG	Electrocardiogram
EDTA	Ethylene diamine tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EOPC	End of Production Cells
EPC	External positive control
EU	Endotoxin unit
FBS	Foetal Bovine Serum
FDA	Food & Drug Administration
FLBx	Florida Biologix
FT-ICR	Fourier transform ion cyclotron resonance
FUSE	Facilities, utilities, systems and equipment
GC	Gas chromatography
GCP	Good Clinical Practice
GLP	Good laboratory practice
GMP	Good manufacturing practice
GOI	Gene of interest
GOI	Gene of interest
HA	Haemagglutinin
hAADC	Human aromatic L-amino acid decarboxylase
HAD	Haemadsorption
HAV	Hepatitis A virus
HBG	Human beta-globin
HBS	HEPES-buffered saline
HBV	Hepatitis B Virus
HCP	Host cell protein
HCV	Hepatitis C Virus
HEK	Human embryonic kidney
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HHV	Human herpes virus

HIAA	Hydroxyindoleacetic acid
HIC	Hydrophobic interaction chromatography
HIV	Human immunodeficiency virus
HMW	High molecular weight
HPLC	High performance liquid chromatography
HPRA	Health Products Regulatory Authority
HRP	Horseradish peroxidase
HVA	Homovanillic acid
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICP	Inductively coupled plasma
ICV	Intraputaminal ventricular infusion
IEC	Independent Ethics Committee
IPA	Isopropanol
IPC	In process control
IPM	In process monitoring
IRB	Institutional Review Board
ISMC	Independent Safety Monitoring Committee
ISO	International Organisation for Standardisation
ITR	Inverted terminal repeat
ITT	Intent-to-Treat
IU	Infectious unit
K	Potassium
KanR	Kanamycin resistance gene
kGy	kiloGray
LAL	Limulus amebocyte lysate
LC-MS	Liquid chromatography – mass spectrometry
L-DOPA	L-3,4-dihydroxyphenylalanine
LDPE	Low-density polyethylene
LFQ	Label-free quantitation
LLOQ	Lower limit of quantitation
LMW	Low molecular weight
LOD	Limit of detection

LOQ	Limit of quantitation
LS	Least square
LTL	Less than lifetime
m/z	Mass-to-charge ratio
MAA	Marketing Authorisation Application
MAO	Monoamine oxidase
MAX	Maximum
MBL	MassBiologics
MCB	Master Cell Bank
MedDRA	Medical Dictionary for Regulatory Activities
MEM	Minimum essential medium
MIN	Minimum
MOI	Multiplicity of Infection
MoS	Margin of safety
MRA	Mutual Recognition Agreement
MRI	Magnetic resonance imaging
MW	Molecular weight
N/a	Not applicable
NA	Sodium
NaOH	Sodium hydroxide
NF	National formulary
NHP	Nonhuman primate
NMT	Not more than
NOR	Normal operating range
nsTEM	negative stain Transmission electron microscopy
NTC	No template control
NTU	National Taiwan University
NTUH	National Taiwan University Hospital
OD	Optical density
OES	Optical emission spectroscopy
OGC	Oculogyric crisis
P	Phosphate
P188	Poloxamer 188

PAR	Proven acceptable range
PBS	Phosphate buffered saline
PD	Pharmacodynamic
PDE	Permitted daily exposure
PDMS-2	Peabody Developmental Motor Scales-Second Edition
PEEK	Polyether ether ketone
PET	Positron emission tomography
PETG	Polyethylene terephthalate glycol
PGTC	University of Florida – Powell Gene therapy Centre
PI	Principle investigator
POD	Peroxidase
PP	Process parameter
PPQ	Process performance qualification
PT	Prothrombin time
PTM	Post translational modification
PTT	Partial thromboplastin time
PV	Process validation
PVDF	Polyvinylidene difluoride
QA	Quality Attribute
QP	Qualified Person
qPCR	Quantitative polymerase chain reaction
rAAV	Recombinant adeno-associated virus
rAAV2	Recombinant adeno-associated virus serotype 2
RAB	Restricted access barrier
rcAAV	Replication competent adeno-associated virus
RS	Reference standard
RSD	Relative standard deviation
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SD	Standard deviation
SmPC	Summary of Product Characteristics
SOC	System organ class
SP	Sulfopropyl

SPC	Statistical process control
SV40	Simian vacuolating virus 40
SV-AUC	Sedimentation velocity analytical centrifugation
SVHC	Substance of very high concern
TA	Test article
TCID50	Tissue culture infectious dose
TEAE	Treatment-emergent adverse event
TEM	Transmission electron microscopy
TFF	Tangential flow filtration
TIC	Total ion current
TP	Total protein
TRIS	Tri(hydroxymethyl)aminomethane
TSB	Tryptone soya broth
TSE	Transmissible spongiform encephalopathy
TTC	Threshold of toxicological concern
UF/DF	Ultrafiltration/ diafiltration
ULOQ	Upper limit of quantitation
USP	United States Pharmacopoeia
UV	Ultraviolet
v/v	Volume/ volume
Vg	Vector genome
VP	Virus protein
WCB	Working Cell Bank
WFI	Water for Injections
WHO	World Health Organization
wtAAV	wild-type adeno-associated virus

1. Background information on the procedure

1.1. Submission of the dossier

The applicant PTC Therapeutics International Limited submitted on 12 January 2020 an application for marketing authorisation to the European Medicines Agency (EMA) for Upstaza, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 26 April 2019.

Upstaza, was designated as an orphan medicinal product EU/3/16/1786 on 18/11/2016 in the following condition: Treatment of Aromatic L-Amino acid decarboxylase deficiency.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Upstaza as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: https://www.ema.europa.eu/en/medicines/human/EPAR/upstaza.

The applicant applied for the following indication:

Upstaza is indicated for the treatment of adult and paediatric patients with aromatic L-amino acid decarboxylase (AADC) deficiency.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application. The applicant indicated that eladocagene exuparvovec was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) EMEA-002435-PIP01-18 on the agreement of a paediatric investigation plan (PIP).

The PDCO issued an opinion on compliance for the PIP EMEA-002435-PIP01-1.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.5. Applicant's request(s) for consideration

1.5.1. Marketing authorisation under exceptional circumstances

The applicant agreed to a marketing authorisation under exceptional circumstances in accordance with Article 14(8) of Regulation (EC) No 726/2004.

1.5.2. New active Substance status

The applicant requested the active substance eladocagene exuparvovec contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

1.6. Protocol assistance

The applicant received the following Protocol assistance on the development relevant for the indication subject to the present application:

Date	**Reference**	**SAWP co-ordinators**
26 April 2018	EMEA/CHMP/SAWP/236092/2018	André Elferink, Rune Kjeken

The Protocol assistance pertained to the following non-clinical and clinical aspects:

- Adequacy of the non-clinical data package to support a MAA.
- Appropriateness of the design, population, comparator, endpoints, study duration, and sample size of the single-arm studies AADC-1601 and AADC-010 to assess the clinical benefit in view of a MAA.
- Adequacy of the proposed safety database to support a MAA.
- The submission of the videos in the MAA as supportive evidence of efficacy.

1.7. Steps taken for the assessment of the product

The CAT Rapporteur and Co-Rapporteur appointed by the CHMP were:

CAT Rapporteur: Maura O'Donovan

CAT Co-Rapporteur: Lisbeth Barkholt

CHMP Coordinator (Rapporteur): Peter Kiely

CHMP Coordinator (Co-Rapporteur): Kristina Dunder

The appointed CAT co-rapporteur had no such prominent role in Protocol assistance relevant for the indication subject to the present application.

The application was received by the EMA on	12 January 2020
The procedure started on	28 January 2020
The CAT Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	20 April 2020

The CAT Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on	20 April 2020
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC members on	4 May 2020
The PRAC agreed on the PRAC Assessment Overview and Advice to CAT during the meeting on	14 May 2020
The CAT agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	20 May 2020
The applicant submitted the responses to the CAT consolidated List of Questions on	15 February 2021
The CAT Rapporteur circulated the Joint Assessment Report on the responses to the List of Questions to all CAT and CHMP members on	23 March 2021
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	8 April 2021
The CAT agreed on a list of outstanding issues in writing to be sent to the applicant on	16 April 2021
The applicant submitted the responses to the CAT List of Outstanding Issues on	6 October 2021
The CAT Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CAT and CHMP members on	21 October 2021
The CAT agreed on a list of outstanding issues in writing to be sent to the applicant on	5 November 2021
The applicant submitted the responses to the CAT List of Outstanding Issues on	14 March 2022
The CAT Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CAT and CHMP members on	31 March 2022
The outstanding issues were addressed by the applicant during an oral explanation before the CAT during the meeting on	13 April 2022
Expert group were convened to address questions raised by the CHMP on The CAT and CHMP considered the views of the Expert group as presented in the minutes of this meeting.	4 April 2022
The CAT, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Upstaza on	13 May 2022
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Upstaza on	19 May 2022

Furthermore, the CAT adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	13 May 2022
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	19 May 2022

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Aromatic L-amino acid decarboxylase deficiency is a rare autosomal recessive disorder of dopaminergic and serotonergic pathways that manifests in young children and most commonly results in complete arrest of motor development (Wassenberg 2017). AADC deficiency is due to the presence of pathological variants in the *DDC* gene that encodes for AADC, the enzyme responsible for the decarboxylation of L-DOPA and 5-HTP to form the neurotransmitters dopamine and serotonin, respectively (Wassenberg 2017).

2.1.2. Epidemiology

Although, the global incidence of AADC deficiency is not well understood, the disease is more prevalent in certain Asian populations, particularly in Taiwan and Japan. This is likely due to a founder splice site variant IVS6+4A>T (also designated as C.714+4A>T) that phenotypically results in complete motor development arrest (Lee 2009, Wassenberg 2017).

The predicted birth rates of individuals with AADC deficiency are estimated to be between 1/42,000 to 1/90,000 in the US (Hyland 2018, Whitehead 2018, Himmelreich 2019), and approximately 1/118,000 in the European Union (Himmelreich 2019). A 1/90,000 birth-rate translates into a current estimate of about 840 living patients with AADC deficiency in the US (Himmelreich 2019). Given only about 123 unique patients have been identified world-wide (Wassenberg 2017), it is clear that most individuals with this genetic disorder have not been diagnosed and that the disease is more prevalent than generally recognized (Whitehead 2018, Himmelreich 2019).

2.1.3. Aetiology and pathogenesis

In the normal human brain, AADC enzyme is present predominately in the brainstem, where it is produced in the substantia nigra of the midbrain (brainstem). Dopaminergic neurons in the substantia nigra project into the striatum, which includes the putamen and caudate nuclei, and dopamine is released from these presynaptic terminals (Himmelreich 2019). Dopamine is required within the putamen to enable motor development and function.

Aromatic L-amino acid decarboxylase is a key component of highly interlinked catalytic and metabolic pathways that regulate levels of dopamine, and in turn, controls essential neurotransmitters such as norepinephrine and epinephrine. Norepinephrine and epinephrine deficiencies affect principally

attention, mood, and sleep. The neurotransmitters affected by AADC are key for control of the sympathetic nervous system, mood, cognition, and motor coordination (Himmelreich 2019).

AADC Genetics

The *DDC* gene is located on chromosome 7p12.1-7p12.3 and contains 15 exons. The AADC enzyme, which has 480 amino acids, is highly conserved across species and is expressed in the brain, sympathetic ganglia, and adrenal medulla. *DDC* gene spans >107 kb and multiple alternatively spliced variants encoding different isoforms of the protein have been identified (Himmelreich 2019). Currently, across the 123 confirmed patients with AADC deficiency, 82 variants have been observed, of which 79 are known to lead to AADC deficiency (Himmelreich 2019). The current list of *DDC* variants includes 58 missenses, 6 frame-shift, 9 splice sites, 1 in-frame, 3 complex, and 2 nonsense mutations. In general, these variants affect the production, folding, conformation, and/or catalytic activity of AADC (Himmelreich 2019).

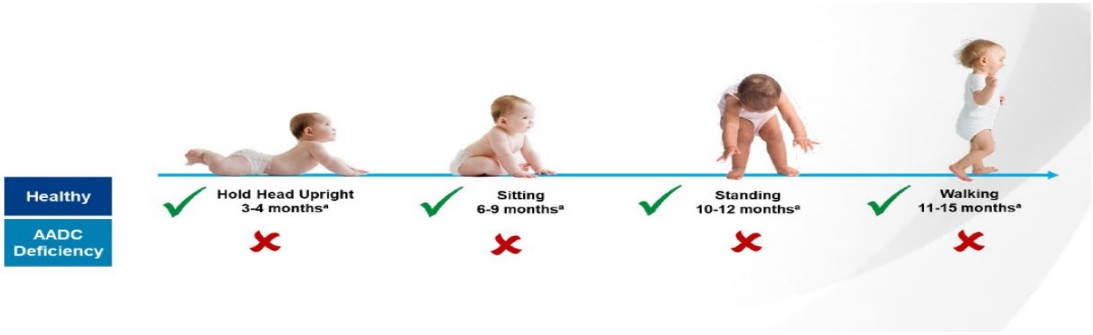
Across the patients, 58 different genotypes have been identified; 19 were homozygous for the same variant and 39 were heterozygous. The most commonly observed genotype (gene frequency of 26.0%) is homozygous for the founder mutation that is common in certain Asian populations (i.e., c.[714+4A>T];[714+4A>T]) (Himmelreich 2019). The founder mutation is a splice site mutation that results in the production of a truncated AADC protein (Lee 2009). Importantly, to date, no genotype-phenotype correlation has been reported (Wassenberg 2017, Himmelreich 2019).

2.1.4. Clinical presentation, diagnosis and stage/prognosis

Patients with AADC deficiency are heterogenous and have a wide range of clinical presentation, likely reflecting the different forms of pathogenic variants (e.g., missense variants, deletions, etc.). Of reported cases of AADC deficiency, all patients had symptoms within the first year of life (onset of symptoms at 2.7 months) (Brun 2010, Wassenberg 2017) However, the median age of diagnosis was about 3.5 years, indicating the difficulties in recognizing, and subsequently delays in diagnosis, of the disease (Brun 2010, Wassenberg 2017).

The AADC enzyme deficiency results in a marked or complete loss of dopamine production in the brain from birth. Consequently, AADC-deficient patients exhibit symptoms related to loss in dopamine signaling, affecting voluntary movements, cognitive functions, and emotion. Patients with AADC deficiency have arrested motor development despite essentially preserved neurophysiology and neuroanatomy as determined by brain imaging. These patients fail to achieve motor milestones typical of healthy children, such as full-head control and ability to sit, stand, or walk. Patients also experience intellectual disability and show irritability, and are at risk of death in the first decade of life (Korenke 1997, Pons 2004, Helman 2014). A systematic literature review of AADC deficiency through December 2015 described and catalogued 117 patients worldwide (Wassenberg 2017). Adequate information was available to assess the overall impact of AADC deficiency on motor development for 103 patients. AADC deficiency that results in complete arrest of motor development, the most common phenotype identified by Wassenberg and colleagues, was observed in 80% of patients (82 of 103 cases) and was also characterized by failure to gain adequate head control or the ability to sit, stand, or walk. AADC deficiency with motor development is the most infrequent phenotype (identified in only 6 of 103 cases, or 6%), and is defined as a delay in developmental milestone acquisition but with eventual achievement of ambulation. Patients with this phenotype still have profound motor deficits, as they continue to be disabled without full motor function and have impaired intellectual function.

Comparison of Milestones of Patients with AADC Deficiency and Healthy Children



The phenotype of AADC deficiency is a spectrum, with disease severity appearing independent of gender (Brun 2010, Wassenberg 2017). Overall, AADC-deficient patients have decreased contact, social interaction, alertness, and sleep disorders. They also have loss of appetite and impairment of memory and learning, body temperature regulation, cardiovascular functions, and hormone secretions. The most common symptoms of AADC deficiency are hypotonia, movement disorders, developmental delays, and autonomic disorders (Himmelreich 2019). The most frequent motor disorders include oculogyric crisis and/or dystonia. Common autonomic symptoms are ptosis and excessive sweating (Brun 2010, Wassenberg 2017).

Less common symptoms include sleep disturbance, epileptic seizures, and sleep apnoea. Symptoms associated with behaviour, such as irritability, dysphoria, excessive crying, and those similar to autism are common and can be the most taxing for patients/caregivers (Brun 2010, Wassenberg 2017).

The most common non-neurologic symptoms are gastrointestinal such as diarrhoea, constipation, feeding difficulties, and gastroesophageal reflux (Brun 2010, Wassenberg 2017).

Typical Signs and Symptoms Reported in Patients with AADC Deficiency

Common	Less Common	Non-neurologic
Hypokinesia	Epileptic seizures	Short stature
Hypotonia (proximal)	Sleep disturbance	Diarrhea
Hypertonia (proximal)	Irritability	Constipation
Oculogyric crises	Dysphoria	Feeding difficulties
Developmental delay	Autism-like symptoms	Nasal congestion
Ptosis	Temperature instability	Gastroesophageal reflux
Excessive sweating		Hypoglycemia
Dystonia		

Abbreviations: ADDC, aromatic L-amino acid decarboxylase
Source: from (Himmelreich 2019)

2.1.5. Management

Current Standard of Care for AADC Deficiency

The consensus paper from Wassenberg detailed a review of the literature that included the systematic reporting by Brun and colleagues to assess AADC-deficient patient responses to dopamine agonists, monoamine oxidase (MAO) inhibitors, and pyridoxine therapies (Brun 2010). Among the 117 AADC-

deficient patients identified in Wassenberg's guideline, 56 received dopamine agonists, with bromocriptine and pergolide being used most frequently. Limited improvement in head control, hypotonia, voluntary movements, OGC, and autonomic symptoms have been reported in some patients. However, the use of dopamine agonists is associated with side effects, including weight loss, failure to thrive, and mild to severe dyskinesia, thus limiting the use of these medications in children with AADC deficiency who already have difficulty gaining weight over time or have failure to thrive. Thirty-one of the 117 patients with AADC deficiency received an MAO inhibitor; all 31 patients also received dopamine agonists and/or pyridoxine (vitamin B6). Most reports of patients with AADC deficiency treated with MAO inhibitors reported modest improvement in a single symptom (e.g., hypotonia), while some reported no clinical improvement or only a temporary improvement, although side effects appeared to be rare. In patients with AADC deficiency with arrested motor development, despite these available therapies, the progression of AADC deficiency was not attenuated.

2.2. About the product

Eladocagene Exuparvovec

Eladocagene exuparvovec is a sterile, parenteral formulation gene therapy indicated for the treatment of patients with AADC deficiency. Eladocagene exuparvovec contains the active biological substance rAAV2-h*AADC* (a recombinant adeno-associated virus serotype 2 [rAAV2] vector containing the human *DDC* gene and coding deoxyribonucleic acid (DNA) (cDNA) that encodes the human aromatic L-amino acid decarboxylase [hAADC]) and compendial excipients. Eladocagene exuparvovec is delivered to cells in the putamen of the brain, resulting in increased production of the enzyme AADC.

Figure 1 Diagram of Eladocagene Exuparvovec Expression Cassette

Abbreviations: CMV IEP, cytomegalovirus immediate-early promoter; HBG2/3, Human β-globin partial intron 2/partial exon 3; hAADC, Human aromatic L-amino acid decarboxylase; Poly A, polyadenylation sequence; ITR, inverted terminal repeat
Note: genetic elements are drawn to approximate proportions.

Vector Selection (AAV2)

Eladocagene exuparvovec is an adeno-associated virus 2 (AAV2) vector with a known safety profile and local persistence at area of administration, i.e., the vector does not integrate into the host genome. The vector will be infused directly to the central nervous system (CNS) and specifically to the putamen to maximize target tissue expression and hence local exposure while limiting off-target tissue effects. AAV is a small, non-enveloped, single-stranded DNA virus commonly used as a gene therapy vector for a variety of applications in the brain given its established safety profile, lack of pathogenicity, and ability to transduce quiescent (non-dividing) cells, particularly neurons (Hocquemiller 2016). Recombinant AAV2 (rAAV2) was chosen as the vector for delivery due to its neurotropic properties, its demonstrated long-term gene expression in the CNS, and the extensive testing of this serotype in nonclinical species and humans, including patients with Parkinson's disease, where AADC activity, dopamine induction, and motor function improvement and safety were demonstrated. In addition,

rAAV2 has shown long-term gene expression in vivo, which offers a treatment for genetic diseases affecting the nervous system.

Recombinant AAVs (rAAV) were developed to package DNA cassettes within a 4.7Kb size constraint flanked by 145 bp inverted terminal repeats (ITR). This sequence ensures that the inserted DNA cassette will become an extrachromosomal episome (Gray 2010). The rAAV2 capsid used to deliver eladocagene exuparvovec to the putamen contains no replication or protein encoding genes of AAV, nor does it contain DNA sequences that allow integration into the host genome, which reduce the risks of recombination events and transformation.

Intraputaminal Route of Administration

Eladocagene exuparvovec is being infused into the putamen to treat AADC deficiency. The rationale for the delivery of the gene therapy into the putamen is as follows:

- Local delivery of eladocagene exuparvovec reduces the chance of expression of AADC enzyme, and possibly mis-expression of dopamine or serotonin, in non-targeted areas of the brain and adverse effects.
- Delivery of rAAV2-*AADC* containing the same AAV2 capsid, transgene promoter, and ITR's as in eladocagene exuparvovec resulted in AADC protein expression, enzyme activity, and dopamine production in mouse, rats, and non-human primates (Sanchez-Pernaute 2001, Muramatsu 2002, Bankiewicz 2006, Forsayeth 2006, Cunningham 2008b).
- Injection of the rAAV2-h*AADC* vector into the bilateral putamen of humans with Parkinson's disease resulted in increased AADC activity and reduction of disease symptoms (Christine 2009, Muramatsu 2010).

The rAAV2-h*AADC* vectors used in these studies contained the same wild-type AAV2 capsid and human *DDC* cDNA as eladocagene exuparvovec. Based on these intra-putaminal dosing studies with the AAV2-h*AADC* vector in Parkinson's disease patients, which induced dopamine induction in these patients, the intra-putaminal route of administration was selected for AADC deficiency clinical studies with eladocagene exuparvovec.

Mechanism of Action

Two main possibilities are posited for the means by which eladocagene exuparvovec achieves appropriate expression of the AADC enzyme: retrograde transport of the virus to transduce substantia nigra neurons (Hadaczek 2016) and/or direct transduction by the virus of cells in the putamen in situ.

Following eladocagene exuparvovec gene therapy, virus particles may be internalized by dopaminergic presynaptic terminals, transported back to the cell body in the substantia nigra, transcribed into mRNA, and synthesized into AADC protein (enzyme). The AADC enzyme then converts L-DOPA to dopamine, which is incorporated into vesicles and stored in the presynaptic terminal for release into the synaptic cleft upon nerve stimulation to modulate neuronal control of motor activity. Alternatively, virus particles may be internalized by cells in the putamen, transcribed and translated to AADC protein (enzyme), which could then convert available L-DOPA to dopamine. Dopamine would then be packaged and released to modulate neuronal control of motor activity.

2.3. Type of Application and aspects on development

This was a complete and independent application.

2.4. Quality aspects

2.4.1. Introduction

Upstaza is a gene therapy medicinal product indicated for the treatment of patients with AADC deficiency. Upstaza contains the rAAV2-h*AADC* (a recombinant adeno-associated virus serotype 2 [rAAV2] vector containing the human *DDC* gene and coding deoxyribonucleic acid (cDNA) that encodes the human aromatic L-amino acid decarboxylase [hAADC]) and compendial excipients. Upstaza is presented as a solution for infusion and delivered to cells in the putamen of the brain, resulting in increased production of the enzyme AADC.

The finished product is presented as a sterile, clear to slightly opaque, colourless to faint-white solution. The finished product formulation is comprised of compendial excipients including potassium chloride, sodium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate and poloxamer 188 in water for injections, at a pH 6.9. Each vial of finished product contains an extractable volume of 0.5 mL with a total of 2.8×10^{11} vector genome copies (vg). Each vial is for single use only and this medicinal product should only be infused with the SmartFlow ventricular cannula.

2.4.2. Active Substance

2.4.2.1. General information

The active substance (AS) is eladocagene exuparvovec which is a rAAV2 comprising a human dopa decarboxylase variant 2 cDNA transcript, which encodes hAADC isoform 1, under the control of the cytomegalovirus immediate-early promoter and SV40 poly A transcription terminator. The wild type AAV2 genome (with the exception of the flanking inverted terminal repeats (ITRs)) has been replaced with the therapeutic transgene expression cassette.

Figure 2 Diagram of Eladocagene exuparvovec expression cassette

Abbreviations: CMV IEP, cytomegalovirus immediate-early promoter; HBG2/3, Human β-globin partial intron 2/partial exon 3; hAADC, Human aromatic L-amino acid decarboxylase; Poly A, polyadenylation sequence; ITR, inverted terminal repeat
Note: genetic elements are drawn to approximate proportions.

The eladocagene exuparvovec gene therapy contains no replication or protein encoding genes of adeno-associated virus (AAV). The infectivity, transgene expression and hAADC enzymatic activity attributes contribute collectively to the overall biological activity of eladocagene exuparvovec. Properties potentially affecting biological activity include primary, secondary, tertiary and higher-order structure of the AAV2 capsid and the sequence of the hAADC expression cassette.

Eladocagene exuparvovec is based on the naturally-occurring AAV serotype 2, which is abundantly circulated and has not been typically associated with human disease. Manufacture of eladocagene exuparvovec requires the use of a dual-plasmid transfection process and the recombinant AAV2 capsids are produced in a human embryonic kidney (HEK) 293 cell expression system.

Recombinant AAVs (rAAV) were developed to package DNA cassettes within a 4.7Kb size constraint. This sequence ensures that the inserted DNA cassette will become an extrachromosomal episome. The rAAV2 capsid used to deliver eladocagene exuparvovec DNA to the putamen contains no replication or protein encoding genes of AAV, nor does it contain DNA sequences that allow integration into the host genome, which reduce the risks of recombination events and transformation.

2.4.2.2. Manufacture, characterisation and process controls

Manufacturers

Several deficiencies were noted regarding valid GMP certificates which resulted in a major objection at Day 120. The active substance manufacturing facility, MassBiologics South Coast, 1240 Innovation Way, Fall River, Massachusetts 02720, USA has now been inspected by the supervisory authority and a valid EU GMP certificate has been issued.

The active substance release and stability testing site has also been inspected by the supervisory authority and an EU GMP certificate has been issued. The major objection has been resolved.

Manufacturing process

The commercial process is process C. The active substance is manufactured by transient transfection of human embryonic kidney 293 (HEK 293) cells with transgene and helper plasmids.

The upstream and downstream manufacturing process has been described in the dossier, including a flow diagram and narrative of how each step is performed. Each upstream manufacturing run is initiated with thawing of HEK 293 cell bank vials followed passaging to expand the cell culture, transfection with plasmids, cell harvest, and washing into cell pellets.

The cell pellets are stored frozen prior to initiating the downstream manufacturing process. The downstream process consists of purifying the harvest pellets with unit operations of cell lysis and clarification, affinity chromatography, hydrophobic interaction chromatography, cation-exchange chromatography, and buffer exchange and concentration by diafiltration/ultrafiltration (DF/UF). The retentate is filtered and transferred to a bag, resulting in the active substance. Samples are taken to perform active substance release testing. The active substance is frozen until initiation of the finished product manufacturing process.

Critical process parameters (CPPs), key process parameters (KPPs), critical process attributes (CPAs) and key process attributes (KPAs) are included in the manufacturing process description.

Control of Materials

All raw materials are sourced from approved suppliers with defined controls and specifications. Specifications have been defined and all raw materials have been qualified for their intended uses. The cell banks and plasmids are defined as starting materials to be used in manufacturing of eladocagene exuparvovec.

Cell banks:

The cell line used in the eladocagene exuparvovec manufacturing process is the HEK 293 cell line.

The source, generation and history of the producer cell banks used for clinical batches and for manufacturing process development have been provided. The master cell bank (MCB) has been characterised in line with the general principles of ICH Q5D, Ph. Eur. 5.2.3 and ICH Q5A. Issues were identified with the proposed WCB, including low viable cell density observed across expansion steps of a production batch, which resulted in termination of the batch and triggered an investigation. The

proposal in the Day 120 responses was to launch commercial production initially with the MCB and then to introduce the WCB once investigation is resolved post-authorisation. In general, the new WCB has been characterised in line with ICH Q5A, ICH Q5D and Ph. Eur. 5.2.3 and may be suitable. However, some data is still awaited before this WCB can be approved for commercial manufacture. At this time, the proposed WCB has been removed from the dossier and will be registered post-authorisation when all necessary data is available. Overall, the major objection is resolved as commercial manufacture from the MCB is sufficiently justified until such time as the WCB can be introduced. It has been confirmed that the WCB will be registered.

Future cell banks for commercial production will be manufactured under GMP. A protocol for monitoring banked cell stability has been provided and the procedure to generate and qualify future production cell banks is acceptable. The cell banks are being stored under GMP.

Plasmids:

Two starting material plasmids are described: the transgene plasmid and the helper plasmid which carries the AAV2 CAP and REP genes and the AdV helper genes necessary for replication and for the packaging of the transgene into the capsid. The structural elements of the plasmids have been described and details of functional components presented. The full nucleotide sequence of both plasmids is presented in the dossier.

Plasmid production is based on a bacterial cell bank system. It is noted that a single-tiered banking system is proposed; this has been justified in the context of anticipated cell bank usage. The information regarding the bacterial MCB generation and qualification is sufficient and, in general, meets the requirements for bacterial cells used for the manufacture of plasmids vectors for human use as specified in Ph. Eur. 5.14. The cell banks have been generated using standard methodology.

In general, the characterisation/qualification of plasmid cell banks described in the dossier follow the principles outlined in the aforementioned general chapter of the Ph. Eur., including full sequencing of the plasmids in the bacterial cell banks. A protocol has been provided describing testing to be performed on bacterial end of production cells banks (EOPCB) which is acceptable. The cell bank stability monitoring program has been registered in the dossier. Adventitious agents screening of the MCBs is in line with Ph. Eur. 5.14 and the bacterial cell banks have also been tested for the absence of lytic and lysogenic bacteriophage particles.

The plasmids for commercial use will be manufactured under GMP. The manufacturing process for the plasmids has been adequately described and includes a risk assessment regarding the suitability of the downstream process for impurity removal. The specifications for the plasmids are presented in the dossier and, in general, are in line with the requirements of Ph. Eur. 5.14.

Raw materials:

A list of compendial raw materials has been provided in the dossier. The specifications presented for non-compendial raw materials used during the manufacturing process are considered to be appropriate. With cross reference to the section on Adventitious agents, the applicant has provided a risk assessment on the potential of the manufacturing process to remove small virus particles and intends to perform a viral clearance study for the process.

Control of critical steps and intermediates

Critical process parameters and in-process controls for the upstream and downstream manufacturing process are described in the dossier. Confirmed excursions to established process control strategy ranges are investigated depending on the classification.

The control strategy for the upstream culture process is acceptable and includes appropriate KPPs and KPAs to control for cell performance and bioburden.

The control strategy for harvest complies with the requirements of Ph. Eur. 5.14: CPAs are in place for identification, vector concentration, extraneous agents (Ph. Eur. 2.6.16) and control cell testing (Ph. Eur. 2.6.16 and Ph. Eur. 5.2.3).

In general, the process parameters and process attributes defined are standard and appropriate. Data was requested and has been provided to support all proposed hold times for intermediates with respect to stability and microbiological purity.

Process validation

The active substance manufacturing process is undergoing process performance qualification (PPQ) at MassBiologics. A concurrent process validation strategy was proposed in the initial dossier.

During the first PPQ run (PPQ1), a number of deviations in the upstream process and downstream process against the validation criteria were identified. Furthermore, the data from the process demonstration runs lacked completeness and clarity and was not sufficient to support the proposed concurrent validation strategy. This formed the basis of a major objection at Day 120. With the response to the major objection, a lack of impact to the validity of PPQ1 was not agreed with respect to several deviations.

Taking into account the above issues, the proposed concurrent validation strategy was still not agreed at Day 170. In addition, it was requested that data from additional full-scale PPQ batches (AS and FP) be provided to support that the entire process performs in a consistent manner and meets the validation acceptance criteria for process parameters and attributes.

In the response to the Day 170 major objection on process validation, the applicant maintained their position that a concurrent validation approach is appropriate based on the serious and highly morbid nature of the disease, coupled with the lack of approved therapies. Full PPQ2 data is provided in the response and detailed justification is presented in relation to all deviations for both PPQ1 and PPQ2. In addition, it is noted that the filter integrity deviation for PPQ1 did not recur in PPQ2 and the microbial control strategy has been tightened. In response to the request for data on filter compatibility using product specific material (AS intermediates, AS and FP), it is proposed to bridge the existing validation data (performed with formulation buffer) to process-specific vector containing matrices post-marketing authorisation (MA). This proposal is acceptable on the grounds that (i) the filter deviations during PPQ1/2 appear to relate to isolated root causes (not related to sample matrix) and (ii) the control strategy has been updated to require that both finished product sterile filters pass filter integrity testing. The study will establish that the relevant physiochemical properties of the formulation buffer are highly comparable to the process matrices and this data can be provided as a recommendation. The data presented for finished product PPQ1 and PPQ2 is supportive of a concurrent validation strategy for the finished product and the data should be provided as part of an Annex II condition.

The other potentially impactful deviations from active substance PPQ1 have been discussed in detail, the root cause was assigned and corrective actions implemented. Although some deviations occurred (with respect to KPPs/KPAs), none were determined to impact product quality, overall process performance or the validity of the PPQ runs. All PPQ protocol acceptance criteria CPPs, CPAs were met, with exception of two deviations relating to operator error. All release criteria were met for PPQ1 and PPQ2, with the exception of residual DNA levels of the host cell derived genes of Adenovirus early region 1A/1B E1A/E1B during PPQ2. All protocol validation criteria (KPPs and KPAs) were met, with the exception of several deviations. Although the number of deviations is notable, it is accepted that appropriate actions have been implemented and that they do not impact on the validity of the active substance PPQ runs.

However, the failure of active substance PPQ2 to meet the active substance release specification for E1A/E1B residual DNA was not considered acceptable as it undermines the validity of this batch. The major objection to the concurrent validation strategy for active substance was maintained at Day 189. Further data was requested to substantiate the claim that the higher observed level of E1A/E1B in PPQ2 was related to method variability and that this batch is consistent with historical batches. The major objection also requested the revision of the method to minimise factors contributing to method variability and the tightening of the proposed specification for E1A/E1B. In the response to the day 189 major objection, data was presented using a new quantitative polymerase chain reaction (qPCR) method for control of residual E1A and E1B DNA. The method includes appropriate standard curve controls and assay validity criteria and has been fully validated in line with ICH Q2. The batch results for PPQ2 are within the range of historical results and the specifications for E1A/E1B were tightened. The major objection is resolved and the concurrent validation approach is accepted. The provision of the data on the final active substance PPQ batch (PPQ3) will be addressed as an Annex II condition. PPQ will be completed using the MCB as starting material.

A risk assessment has been provided regarding the potential for leachables/extractables across all product contact materials used during the manufacturing process. Overall, the formulation buffer components are considered low risk during routine processing and this rationale is agreed. The elements of the filtration assembly are considered low risk given that they are standard pharmacopoeial grade materials used in biopharmaceutical manufacturing.

According to the outlined process validation (PV) strategy, ongoing process verification is proposed. A high-level summary of the process verification strategy has been included. Overall, the approach appears reasonable.

Manufacturing process development

A summary of the process changes made throughout development, and their potential risk to impact product quality, form part of the comparability assessment, and are discussed below in the finished product section (Pharmaceutical development).

The control strategy includes three parameter and attribute tiers: critical, key and monitoring. Process parameters and process attributes were evaluated using a failure modes and effects analysis (FMEA) which is described in the updated dossier. All process parameter and product attribute ranges were defined on the basis of historical manufacturing ranges including from the technology transfer runs with the exception of a select number of parameters whose ranges are supported by development studies. The acceptance criteria for a number of process parameters have been tightened and a more thorough justification has been presented to support the proposed control strategy against historical manufacturing experience.

In the case of the upstream process, the representativeness of runs used to define the control strategy has been justified. In general, the data provided supports the proposed ranges for the CPPs/KPPs for the upstream process. For the downstream process control strategy, multiple partial and at-scale manufacturing runs (representative of commercial process) were executed during process development to evaluate the impact of changes introduced as part of technology transfer. Data has been presented to support the representativeness of these small-scale studies. The majority of parameter and attribute ranges defined for the revised control strategy fall within the ranges observed for the commercial-scale historical batches.

In accordance with ICH Q11, the development of eladocagene exuparvovec has followed a traditional development approach establishing process control ranges based on manufacturing process history and process development studies. The CQAs for the active substance have been appropriately defined. A comprehensive assessment of CQA control points across the process, assessed at the unit operation

level, has also been provided and supports the proposed process control strategy. Overall, the updated control strategy is acceptable and the major objection is resolved. However, the number of batches produced with the commercial process is limited. As such, while the proposed acceptance ranges for process parameters and attributes for routine control and PV seem adequate, they should be revised after a further ten active substance batches have been manufactured.

The revised section 3.2.S.2.6 of the dossier also included additional data to support appropriate process development. The removal of the density gradient ultracentrifugation step from the manufacturing process was queried as this resulted in a significant increase in empty capsids for the process B and process C materials. It has been clarified that PTC Therapeutics International Limited acquired the product after pivotal trials had been completed with the process B material and data has been presented to demonstrate relatively consistent levels of full particles across clinical and process C commercial materials. While the removal of the gradient ultracentrifugation could be seen as a retrograde step in terms of process, it is claimed that the ratio of empty/full capsids for commercial product is comparable to levels dosed in the pivotal clinical trial. This point was not entirely agreed and was further discussed in the response to the major objection on comparability (from D120 list of questions (LoQ)). Further justification was also provided to support downstream clearance of impurities.

Characterisation

Characterisation of active substance includes analysis of primary structure, higher order structure, product-related variants and potency/strength. The use of only a single batch of finished product (pilot scale reference standard) for characterisation of primary structure, higher order structure and potency was considered to be too limited and was queried. The representativeness of the pilot scale reference standard for the commercial process has been demonstrated. Additional characterisation data has now been presented for several further lots representative of the commercial process and is acceptable. The additional characterisation used either active substance or finished product since both have the same formulation. The added data include characterisation of the primary structure, higher order structure, potency of these batches. In addition, consistency of molecular weight/ratio of VPI/VP2/VP3 across commercial batches has been demonstrated. Data on post-translational modifications (PTMs) was requested and has been provided. The detected PTMs were similar across all the lots analysed. The comparability data demonstrated that although there were differences in the levels of PTMs observed amongst the clinical, PPQ and process demonstration lots, there was no clear impact on infectivity or titer. The applicant will test the PTM levels of each PPQ lot manufactured after that data is available. This is acceptable.

Product related impurities have been assessed using a range of methods and the information is, in general, sufficient. According to the results a number of truncated VP3 residues were identified. The relative levels of these truncated VP3 fragments has been presented for process C batches. Levels observed are low and relatively consistent and it has been confirmed that no clinical impact is expected from these impurities. Full capsid vs empty capsids were evaluated using two methodologies. The results are relatively consistent across batches. Given the high percentage of empty/intermediate capsules in the active substance, further characterisation of these species was requested, i.e. the potential for deleted, rearranged, hybrid DNA sequences or co-packaged extraneous DNA to be present was queried. The results of next generation sequencing were provided for several commercially representative batches and supports a low level of co-packaged DNA. The results of support that most of the DNA in the product contains intact vector sequence and there is a very low likelihood of significant rearrangement or recombination. Data was provided on plasmid, E1A/E1B, Rep and Cap DNA for a number of representative batches confirming relatively low levels and the absence of transcriptional potential of contaminating DNA.

The risk for any known oncogenic/tumorigenic sequences present in the production system has been discussed. The only known oncogenic sequences are E1A and E1B and these are controlled in the active substance specifications.

2.4.2.3. Specification

Specifications

The active substance release and shelf life specification include tests for appearance, identity, potency, purity and safety. The release specification established covers most of the relevant characteristics of AAV vectors and complies with the general principles of EMA/CAT/80183/2014 and Ph. Eur. 5.14. It was requested to include a test for genetic characterisation of the vector DNA and the applicant's proposal to verify vector DNA integrity is acceptable. In addition, it was confirmed that the test was performed for active substance as a characterisation test for all batches manufactured to date including the PPQ batches, confirming that the commercial manufacturing process consistently yields a vector that has the expected genomic integrity.

Empty capsids represent a significant product related impurity. A specification for control of full and empty AAV particles and method validation data has been provided. The applicant is recommended to investigate and introduce appropriate process improvements to reduce the levels of empty capsids in the product.

Given the limited amount of clinical data, it is acknowledged that it may be very challenging to determine clinically qualified levels of impurities. However, in general, it was considered that specifications should be narrowed to more closely reflect available batch analysis data and some rationale presented as to how specifications are considered to be clinically justified. In response, the specifications have been further justified and is accepted.

A general recommendation is made that all active substance release specifications should be re-evaluated after 10 further active substance batches are manufactured.

Analytical methods

The analytical methods for control of active substance, as described in the dossier, were lacking in detail and this was queried in the Day 120 list of questions. The requested information has now been registered in the dossier. The identity of the commercial kits has been specified in the dossier and the controls in place to ensure consistency between different lots of commercial kits has been described. The controls in place to ensure consistent cell performance during the cell culture portion of the relevant assays have been justified.

More comprehensive method validation summaries have been provided in response to the Day 120 questions and an updated method validation has been presented for some assays. In general, method validation is in line with ICH Q2.

Batch analysis

Batch analysis data is presented for several commercial scale batches including PPQ batches. All batches meet the specifications.

Reference standard

The reference standard is a commercially representative (process C) pilot scale lot manufactured at process development labs. The representativeness of this material for the commercial process has been demonstrated.

Comparability of the process C material for clinical material has been extensively discussed. A qualification protocol for future reference standards has been provided and is acceptable.

Container closure

The container closure system for the eladocagene exuparvovec active substance is a sterile Bioprocess container. Mechanical and physiochemical suitability, biocompatibility information as well as extractables data have been summarised in the dossier. In general, the specifications for container closure are acceptable and confirmation of compliance with *Ph. Eur. 3.2.2.1 Plastic containers for aqueous solutions for infusion* and *Ph. Eur. 3.2.2 Plastic containers and closures for pharmaceutical use* has been provided.

2.4.2.4. Stability

Long-term data at -80 ± 15°C is available for three batches representative of the commercial scale process demonstration batches and supports the proposed active substance shelf life. Accelerated data (5 ± 3°C) is available for one batch. In the response, it has been agreed to continue the accelerated and stressed studies for 3 months. All quality parameters meet the specification limits after 3 freeze-thaw cycles.

In conclusion, the claimed shelf life for the active substance when stored at the recommended storage condition is acceptable.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

Description of the product

Eladocagene exuparvovec finished product is a sterile, clear to slightly opaque, colourless to faint-white solution. Each vial of finished product contains an extractable volume of 0.5 mL with a total of 2.8×10^{11} vector genome copies (vg). Each mL of solution contains 5.6×10^{11} vg of eladocagene exuparvovec. Each vial is for single use only. This medicinal product should only be infused with the SmartFlow ventricular cannula.

The finished product is comprised of compendial excipients including potassium chloride, sodium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate and poloxamer 188 in water for injections.

Pharmaceutical development

The first batch used in a non-clinical and clinical study was manufactured and is called Process A. Subsequently two further lots were manufactured and were used in an additional non-clinical and two further clinical studies (Process B). The clinical experience with the product is limited. The manufacturing process was transferred to a new site, and the process was significantly changed to develop the current manufacturing process called Process C.

The commercial manufacturing process includes the addition of a new excipient, poloxamer 188. Although poloxamer 188 is part of the formulation of several approved parenteral medicinal products,

none of these products are administered via an intraputaminal route of administration. Poloxamer 188 is therefore classified as a novel excipient due to the new route of administration. Nonetheless, as poloxamer 188 is controlled according to the relevant Ph. Eur. monograph, the quality information provided on this excipient is acceptable. The other excipients are present in other authorised products using routes of administration with direct delivery to the central nervous system.

Apart from the addition of poloxamer 188, many significant and substantial changes were introduced as part of the Process C manufacturing process. A comparability exercise was presented in which the applicant compared several finished product batches from the three manufacturing processes. The Process A and B batches were used in clinical studies and none of the commercial Process C batches have been used in clinical studies. The analytical comparability exercise included tests for potency, identity product-related purity, process-related impurities and safety.

In general, the panel of tests is considered reasonably comprehensive for the purposes of demonstrating comparability of an AAV product. Overall, it remains uncertain whether the commercial batches have comparable biological activity and comparable levels of empty capsids compared to the clinical batches. However, as there are no more samples from Process A and B batches available, no further information which could aid in regulatory decision making can be obtained from these batches. Therefore, no further questions are raised from a quality perspective. The remaining uncertainty on comparability was considered acceptable in the context of the overall benefit/risk decision.

A standard risk assessment approach was used to identify the CQAs based on impact and uncertainty and the final list of CQAs covers the relevant attributes of identity, potency, purity and safety. A summary of the control strategy was provided and includes set points, acceptable ranges and historical ranges for process parameters (PPs), critical process parameters (CPPs), and IPCs. The dossier states that the criticality of each process parameter was set based on the underlying chemistry and biology, the variability and impact to CQAs and regulatory requirements. It is generally expected that a thorough justification should be provided for each process parameter that is deemed to be non-critical. However, in this case the majority of process parameters are designated as critical, the approach is therefore acceptable.

The CE marked SmartFlow cannula was used for the in-use compatibility study. Results were presented from this in-use study for vector titre, purity, and *in vitro* potency; the results showed that interaction with the cannula did not significantly impact these CQAs. A CE certificate for the SmartFlow cannula has been provided and the SmartFlow cannula is a referenced medical device in the SmPC.

2.4.3.2. Manufacture of the product and process controls

Manufacturers

The commercial manufacturing and testing facilities for eladocagene exuparvovec finished product are provided in the dossier. Appropriate evidence of GMP has been provided for the manufacturing and testing sites.

Manufacture

The physicochemical and biological properties of finished product are the same as those described for the eladocagene exuparvovec active substance. The finished product manufacturing process is relatively straightforward and involves active substance thaw, dilution with formulation buffer, sterile filtration, filling and freezing. A flow diagram of the manufacturing process has been provided which includes the relevant CPPs and IPCs associated with each step.

Overall, the manufacturing process is considered straightforward and, in general, has been described in sufficient detail.

Process controls

The process controls include CPPs, KPPs, CPAs, and KPAs. Further details of the definition of these controls can be found in the active substance section. Overall, the control strategy as described in P.3.4 is considered generally acceptable.

Process validation / verification

The applicant proposes to use a concurrent process validation strategy. Overall, given that the finished product manufacturing process is relatively straightforward and given the indication, a concurrent process validation strategy for the remaining finished product PPQ batch is acceptable. Nonetheless, given that a number of deviations have occurred during active substance and finished product process validation, the data from this finished product concurrent process validation batch is formally requested by an Annex II condition.

Data on mixing studies has been provided which shows that the registered number of manual inversions will result in a homogeneous product. A plan for the collection of data on content uniformity in further PPQ batches has been provided and is acceptable.

The proposed hold times are supported by accelerated stability studies. The fill duration is supported by media fill studies.

2.4.3.3. Product specification

Specifications

The proposed specifications include relevant tests for identity, potency, purity and safety. The commercial release and shelf life specifications for finished product.

The proposed panel of tests are generally in line with the requirements of the Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014) and Ph. Eur. 5.14 Gene transfer medicinal products for human use.

A risk evaluation concerning the potential presence of nitrosamine impurities was provided and confirmed that no risks are associated with the manufacturing process, equipment, primary packaging, or excipients. No additional testing is considered necessary.

The potential presence of elemental impurities has been assessed on a risk-based approach in line with the ICH Q3D guideline for elemental impurities. The risk of carryover of elemental impurities from reagents and materials used for manufacture is considered negligible and no additional control is required.

The applicant is recommended to re-evaluate the acceptance criteria after ten batches have been manufactured and revise them if necessary.

Analytical procedures and reference standards

The majority of release tests for the finished product are either described in the active substance section or are compendial.

Batch analysis

Batch data are provided from several batches from the different processes. All batches were well within the specification limits. The data from the several Process C lots show a good degree of consistency

and support the conclusion that the finished product manufacturing process in under a sufficient level of control.

Reference standard

Eladocagene Exuparvovec reference standards are prepared from the active substance and are used for release and stability testing of active substance batches as well as finished product lots. The reference standard is described in Section S.5.

Container Closure System

The container closure system is a Type I borosilicate glass vial, with a siliconised chlorobutyl stopper with coating sealed with an aluminium/plastic cap. The vial complies with Ph. Eur. 3.2.1 and the stopper complies with Ph. Eur. 3.2.9.

Specifications and technical drawings have been provided for the vial stopper and cap and are considered acceptable. Sufficient information on the selection of the container closure system is provided. The selected container closure system supports product stability and is suitable for its intended use.

2.4.3.4. Stability of the product

A 24 month shelf life at the long term storage condition of ≤ -65°C is claimed for the finished product. Stability data for Process C batches have been provided for up to 24 months for four batches, and supportive data of up to 18 months for an additional batch.

No significant trends were observed and the stability data are supportive of the claimed 24 month shelf life at the recommended storage conditions. Adequate data from accelerated, stress, and free-thaw studies was also provided and are acceptable. Once thawed, the medicinal product should not be re-frozen. The filled syringe prepared under aseptic conditions for delivery to the surgical site should be used immediately; if not used immediately, it can be stored at room temperature (below 25°C) and used within 6 hours of starting product thaw.

2.4.3.5. Adventitious agents

The applicant has provided an overview of the facility controls and process controls which may have a role in the reduction or clearance of viruses, as supported by literature sources. In addition, as viral clearance studies are possible for AAV vector products, the applicant has presented a plan for a post-approval viral clearance study in accordance with ICH Q5A using a panel of model viruses.

The downstream process includes a number of steps which are expected to have viral clearance capacity. Moreover, the applicant intends to perform a viral clearance study and will submit results.

2.4.3.6. GMO

Eladocagene exuparvovec is a genetically modified organism (GMO) constructed using recombinant DNA technology from wild-type (wt) AAV virus serotype 2 (wild type AAV2), which is a non-pathogenic, single-stranded DNA genome-containing, helper virus-dependent member of the parvovirus family. For further information, see the non-clinical section.

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

The active substance is eladocagene exuparvovec, a recombinant non-replicating adeno-associated virus serotype 2 vector comprising of a dopa decarboxylase variant 2 cDNA transcript, which encodes human aromatic-L-amino-acid decarboxylase isoform 1. The active substance and finished product manufacturing and testing sites have been inspected by an EU authority and the EU-GMP certificates have been issued. The major objection relating to GMP has been resolved.

A major objection was raised on the control strategy for the active substance manufacturing process at Day 120 but has been resolved. Given the limited manufacturing experience, the proposed acceptance ranges for process parameters and attributes for routine control and PV should be re-evaluated and revised, as appropriate, after a further ten active substance batches have been manufactured.

A major objection was raised regarding the suitability of the cell banks. Sufficient assurance has now been provided regarding the GMP status of the MCB and the failed WCB has been removed from the dossier. On these grounds, the major objection is resolved. A new WCB has been generated but is not yet fully qualified. Given that that sufficient assurance has been provided regarding the GMP status of the MCB, it is acceptable to use the MCB for commercial manufacture and to register the new WCB at a later date through a recommendation.

A major objection was raised in relation to the process validation data at Day 120 and the proposal to use a concurrent validation strategy. The process validation was not considered sufficient to support a marketing authorisation. This major objection is now resolved subsequent to the introduction of a new validated E1A/E1B method and retesting of PPQ2 and historical batches to demonstrate consistent batch results. The proposed concurrent validation approach is acceptable. However, while it is accepted that process consistency has been sufficiently supported at this point, there were numerous deviations and issues with the AS/FP PPQ runs which resulted in a maintained major objection until Day 189. As some of these deviations related to process controls required to meet CQAs, it is considered that process validation should be completed as part of an Annex II condition. This is on the grounds that failure to demonstrate a consistent process capable of meeting CQAs relevant to patient safety would impact on the benefit risk of the product.

The active substance has been appropriately characterised. The release specifications for the active substance cover most of the relevant characteristics of AAV vectors and complies with the general principles of EMA/CAT/80183/2014 and Ph. Eur. 5.14.

The specification for total capsids and full/empty capsids has been tightened as much as feasible and aligned to manufacturing experience. Nonetheless, the applicant is recommended to investigate and introduce appropriate process improvements to reduce the levels of empty capsids in the product. The analytical methods and validation are adequately described. Given the limited batch data available, the active substance release specifications should be re-evaluated after ten further active substance batches are manufactured. The proposed shelf life for the active substance has been supported by real time data.

The finished product contains a total of 2.8×10^{11} vector genome copies (vg) in 0.5 ml. The finished product is comprised of compendial excipients including potassium chloride, sodium chloride, potassium dihydrogen phosphate, anhydrous, disodium hydrogen phosphate, anhydrous, and poloxamer 188 in water for injections.

A major objection was raised at Day 120 on the deficiencies in the comparability exercise, mainly due to the fact that data from only one Process A clinical batch and one Process B clinical batch were available. No further data is available for these batches due to sample depletion. It therefore remains uncertain whether the commercial batches have comparable biological activity and comparable levels

of empty capsids compared to the clinical batches. As there are no more samples from Process A and B batches available, there is no further quality data which could be of use for decision making. Therefore, no further questions were raised from a quality perspective. The remaining uncertainty on comparability was considered acceptable in the context of the overall benefit/risk decision.

Data from finished product PPQ batches have been provided and the proposal to use a concurrent validation approach for manufacture of an additional finished product PPQ batch is acceptable in principle. An Annex II condition was agreed in relation to the results of the next active substance and next finished product concurrent process validation batches, including hold time data for the finished product batch by March 2023.

The proposed finished product specifications include relevant tests for identity, potency, purity and safety. Given the limited available batch data, it is also recommended to re-evaluate the acceptance criteria for finished product release after ten batches have been manufactured and revise them if necessary.

An overview of the facility and process controls which may have a role in reduction or clearance of viruses and a viral risk assessment were provided.

In conclusion, based on the review of the quality data provided, the marketing authorisation application for Upstaza is considered approvable from the quality point of view.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The overall quality of Upstaza is considered acceptable when used in accordance with the conditions as defined in the SmPC. The quality of the active substance and finished product is controlled by adequate test methods and specifications. Adventitious agents' safety including TSE have been sufficiently assured.

The CAT has identified the following measure necessary to address the identified quality developments issues that may have a potential impact on the safe and effective use of the medicinal product:

In order to further assess process consistency and maintain patient's safety, the applicant shall provide the results of the next active substance and next finished product concurrent process validation batches, including hold time data for the finished product batch. This data should be provided by March 2023 (Annex II condition).

The CHMP endorse the CAT assessment regarding the conclusions on the chemical, pharmaceutical and biological aspects as described above.

The applicant agreed to the Recommendations for future quality development.

2.4.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CAT recommended some points for further investigation.

The CHMP endorses the CAT assessment regarding the recommendations for future quality development as mentioned above.

2.5. Non-clinical aspects

2.5.1. Introduction

The nonclinical pharmacology program for eladocagene exuparvovec consists of in vitro studies in human cells to characterize the expression and biological activity of eladocagene exuparvovec. Since reduced dopamine levels in the striatum is also the underlying pathophysiology in Parkinson's disease, preclinical efficacy studies published with rAAV2-hAADC vectors in rat and non-human primate models of Parkinson's disease are supportive of the eladocagene exuparvovec pharmacology program. In addition, a published nonclinical efficacy study in a mouse model of AADC deficiency using rAAV9-hAADC is supportive towards proof of concept for human AADC gene therapy.

2.5.2. Pharmacology

AAV vectors are nonenveloped 25 nm particles with a foreign DNA packaging capacity of 4.6 kb. They have been clinically demonstrated to be safe in the CNS, and certain serotypes display strong neural tropism. Importantly for CNS gene therapy applications, AAV can transduce nondividing cells and has the ability to confer long-term stable gene expression without associated inflammation or toxicity. The AAV-delivered genome persists mostly as an extrachromosomal episome, but the approximately 1% of genomes that integrate in the target genome raises the hypothetical risk of insertional mutagenesis and oncogenesis. (Gray et al., 2010)

Rationale Supporting the Selection of the Vector Serotype, Route of Administration, and Dose of Vector

The use of AAV vectors has been clinically demonstrated to be safe in the CNS, and AAV2 has been studied extensively. AAV2 has neural tropism, and is the choice of the applicant, however AAV9 also has unique characteristics that would also make it an attractive choice for neuronal transduction, such as the ability to transduce more cells than AAV2 (Gray et al., 2010). The applicant does not discuss their choice relative to other AAV serotypes.

The applicant has provided a justification for localised delivery to the putamen. In this justification they raise concerns regarding ectopic over expression of dopamine and serotonin. The amount of AADC enzyme would be considered to be the rate limiting step in the conversion of L-dopa to dopamine, therefore the dose is crucial to not result in overproduction of dopamine.

The choice of localised delivery as opposed to global delivery is endorsed. Lee et al., 2014 clearly demonstrated that ICV administration resulted in widespread but asymmetric staining, and AAV9 AADC transduction following ICV injection does not mirror WT AADC staining, especially in the hippocampus and cortex. Staining in the substantia nigra was not observed.

The applicant states that over expression of eladocagene exuparvovec gene therapy outside of the putamen can result in excessive serotonin production. Dopamine and serotonin are produced by distinct groups of neurons in the brain, and ectopic dopamine expression may be a concern, however the transduction of the serotoninergic neurons was not efficient. Biodistribution studies carried out by the applicant using iPut route of administration, demonstrated that in animals the highest transgene deoxyribonucleic acid (DNA) concentrations were found in the key target therapeutic area, the putamen. The issue will not be pursued further, as using the clinical route of iPut administration, eladocagene exuparvovec is expressed primarily in the putamen and ectopic expression of dopamine and serotonin is unlikely.

Dopamine is normally synthesized and stored in the terminals of neurons that originate in the substantia nigra (SN) and project to the striatum (caudate and putamen). The SN is the source of a clinically important dopaminergic pathway to the striatum, i.e. dopamine released in the putamen originates in SN neurons. In PD treatment AADC is delivered directly to the putamen presumably as there is degradation of the dopaminergic nigrostriatal neurons. The pathophysiology of AADC deficiency is however different and these pathways appear to be unaffected (Lee et al., 2009). The applicant also states that infusions to the putamen did not have the same safety concerns as infusions to the substantia nigra and reference a study by Bartus et al., (2013), in fact the Bartus paper actually states that delivery to the SN and putamen was well tolerated with no safety complications identified over 2 years. Furthermore, it has been proposed that better efficacy could be achieved in AADC deficiency by infusing the SNpC as opposed to the putamen, as when infusion into the putamen is performed AAV-2-AADC transduces medium spiny neurons, and these cells do not synthesise appreciable amounts of L-Dopa, as opposed to the SnPC which does (San-Sebastian et al, 2014). Hadaczek et al., (2016) clearly demonstrated that targeted delivery of AAV1 or AAV2 to the striatum results in robust and global transduction of the striatum as well as many cortical regions via axonal transport. The applicant was asked to further justify the choice of putamen as injection site in AADC deficiency as opposed to the substantia nigra. Furthermore, the applicant was asked if targeting the putamen for delivery of eladocagene exuparvovec results in transduction of other brain structures beyond the striatum, and what are the implications of AADC expression in these locations.

The applicant provided a discussion, three clinical studies have demonstrated that surgical injection of eladocagene exuparvovec to the putamen results in clinically meaningful improvement in motor and cognitive function in aromatic L-amino acid decarboxylase (AADC) subjects and is well tolerated. In contrast, adeno-associated virus serotype 2 delivery to the substantia nigra (SN), a relatively small structure deep within the midbrain in proximity to critical neuronal and vascular structures, presents a practical problem for stereotactic navigation. Expression of the transgene outside of the striatum is expected to be limited with direct administration of AAV2 to the putamen.

Other possible routes of administration that were explored in the planned juvenile toxicity studies, namely intrathecal/intra-cisternal and ICV administration Intraputaminal administration has the advantage of both achieving the highest transgene concentration and limiting non-specific distribution following administration. IT and ICV routes of administration possibly increase safety concerns, however, these routes of administration are more amenable to paediatric dosing. These three routes of administration were explored in a juvenile toxicity study (CRL 1144-023), a 4-week NHP biodistribution study to evaluate 3 routes of administration (RoA) including intraputaminal (iPut), intracerebroventricular (ICV), and intrathecal (IT)/intra-cisternal routes. Monkeys receiving vector via iPut administration yielded the highest levels of vector-derived gene expression (copies/10 ng RNA) in the putamen (1000 to 6000 copies), globus pallidus (4000 copies), and caudate (40 to 500 copies). In contrast, there was no expression in putamen or globus pallidus following ICV or IT administration, and no expression in the caudate following IT administration, with negligible expression (<45 copies) in the caudate following ICV administration. Unlike ICV and IT routes, following iPut dosing, there was no vector-derived gene expression at any level of the spinal cord and no expression in the dorsal root ganglia, greatly diminishing concern regarding this tissue of special toxicological interest for AAV-related therapies. Therefore, based on the results of this study neither IT nor ICV routes appear feasible

Doses used in models of Parkinson's in NHPs were of the order of 10^{11} vg. FIH trials in PD utilised 9 x 10^{10} and 3 x 10^{11} vg. There are differences in the pathophysiology of these two diseases. A higher dose may be required in the complete absence of enzyme, as opposed to degeneration of neurons of the nigrostriatal pathway as occurs in PD.

The applicant has reasoned a child's brain mass (1000g) is approximately 75% that of an adult (1350g) therefore at 60% of the PD trial dose this would equate to 1.8 x 10^{11} vg (the current proposed dose), however this is based on brain mass.

The human brain is 13–18 times larger than the monkey brain. However, this ratio is significantly smaller for striatum (5.7–6.5), caudate nucleus (4.6–6.6) and putamen (4.4–6.6) (Yin et al., 2009). Hadaczek et al., (2010) measured the volume of AADC gene transduction from sequentially stained brain sections in two monkeys, 8 years after the original study, Bankiewicz et al., (2006). The volume of the infused (Vi) vector (6 × 30 µl, in one hemisphere) resulted in a volume of expression (Ve) of 580 and 490 mm^3 of the striatum. Although 35–40% of the striatal volume was transduced, only 5–6% of the neurons within this region expressed the AADC transgene, and only neurons were transduced by AAV2 in the brain. (Total dose 3.6 x 10^{11} vg, in one hemisphere). Considering the volume of the human striatum is ~5 times larger than the monkey striatum, this would require a larger dose to achieve transduction of the same volume. The proposed total dose for humans is 1.8 x 10^{11} vg, in a volume of 4 x 80µl. The applicant justified this dose on the basis that clinical studies in adult PD using rAAV2-hAADC reported that doses up to 3x 10^{11} vg of rAAV2-hAADC in a volume of 4×50 µl were well tolerated following bilateral, intraputaminal infusions during a single operative session. 1.8x10^{11} vg eladocagene exuparvovec for treatment of children with AADC deficiency was expected to improve AADC activity in the putamen based on the relative average brain mass of an adult brain (1350 g), compared to a child brain (1000 g), where the 1.8 x 10^{11} vg dose is approximately 60% of the highest dose tested in adult PD patients. Furthermore, the dose volume (4×80 µL) in the current study (AADC-010) exceeded the dose volume (4×50 µL) in adult PD patients potentially enabling the transduction area in the current study to be larger than the transduction area of the adult PD patients.

Primary Pharmacodynamics

In primary pharmacodynamic studies, the applicant demonstrated expression of the AADC protein in HEK293 cells following transfection with eladocagene exuparvovec and determined the enzymatic activity of the AADC protein expressed. Expression from the vector was achieved however no loading control blot has been provided to demonstrate equal loading of protein, neither has the amount of protein per lane been stipulated.

The applicant has transduced HEK293 cells, these cells are routinely used due to their ease of use. The applicant does not state duration of expression in these cells. No proof that neuronal cells can be transduced with rAAV2-hAADC and express the enzyme has been provided. The applicant was asked to justify the use of these cells. The applicant acknowledges that an *in vitro* demonstration of rAAV2-hAADC transduction of neuronal cells has not been carried out, however positron emission tomography imaging from subjects dosed with eladocagene exuparvovec shows that dopamine is produced in the putamen indicating that rAAV2-hAADC transgene is expressed in neural cells in the putamen. HEK 293 cells appear appropriate for the Potency assay due to permissibility of AAV2 transduction, and HEK293 cells demonstrate no evident tissue-specific gene expression signature and express the markers for renal progenitor cells, neuronal cells and adrenal gland.

The applicant has not carried out any *in vivo* pharmacology studies with eladocagene exuparvovec. However, multiple nonclinical studies in models of Parkinson's disease involving CNS delivery of rAAV2-hAADC, which are relevant to eladocagene exuparvovec, have been reported. These studies were generally conducted with rAAV2-hAADC containing the same capsid, promoter, and encoded protein as eladocagene exuparvovec and are therefore supportive information. In addition, the doses used in these published studies span the previous doses of rAAV2-hAADC reported in human studies and the clinical dose of eladocagene exuparvovec.

Subsequent to the applicant commencing clinical studies, Lee et al., generated a mouse model of AADC deficiency (Lee *et al*., 2013, 2014). They utilised an AAV9-AADC construct and transduced the brain

using ICV, thereby not specifically targeting the putamen. They demonstrated widespread AADC expression in the mouse brain, with normalised DA levels, resolving motor symptoms, however ectopic DA expression was a concern, and transduction of serotonergic neurons was not efficient. Desired effect is in the striatum and/or putamen. In the brain, dopaminergic and serotoninergic neurons also reside in the ventral tegmental area, the dorsal raphe nuclei, and other areas. AAV9 AADC transduction following ICV injection does not mirror WT AADC staining as visualised by immunohistochemistry.

Sánchez-Pernaute *et al*., (2001) demonstrated that a single infusion of AAV2-AADC was sufficient to restore the decarboxylating capacity to 50% of normal striatal activity in 6-OHDA hemiparkinsonian rats. Thereby suggesting that it is possible to restore AADC activity to normal levels if the entire striatum is transduced. The applicant also described a study in rats by Shen et al., (2000) and similar studies in NHPs by Muramatsu et al., (2002) and Sehara et al., (2017), which utilised triple AAV treatment expressing (1) tyrosine hydroxylase (TH) which catalyzes the synthesis of L-DOPA from tyrosine, (2) AADC, and (3) guanosine triphosphate (GTP) cyclohydrolase I (GCH). In these studies it is difficult to appreciate the contribution of AADC as two other enzymes are co-expressed.

In a non-human primate model of Parkinson's disease, long-term clinical improvement after treatment with AAV2-hAADC was observed (Bankiewicz *et al*., 2006).

No secondary pharmacology studies were conducted and this is acceptable due to the targeted delivery of the vector to the brain and its limited distribution outside the brain.

Safety Pharmacology

Stand-alone safety pharmacology studies were not conducted with eladocagene exuparvovec. An evaluation of CNS effects was included in the 6-month GLP toxicology study. No eladocagene exuparvovec related effects on CNS were observed during the FOB evaluation. No stand-alone respiratory or cardiovascular safety pharmacology studies were conducted. Clinical observations and FOB measurements did not indicate any effects on respiratory function. According to ICH S7A, it is sufficient to evaluate safety pharmacology endpoints as part of toxicology and pharmacology studies for biotechnology-derived products that achieve highly specific targeting (such as AAV vectors).

Overall, eladocagene exuparvovec expresses the enzyme AADC, and expression of AADC appears to restore dopamine production. The provided non-clinical pharmacology package could be sufficient to support the marketing authorisation application AADC deficiency provided the other concerns can be adequately addressed.

2.5.3. Pharmacokinetics

The pharmacokinetics (PK) of eladocagene exuparvovec was evaluated as part of a 6-month Good Laboratory Practice (GLP)-compliant toxicity and biodistribution study in rats. Supportive and relevant PK data with other recombinant adeno associate virus type-2-human aromatic l-amino acid decarboxylase (rAAV2-hAADC) vectors were also obtained from published literature.

A series of analytical and bioanalytical methods were developed to support the eladocagene exuparvovec nonclinical program. Methods were appropriately validated and met applicable standards for performance.

The standard ADME studies for conventional medicinal products are not typically relevant for GTMPs, and as such standard pharmacokinetic assessments were not conducted as part of the eladocagene exuparvovec gene therapy program. The distribution of eladocagene exuparvovec was evaluated as part of the nonclinical program in the 6-month GLP toxicity and biodistribution study in rats (Study 8366537, AADC-003). Eladocagene exuparvovec was distributed to the CNS following bilateral infusion

to the putamen, with distribution primarily to the injection site. The AADC transgene was expressed throughout the entire duration of the study in rats. No evidence of shedding of the vector in blood or CSF fluid was observed.

Protocol-specified reverse transcriptase polymerase chain reaction (RT-PCR) analyses were conducted as per protocol and conducted without deviation except for sample-related issues (eg, not collected, insufficient sample).

Primer and probe sequences used in the qPCR and RT-qPCR were provided. The sequences for the quantitative polymerase chain reaction assay were designed based on their specificity for the human dopa decarboxylase (DDC) cDNA sequence. To ensure amplification of vector transgene and not genomic DNA, the forward and reverse primers were designed to span exon-intron boundaries (Exons 6 to 7 and Exons 7 to 8, respectively). Designed primers and probe were tested in 3 different commercial master mix preparations for the detection and quantification of the adeno-associated virus expressing the human aromatic L-amino acid decarboxylase gene plasmid. The primers and probes were analyzed in the presence of human and rat DNA to assess specificity. Additionally, the same primer and probe set was used to develop the reverse transcriptase polymerase chain reaction assay and was tested in 2 different commercial master mixes. Rat and human liver ribonucleic acid were used as a specificity control. Both assays were validated in accordance with the ICH Q2 (R1).

Expression of the DDC gene was measured by qPCR for vector DNA and RT-qPCR for mRNA expression. As expected, the presence of eladocagene exuparvovec DNA was confirmed in the putamen (the dose site) at all time points and dose groups and was at its highest level on Day 7 in high dose (7.5×10^9 vg) animals. However, different volumes of the vector were administered to each group, 0.5 (low dose) – 5 µl (high dose). In calculations provided, the dose-normalized (vg/µg DNA/vg dose) biodistribution at the dose site was equivalent between the high dose and low dose at Day 7 ($4.56x10^4$ versus $4.57x10^4$/µg DNA/vg), thereby suggesting that the volume of the dose did not affect the volume of distribution 1 week after dosing. In addition, the high dose and vehicle had the same dose volume of 5 µL. Since there were no toxicologically significant findings at the high dose, the no observed adverse effect level (NOAEL) was set at the high dose ($7.5x10^9$ vg). Based on these data, the dosing volume was determined to have no impact on the NOAEL.

Eladocagene exuparvovec DNA was detected in control animals and not detected in some Group 3 animals. More DNA copies were detected in lumbar and thoracic spinal cord in control group than in Group 2. The results suggest that differences between groups represent biological variation of vector distribution combined with dose. Even at the high dose (Group 4) there were 2 samples <LLOQ, 3 samples <200 copies, and 1 sample <800 copies, while the remaining 6 (of 12 samples) varied from 5000 to 23000 copies. The applicant states that this was due to cleaning procedures which were not sufficient to remove the vector from the fixed needle syringes used in the bioanalytical procedure. The applicant has further clarified that the issues detected and investigated with respect to insufficient cleaning were not a result of the syringes used for dosing, but instead those used for quantitative polymerase chain reaction (qPCR). The Hamilton syringes used for dose administration were distinct from the fixed-needle syringes utilized during qPCR. Therefore no mis-dosing occurred.

Eladocagene exuparvovec distributed primarily to the CNS but distribution to other tissues cannot be excluded based on the non-clinical data. The applicant was therefore asked to provide a discussion on whether the non-target expression may pose safety concerns. The rat biodistribution/toxicity study (AADC-003) resulted in no evidence of consistent transgene presence in the periphery either in blood (as a transgene transport mechanism) or in peripheral non-neural tissues. Even within the CNS in which distribution occurred (eg, cerebellum and spinal cord), the expression of transgene was minimal to mild even at the highest dose level. The strongest transgene levels were observed in the putamen, the target tissue. It is considered, given negative/negligible vector presence in rat blood and CSF by

Day 7, combined with both low vector presence/transfection within nontarget CNS tissue at Day 7, to be a low probability that a therapeutic dose of eladocagene exuparvovec in human subjects would result in either peripheral tissue distribution of vector or of any transgene expression.

The applicant has also submitted two new PK-studies for biodistribution in cynomolgus (#1144-015 and #1144-023). In the first study (#1144-015), there was an assessment of the distribution of rAAV-eGFP (+/- 0.001% poloxamer 188 [P188]) from a single bilateral intra-putamen injection in cynomolgus monkeys. In the second cynomolgus study (#1144-023), the CNS distribution was assessed for rAAV2-hAADC from a single bilateral intra-putamen (BIP), intracerebroventricular (ICV), or intrathecal (IT; lumbar puncture) injection in cynomolgus monkeys. Considering the limited number of animals in the experimental groups, and the extensive inter-individual difference in vector copy levels, it is difficult to draw strong generalized conclusions beyond noting that the inter-putamen route seems most feasible to introduce high levels of the transgene into the putamen (compared to the ICV and IT routes). There were no signs of vector or poloxamer 188 mediated toxicity beyond the damage caused by the injection procedure into the neural tissues. That being said, one cannot dismiss the possibility that the transgene will spread to adjacent brain regions (e.g. globus pallidus, caudate, amygdala) at least in some individuals. The consequences of this spread are not clear but there were no alarming clinical signs detected in cynomolgus in the present studies.

The applicant argues that non-target expression of eladocagene exuparvovec DNA in the periphery would likely be limited by normal physiological control mechanisms and would not be considered to pose a safety concern. This may or may not be true (especially considering the near putamen levels of the transgene in some brain regions). The applicant also notes that there may be a pharmacological approach to handle to high AADC levels if needed. It is noted that Carbidopa is a peripherally acting decarboxylase inhibitor, which prevents AADC activity systemically and thus increases central efficacy. Thus, theoretically, excessive non-target expression of eladocagene exuparvovec DNA that presumably increases AADC activity to a non-physiological level, could be safely treated with carbidopa and thus minimize any potential safety concerns.

The applicant also described a biodistribution study by Cunningham et al., (2008), where biodistribution of AAV2-hAADC was assessed over a wide range of vector dose in 12 monkeys with parkinsonian syndrome, 6 months after intraputamenal infusion. Biodistribution of rAAV2-hAADC in brain and peripheral tissues was assessed which demonstrated that total copies of vector DNA increased in the brain in a roughly dose-dependent manner. Biodistribution was also assessed in eight peripheral organs, in the serum, and in the CSF to determine whether AAV2-hAADC delivered to the brain can spread to non-neuronal tissues. No vector DNA was detected in fluids or tissues, other than trace amounts in spleen in the two highest-dose groups, 3.4×10^{11} vg/brain and 1×10^{12} vg/brain.

The applicant is relying on publications from the Bankiewicz group as supporting information, however there appear to be differences in their methodology. In Cunningham et al., (2008), AAV2-hAADC was infused bilaterally at four sites (2/hemisphere) into postcommissural PT at the following rates: 0.1 µl/min (10 minutes), 0.2 µl/min (10 minutes), 0.5 µl/min (10 minutes), 0.8 µl/min (10 minutes), and 1.0 µl/min (36 minutes) The applicant in their study 8366537 describes their rate of infusion as 0.5 µl/min. Furthermore, the applicant uses different volumes for each of their groups from 0.5 µl to 5 µl, Cunningham used a consistent volume at each infusion site. The applicant provided a discussion arguing that differences in infusion rates or volumes appears to have minimal differences on final distribution. Distribution of vector is largely a function of the vector 'spread' within the target area at the time of infusion of the liquid volume coupled with subsequent secondary movement of vector, both within and beyond the target area via additional processes (eg, diffusion, active transport). The 'initial spread' of vector is in part a function of hydrodynamics during infusion, along with biological aspects, such as vector adhesion and uptake into cells; a smaller volume (eg, 0.5 µL) would likely result in smaller initial target area 'spread' than a larger volume (eg, 5 µL). However, this would not affect

secondary vector distribution, which would be a function of local vector concentration and therefore of vector dose.

Overall, biodistribution of eladocagene exuparvovec is mostly confined to the CNS following bilateral infusion to the putamen. The applicant has provided new studies, in response to the D120 LoQ, in cynomolgus and a discussion on the potential safety concerns. While there is some spread to adjacent brain regions, there are no indications of vector/transgene or poloxamer 188 related toxicity. While the argument for normal physiological control of excessive levels of transgene expression in non-target tissues is very speculative, the long-term San Sebastian study seems to support a non-toxicity profile (at least for 9 months) and the carbidopa hypothesis seems a testable option.

2.5.4. Toxicology

The nonclinical safety program conducted to characterize the toxicity and biodistribution of eladocagene exuparvovec comprised of two studies, a 30-day study, and a 6 month study, both in rats. Additional published toxicity and biodistribution studies in nonhuman primates with rAAV2-AADC using the same AAV2 capsid, promoter and human dopa decarboxylase (DDC) gene provided supportive toxicity data for the safety assessment of eladocagene exuparvovec.

Comparability of the clinical lots 2004-101 and PBR-0045- 001 to lots made using the intended commercial process was provided. It is clear that in the initial submission no nonclinical studies had been carried out using the intended commercial formulation. Furthermore, the commercial formulation contains 0.001% poloxamer 188 (Pluronic F68). The applicant claims that based a study carried out by San-Sebastian et al., (2014) that the administration of 0.001% pluronic to the brain is safe. However, San-Sebastian (2014) transduced the substantia nigra pars compacta and the VTA, not the putamen., The total volume transduced to these areas was 60 µl, the proposed dose volume in humans is 320 µl. At an absolute level this is ~5 times less than that proposed for injection into human. A toxicological assessment of Poloxamer 188 injected into the brain was requested and provided in response.

Toxicological Assessment of Poloxamer 188 (P188)

The applicant carried out two new nonclinical studies, one in rat, and one in NHP, in support of the use of P188;

- *Poloxamer 188: Single Dose Toxicity Study in Rats via Bilateral intra Putaminal Injection (Study 8446039, GLP).*
- *A non-GLP Safety and Biodistribution Study of rAAV-eGFP With and Without Poloxamer Following Intra-putaminal Infusion in the Non-Human Primate (Study AADC-2020-005, CRL 1144-015)*

In addition, the applicant provided a literature-based risk/toxicity assessment.

- *Poloxamer 188: Single Dose Toxicity Study in Rats via Bilateral intra Putaminal Injection (Study 8446039, GLP)*

This study was intended to evaluate the safety of P188 in rats at 0.003% and 0.010% concentrations administered once via bilateral intracranial injection into the putamen.

No P188-related mortality occurred. There were no adverse effects on clinical observations, FOB, ophthalmic exam, body weight or body weight gain, food consumption, clinical pathology (hematology, clinical chemistry), organ weights, macroscopic observations, or microscopic examination

The NOAEL for P188 was 0.010% (w/v). Based on the concentration of P188 (0.010%) and dose volume of administration (5 µL/side), this NOAEL value is equivalent to a dose of 0.5 µg/side of P188 or 1 µg total per animal.

- *A non-GLP Safety and Biodistribution Study of rAAV-eGFP With and Without Poloxamer Following Intra-putaminal Infusion in the Non-Human Primate (Study AADC-2020-005, CRL 1144-015)*

This study was intended to evaluate distribution of a recombinant AAV2-delivered reporter gene (green fluorescent protein gene [GFP]) in absence or presence of 0.001% P188, as well as evaluate dose responsiveness in presence of 0.001% P188. The recombinant adeno-associated virus, serotype 2 (rAAV2) vector was identical to that used for eladocagene exuparvovec and this study also evaluated limited toxicological parameters.

The biodistribution of the recombinant adeno-associated virus (rAAV)-enhanced GFP was evaluated following a single intraputaminal infusion in monkeys. Intraputaminal injection was performed at a rate of 5 µL/minute. The following parameters and endpoints were evaluated in this study: mortality, detailed clinical signs, body weights, clinical pathology parameters (haematology, clinical chemistry), bioanalysis (anti-drug antibody [ADA], cerebral spinal fluid [CSF] biodistribution), gross necropsy findings, histopathologic examination, and tissue biodistribution.

There was no effect due to the presence of 0.001% P188 in the dosing formulation (compared to Control also with P188 or to the active comparator without P188) on procedure-related (infusion needle) brain parenchymal microscopic findings, or anti-AAV2 antibody levels, or rAAV2-GFP dose-responsive vector biodistribution. Additionally, there were no effects in this study (with or without P188) on mortality, detailed clinical signs, body weights, food consumption, clinical pathology parameters (hematology, clinical chemistry), gross necropsy findings, histopathologic examination.

The biodistribution results from the tissues analysed revealed a general biodistribution pattern although variability was observed. Despite such variation, overall, the highest concentrations of GFP viral vector at Day 8 were consistently detected in the putamen (dorsal/ventral) and globus pallidus. Vector levels in these regions were dose responsive and durable (lasting to Day 30).

Poloxamer 188 Risk Analysis

The inclusion of P188 in pharmaceutical formulations is not novel. As of 2020, P188 has been approved by FDA as an inactive ingredient in 21 marketed products. Due to its amphiphilic chemical properties, P188 has beneficial manufacturing properties (surfactant, dispersing agent, prevention of vector aggregation) necessary for production of a quality drug product, and potential beneficial pharmaceutical properties (reduction of trauma-induced inflammation/damage of neuronal tissue) (Patel 2009).

Pharmacokinetic and metabolism data from both animals and humans provide valuable insight into these general topics from which reasonable extrapolations can be made regarding likely events and safety in the brain parenchyma. It is reasonable to conclude the following regarding P188 in the putamen:

1. It is primarily distributed in extracellular fluid and does not bind to membrane extracellular surfaces, likely including neuronal tissue (Grindel 2002).
2. It is freely diffusible in the extracellular compartment and rapidly equilibrates between CSF and other body fluids (lymph, interstitial fluid, blood) (Willcox 1978).
3. It is expected to redistribute from brain to blood circulation as mostly free/unbound P188 (Willcox 1978).

4. It is likely not metabolised in the brain since whole body clearance (half-life in humans of 7 to 8 hours) is primarily via renal mechanisms without metabolism (Grindel 2002).
5. Tissue distribution of P188 in animals suggests an approximate ratio of 10:1 for plasma to-brain P188 levels (Willcox 1978).
6. based on animal data for tissue and brain partitioning, the estimated human brain Cmax could be as high as 73 µg/mL (or 73 µg/g brain weight based on partitioning to the brain at 8% of the plasma value).

The absolute amount of intraputaminally administered P188 in the rat GLP toxicity study at the NOAEL of 0.010% P188 is equal to 1 µg P188 total.

In the proposed clinical formulation of 0.001% P188, dosed in a volume of 320 µl, the total amount of P188 administered is 3.2 µg. In comparison to the rat dose of 1 µg P188 per 2 g brain = 500 ng/g, this provides a margin of 156 for a child's brain (1000g) and 210 for an adult brain (1350 g).

Single dose toxicity

Two single-dose studies were conducted to assess toxicity at 30-days and 6 months. The 30-day toxicology study evaluated the potential toxicity of eladocagene exuparvovec in rats following a single direct injection into the putamen at dose levels of 0, 3×10^9, and 7.5×10^9 vg or into the substantia nigra at a dose level of 1.5×10^9 vg. The study was conducted in support of clinical development, and infusions to both putamen and substantia nigra were explored as potential routes of administration. Both routes were shown to be well tolerated.

In the 6-month study a single eladocagene exuparvovec administration via bilateral IPI at doses up to 7.5×10^9 vg was generally well tolerated with no obvious signs of toxicity. Based on the study results, the highest dose administered (7.5×10^9 vg) in the study provides a safety margin of 21 over the human dose used in clinical studies, as calculated by the applicant. The dose is chosen based on vector genomes (vg) per gram of brain weight, since eladocagene exuparvovec is injected directly into brain tissue [average brain weights: 2 g (rats) and 1,000 g (child)]. Based on brain weight the ratio of human:rat is 500:1, however based on structure volume an adult putamen = 3.5 cm^3 (Yin et al., 2009) and rat striatum = 0.045cm^3 (Andersson et al., 2002), an approximate ratio is 77:1. Other studies referenced in the dossier calculate dose based on volume of the structure targeted (San-Sebastian, 2012 and 2014). Due to large differences in brain volume, and limited diffusion/ active transport of vector, the applicant was asked to discuss if this is appropriate method of scaling. The applicant referenced three papers in support of their approach, these papers describe volumetric errors with respect to stereotactic surgical procedures where accuracy and precision are essential. Yin et al (2009) state they have taken these very concerns into account and while absolute volume data can be affected by error, the volumetric ratios between NHP and humans would remain valid. The calculations provided by the applicant have demonstrated more conservative safety margins when calcuations are based on structural volume. Sufficient acceptable margins are still achieved using this method of calculation.

Blood samples were collected for anti-drug antibody (ADA) evaluation against the vector capsid. The formation of anti-drug antibodies against the AAV2 vector capsid was dose dependent with the highest incidence observed in Group 4 high-dose animals at Days 30 and 180. Anti-drug antibodies were not evaluated against the transgene product, aromatic L-amino acid decarboxylase (AADC) at the time of the study because an antibody assay against AADC was not available at that time. The product was reported in the sub-report submitted on 18 October 2018, which shows that the presence of anti-drug antibodies had no effect on the biodistribution or expression.

Eladocagene exuparvovec is a single dose gene therapy with persistent gene expression through 6 months in rodents (Leidos 8366537, AADC-003) which provides chronic exposure and, therefore, no repeat-dose studies were conducted.

Genotoxicity and carcinogenicity

It was previously agreed with CHMP that genotoxicity, carcinogenicity and evaluation of insertional mutagenesis studies were not required (EMEA/CHMP/SAWP/236092/2018). The applicant has provided a discussion on the risk of insertional mutagenesis and carcinogenic potential. Based on the weight of evidence discussed regarding episomal AAV2 vector biology, integration sites, clinical experience with recombinant AAV2 vectors, the lack of any histologic or clinical evidence for tumor formation in eladocagene exuparvovec or rAAV2-AADC chronic toxicology studies in rats, carried out by the applicant, and supportive data from nonhuman primates, there appears to be a low risk for genomic integration or tumorigenicity in post-mitotic neuronal cells. Thus, the risks for DNA insertions and deletions are similar to spontaneous rates observed in normal cells.

Reproductive and developmental toxicity

No specific developmental and reproductive toxicology studies were conducted by the applicant with eladocagene exuparvovec, in agreement with CHMP. Given the local administration of eladocagene exuparvovec to the brain and lack of systemic exposure, and the absence of biodistribution to the gonads, the risk for germline transmission is low.

Juvenile toxicity

The applicant has agreed a paediatric investigation plan and been granted a deferral for recombinant adeno-associated viral vector serotype 2 carrying the gene for the human aromatic L-amino acid decarboxylase protein (EMEA-002435-PIP01-18) in accordance with Regulation (EC) No 1901/2006 of the European Parliament and of the Council. Two further nonclinical studies are planned to determine the biodistribution and toxicity of eladocagene exuparvovec in Cynomolgus monkeys. An update was requested in the D120 LoQ regarding the timelines of these studies.

The first of these two studies (CRL 1144-023) in juvenile monkeys is now complete; final study report dated 31 August 2020, and was submitted with the D120 responses, in response to Q160. Based on the data obtained in the initial RoA study demonstrating superior expression in the target putamen region with iPut administration despite using a 10-fold lower dose as compared to ICV and IT administration, a waiver (EMEA-002435-PIP01-18-M02 [eladocagene exuparvovec]) for the second study was submitted to PDCO and accepted 16 December 2020.

- *A 4 Week Biodistribution Study of rAAV2-hAADC by Multiple Routes of Administration in Cynomolgus Monkeys (Study No. 1144-023)*

In the initial biodistribution study (CRL 1144-023), the iPut group was dosed at 1.2x 10^{10} vg. The doses in both the ICV and IT dose groups were increased 10-fold (to 1.2x 10^{11} vg) to potentially achieve a comparable distribution to target brain nuclei as that attained via the iPut route, given the assumed greater biodistribution of vector via the cerebrospinal fluid (CSF) of these 2 routes.

The 1.2x 10^{10} vg dose for iPut administration was selected to represent a pharmacologically active dose (PAD) in monkey and determined via a brain weight scaling of a clinical human dose of 1.8x10^{11} vg (as per Studies AADC-010 and AADC-011 [for subjects >3 years old]). Dose scaling was based on brain weight. Similar dose scaling results was demonstrated based on putamen volume.

Overall, bilateral intra-putaminal, unilateral intracerebroventricular, or intrathecal dosing was well tolerated. All animals received a full dose of test article.

Animals receiving the rAAV2-hAADC viral vector via intra-putamen dose yielded the highest levels of vector derived gene expression in the putamen, caudate, and globus pallidus as compared to either the ICV or IT lumbar puncture routes of administration for which there was generally no (BLOD) expression.

Unlike ICV and IT dosing, the intra-putamen route produced no detectable vector in blood. All three routes of administration resulted in comparable CSF vector concentrations, though the ICV route trended higher than both IT and intra-putamen routes.

The concentration of the rAAV2-hAADC viral vector in the blood and CSF between the Day 2 timepoint and the Prior to Necropsy collections decreased in all animals with the exception of the Group 2 Animal Number 2002M. In this animal, the concentrations of the vector increased between the 2 intervals with the Prior to Necropsy sample being significantly higher than all other animals. Group 1 exhibited no (i.e., BLOD) vector in the blood on either Day 2 or at necropsy. In contrast, both Groups 2 and 3 exhibited detectable vector (up to 757 and 1513 copies/µg DNA, respectively) on Day 2 in blood, which decreased to none (BLOD) by necropsy. With regards to CSF vector (copies/µg DNA), all 3 routes of administration resulted in slight to moderate levels of vector on Day 2 with a range of values: 314-33838 copies (Group 1), 29483-58560 copies (Group 2), and 224-57531 copies (Group 3). Based on group mean values the rank order of CSF vector was ICV (42293 copies) > IT (18186 copies) > intra-putamen (11235 copies).

ICV administration resulted in mild to moderate (< 100000) copies in the caudate, though with maximum levels still less than that attained via intra-putamen administration. Vector concentration (copies/µg DNA) attained in the caudate following ICV administration ranged from 1752-48811 copies (c.f. intra-putamen range up to 437897 copies).

Vector copy number detected in all target areas following IT administration were significantly less than those attained following intra-putamen dosing.

The monkeys in this study were approx. 1.5 year old. 2 year old monkeys cover 5-12 year old humans. A 6 month old monkey is the developmental equivalent of a 2 year old human child, and ethically the youngest age monkeys can be used in studies is 10 months. It is therefore acknowledged that studies in pre-weaning NHPs are difficult to perform.

The applicant was asked to clarify if the brain weight of these young monkeys (~1.5 year old) is similar to that of 74g quoted by Pardo et al., (2012) for 2.5-5 year old monkeys. The applicant confirmed that the brain weight of the monkeys used was similar to those referenced in Pardo et al., (2012). Maximum brain weight appears to be achieved by approximately 3 months of age in monkeys (Sakamoto et al., 2014).

Overall, in the toxicity studies eladocagene exuparvovec was generally well tolerated with no obvious signs of toxicity.

2.5.5. Ecotoxicity/environmental risk assessment

The evaluation of Environmental Risk Assessment was conducted in consultation with national bodies responsible for release of genetically modified organisms into the environment.

Eladocagene exuparvovec is a replication-incompetent virus derived from AAV2. The genetic modifications do not affect its natural host and tissue tropism. Eladocagene exuparvovec is unable to replicate independently, even in the presence of a helper virus, since it lacks the *REP* and *CAP* genes required for rescue/packaging. None of the genetic modifications made to wild-type AAV2 during construction of eladocagene exuparvovec would be expected to enable the transfer or maintenance of

genetic material into the environment (outside its obligate host species), or have an effect on sensitivity to inactivating agents or survivability in the environment.

Taking into account the data of follow-up for 10 years in a study of dogs treated with AAVs (Giang N. Nguyen, BS *et al*., Blood (2019) 134 (Supplement_1): 611), it is concluded that there is a greater risk of integration in the genome than previously assumed and it would advisable to increase the follow-up time of the patients. The applicant plans a long-term PAES study PTC-AADC-MA-407, for a minimum of 10 years following gene therapy. There are two additional post authorisation studies planned. A long-term natural history study PTC-AADC-MA-406, to document the natural history of the disease, and the final post authorisation study planned is the completion of the ongoing long term follow-up study (AADC-1602) of subjects treated in the clinical trial programme. Subjects will be followed up for 10 years.

Based on the nature of the GMO, the parental organism and the receiving environment, the deliberate release of eladocagene exuparvovec is not anticipated to have any potential for direct effects on the environment.

2.5.6. Discussion on the non-clinical aspects

The nonclinical pharmacology program for eladocagene exuparvovec consists of *in vitro* studies in human cells to characterize the expression and biological activity of eladocagene exuparvovec. The applicant carried out these studies using HEK293 cells and not neuronal cells, however HEK cells express markers for renal progenitor cells, neuronal cells and adrenal gland. No *in vivo* pharmacology studies were carried out in support of this application, and this is acceptable. The applicant refers to studies published in the literature as supportive information.

The applicant provided a rationale in support of the selection of vector serotype, route of administration and dose of vector. Using the clinical route of intra-putamen (iPut) administration, eladocagene exuparvovec is expressed primarily in the putamen and ectopic expression of dopamine and serotonin is unlikely. The putamen is the preferred target, rather than the substantia nigra. Expression of the transgene outside of the striatum is expected to be limited with direct administration of AAV2 to the putamen. Intraputaminal administration has the advantage of both achieving the highest transgene concentration and limiting non-specific distribution following administration. IT and ICV routes of administration possibly increase safety concerns, however, these routes of administration are more amenable to paediatric dosing. However based on the results from a juvenile animal study exploring these alternative RoA, neither ICV nor IT routes appear feasible due to low vector copy number detected in the target area following administration via these routes. Stand-alone safety pharmacology studies were not conducted with eladocagene exuparvovec. An evaluation of CNS effects was included in the 6-month GLP toxicology study.

The distribution of eladocagene exuparvovec was evaluated as part of a 6-month Good Laboratory Practice (GLP)-compliant toxicity and biodistribution study in rats. Eladocagene exuparvovec was distributed to the CNS following bilateral infusion to the putamen, with distribution primarily to the injection site. The AADC transgene was expressed throughout the entire duration of the study in rats. No evidence of shedding of the vector in blood or CSF fluid was obtained.

Primer and probe sequences used in the qPCR and RT-qPCR are given. Sequences for the quantitative polymerase chain reaction assay were designed based on their specificity for the human dopa decarboxylase (DDC) cDNA sequence. To ensure amplification of vector transgene and not genomic DNA, the forward and reverse primers were designed to span exon-intron boundaries.

Eladocagene exuparvovec DNA was detected in other tissues apart from the CNS. The non-CNS tissues evaluated via quantitative polymerase chain reaction (qPCR) on Day 7 showed vector levels up to 300 copies/µg DNA Mean tissue vector copy values <1000 copies/µg DNA represent biodistribution that is negligible and/or artifactual. As expected, the presence of eladocagene exuparvovec DNA was confirmed in the putamen (the dose site) at all time points and dose groups and was at its highest level on Day 7 in high dose (7.5×109 vg) animals. Different dosing volumes did not result in different distribution patterns. Eladocagene exuparvovec is distributed primarily to the CNS but distribution to other tissues cannot be excluded based on the non-clinical data. Distribution studies in cynomolgus monkeys demonstrated some spread to adjacent brain regions, however there were no indications of toxicity from either the transgene or the excipient poloxamer 188. No evidence of shedding of the vector in blood or CSF fluid was obtained. Supportive and relevant PK data with other recombinant adeno associate virus type-2-human aromatic l-amino acid decarboxylase (rAAV2-hAADC) vectors were also obtained from published literature.

The nonclinical safety program conducted to characterize the toxicity and biodistribution of eladocagene exuparvovec comprised of two studies, a 30-day study, and a 6 month study, both in rats. Additional published toxicity and biodistribution studies in nonhuman primates with rAAV2-AADC using the same AAV2 capsid, promoter and human dopa decarboxylase (DDC) gene provided supportive toxicity data for the safety assessment of eladocagene exuparvovec.

Two new non-clinical studies were carried out to characterise the administration of the Poloxamer 188 excipient to the putamen of rats and monkeys. No toxicity concerns arose in these studies regarding poloxamer 188.

In the studies carried out eladocagene exuparvovec was generally well tolerated with no obvious signs of toxicity. Anti-drug antibodies were evaluated against the vector capsid. Anti-drug antibodies were not evaluated against the transgene product, aromatic L-amino acid decarboxylase (AADC) at the time of the study because an antibody assay against AADC was not available at that time. The presence of anti-drug antibodies had no effect on the biodistribution or expression Studies evaluating genotoxicity and carcinogenicity were not carried out, in agreement with CHMP. The applicant has provided a discussion on the risk of insertional mutagenesis and carcinogenic potential. Based on the weight of evidence discussed there appears to be a low risk for genomic integration or tumorigenicity in post-mitotic neuronal cells.

No specific developmental and reproductive toxicology studies were conducted by the applicant with eladocagene exuparvovec, in agreement with CHMP. Given the local administration of eladocagene exuparvovec to the brain and lack of systemic exposure, and the absence of biodistribution to the gonads, the risk for germline transmission is low.

Two further nonclinical studies are planned to determine the biodistribution and toxicity of eladocagene exuparvovec in Cynomolgus monkeys, in line with the proposed PIP. Two alternate routes of administration (RoA), besides intraputaminal (iPut), were evaluated in an initial biodistribution study, intracerebroventricular (ICV) and intrathecal (IT). Based on the data obtained in the initial RoA study demonstrating superior expression in the target putamen region with iPut administration despite using a 10-fold lower dose as compared to ICV and IT administration, a waiver (EMEA-002435-PIP01-18-M02 [eladocagene exuparvovec]) for a second study was submitted to PDCO and accepted 16 December 2020.

The CHMP endorse the CAT discussion on the non-clinical aspects as described above.

2.5.7. Conclusion on the non-clinical aspects

The non-clinical studies are adequate to support the Marketing Authorisation Application.

The CHMP endorse the CAT conclusions on the non-clinical aspects as described above.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- **Tabular overview of clinical studies**

Three clinical trials are submitted in support of this MAA.

The first trial AADCCU-1601 is complete. Efficacy data for the primary efficacy endpoint at 24 months is presented for this trial, as is follow-up data on subjects to 60 months post-treatment. Eight subjects took part in this trial. This trial started as a compassionate use access programme.

In the second trial AADC-010, ten subjects were treated and data for the assessment of the primary efficacy endpoint at 24 months is available on all subjects. The trial is ongoing and follow-up for 60 months is not completed on all subjects to date.

Efficacy Data at 12 months is available from the 8 subjects treated in AADC-011. Five subjects in this trial were treated with a 30% higher dose. This was because of elimination of a dilution step in the preparation of the active for administration. This trial is still ongoing. This data is not included in the assessment of the primary endpoint at 24 months. Data from the trial is presented in support of the MAA. Further efficacy & safety data on subjects treated in this trial should be available and presented as requested in the day 121 responses.

Figure 3

Study ID	Number of Study Centers/ Location	Study Start, Enrollment Status, Total Enrollment/ Enrollment Goal	Design Control Type	Study Drug Dose & Regimen	Study Objective	# Subjects by Dose Entered/ Completed	Duration (Months)	Gender M/F Mean Age (Range) (Months)	Diagnosis Inclusion Criteria	Primary Endpoint
AADC-CU/ 1601	1 NTUH	27 Feb 2010 8/NA Completed	Interventional, observational	Eladocagene exuparvovec $1.8x10^{11}$ vg intraputaminal	Efficacy and safety	8/6	60	3/5 58.8 (24.0, 99.0)	AADC deficiency, classic clinical symptoms, >2 YOA	PDMS-2 motor milestones at 60 months
AADC-010	1 NTUH	22 Oct 2014 10/NA Ongoing	Prospective; historical	Eladocagene exuparvovec $1.8x10^{11}$ vg intraputaminal	Efficacy and safety	10/0	60	5/5 52.5 (21.0-102.0)	AADC deficiency, classic clinical symptoms, >2 YOA or head circumference big enough for surgery	PDMS-2 motor milestones at 24 months
AADC-011	1 NTUH	09 Nov 2016 8/10 Ongoing	Prospective;	Eladocagene exuparvovec $1.8x10^{11}$ vg for patients ≥3 YOA $2.4x10^{11}$ vg for patients <3 YOA intraputaminal	Efficacy and safety	3/3 ($1.8x10^{11}$ vg group) 5/4 ($2.4x10^{11}$ vg group)	12	5/3 36.1 (21.0-70.0)	Documented AADC deficiency, classic clinical symptoms, >2 YOA or head circumference big enough for surgery, ≤6 YOA	PDMS-2 motor milestones at at 12 months

Abbreviations: AADC, aromatic L-amino acid decarboxylase; CU, compassionate use; F, female; ID, identification; M, male; N/A, not applicable; NTUH, National Taiwan University Hospital; PDMS-2, Peabody Developmental Motor Scale, second edition; YOA, years of age; vg, vector genomes
Source: CSRs

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

No dedicated clinical PK studies were conducted.

Bioanalytical Methods

An enzyme-linked immunosorbent assay (ELISA) method was used for screening of anti-AAV2 antibodies in human serum using a commercially available kit. Anti-AAV2 antibodies in diluted serum sample bind to the AAV-2 particles coated onto a microtitre plate. With this commercial kit, the AAV2 particles are produced in HEK 293 cells transfected with 3 plasmids containing the AAV-2 rep and cap genes, the adenovirus genome and a β-galactosidase expression cassette. The optical density (OD) cut-off used to assign positive samples is based on a research paper where samples with a value greater than the 0.5 OD were also positive for neutralising antibodies to β-galactosidase. It is not considered relevant to extrapolate data from the Ito et al study as it has not been shown that neutralisation of β-galactosidase correlates with positive ADAs against eladocagene exuparvovec. Furthermore, antibody titres were estimated by extrapolation of OD values from the Ito et al paper, which is also not agreed to.

During the course of the evaluation, the applicant was requested to present validation results from a new anti AAV2 antibody assay and an anti AADC assay and the issue remained at day 170. In the response to the day 170 list of issues, method validation is presented for two new assays to anti-AAV2 antibodies and neutralising anti-AAV2 antibodies. The approach for development of assay cut points is generally acceptable although the use of serum from patients with advanced metastatic tumours to determine the cut points for the total anti-AAV2 method should be justified. In general, the methods

have been appropriately validated in line with the EMA guideline for bioanalytical method validation (EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2) addressing sensitivity, intra- and inter- assay precision, matrix selectivity, hook effect, specificity and sample stability. Drug tolerance data is presented for the total anti-AAV2 method but has been omitted for the neutralising antibody method and this data is requested.

The total and neutralising anti-AAV2 antibody assays were used to analyse samples for subjects enrolled in the eladocagene exuparvovec clinical trial AADC-011 who were either treated with 1.8×10^{11} vg (low dose group) or 2.4×10^{11} vg (high dose group). For the total antibody assay, samples were first screened (screening tier) for being positive for total anti-AAV2 antibody. If found positive, they were re-assayed (confirmatory tier), and if confirmed, the titre of samples was subsequently determined. A sample was determined negative for the total anti-AAV2 antibody assay if antibodies were not detected at the minimum required dilution of 1:50. Across the five subjects tested, an increase in total antibody titre was observed following administration of gene therapy. In general, the titre was either maintained or decreased over 12 months. The 2 patients treated with the higher dose of 2.4×10^{11} vg showed higher titres of total anti-AAV2 antibody than those of the lower dose group. A sample was determined negative for neutralising anti-AAV2 antibody assay if antibodies were not detected at the minimum required dilution of 1:20. After treatment, the levels of nAbs transiently increased and, in all cases, decreased by Month 12. Overall, the results are consistent with the results presented from the original ELISA method with the exception of subject AADC-307 who at baseline was positive for total anti-AAV but negative for neutralising antibodies and subject AADC-301 who maintains positive nAB status up to 12 months according to the updated assay method.

While the method validation is generally acceptable, it is noted that pre-screening samples from patients have not be re-analysed using the updated methods. Data is provided from baseline (visit 2) onwards only. The pre-screening samples for subjects included in trial AADC-011 should be re-analysed using the new anti-AAV2 and neutralising assays in order to justify the threshold criteria for exclusion of patients from treatment (defined in SmPC sections 4.2 and 4.8) or the applicant should otherwise justify how the cut off of ">1:20" is justified.

A method for detection of anti-AADC antibodies is under development but has not yet been validated. The validation protocol presented for the method is generally acceptable.

PTC plans to monitor levels of anti-AAV2 and anti-AADC antibodies from subjects in the proposed patient registry study.

A real time polymerase chain reaction (RT-PCR) assay is used to quantify AAV2-hAADC in blood and urine. An overview of the PCR procedure has been provided including reaction components, cycle conditions and primer sequences. Linearised p-AAV2-hAADC plasmid DNA was used to prepare standard curves and QC samples. Patient DNA was extracted from peripheral blood and urine. A sample was classified as positive for vector genomes if there were ≥100 copies in 250 ng of DNA. Details of method validation have been provided and are acceptable.

A HPLC method was used to detect5-hydroxyindoleacetic acid (5-HIAA), 3-methoxytyrosine (3MT, also known as 3OMD) and homovanillic acid (HVA) in human CSF samples. The method was initially described in a 1992 paper from Hyland et al. paper. A validation report has been provided from the testing site demonstrating linearity, precision, accuracy, stability and matrix effects. The assay can be considered acceptable for testing HVA, 5-HIAA, and 3MT.

Viral shedding assessment

The risk of eladocagene exuparvovec viral vector shedding was evaluated in each clinical study via a real time-polymerase chain reaction method. Eladocagene exuparvovec viral vector was not detected in any patient's blood or urine samples prior to or up to 12 months post-surgery in all clinical studies.

2.6.2.2. Pharmacodynamics

Mechanism of action

Eladocagene exuparvovec is a sterile, parenteral formulation gene therapy indicated for the treatment of patients with AADC deficiency. Eladocagene exuparvovec contains the active biological substance rAAV2-h*AADC* (a recombinant adeno-associated virus serotype 2 [rAAV2] vector containing the human *DDC* gene and coding deoxyribonucleic acid (DNA) (cDNA) that encodes the human aromatic L-amino acid decarboxylase [hAADC]) and compendial excipients. After injection into the putamen, the product results in the expression of the AADC enzyme resulting in production of dopamine, and consequently, restoration of motor function in AADC deficient patients.

Primary and Secondary pharmacology

Measurement of putaminal-specific uptake

Expression and activity of the AADC enzyme in the putamen was assessed by PET imaging using ^{18}F-DOPA, a positron-emitting fluorine-labelled version of levodopa, which is a substrate for AADC. The ^{18}F-DOPA is administered intravenously, crosses the blood-brain barrier, and is taken up by the pre-synaptic nigro-striatal dopaminergic neurons in the putamen and converted by AADC to dopamine. Therefore, increased ^{18}F-DOPA putamen uptake over time objectively demonstrates newly produced dopamine and the presence of functional AADC enzyme.

PET was evaluated in each clinical study and typically demonstrated a statistical significance in putaminal-specific uptake of ^{18}F-DOPA increases over time while no statistical significance was determined in association with patient age, suggesting that the observed increases in putaminal-specific uptake of ^{18}F-DOPA were a result of eladocagene exuparvovec administration. However, in Study AADC-011, mean putaminal-specific uptake on PET imaging was relatively unchanged during the study. Summary statistics of PET-specific uptake by time point for studies AADC-CU/1601, AADC-010 and AADC-011 are provided in tables below.

Table 1

Table 11. Summary Statistics for Putaminal-Specific Uptake by Timepoint (ITT Population)

PET Parameter	n	Data Type	Timepoint	Mean (SD)	LS Mean (SE)	95% CI of LS Mean
Specific uptake	8	Raw	BL	0.13 (0.07)	0.12 (0.06)	-0.01, 0.24
	4	CFB	Month 6	0.25 (0.38)	0.32 (0.07)	0.17, 0.47
	4	CFB	Month 12	0.17 (0.09)	0.29 (0.09)	0.10, 0.48
	2	CFB	Month 60	0.42 (0.09)	0.54 (0.10)	0.32, 0.76
	2	CFB	After Month 60[a]	0.35 (0.11)	0.50 (0.13)	0.23, 0.77

Abbreviations: BL, baseline; CFB, change from baseline; CI, confidence interval; ITT, Intent-To-Treat; LS, least squares; SD, standard deviation; SE, standard error

[a] For these patients, PET data were the only data obtained after Month 60 as this was the only time when the patients were able to obtain an imaging examination.

Table 2

Table 10. PET Specific Uptake by Time Point (ITT Population)

	Baseline N=10	CFB at Month 12 N=9	CFB at Month 24 N=9
Mean (SD)	0.22 (0.11)	0.42 (0.21)	0.47 (0.22)
LS Mean (SE)	0.2 (0.05)	0.6 (0.06)	0.7 (0.06)
95% CI of LS Mean	0.1, 0.3	0.5, 0.7	0.5, 0.8

Abbreviations: CFB, change from baseline; LS, least squares; PET, positron emission tomography; SE, standard error

Table 12. PET-Specific Uptake by Time Point (1.8×10^{11} vg)

	Baseline n=3	Change from Baseline at Month 12 n=3
Mean (SD)	0.52 (0.05)	-0.03 (0.07)
Median (Min, Max)	0.54 (0.46, 0.55)	-0.06 (-0.08, 0.04)

Abbreviations: Max, maximum; Min, minimum; PET, positron emission tomography; SD, standard deviation; vg, vector genome

Measurement of neurotransmitter metabolites

The precursors L-3, 4-dihydroxyphenylalanine (L-DOPA) and 5-hydroxy-tryptophan (5-HTP) are decarboxylated by AADC to form dopamine and serotonin, respectively. After action at the nerve synapse, these neurotransmitters are reabsorbed into the presynaptic nerve terminal where they can either be repackaged into vesicles for future neurotransmitter release or metabolized to HVA (from dopamine) and 5-HIAA (from serotonin). In the presence of AADC deficiency, HVA and 5-HIAA levels in the CSF are very low or below the limits of detection due to the abnormally low dopamine and serotonin production, respectively. Upon restoration of AADC activity, concentrations of 5-HIAA and HVA in the CSF rise (Hyland 2006, Hyland 2007).

The metabolites HVA and 5-HIAA were evaluated in each clinical study via a high-performance liquid chromatography (HPLC) method. At baseline, the concentrations of both metabolites were near or below the lower limit of quantitation in all patients with AADC deficiency, indicating little or no AADC enzyme activity. In each clinical study, the CSF concentration of HVA at 12 months after treatment was increased from baseline. Few patients showed increased CSF concentrations of 5-HIAA at 12 months, which was expected since infusion of eladocagene exuparvovec was not into the region of the brain that produces serotonin. Summary statistics for neurotransmitter metabolites by time point in studies AADC-CU/1601, AADC-010 and AADC-011 are provided below.

Table 3

Table 10. Summary Statistics for Neurotransmitter Metabolites by Time Point (ITT Population)

Variable Statistic	BL (n=5)	CFB Month 6 (n=3)	CFB Month 12 (n=3)
HVA[a] (nmol/L)			
Mean (SD)	4.60 (2.27)	27.50 (5.41)	11.17 (7.59)
Median (Min, Max)	5.00 (2.50, 8.00)	29.00 (21.50, 32.00)	12.50 (3.00, 18.00)
5-HIAA[b]			
Mean (SD)	2.50 (0.00)	0.83 (1.44)	0.00 (0.00)
Median (Min, Max)	2.50 (2.50, 2.50)	0.00 (0.00, 2.50)	0.00 (0.00, 0.00)

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; BL, baseline; CFB, change from baseline; HVA, homovanillic acid; ITT, intent -to-treat; Max, maximum; Min, minimum; SD, standard deviation

[a] Reference ranges varied based on lab and included 176-955 nmol/L, 218-852 nmol/L, 478-895 nmol/L, or 233-929 nmol/L.

[b] Reference ranges varied based on lab and included 63-503 nmol/L, 66-338 nmol/L, 231-618 nmol/L, and 74-345 nmol/L.

Note: Not all patients had baseline and postbaseline data. The following patients had data at baseline and 6 months: 04, 05, and 07. The following subjects had data at baseline and 12 months: 04, 07, and 08.

Table 4

Table 9. Neurotransmitter Metabolites by Time Point (ITT Population)

	Baseline N=10	CFB at Month 12 N=9
HVA		
Mean (SD)	5.65 (7.95)	26.56 (21.57)
Median (min, max)	2.50 (2.50, 28.00)	19.50 (0.00, 54.50)
5-HIAA		
Mean (SD)	3.10 (1.29)	6.56 (12.72)
Median (min, max)	2.50 (2.50, 6.00)	0.00 (0.00, 34.50)

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; CFB, change from baseline; HVA, homovanillic acid; max, maximum; min, minimum

Table 5

Table 11. Summary Statistics for Neurotransmitter Metabolites by Time Point (1.8×10^{11} vg)

Variable Statistic	Baseline (n=3)	Change from Baseline at Month 12 (n=3)
HVA (nmol/L)		
Mean (SD)	16.17 (17.58)	15.17 (3.88)
Median (Min, Max)	10.00 (2.50, 36.00)	14.00 (12.00, 19.50)
5-HIAA		
Mean (SD)	15.17 (10.98)	-12.67 (10.98)
Median (Min, Max)	21.00 (2.50, 22.00)	-18.50 (-19.50, 0.00)

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid; Max, maximum; Min, minimum; SD, standard deviation; vg, vector genome

Immunogenicity assessment

Immunogenicity was evaluated in each clinical study for anti-AAV2 antibodies in serum via an enzyme-linked immunosorbent assay (ELISA) method. A positive titre was indicated by an optical density (OD) ≥0.5.

Study AADC-CU/1601

The presence of anti-AAV2 antibodies was detected from Month 2 post-surgery. At Month 12, 2 out of the 6 patients who were evaluated had a positive anti-AAV2 antibody titre (Table 6).

Table 6

Table 2. Study AADC-CU/1601 Patients with Positive Anti-AAV2 Antibody Titers[a]

Timepoint (N=8)	Number of Patients Positive (%)
Enrollment (n=4)	0 (0.0)
Pre-surgery (n=8)	0 (0.0)
Month 1 (n=7)	0 (0.0)
Month 2 (n=8)	1 (12.5)
Month 3 (n=6)	2 (33.3)
Month 4 (n=6)	4 (66.7)
Month 5 (n=6)	3 (50.0)
Month 6 (n=7)	3 (42.9)
Month 9 (n=7)	3 (42.9)
Month 12 (n=6)	2 (33.3)

[a] Positive anti-AAV2 antibody titer corresponds to optical density (OD) >0.5

Note: Patient 1601-01 did not have data at Enrollment or Month 5, Patient 1601-02 did not have data at Baseline, Enrollment, or Month 12, Patient 1601-03 did not have data at Enrollment or Months 3-4, Patient 1601-04 did not have data at Baseline, Patient 1601-05 and Patient 1601-06 did not have data at Baseline, Patient 1601-07 did not have data at Baseline, Month 1 or Month 12, Patient 1601-08 did not have data at Baseline, Enrollment, or Months 3-9.

In a repeated measures analysis, anti-AAV2 OD value was not a statistically significant factor in the model, indicating that the magnitude of the change from baseline in PDMS-2 total scores was not associated with the anti-AAV2 OD values (p=0.3843). There was also no correlation between anti-AAV2 antibody titre and efficacy as measured by changes in PDMS-2 total score (Pearson correlation coefficient = -0.058).

Study AADC-010

The presence of anti-AAV2 antibodies was detected from Month 3 post-surgery. At Month 12, 4 out of the 10 patients who were evaluated had a positive anti-AAV2 antibody titre but showed that titres were generally decreasing (Table 7).

Table 7

Table 5. Study AADC-010 Patients with Positive Anti-AAV2 Antibody Titers[a]

Timepoint	Number of Patients (%) (N=10)
Baseline	0 (0.0)
Month 3	4 (40.0)
Month 6	8 (80.0)
Month 9	7 (70.0)
Month 12	4 (44.4)

[a] Positive anti-AAV2 antibody titer corresponds to optical density (OD) >0.5
Note: Patient 1007 did not have post baseline data at Month 12.

In a repeated measures analysis, anti-AAV2 OD value was a statistically significant factor in the model, indicating that the magnitude of the change from baseline in PDMS-2 total scores was associated with the anti-AAV2 OD values (p=0.0003). There was moderate positive correlation between anti-AAV2 antibody titre and increases in PDMS-2 total score (Pearson correlation coefficient = 0.595). However, the increasing PDMS-2 total score indicates that over time, antibodies had no adverse impact on efficacy.

Study AADC-011

Anti-AAV2 antibody data at 3 months post-surgery was reported for 5 patients, 3 patients receiving 1.8×1011 vg and 2 patients receiving 2.4×1011 vg. The presence of anti-AAV2 antibodies was detected in all 5 patients at Month 3. At Month 6, data reported for 4 patients showed that antibody titres were generally decreasing. Only 1 patient had an evaluation at Month 9 and their antibody titre remained stable from Month 6 (Table 8).

Table 8

Table 10. Anti-AAV2 Antibody Data, Safety Population

	Time Point				
Patient	Screening	Baseline	3 Months	6 Months	9 Months
$1.8x10^{11}$ vg					
301	0.025	0.029	0.601	0.355	0.371
303	0.034	0.034	0.995	1.187	
305	0.016	0.018	0.929	0.354	
$2.4x10^{11}$ vg					
304	0.064	0.058	1.33	1.244	
306	0.059	0.061	1.195		

Abbreviations: AAV2, adeno-associated virus serotype 2; ITT, intent-to-treat; vg, vector genome

In a repeated measures analysis, anti-AAV2 OD value was not a statistically significant factor in the model, indicating that the magnitude of the change from baseline in PDMS-2 total scores was not associated with the anti-AAV2 OD values (p=0.1166). There was moderate positive correlation between anti-AAV2 antibody titre and increases in PDMS-2 total score (Pearson correlation coefficient = 0.713). However, the increasing PDMS-2 total score indicates that over time, antibodies had no adverse impact on efficacy.

Study AADC-1602

Study AADC-1602, a 10-year, long-term follow-up study of clinical trial subjects was set up to collect long-term safety and efficacy data from these 3 studies for subjects who consented to participate. As part of this ongoing study, anti-AAV2 antibody data was available for 4 subjects at 24 months post-administration of gene therapy as of the most recent data cut-off (26 February 2020). All 4 subjects were positive (optical density [OD]>0.5) (Ito 2009) for anti-AAV2 antibodies within 3 months following gene therapy and, in general, continual decrease in antibody titre was observed over the course of 24 months. At 24 months, 2 of the 4 subjects were no longer considered to be positive for anti-AAV2 antibodies (Table below). Although limited, the available 24-month data suggest that antibody titres appear to continue to decline over time.

Table 9: Anti-AAV2 Antibody Titres Up to 24 Months After Eladocagene Exuparvovec Therapy[a]

	Timepoint (OD)[b]					
Subject	Baseline/ Pre-Surgery	Month 3	Month 6	Month 9	Month 12	Month 24
011-304	0.058	1.33	1.244	0.918	0.698	0.594
011-305	0.018	0.929	0.354	0.751	0.53	0.223
011-306	0.061	1.195	1.29	1.009	0.745	0.37
011-307	0.127	1.693	1.797	1.51	1.198	0.665

Abbreviations: AAV2, adeno-associated virus, serotype 2; OD, optical density
[a] Only subjects with 24 month data from Study AADC-1602 are included
[b] Positive anti-AAV2 antibody titer corresponds to optical density (OD)>0.5
Source: ISE Listing 11

Importantly, in these 3 clinical studies, clinical benefit was comparable and maintained, and adverse events generally occurred with a similar frequency, between subjects who had positive antibody titres and those who did not have positive antibody titres, indicating that the presence of anti-AAV2 antibodies does not appear to impact the efficacy or safety of eladocagene exuparvovec.

2.6.3. Discussion on clinical pharmacology

Bioanalytical methods

An ELISA method is used to detect anti-AAV2 antibodies in human serum. The assay is not product specific and instead uses AAV2 particles from a virus expressing beta galactosidase. The cut-off used in the assay to designate a positive sample is based on the level of neutralising antibodies to beta galactosidase. This approach is not supported. The applicant was requested to provide immunogenicity data from an appropriate assay which is validated in accordance with the EMA Guideline bioanalytical method validation. In the response to the day 170 list of issues, method validation is presented for two new assays to anti-AAV2 antibodies and neutralising anti-AAV2 antibodies. As discussed above in section 3.3.1, the total and neutralising anti-AAV2 antibody assays were used to analyse samples for subjects enrolled in the eladocagene exuparvovec clinical trial AADC-011 who were either treated with 1.8×10^{11} vg (low dose group) or 2.4×10^{11} vg (high dose group).

A real time PCR assay was used to quantify AAV2-hAADC in blood and urine. Sufficient details of the assay were provided. Details of method validation have been provided and are acceptable.

A HPLC method was used to detect homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), and 3-methoxytyrosine (3MT) in human CSF samples. The method is sufficiently validated and is considered acceptable.

Pharmacokinetics

No dedicated clinical PK studies were conducted. Conventional clinical pharmacokinetic studies may not be relevant for gene therapy products (EMA/CAT/80183/2014). The lack of dedicated clinical PK studies therefore is acceptable.

There is a lack of information about biodistribution of the vector and active throughout the brain. The presumed rationale for not conducting biodistribution studies is that the active is administered intraputamen and not administered systemically. Shedding studies would indicate lack of systemic biodistribution of the active but preclinical studies in rats and immune response in humans indicate some systemic exposure. Biodistribution studies in the rat indicate a low risk of transmission to the gonads.

Biodistribution of the active throughout the brain is also of interest but would involve biopsy of the brain which is considered too invasive. Indirect evidence of biodistribution is provided by the PD studies looking at putaminal specific uptake. Further data on biodistribution of the vector in the brain may be provided in the results of the post-mortem (if one was performed) on the child that died of encephalitis.

For the population treated in this trial, germline transmission may not be of concern. However, for treatment of other populations i.e. adults, milder phenotypes, information about potential germline transmission may be of value.

Samples of blood and urine were collected to assess viral shedding. The assessment schedule as outlined in the studies is acceptable. Overall, AAV2-hAADC vector viral shedding was not detected in any AADC patient blood or urine samples throughout the clinical development of eladocagene exuparvovec. These results indicate a low risk of transmission to third parties involved in caring for these children.

The target population of Upstaza is considered a special population by itself. Clinical studies did not include neonates and infants less than 18 months, or subjects with impaired renal or hepatic function, but children from 18 months up to 8 years and 6 months of age and of both genders. The population studied only included one Caucasian subject, with the majority of subjects being of Asian races/ethnicities. There are no data presented for European subjects treated with the active. Given that the gene therapy replaces an enzyme that has a similar structure in all populations and the mechanism of action is similar for all populations and the target organ i.e. putamen in the brain is similar in all populations, it is expected that the effects may be similar in all populations including the European population. However, the causative mutation and the clinical phenotype may vary in different populations.

In such an ultra rare orphan condition it is not always possible to recruit subjects with hepatic and renal impairment. As the new P188 excipient added to the commercial product is excreted by the kidney, it is not clear whether renal impairment might impact renal excretion of the excipient. Also, subjects with impaired hepatic and renal function may be prone to more adverse effects such as hypotension or gastrointestinal haemorrhage after surgery. The SmPC should be updated to include a statement in that there are no data available on treatment in subjects with renal or hepatic impairment.

All Subjects treated after MAA will be followed with a Registry study so additional data relating to safety and efficacy in the European population, non-Asian subjects and patients with renal or hepatic impairment with AADC will become available post marketing.

Pharmacodynamics

Pharmacodynamic investigation is limited by the specific nature of the product, its primary target cell type and the intracellular localization of the expressed transgene, and the paediatric target population.

The PD assessments of putaminal uptake is based on measurement of the activity of the enzyme that has been produced as a result of translation of the expression cassette. The substrate for the enzyme is L-Dopa which is decarboxylated by the AADC enzyme. The applicant provided available literature on 18F-DOPA in the putamen of normal non-AADC children. After treatment with Upstaza, uptake of 18F-DOPA increases but remains significantly lower than that observed in the non-AADC population.

In studies AADC-CU/1601 and AADC-010, increases in putaminal-specific dopamine uptake was evident during follow-up. However, in study AADC-011, mean putaminal-specific uptake was relatively unchanged during follow-up. However, there was no clinically significant difference in milestone achievement in this group and those treated in other trials. The lack of uptake may reflect the non-sensitivity of the assay. The increase in uptake of F-DOPA does not correlate with achievement of motor milestones.

Concentrations of HVA and 5-HIAA in the CSF, as downstream metabolites of dopamine and serotonin, respectively, are appropriate as objective biomarkers for AADC activity. In all subjects, levels of these metabolites in the CSF were low or below the LLOQ at baseline.

In all studies, there was an increase in the concentration of HVA in the CSF and this increase was maintained over time. This suggests increased AADC activity following treatment with eladocagene exuparvovec. By contrast, CSF 5-HIAA was relatively unchanged following treatment. The applicant explained that this is expected since the targeted region of the brain (putamen) does not contain serotonergic neurons. The main aim of treatment with eladocagene exuparvovec is to improve motor function.

All AADC patients in the 3 clinical studies were assessed for detection of anti-AAV2 antibodies. The data suggest that there may be a risk of developing anti-AAV2 antibodies after administration of eladocagene exuparvovec (post-surgery). Further, anti-AAV2 antibodies appear to persist to 24 months in some patients. Overall, the presence/development of antibodies to AAV2 does not appear to affect the efficacy of eladocagene exuparvovec, as measured by changes in PDMS-2 score. However, this observation is based on a limited number of patients.

The relevant sections of the SmPC were updated stating that there is no safety or efficacy data for subjects whose pre-treatment antibody levels to AAV2 was greater than 1:20.

Although patient from different ethnicities have been enrolled into the clinical studies, available data of patients treated with Upstaza at the proposed therapeutic dose that have completed the interventional study is too limited to allow for definite statements on genetic differences to PD response.

2.6.4. Conclusions on clinical pharmacology

The clinical pharmacology sections of the dossier are adequate to support this Marketing Authorisation Application.

The CHMP endorse the CAT assessment regarding the conclusions on the Clinical pharmacology as described above.

2.6.5. Clinical efficacy

2.6.5.1. Dose response study(ies)

As AADC deficiency is an ultra-rare paediatric disease; a formal dose-ranging study is not possible as this requires a greater number of patients than is available within this small population, as an estimated 60 patients would be required (assuming around 20 patients per dose group). Instead, strong nonclinical data in non-human primate models of Parkinson's disease and clinical data in patients with Parkinson's disease with rAAV2-hAADC vectors delivered to the putamen were used to guide the dose selection for a one-time administration of eladocagene exuparvovec for treating AADC deficiency. The intended dose of $1.8x10^{11}$ balances positive efficacy assessments with safety.

Parkinson's disease has been extensively studied and prior to recent work in AADC deficiency was the only other disease model to investigate the ability of the rAAV2-hAADC vector infused into the putamen to produce active AADC enzyme. Therefore, it was anticipated that this initial dose in children with AADC deficiency had a high probability of increasing AADC activity, which would be of direct clinical benefit in improving motor function and milestone achievement. The clinical benefit observed with eladocagene exuparvovec supports this expectation.

Dose ranges and efficacy

Intrastriatal (caudate/putamen) and intraputaminal (putamen only) delivery of rAAV2-hAADC vectors in a non-human primate model of Parkinson's disease resulted in AADC protein expression and improvements in response to L-DOPA therapy as evaluated by neurological function (Muramatsu 2002, Bankiewicz 2006, Forsayeth 2006, Cunningham 2008). Doses tested in the different studies were from 6×10^{9} to 3.6×10^{11} vg.

Forsayeth et al (2006) assessed multiple doses within a single preclinical Parkinson's disease study (Forsayeth 2006). They evaluated the relationship rAAV2 hAADC dose infused into the putamen and efficacy including AADC enzyme activity in hemi-parkinsonian monkeys. The doses tested ranged from 6×10^{9} to 5×10^{11} vg. The study found that vector dose produced a linear increase in AADC enzyme activity in brain tissue at doses $<5.5\times10^{10}$ vg followed by a plateau starting at dose 1.7×10^{11} vg, indicating a saturation phenomenon in which higher doses showing little additional AADC enzyme activity. These results suggest that doses above 1.7×10^{11} produce little additional increase in expression of rAAV2 hAADC transgene (Forsayeth 2006). In the context in which there are no demonstrable gains in efficacy at higher doses, it becomes imperative to ensure safety. Based on the dose response efficacy observed in non-human primate Parkinson's disease studies, rAAV2-hAADC was subsequently tested in the first-in-human clinical trials of patients with Parkinson's disease at dose levels of 9×10^{10} and 3×10^{11} vg via bilateral intra-putaminal dosing (Christine 2009, Muramatsu 2010). Both doses were biologically active and well tolerated (Christine 2009). The prior findings in Parkinson's disease patients were used to establish a dose that was expected to improve AADC activity in the putamen of children with AADC deficiency. Based on the relative average mass of a child brain (1000 g) being approximately 60% that of an adult brain (1350 g), and using brain weight scaling to a child brain, the dose of 1.8×10^{11} vg of eladocagene exuparvovec used in the AADC clinical trials in children is equal to approximately 60% of the highest dose used in Parkinson's disease clinical trials in adults, which again was well tolerated.

Dose ranges and safety

When selecting the dose, the potential of adverse effects was considered. Higher doses than the intended 1.8×10^{11} would not be anticipated to meaningfully improve efficacy, as described above, but potentially could risk increased toxicity due to dopamine excess particularly in regions outside of the

putamen. Excess dopamine can also result in medical and psychiatric consequences (Wichmann 1998, Bezard 2001).

Intended dose

The intended dose of 1.8×10^{11} vg is based on the fact that AADC activity and clinical benefit and safety have been demonstrated in 3 separate clinical studies with a follow up of 5 years' post-gene-transfer with eladocagene exuparvovec. Eladocagene exuparvovec will be administered as a single infusion at a dose of 0.45×10^{11} vg and a volume of 80 µl per site to 4 sites (2 per putamen), for the total dose of 1.8×10^{11} vg and a total volume of 320 µl per patient. In Study AADC-011, 5 patients (ages ≥2 and <3 years) received a slightly higher dose of eladocagene exuparvovec at 2.4×10^{11} vg. The higher dose was selected for logistical reasons to remove a dilution step and simplify the administration of the study drug. The treatment benefit from this dose was similar to that observed with the lower 1.8×10^{11} vg dose, which was not unexpected as the difference in dose is only 30%. Only the efficacy and safety findings for the lower dose which is the intended dose are presented below; see Summary of Clinical Efficacy 2.7.3 and Summary of Clinical Safety 2.7.4 for description of the 2.4×10^{11} vg dose result. These results are presented for both doses and demonstrate comparable efficacy and safety.

2.6.5.2. Main study(ies)

The eladocagene exuparvovec clinical program is comprised of 3 studies (1 completed study and 2 ongoing studies) in patients with AADC deficiency. All studies were conducted according to Good Clinical Practice standards. All studies were single-arm trials in which patients received treatment with eladocagene exuparvovec. The 3 studies were conducted at the same centre at the National Taiwan University Hospital.

- AADC-CU/1601 – completed; retrospective, compassionate use
- AADC-010 – ongoing; prospective, Phase 1/2
- AADC-011 – ongoing; prospective, Phase 2b

The completed study (AADC-CU/1601, 8 patients), is an observational study that summarizes data from a compassionate use program of patients with AADC deficiency followed for 60 months (5 years). Evidence of durable clinical benefit and a favourable safety profile in the follow-up period of Study AADC-CU/1601 have justified continued clinical development of eladocagene exuparvovec for treatment of AADC deficiency.

Each study & results is described below and in the integrated summary of efficacy presented. The studies that contribute to the primary efficacy endpoint are studies AADC-010 & study AADC-CU/1601. Study AADC-011 is considered a supportive study as data does not contribute to the primary endpoint at 24 months, as there is only data available at the 12-month time-point.

Study AADC-010

AADC-010 is a single-centre, prospective, single-arm, Phase 1/2 evaluation of the safety and efficacy of eladocagene exuparvovec. A single-arm design was utilized because a placebo control was not ethically feasible, spontaneous improvement in participants was not expected, the treatment intervention required a neurosurgical procedure, and the natural history of the disease was well characterized in this paediatric patient population with a rare disease. A natural history group served as a control for AADC-010. Study enrolment required a confirmed diagnosis of AADC deficiency. Patients were followed every 3 months for safety and efficacy assessments through the first year after treatment. The initial planned observation period was 1 year; however, patients voluntarily returned every 6 months to complete developmental tests and adverse event (AE) reporting. In addition,

patients were recommended to have PD testing of central nervous system (CNS) AADC activity over time that included CSF neurotransmitter metabolites and L-6-[18F] fluoro-3,4-dihydroxyphenylalnine (18F-DOPA)PET at baseline and 1, 2, and 5 years post-treatment. The protocol was formally amended and approved by the IRB to collect study data through 5 years of follow-up.

Methods

Study Participants

The study was expected to recruit 10 subjects with an evaluation period of 13 months. All subjects were recruited at a single hospital centre in Taiwan, which specialises in the treatment of children with AADC deficiency.

Inclusion criteria

1. With a confirmed diagnosis of AADC, including cerebrospinal fluid analysis to show reduced levels of neurotransmitter metabolites, HVA and 5-HIAA, and higher L-Dopa, together with more than one mutation within AADC gene.
2. Classical clinical characteristics of AADC deficiency, such as oculogyric crises, hypotonia and developmental retardation.
3. The sick child has to be over 2 years old or a head circumference big enough for surgery.
4. Participating patients must cooperate completely for all evaluations and examinations before, during and after the whole trial.
5. Parents or guardians must sign to agree on this informed consent.

Exclusion Criteria

1. Significant brain structure abnormality
2. Patients with any health or neurological doubts that may increase the risk of surgery cannot join this trial. PI has the right to evaluate the feasibility of subjects for this trial based on his/her health condition.
3. Since high-level neutralizing antibodies may disturb the therapeutic effect of gene therapy, patients with anti-AAV2 neutralizing antibody titre over 1,200 folds or an ELISA OD over 1 cannot be enrolled into this trial.
4. Subjects enrolled in this clinical trial cannot take any medications that may affect this trial.

Treatments

Surgical procedure

This surgery was performed by the Departments of Neurology and Neurosurgery, National Taiwan University Hospital and under general anaesthesia. Prior to the surgery, putamen location was ensured by Magnetic Resonance Imaging (MRI) and Computed Tomography (CT). After the injection site was determined stereotactically, AAV-AADC was injected into putamen through the holes drilled in the skull outside of the putamen. After the surgery, head CT scan and MRI was performed to check for complications, such as bleeding.

Dosage and injection of viral vector

Eladocagene exuparvovec was administered during a single operative session at a dose of 0.45×1011 vg and a volume of 80 µL per site to 4 sites (2 per putamen), for a total dose of 1.8×1011 vg and a total volume of 320 µL per patient.

Objectives

The objectives of AADC-010 were:

- To understand if the expression of hAADC gene transferred by AAV2 vector may facilitate the conversion from L-DOPA to dopamine to improve the motor function of patients.
- To ensure the safety of hAADC gene transfer by AAV2 vector for children with AADC deficiency.

Outcomes/endpoints

Primary

Achievement of key motor milestones at the 2-year time point was the primary measure of efficacy. The primary efficacy endpoints related to the achievement of such milestones were:

- Proportion of patients achieving full head control, as measured using the Peabody Developmental Motor Scales – Second Edition (PDMS-2)
- Proportion of patients able to sit unassisted, as measured using the PDMS-2
- Proportion of patients able to stand with support, as measured using the PDMS-2
- Proportion of patients able to walk with assistance, as measured using the PDMS-2

The proportions calculated at 1-year post gene therapy were provided as supportive analyses.

The **secondary efficacy** endpoints were:

- Raw scores for the PDMS-2 total and subscales
- Raw scores for the Alberta Infant Motor Scale (AIMS) total and subscales
- Raw scores for the Bayley Scales of Infant Development – Third Edition (Bayley-III) total and subscales
- Change from baseline in body weight
- Neurologic examination findings with respect to muscle tone (i.e. floppiness), OGC episodes, dystonia, muscle power, and deep tendon reflex (DTR) response Immunogenicity Endpoints
- Anti-adeno-associated virus serotype 2 (AAV2) optical density (OD) values.

Pharmacodynamic Endpoints

The pharmacodynamic (PD) endpoints were:

- Change from baseline in the neurotransmitter metabolites homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (CSF)
- Putaminal signal in positron emission tomography (PET)

Safety Endpoints

- All treatment-emergent adverse events (TEAEs)
- Neurologic examination findings (excluding muscle tone, OGC episodes, dystonia, muscle power, and DTR response)

- Viral shedding

Randomisation and blinding (masking)

This was planned as an uncontrolled open-label study.

Statistical methods

Statistical Analysis Plan

The SAP described the statistical methods used in the reporting and in the analyses of data during the study. Version 3.0 of the SAP was approved by the Sponsor on 3 April 2019. A Supplemental Statistical Analysis Plan (version 1.0) detailing additional analyses of secondary efficacy endpoints was approved on 6 September 2019.

This SAP was to cover all data collected through the 12-month visit, as well as the motor development tests, PET data, adverse events, and concomitant medications collected after 12 months.

Analysis Populations

The following analysis populations were defined for the study:

The **Intent-to-Treat (ITT) Population** was to include all enrolled patients. All efficacy analyses were to be performed on the ITT population.

The **Safety Population** was to include all enrolled patients who had been treated by AAV2-hAADC gene therapy. All safety analyses were to be performed on the safety population.

Missing Data Conventions

No missing value imputation was to be used - all analyses were to be based on the observed data. In the instance where lab data are recorded as below the lower limit of quantitation (LLOQ), then the data was to be analysed as (1/2)*LLOQ.

Analysis of primary efficacy endpoints

At the 2-year time-point, the number and proportion of patients achieving each key motor milestone was computed, along with exact binomial (Clopper-Pearson) 95% confidence intervals (CIs). Similar analysis at 1 year were to be provided as supportive analysis.

The following tests were conducted on the primary efficacy endpoint milestone data at the 2-year time-point under a sequential gatekeeping procedure, using a 1-sample exact binomial test at a 1-sided α=0.025 level of significance:

1) H_0: $p_{HC} = p_{0(HC)}$ vs. H_1: $p_{HC} > p_{0(HC)}$

2) H_0: $p_{SU} = p_{0(SU)}$ vs. H_1: $p_{SU} > p_{0(SU)}$

3) H_0: $p_{SS} = p_{0(SS)}$ vs. H_1: $p_{SS} > p_{0(SS)}$

4) H_0: $p_{WA} = p_{0(WA)}$ vs. H_1: $p_{WA} > p0_{(WA)}$

where

p_{HC} is the proportion of patients achieving head control;

p_{SU} is the proportion of patients able to sit unassisted;

p_{SS} is the proportion of patients able to stand with support; and

p_{WA} is the proportion of patients able to walk with assistance.

The chosen testing order of these hypotheses reflected the hierarchical order in which these milestones are typically achieved.

The respective null values for each test, $p_{0(HC)}$, $p_{0(SU)}$, $p_{0(SS)}$, and $p_{0(WA)}$ will be the upper limit of the two-sided exact 95% confidence interval of the proportion of patients achieving the respective milestone from the natural history control group described in Wassenberg et al. (2017). This external control group is comprised of 82 patients, identified as severe by these authors, defined as having no or very limited developmental milestones and being fully dependent AADC deficient patients, and is claimed to represent the natural history of the disease in untreated patients.

Medical history of the patients enrolled in this study will be evaluated for the achievement of these milestones prior to gene therapy. The number and proportion of patients achieving each key motor milestone prior to treatment will be computed, along with exact binomial (Clopper-Pearson) 95% confidence intervals (CIs). The applicant argues that this analysis would support the use of the natural history control group in that the pre-treatment milestone achievement rates in the AADC-010 patients are expected to be comparable to the rates in patients described in Wassenberg et al. (2017).

An additional supportive analysis of key motor milestones at the 2-year time point was to involve a nonparametric approach, treating the motor milestones as ordered categories. Each patient will provide data as to the highest motor milestone achieved. That is, a patient will have one of the following responses: none, head control, sit unassisted, stand with support, walk with assistance. Note that since these are ordered categories, a patient cannot achieve the next milestone without first achieving all prior milestones based on the predefined ranking.

The Wilcoxon-Mann-Whitney test was to be conducted at a two-sided $\alpha=0.05$ level of significance to compare the AADC-010 patients to the natural history control group according to the following hypotheses:

H_0: The distribution of motor milestone achievement of patients treated with AADC gene therapy is equal to the distribution of untreated patients.

H_1: The distribution of motor milestone achievement of patients treated with AADC gene therapy is not equal to the distribution of untreated patients.

This test was to be conducted irrespective of the results of the individual milestone testing described above.

Analysis of secondary efficacy endpoints

Motor Development Tests

Summary statistics were to be computed for PDMS-2, AIMS, and Bayley-III on the raw and change from baseline (CFB) scores by time point for each total score and/or subscale score. In addition, a 95% confidence interval (CI) on the mean (raw and CFB) was to be calculated for each time point for each scale and/or subscale.

Each total score and subscale score was also to be evaluated by a repeated measures analysis using SAS PROC MIXED with fixed effects terms for time point, age at gene therapy (in months), and baseline score. A repeated statement was to be included to specify time point as the repeated effect, with patient identified in the patient option. The least squares (LS) mean CFB was to be estimated for each time point for each scale and/or subscale, along with a 95% CI.

Body Weight

Body weight was to be recorded at each visit. Summary statistics were to be computed on the raw and CFB data by time point for body weight. In addition, a 95% CI on the mean weight was to be calculated for each time point.

Neurological Exam Findings

The presence of floppiness episodes, OGC episodes, limb dystonia, and stimulus-provoked dystonia was to be evaluated monthly for the first year of follow up. The number and proportion of patients with OGC episodes will be computed by time point, along with exact binomial (Clopper-Pearson) 95% confidence intervals (CIs). A similar analysis was to be performed for floppiness, limb dystonia, and stimulus-provoked dystonia.

Muscle power data were collected on a 0-5 scale, and DTR data were collected on a 0-4 scale. The number and proportion of patients reporting each muscle power and DTR response (i.e., value on the 0-5 or 0-4 categorical scale) were to be computed by time point. In addition, summary statistics (mean, standard deviation, median, range, and number of non-missing responses) for muscle power responses were to be computed by time point.

Anti-AAV2 Optical Density Values

Anti-AAV2 OD values were to be obtained at baseline, Months 1-6, Month 9, and Month 12. The number and proportion of patients with an OD value > 0.5 (i.e., OD values corresponding to a positive neutralizing antibody titre; Ito et al. 2009) will be computed by time point. A shift table was to be constructed to show the counts of patients in each shift category (negative/negative, negative/positive, positive/negative, positive/positive) from baseline to Month 12.

To evaluate the potential relationship between the OD values and the PDMS-2 total score, a repeated measures analysis was to be conducted using SAS PROC MIXED with PDMS-2 total score change from baseline as the response and with fixed effects terms for time point, OD value, and baseline score. A repeated statement was to be included to specify time point as the repeated effect, with patient identified in the patient option. The significance of the OD values as an explanatory variable for the variability in PDMS-2 scores over time was to be reported via the tests of fixed effects table. In addition, Pearson's correlation between the PDMS-2 scores and OD values was be computed by patient and overall.

Analysis of pharmacodynamics endpoints

CSF Neurotransmitter Metabolites

The neurotransmitter metabolites were to be evaluated at baseline and Month 12. Summary statistics will be computed on the raw and CFB data by time point for each of these metabolites. In addition, a 95% CI on the mean values was to be calculated for each time point.

Putaminal Signal in PET

PET 18 F-fluorodopa uptake in the left putamen, right putamen, and occipital lobe was to be recorded at baseline, Month 12, and Month 24. These values were to be used to compute the specific uptake at each time point as follows:

$$\text{specific uptake} = \frac{((\text{left putamen-occipital lobe}) + (\text{right putamen-occipital lobe}))/2}{\text{occipital lobe}}$$

Summary statistics for each PET parameter were to be computed on the raw and CFB data by time point. In addition, a 95% CI on the mean values was to be calculated for each time point.

Specific uptake was also to be evaluated by a repeated measures analysis using SAS PROC MIXED with fixed effects terms for time point and age at gene therapy (in months). A repeated statement was to be included to specify time point as the repeated effect, with patient identified in the patient option. The least squares (LS) mean will be estimated for each time point, along with a 95% CI.

Patient Completion

A table was to be constructed with counts and percentages of patients who completed the study through Month 12 and patients who withdrew from the study. Of the patients who withdrew from the study, the number and percent of patients with each withdrawal reason was to be reported.

Counts and percentages of patient completion were also to be provided for the following duration of follow up categories: 0 to 3 months, 3 to 6 months, 6 to 9 months, 9 to 12 months, and 12 to 24 months. Patients will be counted once in this analysis according to their entire duration of follow up.

External Control

The natural history control was based on a systematic literature review of all available reported AADC deficiency cases, which was performed by Wassenberg et al (Wassenberg 2017). This review identified 117 confirmed AADC deficiency cases, of which 103 had sufficient information to adjudicate severity. From these 103 cases, a total of 82 AADC-deficient patients were considered as having a severe phenotype, which was defined as having no or very limited developmental milestones and being fully dependent AADC deficient patients. The applicant claims that this group of patients is comparable to the patients enrolled in the 3 eladocagene exuparvovec clinical studies and therefore suitable for use as a natural history control.

Interim analysis

No formal interim analyses were planned for this study.

Results

Participant flow

All subjects who were screened were included in the trial and treated with the active. All subjects had their primary efficacy endpoint measured at 24 months. One subject was lost to follow-up due to death secondary to encephalitis. The trial is currently ongoing.

Baseline data

The mean age was 52.5 months (range 21.0 to 102.0 months), there were 5 males and 5 females, 9 patients were Asian-Chinese, and 1 was White. As of the data cut-off (27 March 2019), the mean duration of follow-up was 39.9 months and 9 patients completed follow-up through Month 24.

Prior Medications in 2 or More Patients (Safety Population)

Most patients received prior medications. The most frequently administered prior medications included hypnotics and sedatives (9 patients, 90%), dopaminergic agents and plain vitamin preparations (8 patients each, 80%), and expectorants excluding combinations with cough suppressants (7 patients, 70%).

Concomitant Medications

The most common classes of concomitant medication administered to all patients within 12 months of eladocagene exuparvovec gene therapy were anti-inflammatory agents, antipsychotics, expectorants (excluding combinations with cough suppressants), hypnotics and sedatives, and other analgesics and antipyretics. The use of most classes of medication was stable from within 12 months of treatment to within 24 months of treatment.

There was an increase in use of IV solutions (from 4 to 8 patients) and nasal decongestants for systemic use (from 2 to 4 patients); Use of dopaminergic agents for AADC deficiency after treatment was recorded in a total of 4 patients. In 8 patients, dopaminergic agents were taken prior to eladocagene exuparvovec therapy as standard of care for AADC. As directed by the Investigator, these agents were then stopped within 1 month prior to the gene therapy or 10 days after therapy. In 4 patients, treatment with dopaminergic agents (either the original agent or a new agent) was started within 24 months of eladocagene exuparvovec treatment for alleviation of AADC deficiency-associated symptoms in accordance with standard of care.

Figure 4 Demographics and Baseline Data Summary Statistics for the three trials

Variable	AADC-CU/1601 (N=8)	AADC-010 (N=10)	AADC-011 (N=8)
Age at baseline, months			
Mean (SD)	58.75 (24.84)	52.50 (30.84)	36.13 (17.38)
Age at diagnosis, months			
Mean (SD)	15.84 (9.72)[a]	11.40 (7.04)	14.13 (9.51)
Baseline height, cm			
Mean (SD)	96.00 (8.35)	98.60 (17.99)	85.53 (10.73)
Median (min, max)	97.50 (85.0, 109.0)	93.00 (79.0, 126.0)	84.10 (75.0, 107.0)
Baseline weight, kg			
Mean (SD)	11.49 (2.67)	12.65 (4.67)	9.59 (1.59)
Median (min, max)	10.45 (8.6, 17.0)	10.51 (7.7, 20.5)	9.65 (7.5, 12.0)
Gender, n (%)			
Male	3 (37.5%)	5 (50.0%)	5 (62.5%)
Female	5 (62.5%)	5 (50.0%)	3 (37.5%)
Race, n (%)			
Asian-Chinese	0 (0.0%)	9 (90.0%)	6 (75.0%)
Asian-Other	8 (100%)	0 (0.0%)	2 (25.0%)
White	0 (0.0%)	1 (10.0%)	0 (0.0%)
Genotype, n (%)			
Homozygous founder mutation	7 (87.5%)	6 (60.0%)	3 (37.5%)
Heterozygous founder mutation	1 (12.5%)	4 (40.0%)	5 (62.5%)
PDMS-2 total score at baseline			
Mean (SD)	8.75 (5.42)	9.50 (3.92)	13.25 (6.04)
Median (min, max)	7.50 (2.00, 16.00)	10.0 (4.00, 15.00)	11.50 (7.0, 26.0)
95% CI for the mean	4.22, 13.28	6.69, 12.31	8.20, 18.30
AIMS total score at baseline			
Mean (SD)	2.60 (2.07)[b]	1.60 (0.97)	2.63 (2.26)
Median (min, max)	3.00 (0.00, 5.00)	1.00 (1.00, 4.00)	2.00 (1.0, 8.0)
95% CI for the mean	0.03, 5.17	0.91, 2.29	0.73, 4.52

Abbreviations: AADC, aromatic L-amino decarboxylase; AIMS, Alberta Infant Motor Scale; CI, confidence interval; CU, compassionate use; Max, maximum; min, minimum; PDMS-2, Peabody Developmental Motor Scale - version 2.0; SD, standard deviation.
[a] N=7; one patient did not provide age at baseline.
[b] N=5; AIMS score was not collected for 3 patients at baseline.

Figure 5 Patient Completion Summary

	All Patients N=10
Completed through Month 12	10 (100.0%)
Withdrawn after Month 12 (Death from Encephalopathy due to Influenza B)	1 (10.0%)
Duration of follow-up (months)	
Mean (SD)	39.9 (12.03)
Median	45.7
Min, max	12.2, 49.1
Maximum Duration of follow-up (months)	
12 to <24	1 (10.0%)
24 to <36	1 (10.0%)
36 to <48	5 (50.0%)
48 to <60	3 (30.0%)

Abbreviations: Max, maximum; min, minimum

Numbers analysed

There were 10 subjects recruited, 10 subjects treated and 10 subjects in the ITT analysis.

Outcomes and estimation

For Study AADC-010, at 24 months, 5 (55.6%) patients had mastered head control, 3 (33.3%) were able to sit unassisted, and 2 (22.2%) were able to stand with support. In contrast, none of the natural history control group patients achieved any motor milestones. Achievements for head control (p value<0.0001) and sitting unassisted (p=0.0059) were highly statistically significant compared with the natural history control group.

Figure 6 Key Motor Milestone Acquisition from Study AADC-010 at Month 24 (ITT Population, N=10)

Key Motor Milestone[a]	ITT Population No. (%)	95% CI for Proportion	p valued
Head Control	5 (55.6)	(0.2120, 0.8630)	<0.0001[b]
Sitting Unassisted	3 (33.3)	(0.0749, 0.7007)	0.0059[b]
Standing with Support	2 (22.2)	(0.0281, 0.6001)	0.0567
Walking with Assistance	0	(0.0000, 0.3363)	N/A

Abbreviations: AADC, aromatic L-amino decarboxylase; CI, confidence interval; ITT, Intent-to-treat; N/A, not applicable
[a] One-sided p value for testing H_0: proportion > upper CI limit for historical control data for each milestone at the primary efficacy timepoint for measurement of key motor milestones.
[b] Statistically significant
Note: ITT population refers to all enrolled patients.

Figure 7 Patients Achieving Key Motor Milestones (ITT Population)

Motor Milestone	Timepoint	Number (%) of Patients N=10	95% CI for Proportion	Natural History Control Proportion (95% CI) N=82	P Value[a]
Head control	Pretreatment	0 (0.0000)	(0.0000, 0.3085)	--	--
	1 Year[b]	1 (0.1111)	(0.0028, 0.4825)	--	--
	2 Years	5 (0.5556)	(0.2120, 0.8630)	0 (0.0000, 0.0440)	<0.0001
Sitting unassisted	Pretreatment	0 (0.0000)	(0.0000, 0.3085)	--	--
	1 Year[b]	1 (0.1111)	(0.0028, 0.4825)	--	--
	2 Years	3 (0.3333)	(0.0749, 0.7007)	0 (0.0000, 0.0440)	0.0059
Standing with support	Pretreatment	0 (0.0000)	(0.0000, 0.3085)	--	--
	1 Year[b]	0 (0.0000)	(0.0000, 0.3363)	--	--
	2 Years	2 (0.2222)	(0.0281, 0.6001)	0 (0.0000, 0.0440)	0.0567
Walking with assistance	Pretreatment	0 (0.0000)	(0.0000, 0.3085)	--	--
	1 Year[b]	0 (0.0000)	(0.0000, 0.3363)	--	--
	2 Years	0 (0.0000)	(0.0000, 0.3363)	0 (0.0000, 0.0440)	N/A

Key motor milestone acquisition continued after the primary efficacy assessment at the 2-year time-point. Full head control was achieved at Month 30 by Patient 1005 and at Month 36 by Patient 1001, bringing the number of patients who achieved this milestone after eladocagene exuparvovec gene therapy to 7 (70%). In addition, Patient 1002 was able to sit unassisted at Month 48, and Patient 1004, who achieved head control, sitting unassisted, and standing with support by Month 18, was able to walk with assistance at Month 36.

LS Means of Post-treatment PDMS-2 Total Scores for Studies AADC- 010 (ITT Population)

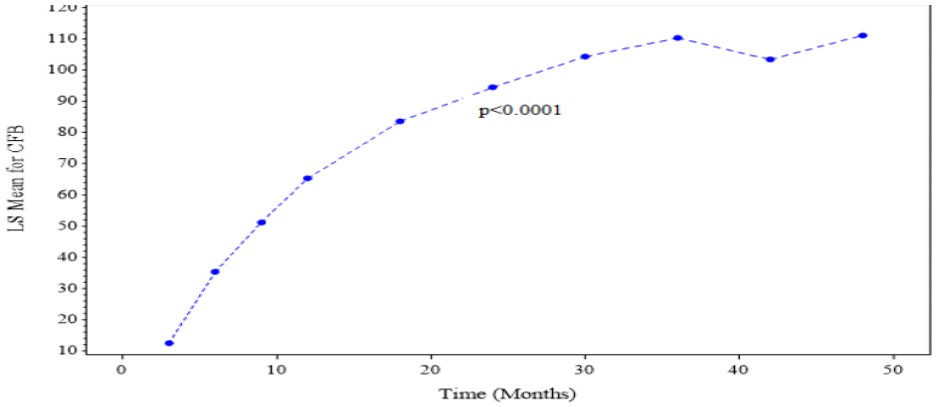
Eladocagene exuparvovec-treated patients showed statistically significant increases in PDMS-2 total scores over time. All patients achieved clinically meaningful improvement in PDMS-2 total scores that were maintained or continued to improve over the course of the study. The onset of improvement was also observed within months of gene therapy, and the LS mean change from baseline was PDMS-2 total score was statistically significant (p value<0.0001) at the 24-month time-point and continued to improve up to 4 years, which was the length of available follow-up at the data cut-off. The distribution of motor milestone achievement in the eladocagene exuparvovec gene therapy patients at 2 years post treatment was significantly different compared to the natural history control group (p<0.0001).

PDMS-2 total and subscale scores, when assessed by visit using a repeated measures mixed effects model that tested fixed effects, were consistently statistically significant (p≤0.05).

Eladocagene exuparvovec-treated patients showed increases in mean PDMS-2 subscale scores from baseline to Year 2, with the exception of reflexes and object manipulation. The increases in mean PDMS-2 total and subscale scores relative to baseline were evident as early as Month 3. Similar results were demonstrated at the 1-year time-point.

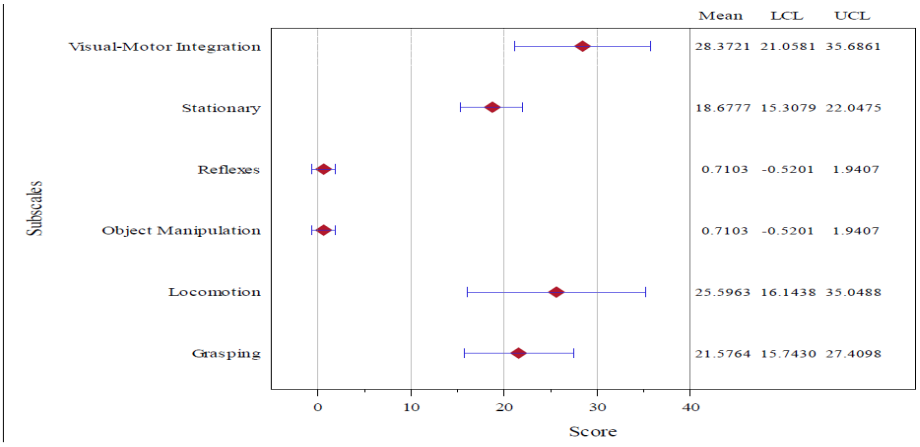
Eladocagene exuparvovec-treated patients also demonstrated improvement of specific skills on the PDMS-2 subscales that represent additional evidence of clinical benefit and development toward more independent motor function, including sitting, symmetrical posture, rolling, manipulating a rattle and paper, engaging one's own fingers, reaching for a rattle, removing socks, and turning pages. These observations are detailed in the individual patient profiles.

Figure 8 PDMS-2 Total Scores Up To 4 Years After Eladocagene Exuparvovec Administration (ITT Population)



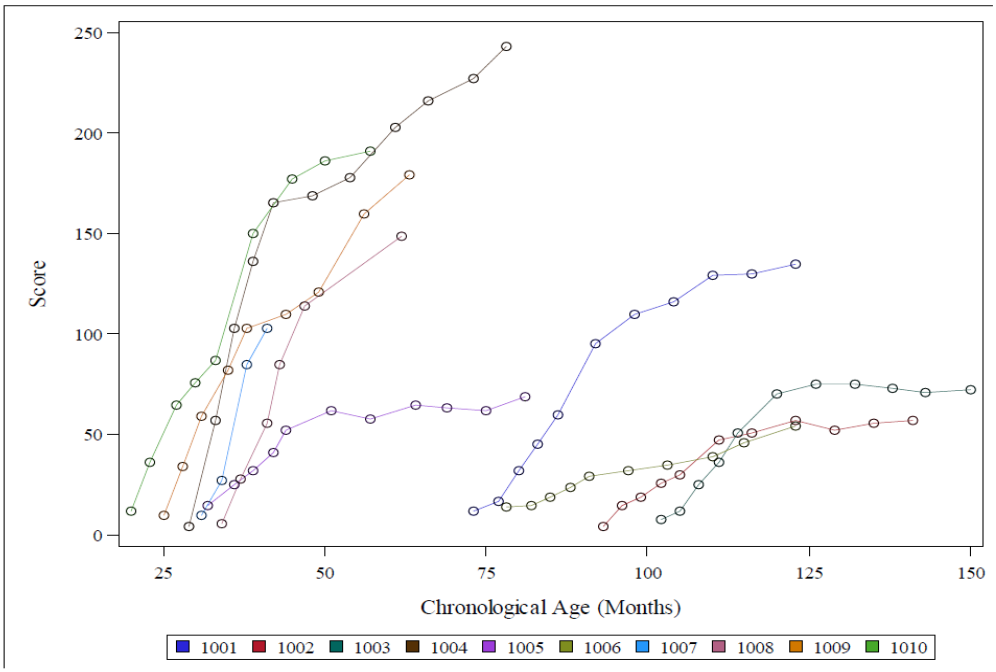
Abbreviations: CFB, change from baseline; ITT, intent-to-treat; LS, least squares; PDMS-2, Peabody Development Motor Scales-2nd Edition

Figure 9 LS Means for PDMS-2 Subscales at 2 Years After Eladocagene Exuparvovec Administration (ITT Population)



Abbreviations: ITT, intent-to-treat; LCL, lower confidence limit; LS, least squares; PDMS-2, Peabody Development Motor Scales-2nd Edition; UCL, upper confidence limit

Figure 10 PDMS-2 Total Scores by Patient and Chronological Age (Months) (ITT Population)

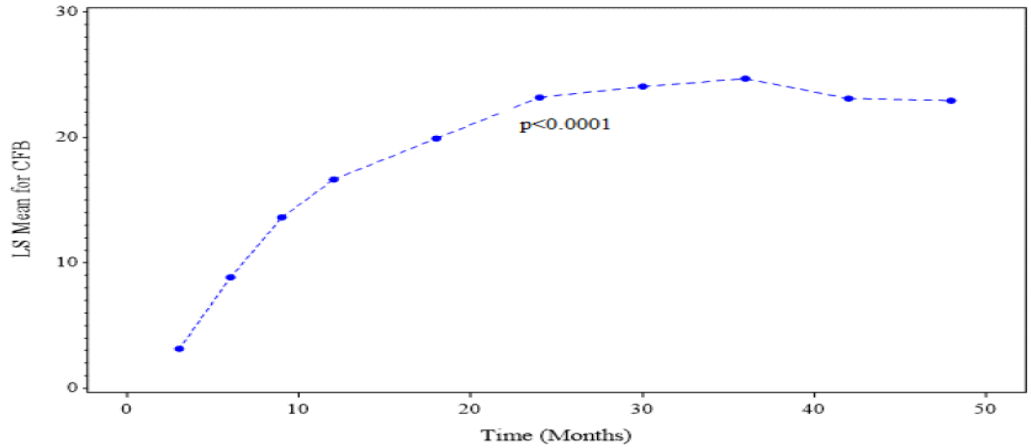


Abbreviations: ITT, intent-to-treat; PDMS-2, Peabody Development Motor Scales-2nd Edition

Motor Development: AIMS

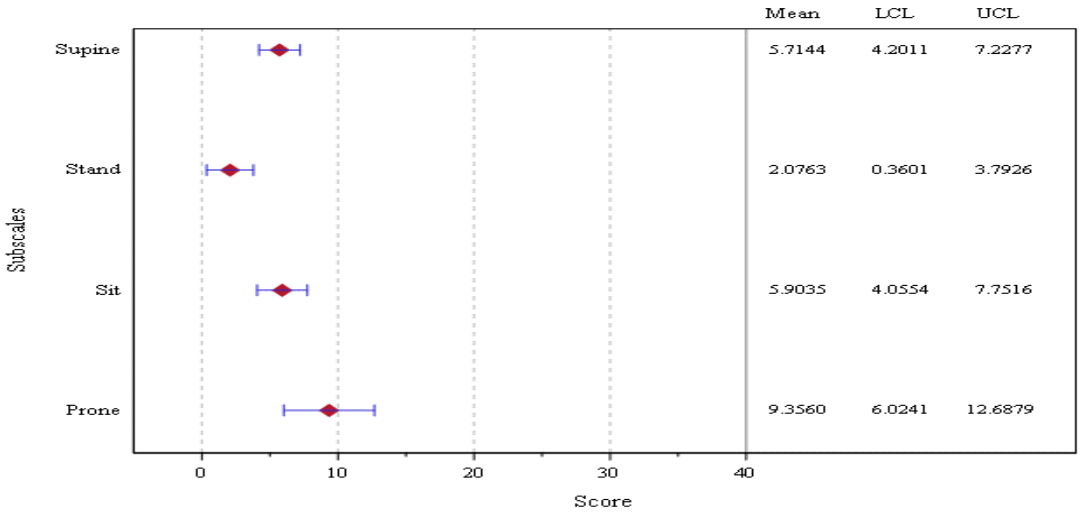
Eladocagene exuparvovec-treated patients showed increases in mean AIMS total scores from baseline over time that were statistically significant (p<0.0001) at the 2-year time-point and that were maintained up to 4 years, which was the length of available follow-up at the data cut-off. Six patients (60%) achieved and maintained AIMS total score increases of approximately 20 to 40 points.

Figure 11 AIMS Total Scores Up To 4 Years After Eladocagene Exuparvovec Administration (ITT Population)



Abbreviations: AIMS, Albert Infant Motor Scale; CFB, change from baseline; ITT, intent-to-treat; LS, least squares

Figure 12 LS Means for AIMS Subscales at 2 Years After Eladocagene Exuparvovec Administration (ITT Population)



Improvement in Cognitive Function

Cognitive function was assessed by the Bayley-III in Study AADC-010.

Bayley-III

At the 24-month time-point, the LS mean change from baseline in Bayley-III total scores for patients in Study AADC-010 was statistically significant (p value<0.0001) and continued to improve up to 48 months (4 years). Increases from baseline in all mean Bayley-III subscales were also observed at 12 months and were evident as early as Month 3.

At baseline in Study AADC-010 (n=10), most patients exhibited minimal cognitive skills including calming when being picked up, continuous gazing at an object, responding to stimuli (rattle), responding to a bell, and recognizing caregivers.

Over the 24-month period, examples of cognitive skills newly developed include the following:

- 6 patients explored objects using shaking, using their mouths, or other activity
- 3 patients reacted to the disappearance of a caregiver's face
- 7 patients persistently reached for objects
- 9 patients responded to their image in the mirror by smiling, laughing, patting, etc.
- 5 patients played with a string (picking it up, chewing, manipulating, pulling)
- 6 patients looked for a fallen toy on the floor
- 5 patients held a bell by the handle and rang it on purpose
- 5 patients reached for an additional block while already holding one

Examples of receptive language skills newly developed over the 24-month period include the following:

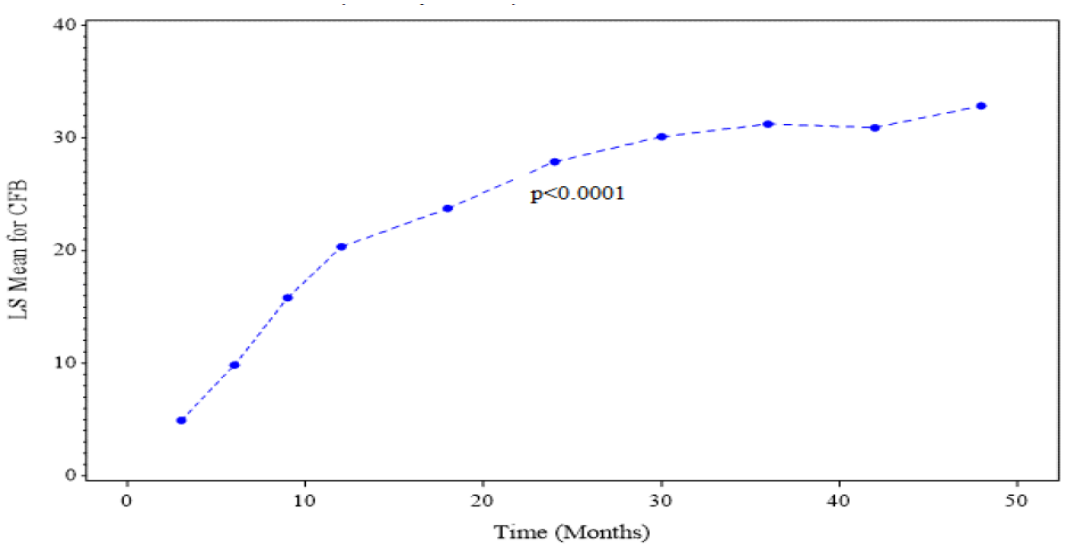
- 6 patients turned their head toward sound
- 7 patients interacted with an object for at least 60 seconds
- 4 patients stopped reaching for an object in response to a "no-no"

- 3 patients-maintained attention and enjoyed interacting/playing with a caregiver for at least 60 seconds
- 9 patients responded in an appropriate manner to at least one request

Examples of expressive language skills newly developed over the 24-month period include the following:

- 3 patients used 2-vowel sounds
- 5 patients used 2 consonant sounds
- 9 patients imitated at least one consonant-vowel combination
- 5 patients actively participated in at least 1 play routine
- 8 patients jabbered expressively
- 6 patients used one-word approximations

Figure 13 Bayley-III Total Scores Up To 4 Years After Eladocagene Exuparvovec Administration (ITT Population)

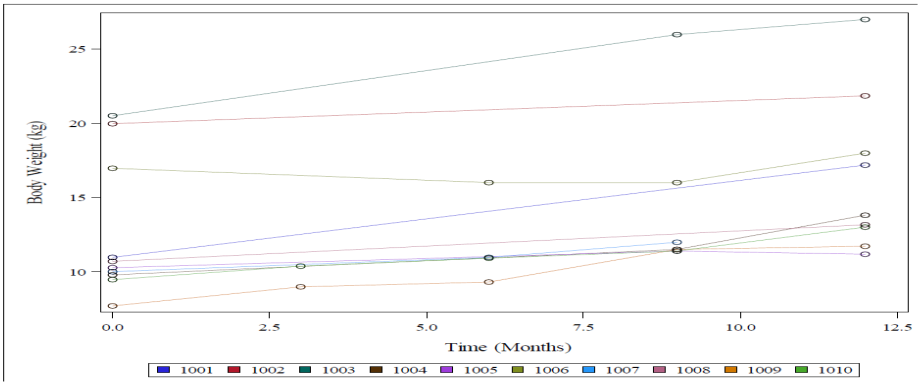


Abbreviations: Bayley-III, Bayley Scales of Infant Development – Third Edition; CFB, change from baseline; ITT, intent-to-treat; LS, least squares

Body Weight

There was a statistically significant increase from baseline in mean body weight at Year 1 (p=0.0011)

Figure 14 Body Weight by Patient Over Time (ITT Population)



Abbreviations: ITT, intent-to-treat

Neurologic Examination Findings

Following eladocagene exuparvovec gene therapy, the number of patients with floppiness, OGC episodes, limb dystonia and stimulus-provoked limb dystonia decreased during the first year. In most cases, reductions in the number of patients with these neurologic findings were apparent as early as Month 3 following treatment. Limb dystonia and stimulus-provoked limb dystonia did not occur in any patient at the Month 9 and Month 6 neurologic examination, respectively, and thereafter through Month 12. The majority of the patients had neurologic findings on examination pre-surgery.

Immunogenicity

As anticipated, no patients had a positive antibody titre at baseline. The presence of anti- AAV2 antibodies was detected in 4 (40%) patients at Month 3, 8 (80%) patients at Month 6, and 7 (70%) patients at Month 9 following treatment with eladocagene exuparvovec gene therapy. At Month 12, 4 patients (40%) had a positive anti-AAV2 antibody titre (OD value >0.5). Change from baseline in PDMS-2 total scores was evaluated by a repeated measures analysis with anti-AAV2 OD values as a fixed effect. The anti-AAV2 OD value was a statistically significant factor in the model, indicating that the magnitude of the change from baseline in PDMS-2 total scores was associated with the anti-AAV2 OD values (p=0.0003). A Pearson correlation coefficient was calculated for these data. The overall correlation was 0.59510, indicating a moderate positive correlation between anti-AAV2 antibody titre and increases in PDMS-2 total score. However, the increasing PDMS-2 total score indicates that over time, antibodies had no adverse impact on efficacy.

Pharmacodynamic Endpoints

Change from Baseline in Neurotransmitter Metabolites

The presence of neurotransmitter metabolites HVA (the metabolite of dopamine) and 5-HIAA (the metabolite of serotonin) was measured in CSF during the first year of follow up. At Month 12, the concentration of both HVA and 5-HIAA were increased compared with Baseline.

Figure 15

	Baseline N=10	CFB at Month 12 N=9
HVA		
Mean (SD)	5.65 (7.95)	26.56 (21.57)
Median (min, max)	2.50 (2.50, 28.00)	19.50 (0.00, 54.50)
5-HIAA		
Mean (SD)	3.10 (1.29)	6.56 (12.72)
Median (min, max)	2.50 (2.50, 6.00)	0.00 (0.00, 34.50)

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; CFB, change from baseline; HVA, homovanillic acid; max, maximum; min, minimum
Source: Data Table 23

Putaminal-Specific Uptake

An increase in mean putaminal-specific uptake of 18F-DOPA on PET imaging was evident at Month 12 and further increased through Month 24.

Figure 16 PET Specific Uptake by Time Point (ITT Population)

	Baseline N=10	CFB at Month 12 N=9	CFB at Month 24 N=9
Mean (SD)	0.22 (0.11)	0.42 (0.21)	0.47 (0.22)
LS Mean (SE)	0.2 (0.05)	0.6 (0.06)	0.7 (0.06)
95% CI of LS Mean	0.1, 0.3	0.5, 0.7	0.5, 0.8

Abbreviations: CFB, change from baseline; LS, least squares; PET, positron emission tomography; SE, standard error

In a repeated measures analysis of PET imaging of putaminal-specific 18F-DOPA uptake with fixed effect terms for time-point and age at gene therapy, age at the time of treatment was determined not to be statistically significant, indicating that the magnitude of the change in putaminal specific uptake was not associated with age (p=0.3760). The repeated measures analysis of putaminal-specific uptake by time-point was statistically significant (p=0.0002), indicating that putaminal-specific uptake of 18F-DOPA increases over time.

Summary of main efficacy results

Table 10: Summary of efficacy for trial AADC-010

Title: A Phase 1/2 Clinical Trial for treatment of Aromatic l-Amino Acid Decarboxylase (AADC) Deficiency using AAV2-hAADC	
Study identifier	AADC-010
Design	Study AADC-010 is an ongoing single-center, prospective, single-arm, Phase 1/2 evaluation of the safety and efficacy of intraputaminal infusion of eladocagene exuparvovec (AAV2-hAADC) gene therapy in children with AADC deficiency for a period of up to 5 years after study drug administration. Eladocagene exuparvovec gene therapy at a total dose of 1.8×10^{11} vector genomes (vg) was administered during a single operative session.

	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		12 months not applicable Subjects returned voluntarily every 6 months after the first 12 months for a total of 5 years.
Hypothesis			
Treatments groups	Treated patients		Eladocagene exuparvovec, Total dose of 1.8×10^{11} vector genomes (vg), administered by intraputaminal injection during 1 operative session in 10 patients.
Endpoints and definitions	Primary endpoint	Achievement of motor milestones	Achievement of motor milestones as assessed by Peabody Developmental Motor Scale, second edition (PDMS-2) (full head control, sitting unassisted, standing with support, and walking with assistance) **at the 2-year time-point.**
	Secondary endpoint	PDMS-2 total and subscale scores	PDMS-2 is a validated instrument used to measure motor skills and developmental milestone achievement in infants and children. Patients received a numerical score that correlated with achievement of motor milestones. The raw total PDMS-2 scores were calculated by adding the subscale scores.
	Secondary endpoint	AIMS total and subscale scores	The AIMS is a 58-item observational measure that assesses sequential development of motor milestones. Patients received a numerical score that correlated with achievement of motor milestones. The total AIMS scores were calculated by adding the subscale scores.
	Secondary endpoint	Bayley-III total and subscale scores	The Bayley-III is a standardized assessment of cognitive, language, and motor development for children between 1 and 42 months of age. The whole CDIIT scores were calculated by adding the subtest scores.
	Secondary endpoint	Neurological exam findings	Neurological exam findings associated with AADC deficiency, including floppiness, oculogyric crisis (OCG) episodes, stimulus-provoked dystonia, and limb dystonia were evaluated throughout the study.
	Secondary endpoint	Change from baseline in body weight	Body weight was measured at baseline and throughout the study and the change from baseline was calculated.
	Secondary endpoint	Change in from baseline ^{18}F-DOPA PET scan data	Expression and activity of the AADC enzyme in the putamen was assessed by PET imaging using ^{18}F-DOPA, a positron-emitting fluorine-labelled version of levodopa, which is a substrate for AADC that is incorporated into de novo dopamine.

	Secondary endpoint	Change from baseline in CSF neurotransmitter metabolites HVA and 5-HIAA	CSF samples were collected at baseline and 12 months after eladocagene exuparvovec administration and evaluated for levels of HVA and 5-HIAA, which are metabolic products of dopamine and serotonin, respectively.
Database lock	27 March 2019 (interim cut-off date)		

Results and Analysis

Primary Analysis: Number and proportion of patients achieving key motor milestones at 2 Years

Analysis description	**Primary Analysis: Number and proportion of patients achieving key motor milestones at 2 Years**		
Analysis population and time point description	Intent-to-treat (ITT) Population (all patients) 2 years		
Descriptive statistics and estimate variability	Treatment group	ITT Population	Natural History Cohort
	Number of subjects	N=10	N=82
	Motor milestone: head control at 2 years (number of subjects achieving motor milestone)	N=5	N=0
	95% Confidence interval (CI)	(0.2120, 0.8630)	(0.0000, 0.0440)
	Motor milestone: sitting unassisted at 2 years (number of subjects achieving motor milestone)	N=3	N=0
	95% CI	(0.0749, 0.7007)	(0.0000, 0.0440)

	Motor milestone: standing with support at 2 years (number of subjects achieving motor milestone) 95% CI	N=2 (0.0281, 0.6001)	N=0 (0.0000, 0.0440)
	Motor milestone: walking with assistance at 2 years (number of subjects achieving motor milestone) 95% CI	N=0 (0.0000, 0.3363)	N=0 (0.0000, 0.0440)
Effect estimate per comparison	Primary endpoint (head control)	Comparison groups	ITT population vs natural history cohort
		P-value (one-sided test)	p<0.0001
	Primary endpoint (sit unassisted)	Comparison groups	ITT population vs natural history cohort
		P-value (one-sided test)	p=0.0059
	Primary endpoint (stand with support)	Comparison groups	ITT population vs natural history cohort
		P-value (one-sided test)	P=0.0567
	Primary endpoint (walk with assistance)	Comparison groups	ITT population vs natural history cohort
		P-value (one-sided test)	n/a
Analysis description	**Secondary analysis:**		
Analysis population and time point description	Intent-to-treat (ITT) Population (all patients) 24 months		

Descriptive statistics and estimate of variability	Number of subjects N=10 **PDMS score**-Least squares (LS) mean change from baseline at 2 years =94.5, 95% CI (72.1-116.9). **AIMS Total Score**- Least squares (LS) mean change from baseline at 2 years =23.1, 95% CI (15.7, 30.5). **Bayley-III Total Score-**Least mean squares (LS) mean change from baseline at 2 years=27.9, 95% CI (23.3, 32.5). **Neurological exam findings, OCG episodes at 12 months-**Number and proportion with limb dystonia at 12 months =0, 95% CI (0.0000, 0.4096). Number of patients with OGC episodes at 12 months= 5 95% CI (0.2904, 0.9633). **Mean change in body weight at 12 months-**3.39kg (min max kg-0.9, 6.5) p=0.0011 **Mean change from Baseline in Putaminal-specific uptake of F-DOPA PET, at 24 months N=8-**0.6 CI (0.5, 0.7). **Mean change from baseline in neurotransmitter metabolites, 12 months N=10** HVA: 25.56 (21.57) HIAA: 6.56(12.72).

Study AADC-CU1601

Study AADC-CU/1601 is a single-centre, observational study that summarized and analysed data from a single arm, compassionate use interventional study to evaluate the safety and efficacy of intraputaminal infusion of eladocagene exuparvovec gene therapy in children with AADC deficiency for a period of up to 60 months after study drug administration. Eladocagene exuparvovec gene therapy at a total dose of 1.8×10^{11} vg was administered during a single operative session. The planned duration of the study was 1 year, and patients returned voluntarily every 6 months to complete developmental tests (PDMS-2, AIMS, and CDIIT) and adverse event (AE) reporting for up to 60 months.

Efficacy Data from this study at 24 months is used in the assessment of the primary endpoint. Therefore, the study is presented as one of the main studies.

This was the first study started by the neurosurgical centre in Taiwan. After the start of the compassionate use programme, a study with an approved protocol was started. Eight patients were treated as part of the compassionate use programme. There is 5 year's follow-up on 6 of them.

Treatments

Surgical procedure

This surgery was performed by the Departments of Neurology and Neurosurgery, National Taiwan University Hospital, and under general anaesthesia. Prior to the surgery, putamen location was ensured by Magnetic Resonance Imaging (MRI) and Computed Tomography (CT). After the injection site was determined stereotactically, AAV-AADC was injected into putamen through the holes drilled in the skull outside of putamen. After the surgery, head CT scan and MRI was performed to check for complications, such as bleeding.

Treatment Administered

AAV2-h*AADC* (eladocagene exuparvovec) was administered under the AADC-CU treatment plan during a single operative session at a dose of 0.45×1011 vg and a volume of 80 µl per site to 4 sites (2 per putamen), for a total dose of 1.8×1011 vg and a total volume of 320 µl per patient.

Objectives

The objective of the AADC-compassionate use treatment plan was to evaluate the safety and long-term benefits of administration of the hAADC gene with the AAV2 vector to patients with AADC deficiency. The primary objective of the AADC-CU/1601 protocol was to collect data from patients with AADC deficiency who received humanitarian assistance treatment following AAV2-h*AADC* administration via intraputaminal injection, and further to observe the safety and efficacy fora period of up to 60 months (5 years) after administration of eladocagene exuparvovec.

The objectives from the 2 studies were integrated in the statistical analysis plan as follows: The primary objective of this single-arm, interventional study was to retroactively evaluate the safety and efficacy of intraputaminal infusion of eladocagene exuparvovec in children with AADC deficiency for a period of up to 60 months after study drug administration.

Inclusion Criteria for AADC-CU study

Patients were included in the AADC-CU study if all of the following inclusion criteria were fulfilled:

1. Patient had a confirmed diagnosis of AADC deficiency, documented by CSF analysis of neurotransmitter metabolites HVA and 5-HIAA and confirmed by enzyme activity test or screening of AADC gene mutation
2. Patient had classical clinical characteristics of AADC deficiency, such as oculogyric crises, hypotonia, and developmental retardation
3. Patient was greater than 2 years of age
4. Patient agreed to cooperate completely for all evaluations and examinations before, during, and after the whole trial.
5. Parents or guardians agreed to sign informed consent.

Inclusion Criteria for AADC-1601 Study

Male or female children were included in the AADC-1601 study if all of the following inclusion criteria were fulfilled:

1. Patient had a documented diagnosis of AADC deficiency and was receiving humanitarian assistance treatment following AAV2-h*AADC* administration via intraputaminal injection
2. Parent(s) or legal guardian(s) must have provided written informed consent prior to data abstraction, unless all of the following applied:
    a. The patient was deceased
    b. The responsible IRB/IEC/Research Ethics Board did not require informed consent per a review of their documented local policies for collecting data on patients who are deceased
    c. Written confirmation was received from the responsible IRB/IEC/REB confirming that the abstracted data could be analysed and used to support regulatory filings by the Sponsor.

Exclusion Criteria for AADC-CU Study

Patients were excluded from the AADC-CU study for any of the following reasons:

1. Patient had any health or neurological concerns that may have increased the risk of surgery. The investigator had the right to evaluate the feasibility of a patient for this trial based on his or her health condition.
2. The patient was taking any medications that may affect the trial.
3. The patient has had a severe allergic reaction to the components of the vector preparation/solution used in the preparation of vector.

Note: In version 1.2 of the AADC-CU treatment plan, an additional exclusion criterion was added: the patient has anti-AAV2 neutralizing antibody titre over 1200-fold. In version 1.3, this criterion was clarified: the patient has anti-AAV2 neutralizing antibody titre over 1200-fold or an ELISA OD over 1.

Exclusion Criteria for AADC-1601 Study

Patients were excluded from the AADC-CU/1601 study for any of the following reasons:

1. Subjects for whom informed consent was not obtained, as described in Inclusion criteria.

Outcomes/endpoints for AADC-1601 Study

Achievement of key motor milestones at the 60-month time-point was the primary measure of efficacy.

Primary efficacy endpoints related to the achievement of such milestones are as follows:

-Proportion of patients who achieved full head control, as measured using the Peabody Developmental Motor Scales-Second Edition (PDMS-2)

-Proportion of patients who were able to sit unassisted, as measured using the PDMS-2

-Proportion of patients who were able to stand with support, as measured using the PDMS-2

-Proportion of patients who were able to walk with assistance, as measured using the PDMS-2.

Secondary efficacy endpoints were:

- Raw scores for the PDMS-2 total and subscales (Month 60)
- Raw scores for the Alberta Infant Motor Scale (AIMS) total and subscales (Month 60)
- Raw scores for the Comprehensive Developmental Inventory for Infants and Toddlers (CDIIT) whole test and subtests (Month 60)
- Change from baseline in **body weight** (collected at each visit)
- **Neurological examination** findings with respect to muscle tone (i.e. floppiness), oculogyric crisis (OGC) episodes, dystonia, muscle power, and deep tendon reflex (DTR) response (every month for the first year of follow-up).

Change from baseline in body weight (collected at each visit)

Pharmacodynamic Endpoints

- Change from baseline in the neurotransmitter metabolites homovanillic acid (HVA) and 5-HIAA in cerebrospinal fluid (CSF) at Months 6, 12, and 60
- Change from baseline in positron emission tomography (PET) imaging of putaminal-specific L-6-[18F] fluoro-3,4-dihydroxyphenylalnine (18F-DOPA) PET uptake at Months 6, 12, and 60.

Safety Endpoints

The safety endpoints were:

- All treatment-emergent adverse events (TEAEs) (from surgery start time to Month 60)
- Neurological exam findings (excluding muscle tone, OGC episodes, dystonia, muscle power, and DTR response) (collected monthly for the first year of follow-up)
- Viral shedding

Sleep Study

Sleep parameters, including total sleep time, total sleep period, sleep efficiency, obstructive/central/mixed apnea, Apnea Hypopnea Index, and hypopnea were measured prior to surgery and at 12 weeks after administration of study treatment.

Sample size

There was no formal sample size calculation for this study as the recruitable subject pool was small. Eight patients were enrolled in the study and received eladocagene exuparvovec infusion, and therefore comprise both the Intent-to-Treat (ITT) and Safety populations. The majority of patients (6 [75%]) completed the study.

Randomisation

This was an uncontrolled open-label study.

Blinding (masking)

This was an uncontrolled open-label study.

Statistical methods

Similar statistical analysis methods to trial AADC-010. The analysis of data was retrospective as the study had started and data was being collected before the protocol and SAP had been finalised.

Results

Participant flow

Eight patients were enrolled into this study, received eladocagene exuparvovec infusion and comprise both the ITT Population and the Safety Population. The majority of patients (6) completed the study through Month 60. The mean (standard deviation) duration of follow-up was 62.5 months) (range: 59.9 to 68.3 months). One hundred percent of patients completed visits through Month 12. Two

patients completed the study per AADC-CU protocol but did not return for voluntary assessments after Month 24.

Figure 17 Patient Completion Summary

	Number of patients (%) (N=8)
Screen failure	0 (0.0%)
Completed through Month 12	8 (100.0%)
Completed through Month 24	8 (100.0%)
Completed through Month 60	6 (75.0%)
Withdrawn prior to Month 60	2 (25.0%)
Reason for withdrawal	
Unable to attend Month 60 Visit	2 (25 %)
Duration of follow-up (months)	
Mean (SD)	62.5 (2.70)
Median	62.2
Min, max	(59.9, 68.3)

Abbreviations: Max, maximum; min, minimum; SD, standard deviation
Note: Summary data for patients completed through Month 12, 24, and 60 are cumulative, meaning that patients are counted in all applicable groups. Patients are counted in one category only for duration of follow-up.

Recruitment

First patient dosed 27 February 2010; last patient visit 21 August 2017.

Conduct of the study

Protocol Deviations

All patients received treatment in accordance with the AADC-CU treatment plan. Protocol deviations were not explicitly collected in AADC-CU or in observational study AADC-CU/1601.

Baseline data

Demographics and Baseline Data Summary Statistics for study AADC-1601 (Safety Population) provided in table above

Prior and Concomitant Medications

The majority of subjects (8 [100.0%]) received prior medications. The most frequently administered prior medications included anaesthetics, general, hypnotics and sedatives (7 patients [87.5%] each), other beta-lactam antibacterials (5 patients [62.5%]), anti-inflammatory agents, dopaminergic agents, expectorants, excluding combinations with cough suppressants, and vitamin B12 and folic acid (4 patients each [50.0%]), adrenergics (inhalants), cardiac stimulants excluding cardiac glycosides, drugs for peptic ulcer and gastro-oeosophageal reflux, muscle relaxants (peripherally acting agents) (3 patients [37.5%] each). The remaining prior medications were taken by 2 patients or less.

Number and Percent of Subjects Taking Concomitant Medications

The most frequently administered concomitant medications included blood and related products (8 patients; albumin was used to prime the tubing prior to infusion), antipsychotics (haloperidol, risperdal, and risperidone) and drugs for peptic ulcer and gastro-oesophageal reflux (5 patients each), and dopaminergic agents and other beta-lactam antibacterials (4 patients).

Concomitant medications that were newly administered included anticholinergics, antipsychotics, antithrombotics, blood and related products, corticosteroids plain, lipid-modifying agents plain, muscle relaxants centrally acting agents, mydriatics and cycloplegics, other gynaecologicals, and other systemic drugs for obstructive airway diseases.

Concomitant medications that increased after administration of eladocagene exuparvovec included drugs for functional bowel disorders, drugs for peptic ulcer and gastro-oesophageal reflux, muscle relaxants centrally acting agents, mydriatics and cycloplegics.

Concomitant medications that decreased after administration of eladocagene exuparvovec included adrenergics inhalants, expectorants, hypnotics and sedatives, IV solution additives, nasal decongestants for systemic use, opioids, other analgesics and antipyretics, other anti-anaemic preparations, other beta-lactam antibacterials, vitamin B12 and folic acid, and vitamin K and other haemostatics. Of note, use of dopaminergic agents for AADC deficiency was recorded in a total of 4 patients. In all 4 patients, dopaminergic agents were taken prior to eladocagene exuparvovec infusion as standard of care for AADC. As directed by the Investigator, these agents were then stopped within 1 month prior to the eladocagene exuparvovec infusion or 10 days after the infusion. However, in all 4 patients, treatment with dopaminergic agents (either the same agent as originally or a new agent) was reinstated within 12 months after the eladocagene exuparvovec infusion for alleviation of AADC deficiency-associated symptoms in accordance with standard of care.

Numbers analysed

There were 8 subjects in the ITT population.

Outcomes and estimation

Primary Efficacy Endpoint

Treatment with eladocagene exuparvovec demonstrated clinical benefit in patients with AADC deficiency. At month 60, 50% of patients mastered the motor milestones of head control and sitting unassisted (4 of 8 patients), resulting in clinically and statistically significant (p=0.0002 for both comparisons) benefits compared with natural history control. At month 60, two patients (25%) were able to stand with support.

Figure 18 Number and Proportion of Patients Achieving Key Motor Milestone

(ITT Population)

Motor Milestone	Timepoint	All ITT Patients (N=8)	95% CI for Proportion	Natural History Control Proportion (95% CI) (N=82)	P -Value
Head control	Pretreatment	0 (0.0000)	(0.0000, 0.3694)	--	--
	12 months	4 (0.5000)	(0.1570, 0.8430)	--	--
	24 months	4 (0.5000)	(0.1570, 0.8430)	--	--
	60 months	4 (0.5000)	(0.1570, 0.8430)	0 (0.0000, 0.0440)	0.0002
Sitting unassisted	Pretreatment	0 (0.0000)	(0.0000, 0.3694)	--	--
	12 months	2 (0.2500)	(0.0319, 0.6509)	--	--
	24 months	4 (0.5000)	(0.1570, 0.8430)	--	--
	60 months	4 (0.5000)	(0.1570, 0.8430)	0 (0.0000, 0.0440)	0.0002
Standing with support	Pretreatment	0 (0.0000)	(0.0000, 0.3694)	--	--
	12 months	0 (0.0000)	(0.0000, 0.3694)	--	--
	24 months	0 (0.0000)	(0.0000, 0.3694)	--	--
	60 months	2 (0.2500)	(0.0319, 0.6509)	0 (0.0000, 0.0440)	0.0454
Walking with assistance	Pretreatment	0 (0.0000)	(0.0000, 0.3694)	--	--
	12 months	0 (0.0000)	(0.0000, 0.3694)	--	--
	24 months	0 (0.0000)	(0.0000, 0.3694)	--	--
	60 months	0 (0.0000)	(0.0000, 0.3694)	0 (0.0000, 0.0440)	N/A

Abbreviations: CI, confidence interval; ITT, Intent-to-Treat; N/A, not applicable

Secondary Efficacy Endpoints

PDMS-2

Eladocagene exuparvovec-treated patients showed increases in PDMS-2 total scores over time. Onset of improvement was observed as early as 3 months. There was a statistically significant change from baseline in LS means for PDMS-2 total score ($p<0.0001$compared to baseline) at 60 months post-eladocagene exuparvovec treatment. PDMS-2 total and subscale scores, when assessed by visit using a repeated measures mixed effects model that included a combination of fixed and random effects, were consistently statistically significant ($p\leq0.003$) for all models assessed.

Eladocagene exuparvovec-treated patients showed increases in mean PDMS-2 subscale scores from baseline to 60 months with the exception of reflexes (data captured for 3 patients only). The increases in mean PDMS-2 total and subscale scores relative to baseline were evident from as early as Month 3. Similar results were demonstrated at the 12- and 24-month time-points. Eladocagene exuparvovec-treated patients also demonstrated improvement of specific skills on the PDMS-2 subscales that represent additional evidence of clinical benefit and development toward more independent motor function, including sitting, rolling, grasping a rattle or cube, removing pegs, and placing cubes. These observations are detailed in the individual patient narratives.

AIMS

There was a statistically significant change from baseline in LS means for AIMS total score ($p<0.0001$) at 60 months post-eladocagene exuparvovec administration. Three patients achieved and maintained AIMS total score increases of 30 or more points Similar to PDMS-2 subscale scores, increases from baseline in all mean AIMS subscale scores were also observed at 60 months.

The increases in mean AIMS total and subscale scores were evident from as early as 3 months and were sustained throughout the study. AIMS subscale scores at 12 and 24 months post eladocagene exuparvovec administration showed a similar pattern to PDMS-2 subscale scores in that they increased from baseline and were consistent with the 60-month data.

Cognitive/Language Developmental Test: CDIIT

There was a statistically significant increase in change from baseline in LS mean CDIIT total test scores for eladocagene exuparvovec-treated patients at 60 months ($p<0.0001$). Increases from baseline in mean CDIIT subscale scores were also observed at 60 months. Increases from baseline in mean CDIIT total score and all subscales were also observed at 12 and 24 months. The increases in mean CDIIT total test and subscale scores were evident from as early as Month 6.

Eladocagene exuparvovec-treated patients also demonstrated specific skills on the CDIIT that represent additional evidence of clinical benefit and development toward independent motor function, such as grasping a food item or toy when not previously able. These details are described for each patient in the individual patient narratives provided. CDIIT subscale scores at 12 and 24 months post administration of eladocagene exuparvovec increased from baseline and were consistent with the 60-month subscale data. Change from baseline data for CDIIT total and subscale scores as assessed by fixed effect of visit, age (months) at gene therapy administration, and baseline CDIIT score are summarized.

Body Weight

Body weight increased for the majority of patients in the study. There was a statistically significant increase in mean body weight through 60 months ($p=0.0270$).

Neurologic Examination Findings

The majority of the patients had neurologic findings on examination pre-surgery. Following treatment with eladocagene exuparvovec, the number of patients with floppiness, OGC episodes, limb dystonia, and stimulus provoked- dystonia decreased during the first year. No subjects showed floppiness at 12 months after eladocagene exuparvovec administration, however, the number of patients evaluated at the 12-month time-point was small (N=8 at baseline decreased to N=2 patients at the 12-month time-point). In most cases, reductions in the number of patients with these neurologic findings were apparent as early as Month 1 following treatment with eladocagene exuparvovec. The two patients evaluated at Month 12 who reported OCG episodes had also reported these events at baseline, and 1 reported limb dystonia that had not been reported at baseline, and neither reported floppiness or stimulus-provoked dystonia.

Immunogenicity

As anticipated, there were no patients with a positive antibody titre at baseline. The presence of anti-AAV2 antibodies was detected from Month 2 following treatment with eladocagene exuparvovec. At Month 12, 2 out of the 6 patients who were evaluated had a positive anti-AAV2 antibody titre (OD value >0.5).

There was no correlation between anti-AAV2 antibody titre and efficacy as measured by changes in PDMS-2 total score. Change from baseline in PDMS-2 total scores was evaluated by a repeated measures analysis with anti-AAV2 OD values as a fixed effect. The anti-AAV2 OD value was not a statistically significant factor in the model, indicating that the magnitude of the change from baseline in PDMS-2 total scores was not associated with the anti-AAV2 OD values ($p=0.3843$) A Pearson correlation coefficient was calculated for these data. The overall correlation was -0.05758, indicating

no correlation between anti-AAV2 antibody titre and efficacy as measured by changes in PDMS-2 total score.

Pharmacodynamic Endpoints

Change from Baseline in Neurotransmitter Metabolites. The presence of neurotransmitter metabolites HVA (the metabolite of dopamine) and 5-HIAA (the metabolite of serotonin) was measured in CSF during the first year of follow-up. The concentration of HVA at Month 6 and Month 12 was increased compared with baseline. The concentration of 5-HIAA was slightly increased at Month 6, with no change from baseline at Month 12.

Figure 19 Summary Statistics for Neurotransmitter Metabolites by Time Point

Variable Statistic	BL (n=5)	CFB Month 6 (n=3)	CFB Month 12 (n=3)
HVA[a] (nmol/L)			
Mean (SD)	4.60 (2.27)	27.50 (5.41)	11.17 (7.59)
Median (Min, Max)	5.00 (2.50, 8.00)	29.00 (21.50, 32.00)	12.50 (3.00, 18.00)
5-HIAA[b]			
Mean (SD)	2.50 (0.00)	0.83 (1.44)	0.00 (0.00)
Median (Min, Max)	2.50 (2.50, 2.50)	0.00 (0.00, 2.50)	0.00 (0.00, 0.00)

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; BL, baseline; CFB, change from baseline; HVA, homovanillic acid; ITT, intent -to-treat; Max, maximum; Min, minimum; SD, standard deviation
[a] Reference ranges varied based on lab and included 176-955 nmol/L, 218-852 nmol/L, 478-895 nmol/L, or 233-929 nmol/L.
[b] Reference ranges varied based on lab and included 63-503 nmol/L, 66-338 nmol/L, 231-618 nmol/L, and 74-345 nmol/L.
Note: Not all patients had baseline and postbaseline data. The following patients had data at baseline and 6 months: 04, 05, and 07. The following subjects had data at baseline and 12 months: 04, 07, and 08.

An increase in mean putaminal-specific uptake on PET imaging was evident as early as Month 6 and further increased through Month 60. In a repeated measures analysis of PET imaging of putaminal-specific uptake with fixed effect terms for visit and age at gene therapy, age at the time of treatment was determined not to be statistically significant, indicating that the magnitude of the change in putaminal specific uptake was not associated with age (p=0.2516). The repeated measures analysis of putaminal-specific uptake by time point was statistically significant (p=0.0134), indicating that putaminal-specific uptake of 18F-DOPA increases over time. An increase in LS mean putaminal-specific uptake was evident as early as Month 6 and continued through Month 12 and Month 60

Ancillary analyses

Sleep Study

Two patients completed their sleep studies. Results from one of the patients, suggested abnormal results prior to surgery and normal results after administration of gene therapy. Results from the other patient were considered normal before surgery and after administration of gene therapy.

The sleep study was a small study with 2 patients, which demonstrated that treatment with gene therapy did not appear to have a detrimental effect on sleep pattern in those treated and may have a beneficial effect on subjects' sleep patterns. More data on sleep in these subjects before and after treatment would be needed before any firm conclusions can be drawn.

Tabulated Summary of main efficacy results

Table 11: Summary of efficacy for trial AADC/CU/1601

Title: Compassionate Use Treatment With AGIL-AADC In Patients With AADC Deficiency			
Study identifier	AADC-CU/1601		
Design	Study AADC-CU/1601 is a single-center study that summarized and analysed data from a single arm, compassionate use interventional study to evaluate the safety and efficacy of intraputaminal infusion of eladocagene exuparvovec (AGIL-AADC) gene therapy in children with AADC deficiency for a period of up to 60 months after study drug administration. Eladocagene exuparvovec gene therapy at a total dose of 1.8×10^{11} vg was administered during a single operative session. The planned duration of the study was 1 year, and patients returned voluntarily every 6 months to complete developmental tests to measure efficacy (Peabody Developmental Motor Scale [PDMS-2], Alberta Infant Motor Scale [AIMS], and Comprehensive Developmental Inventory for Infants and Toddlers [CDIIT]) and positron emission tomography (PET) imaging of putaminal-specific L-6-[18F] fluoro-3,4-dihydroxyphenalaynine (^{18}F-DOPA) uptake in the putamen, and capture adverse event (AE) reporting for up to 60 months. This study used a natural history control as the comparator for the primary efficacy endpoint.		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		12 months not applicable Follow-up for a total of 60 months
Treatments groups	Treated patients		Eladocagene exuparvovec, Total dose of 1.8×10^{11} vector genomes (vg), administered by intraputaminal injection during 1 operative session in 8 patients
Endpoints and definitions	Primary endpoint	Achievement of motor milestones at 60 months after admin-istration of eladocagene exuparvovec	Achievement of motor milestones as assessed by Peabody Developmental Motor Scale, second edition (PDMS-2) (full head control, sitting unassisted, standing with assistance, and walking with assistance) at the 60-month time-point.
	Secondary endpoint	PDMS-2 total and subscale scores	Achievement of motor milestones as assessed by PDMS-2 (full head control, sitting unassisted, standing with assistance, and walking with assistance) at the 12 and 24-month time-points.
	Secondary endpoint	Alberta Infant Motor Scale (AIMS) total and subscale scores	The AIMS is a 58-item observational measure that assesses sequential development of motor milestones. Patients received a numerical score that correlated with achievement of motor milestones. The total AIMS scores were calculated by adding the subscale scores.

	Secondary endpoint	Compre-hensive Develop-mental Inventory for Infants and Toddlers (CDIIT) whole and subtest scores	The CDIIT evaluates development in infants and toddlers in the domains of cognition, language, motor skills, social skills, and self-care skills. Patients received a numerical score that correlated developmental milestones. The whole CDIIT scores were calculated by adding the subtest scores.
	Secondary endpoint	Neurological examination findings	Neurological exam findings associated with AADC deficiency, including floppiness, oculogyric crisis (OCG) episodes, stimulus-provoked dystonia, and limb dystonia were evaluated throughout the study.
	Secondary endpoint	Change from baseline in body weight	Body weight was measured at baseline and throughout the study and the change from baseline was calculated.
	Secondary endpoint	Change in from baseline ^{18}F-DOPA positron emission tomography (PET) scan data	Expression and activity of the AADC enzyme in the putamen was assessed by PET imaging using ^{18}F-DOPA, a positron-emitting fluorine-labelled version of levodopa, which is a substrate for AADC that is incorporated into de novo dopamine.
	Secondary endpoint	Change from baseline in cerebrospinal fluid (CSF) neurotransmitter metabolites homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA)	CSF samples were collected at 6 and 12 months after eladocagene exuparvovec administration and evaluated for levels of HVA and 5-HIAA, which are metabolic products of dopamine and serotonin, respectively.
Database lock	07 August 2018		

Results and Analysis

Analysis description	**Primary Analysis Number of patients achieving key motor milestones at 24 & 60 months after administration of eladocagene exuparvovec**		
Analysis population and time point description	**Intent-to-treat (ITT) Population (all patients) 24 months & 60 months**		
Descriptive statistics and estimate variability	Treatment group	ITT Population At 24 months At 60 months	Natural History Cohort At 24 months At 60 months
	Number of subjects	N=8(24 months) N=8(60 months)	N=82

	Motor milestone: head control at 24 months & at 60 months (number of subjects achieving motor milestone)	N=4 N=4	N=0
	95% Confidence interval (CI)	(0.1570, 0.8430)	(0.0000, 0.0440)
	Motor milestone: sitting unassisted at 24 and at 60 months (number of subjects achieving motor milestone)	N=4 N=4	N=0
	95% CI	(0.1570, 0.8430)	(0.1570, 0.8430)
	Motor milestone: standing with support at 24 months and at 60 months (number of subjects achieving motor milestone) 95% CI at 24 months 95% CI at 60 months	N=0 N=2 (0.0000, 0.3694) (0.0000, 0.3694)	N=0 (0.0000, 0.0440)
	Motor milestone: walking with assistance at 24 & 60 months (number of subjects achieving motor milestone) 95% CI at 24 months 95% CI at 60 months	N=0 (0.0000, 0.3694) (0.0000,0.3694)	N=0 (0.0000, 0.0440)

Effect estimate per comparison	Primary endpoint (head control)	Comparison groups	ITT population vs natural history cohort
		P-value (one-sided exact test)	P=0.0002
	Primary endpoint (sit unassisted)	Comparison groups	ITT population vs natural history cohort
		P-value (one-sided exact test)	P=0.0002
	Primary endpoint (stand with support)	Comparison groups	ITT population vs natural history cohort
		P-value (one-sided exact test)	P=0.0454
	Primary endpoint (walk with assistance)	P-value (one-sided exact test)	n/a
Analysis description	**Secondary analysis:**		
Treatment group	ITT population		
Number of subjects	N=8		
	PDMS score-Least squares (LS) mean change from baseline at 24 months & 60 months years =93.7,31.1 95% CI (68.4, 118.9) (23.8, 38.4) **AIMS Total Score**- Least squares (LS) mean change from baseline at 24 months & 60 months= 23.0,31.7 95% CI (17.5, 28.6) (26.2, 37.2) **CDIIT Motor Total Score-**Least mean squares (LS) mean change from baseline at 24 months & 60 months years=8.3,17.9 95% CI (4.4, 12.1) (14.2, 21.5) **Neurological exam findings, OCG episodes at 12 months-**Number and proportion with limb dystonia at 24 months & 60 months= 0 (0.10, 0.48), (0.32, 0.76) **Mean change in body weight at 12 months n=7=**3.99kg (range -0.30,10.5) **Mean change from Baseline in Putaminal-specific uptake of F-DOPA PET, at 12 months (n=4) and 60 months (n=2)=** 0.29,0.54 95%CI (0.10, 0.48), (0.32, 0.76). **Mean change from baseline in neurotransmitter metabolites at 12 months(n=3)=**11.17 SD(7.59)		

2.6.5.3. Analysis performed across trials (pooled analyses and meta-analysis)

Integrated Efficacy Results

Primary Analysis: Acquisition of Key Motor Milestones

As of the cut-off date for this submission (27 March 2019), 8 patients from Study AADC-CU/1601 and 10 patients from Study AADC-010 contributed data to the primary endpoint, which was assessed at 24 months (2 years). The primary analysis evaluated the percentage of patients achieving the sequential motor milestones of full-head control, sitting unassisted, standing with support, and walking with assistance. These are consistent with the key motor milestones reported in the medical literature as clinically meaningful in children (Bertenthal 1998, WHO Multicenter Growth Reference Study (MGRS) Group 2006).

Patients from Study AADC-011 do not contribute data to the primary efficacy endpoint because that study is a 12-month study. The data cut-off date for ongoing studies that contributed to integrated analysis is 27 March 2019 (Study AADC-010). Treatment with eladocagene exuparvovec demonstrated clinical benefit in patients with AADC deficiency. At 24 months after gene therapy, 9 patients (50.0%) were able to master full-head control, 7 (38.9%) were able to sit unassisted, and 2 (11.1%) were able to stand with support; this is in contrast to no key motor milestone acquisition in the natural history control group. The achievements for head control and sitting unassisted were highly significant for patients treated with eladocagene exuparvovec gene therapy compared with the natural history control group (p value<0.0001 for both comparisons). These results are further supported by nonparametric analysis, which indicated a significant difference in the highest motor milestone achieved by each patient between eladocagene exuparvovec-treated patients compared with natural history controls (p value<0.0001).

Figure 20 Number and Proportion of Patients Achieving Key Motor Milestones a Month 24 (FAS)

Key Motor Milestone[a]	Summary of Milestone Acquisition[b] (N=18) n (%) [95% CI]	Natural History Control Proportion[c] n (%) [95% CI]	p value[d]
Head Control	9 (50.0) [0.2602, 0.7398]	0 [0.0000, 0.0440]	<0.0001[e]
Sitting Unassisted	7 (38.9) [0.1730, 0.6425]	0 [0.0000, 0.0440]	<0.0001[e]
Standing with Support	2 (11.1) [0.0138, 0.3471]	0 [0.0000, 0.0440]	0.1865
Walking with Assistance	0 [0.0000, 0.1853]	0 [0.0000, 0.0440]	0.555032

Abbreviations: FAS, full analysis set; PDMS-2, Peabody Developmental Motor Scale, Second Edition
[a] Based on results of the PDMS-2.
[b] Excludes AADC-011 patients who had only 12 months of follow-up. Data from the cut-off date of 27 March 2019 for patients from Study AADC-010.
[c] Wassenberg 2017
[d] One-sided p value for testing H0: proportion = historical control rate.
[e] Statistically significant

Acquisition of motor milestones was assessed 12 months following surgery. At 12 months post gene therapy, 5 patients in Study AADC-CU/1601 and Study AADC-010 had mastered head control and 3 patients could sit unassisted (purple shading indicates milestone achievement, grey indicates no milestone achievement, and yellow indicates emerging skills).

Importantly, in patients who achieved head control, key motor milestone acquisition was maintained, and in some patients additional milestones were achieved after the primary efficacy assessment at the 24-month time-point, as some patients achieved the ability to stand and walk with assistance. For example, between Months 24 and 48 follow-up time-point, several patients achieved new milestones:

- 3 patients obtained full-head control
- 2 patients achieved the ability to sit unassisted
- 1 patient was able to stand with support
- 1 patient achieved the ability to walk with assistance

From the Month 54 to Month 60 follow-up time-points, another patient developed the ability to stand with support.

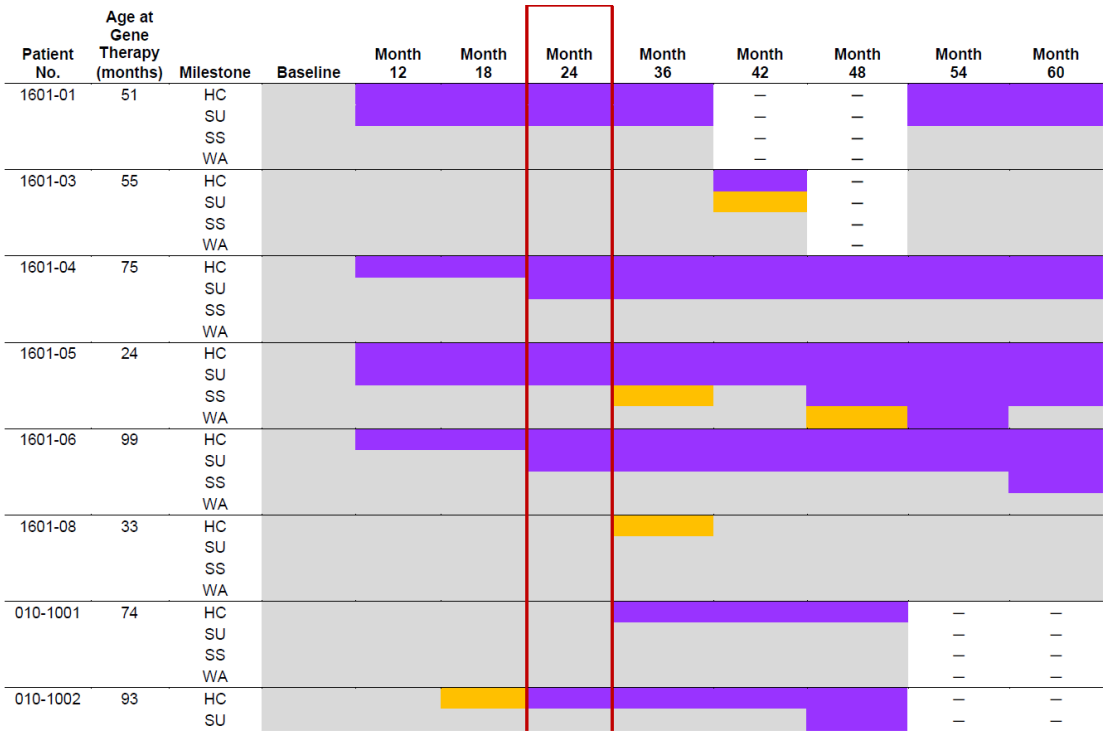
As described above attaining a motor milestone was defined as a patient obtaining a score of 2 on an individual item in PDMS-2. Achieving a score of 2 indicates "mastery" of the skill item and sets a "high bar" for evaluating treatment efficacy. The PDMS-2 also assesses emerging skills that indicate a child has demonstrated the development of a given function but has not achieved mastery; patients with emerging skills would have scored a 1.

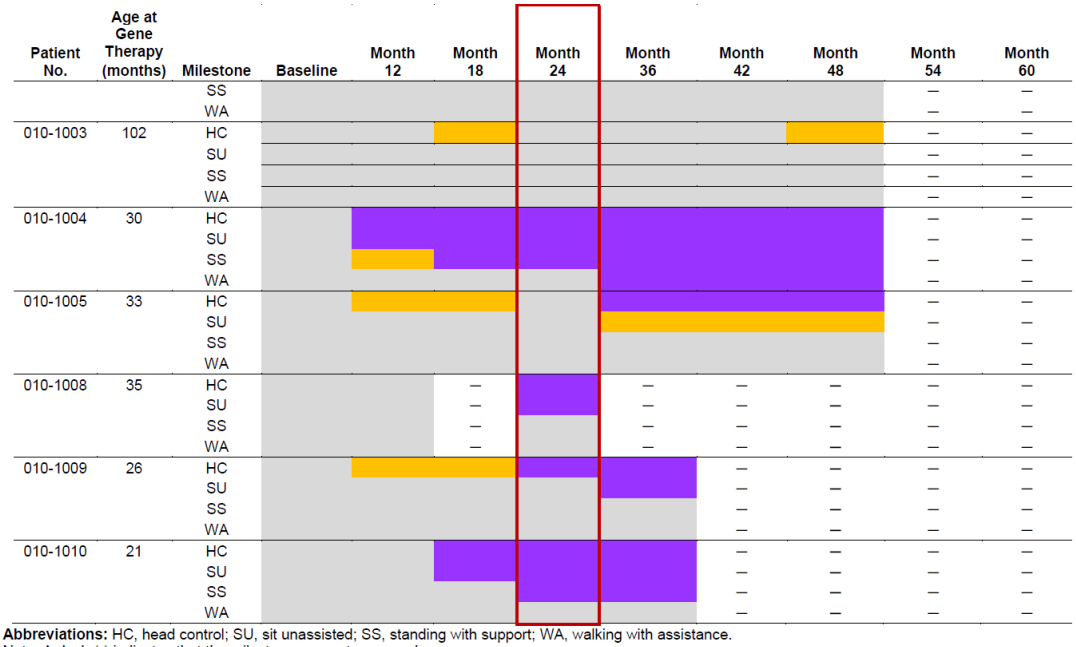
Eight patients in Studies AADC-CU/1601 and AADC-010 demonstrated emerging skills for a milestone at the time of data cut-off (27 March 2019) (yellow shading). For example, one patient (010-1003) scored 1 for head control (ISE Listing 3) at Months 18 and 48 and one patient (010-1005) scored 1 for sitting unassisted at Months 36, 42, and 48. Interestingly, exhibiting an emerging skill (achieving a score of 1) at a given time was often indicative of mastering a skill in the following month(s). Although not part of the primary endpoint analysis, some patients in Study AADC-011 did achieve motor milestones within the 1-year follow-up period. Of the patients in StudyAACD-011 that have been followed for 12 months (n=7), 4 patients developed full-head control within that time frame.

Sensitivity Analysis to Support Natural History Comparison

The primary analysis was a comparison against 82 patients with AADC deficiency described by Wassenberg as severe, having achieved "no or very limited developmental milestones". For the purposes of this analysis, we assumed that these patients achieved less than the study-defined PDMS-2-based milestones (see Section 1.4.2 for full details).A sensitivity analysis was performed and further supports the findings that eladocagene exuparvovec therapy results in meaningful clinical benefit in patients with AADC deficiency compared with the natural history control. The sensitivity analysis used a conservative approach that assumed the moderate patients in the natural history control group achieved all motor milestones. This analysis was consistent with the primary natural control analysis and found that a greater proportion of patients treated with eladocagene exuparvovec achieved full-head control compared with the natural history control (50% vs. 15%, respectively; p=0.0157). No difference was observed between groups for sitting unassisted, standing with support, or walking with assistance (p values ≥0.1217). Mild patients were not included in the sensitivity analysis as they were defined by Wassenberg, et al, as having mild delay in development of motor milestones, ambulatory without assistance, and mild intellectual abilities.

Figure 21 Acquisition and Maintenance of Key Motor Milestones up to Month 60 (Studies AADC-CU/1601 and AADC-010)





Abbreviations: HC, head control; SU, sit unassisted; SS, standing with support; WA, walking with assistance.
Note: A dash (-) indicates that the milestone was not assessed.
Note: Purple shading indicates the primary endpoint of mastery of a milestone (score of 2) was achieved. Yellow indicates having an emerging skill (score of 1). Grey shading indicates the milestone was not achieved (score of 0) or the milestone was not evaluated because the previous milestone was not achieved.
Note: Study AADC-CU/1601 collected data up to 60 months. Study AADC-010 collected data up to 48 months and is still ongoing

Eight patients from Study AADC-CU/1601 were evaluable at the 60-month time-point. At that time, 4 patients (50%) had head control and could sit unassisted and 2 patients (25%) could stand with support. These collective results demonstrate continued improvement of motor milestone acquisition over a longer follow-up period. Additional information about persistence of efficacy beyond the 24-month time-point can be found in below.

Secondary Endpoints

PDMS-2 Total Scores and Subscale Scores Over Time

All patients treated with eladocagene exuparvovec showed clinically meaningful increases in mean PDMS-2 total scores over time, with some as early as 3 months. At the 24-month time-point, the LS mean of change from baseline in PDMS-2 total scores (94.3, 95% CI 75.6, 112.9) was significant (p value<0.0001). Consistent with the PDMS-2 total score results at Month 24, the LS mean change from baseline at Month 12 (63.3, 95% CI 45.4, 81.1) and Month 60 (113.1, 95% CI 90.0, 136.1) were also clinically meaningful. Increases LS means for PDMS-2 subscale scores were observed, with positive changes observed at 24 months post therapy.

Figure 22 Summary of Endpoints available at 3 time points for each study

Endpoints	AADC-CU/1601			AADC-010			AADC-011		
	12 m	24 m	60 m	12 m	24 m	60 m	12 m	24 m	60 m
PDMS-2	X	X	X	X	X	NC	X	NC	NC
AIMS	X	X	X	X	X	NC	X	NC	NC
CDIIT	X	X	X	NC	NC	NC	NC	NC	NC
Bayley-III	NC	NC	NC	X	X	NC	X	NC	NC

Abbreviations: AADC, aromatic L-amino acid decarboxylase; AIMS, Alberta Infant Motor Scale; CDIIT, Comprehensive Development Inventory of Infants and Toddlers; NC, not collected; PDMS-2, Peabody Developmental Motor Scale, second edition, X, collected
Note: The CDIIT was only administered in Study AADC-CU/1601. The Bayley-III was administered in Studies AADC-010 and -011, with data available up to 24 and 12 months after gene therapy, respectively.

Figure 23 LS Mean Change from Baseline in PDMS-2 Subscale Score by Timepoint

PDMS-2 Score, LS mean (SE)	Timepoint		
Subscale	**12 Months**	**24 Months**	**60 Months**
Total Score	63.3 (8.70)	94.3 (9.18)	113.1 (11.55)
Grasping	13.8 (2.04)	20.6 (2.21)	23.6 (2.99)
Locomotion	16.8 (2.98)	24.6 (3.15)	32.8 (3.97)
Object Manipulation	0.4 (0.41)	1.2 (0.47)	3.2 (0.85)
Reflexes	2.0 (0.50)	NA	NA
Stationary	14.0 (1.69)	20.2 (1.79)	25.0 (2.29)
Visual-Motor Integration	17.9 (2.62)	27.6 (2.80)	29.6 (3.65)

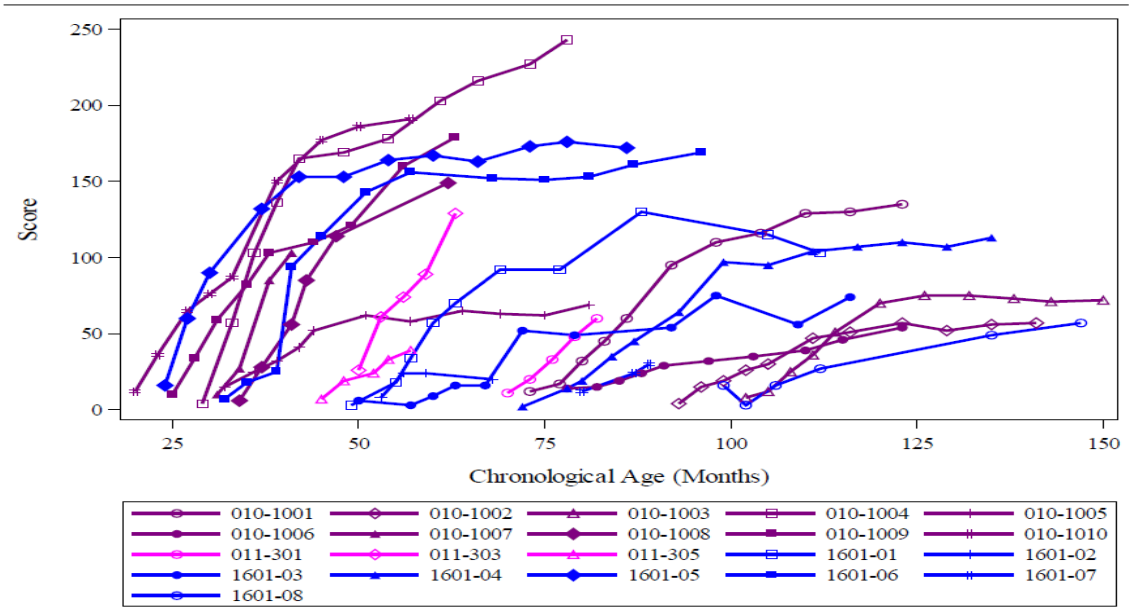
Abbreviations: LS, least square, NA, not assessed; PDMS-2, Peabody Development Motor Scale, second edition; SE, standard error.

Figure 24 LS Mean Change from Baseline in AIMS Subscale Score by Timepoint

AIMS Score, LS mean (SE)	Timepoint		
Subscale	**12 Months**	**24 Months**	**60 Months**
Total score	15.6 (2.65)	21.9 (2.79)	29.3 (3.80)
Prone	6.1 (1.20)	8.5 (1.27)	11.9 (1.77)
Sit	3.6 (0.66)	6.2 (0.73)	8.4 (1.19)
Stand	1.2 (0.62)	1.9 (0.67)	2.6 (0.96)
Supine	5.2 (0.57)	5.8 (0.61)	6.8 (0.91)

Abbreviations: AIMS, Alberta Infant Motor Scale; LS, least square; SE, standard error.

Figure 25 Mean PDMS-2 Total Scores by Patient and Chronological Age – Through Month 60 (FAS)

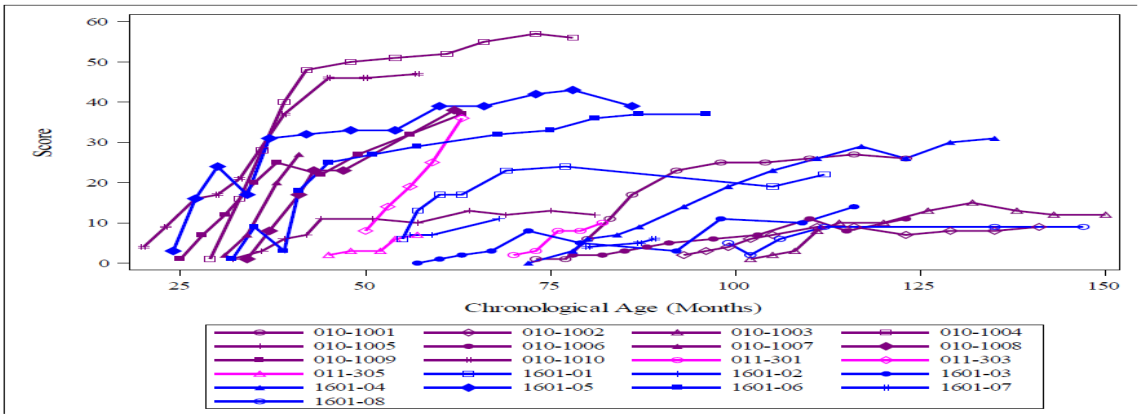


Abbreviations: FAS, full analysis set; PDMS-2, Peabody Developmental Motor Scale, 2nd edition

At the 24-month time-point, the LS mean of change from baseline in AIMS total scores (21.9, 95% CI 16.1, 27.6) was statistically significant (p<0.0001) compared with baseline scores. Of the 14 patients with evaluable AIMS data at the 24-month time-point, all patients showed increases in AIMS total score of 5 points or greater, which was designated as a meaningful change by the study investigator (ISE, Listing 5). Consistent with the AIMS total score results at Month 24, the LS mean change from baseline at Month 12 (15.6, 95% CI 10.1, 21.2) and Month 60 (29.3, 95% CI 21.7, 36.9) were also clinically meaningful, and continued improvement was observed with time.

The AIMS subscale scores evaluated at 12- and 24-months post eladocagene exuparvovec administration showed a similar pattern to PDMS-2 subscale scores in that they increased from baseline and were consistent with the 60-month data. LS Mean AIMS subscale scores at 24 months

Figure 26 Mean AIMS Total Scores by Patient and Chronological Age (FAS Population)



Abbreviations: AIMS, Alberta Infant Motor Scale; FAS, full analysis set

Neurologic Examination Findings

Eladocagene exuparvovec therapy was associated with improvement in neurologic performance, including reduction in number of patients with floppiness, OGC episodes, dystonia, and stimulus-provoked dystonia. Most patients had neurologic examination findings at baseline, with floppiness (13 patients [68.4%]), OGC episodes (15 patients [78.9%]), limb dystonia (15 patients [78.9%]), and stimulus-provoked dystonia (7 patients [36.8%]) present. Overall, the proportion of patients with neurologic exam findings decreased upon treatment. The most marked improvements were seen in floppiness and dystonias, with no events of stimulus-provoked dystonia observed from evaluable patients after Month 3.

Muscle Power and DTR Response

Muscle power was assessed using a 6-point scale in 4 muscle groups (muscle power group I [right upper extremity], II [left upper extremity], III [right lower extremity], and IV [left lower extremity]). Patients were assigned scores that ranged from 1, indicating no movement, to 6, indicating normal movement. No patients demonstrated the ability of a muscle group to overcome resistance of the examiner (normal muscle strength, response 6) for any of the muscle power groups at 1 month after eladocagene exuparvovec administration. Twelve months after eladocagene exuparvovec administration, some patients developed normal strength in upper and lower extremities (1 patient [4.8%] in muscle power group I, 2 patients [7.7%] in muscle power groups II and III, and 3 patients [11.5%] in muscle power group IV). At 12 months post gene therapy, no patient had response <3 in any of the muscle groups evaluated, indicating they all had developed some ability to use muscle groups in their arm or leg. On average, over the 12-month period following eladocagene exuparvovec administration, patients also developed the ability to move the upper arm against some resistance from the examiner (response 5) (7 patients [26.9%] for muscle power I and II). Similarly, 6 patients (23.1%, for muscle power III) and 7 patients (26.9%, for muscle power IV) developed the ability to move a lower extremity against some resistance from the examiner.

Figure 27 Summary statistics for muscle power are presented below.

Evaluation (Muscle power group)	Timepoint	Response n (%)					
		1	2	3	4	5	6
I	Month 1	0	3 (11.5)	3 (11.5)	2 (7.7)	0	0
	Month 12	0	0	0	4 (15.4)	7 (26.9)	1 (3.9)
II	Month 1	0	3 (11.5)	3 (11.5)	2 (7.7)	0	0
	Month 12	0	0	1 (3.9)	2 (7.7)	7 (26.9)	2 (7.7)
III	Month 1	1 (3.9)	2 (7.7)	2 (7.7)	3 (11.5)	0	0
	Month 12	0	0	0	4 (15.4)	6 (23.1)	2 (7.7)
IV	Month 1	1 (3.9)	2 (7.7)	2 (7.7)	3 (11.5)	0	0
	Month 12	0	0	1 (3.9)	1 (3.9)	7 (26.9)	3 (11.5)

Abbreviations: FAS, full analysis set

Improvement in Body Weight

Increase in body weight is a positive indicator for AADC-deficient patients, who typically exhibit feeding and swallowing problems as well as gastrointestinal problems throughout life, which may contribute to

nutritional absorption deficiencies and low body weight. Bodyweight increased from baseline for the majority of patients at Month 12.

Cognitive Development

Cognitive development was evaluated using the CDIIT in Study AADC-CU/1601 and the Bayley-III instrument in Studies AADC-010 and AADC-011. Across all 3 studies, eladocagene exuparvovec was associated with clinically meaningful improvement compared with natural history control in cognitive function.

Measures of Neurotransmitter Metabolite Activity

Neurochemistry measurements of HVA and 5-HIAA in CSF, as downstream metabolites of dopamine and serotonin production, respectively, are objective measurements of de novo neurotransmitter production in the brain and support that the clinical benefit patients obtained in motor function over time. At baseline, the concentrations of both metabolites were near or below the lower limit of quantitation in patients with AADC deficiency, indicating little or no AADC enzyme activity. The CSF concentration of HVA at 12 months after treatment was increased from baseline. Few patients showed increased CSF concentrations of 5-HIAA at 12 months, which is not unexpected given the targeted region of the brain (putamen) does not contain serotonergic neurons.

Putaminal-Specific Uptake

Measurement of 18F-DOPA uptake in the putamen via PET imaging following treatment demonstrated that eladocagene exuparvovec therapy is associated with de novo dopamine synthesis in the brain and that dopamine production is maintained over extended periods of time.

Most patients in the eladocagene exuparvovec clinical trials demonstrated generally continuous increases in putaminal-specific uptake over time. An increase was evident as early as 6 months after treatment (4 patients evaluated; mean change from baseline [SD] 0.25 [0.38]), was further increased at 12 months (16 patients, mean change from baseline 0.27[0.25]) and 24 months (8 patients, mean change from baseline 0.47 [0.22]), and sustained up to 60 months (2 patients, mean change from baseline 0.42 [0.09]).At the 24-month time-point (n=8), the LS mean 18F-DOPA-specific uptake was 0.7, 95% CI 0.5, 0.8; at the 60-month time-point (n=2), the LS mean 18F-DOPA -specific uptake was 0.6, 95% CI 0.3, 0.8.

Comparison of Results in Subpopulations

Analyses for the primary endpoint (motor milestone acquisition at Month 24) and select secondary endpoints (PDMS-2 and AIMS total scores) were conducted in the FAS population to determine whether a particular subgroup within the study population of patients with AADC deficiency received preferential treatment benefit than the overall study population. Subpopulations of gender and age at eladocagene exuparvovec administration were analysed in patients from both dose groups. Results presented in this study include patients who received the 1.8×10^{11} vg dose.

Subgroup Analysis by Gender

Primary Analysis: Acquisition of Key Motor Milestones

Clinically meaningful improvement in head control, sitting unassisted, and standing with support was seen in all patients who received gene therapy, independent of gender.

Secondary Endpoints

As previously described, clinically meaningful improvements in PDMS-2 and AIMS were observed in all eladocagene exuparvovec-treated patients. Analysis of PDMS-2 total scores at Month 24 by gender showed similar mean increases from baseline in males (102.4, 95% CI 31.6, 173.2) and females (96.7, 95% CI 59.1, 134.2). Similarly, the mean increase from baseline in AIMS scores at Month 24 was the same in males (24.4, 95% CI 2.6, 46.2) and females (23.9, 95% CI 11.7, 36.0).

Subgroup Analysis by Age

Primary Analysis: Acquisition of Key Motor Milestones

Clinically meaningful improvement in head control, sitting unassisted, and standing with support was seen in all patients who received gene therapy, independent of age.

Figure 28 Key Motor Milestone Acquisition at Month 24 by Age at Time of Gene Therapy -

(FAS Population)

Subgroup	Head Control[a]	Sitting Unassisted[a]	Standing with Support[a]	Walking with Assistance[a]
<2 Years				
Proportion (n/m)[b]	1/1 (1.0000)	1/1 (1.0000)	1/1 (1.0000)	0/1 (0.0000)
2 to <6 Years				
Proportion (n/m)[b]	6/8 (0.7500)	5/8 (0.6250)	1/8 (0.1250)	0/8 (0.0000)
6 to <12 Years				
Proportion (n/m)[b]	2/5 (0.4000)	1/5 (0.2000)	0/5 (0.0000)	0/5 (0.0000)

Abbreviations: FAS, full analysis set; m, denominator
[a] Based on results of the PDMS-2.
[b] The denominators (m) are the number of FAS patients in the respective subgroup that were evaluable at Month 24.

Secondary Endpoints

When comparing age at time of treatment, only 1 patient received eladocagene exuparvovec treatment at <2 years of age. However, analysis of the 2 to <6-year age group compared with the 6 to <12-year age group suggests that although clinically meaningful improvements were observed in both subgroups, earlier administration may be more advantageous. The mean change from baseline in the PDMS-2 total score at Month 24 was almost double in the 2 to <6 year age group (111.1, 95% CI 70.1, 152.1) than what was observed in the 6 to <12 year age group (65.6, 95% CI 25.1, 106.1) although these findings are limited by the small sample size and high variability. Similar results were observed with AIMS, where the mean change from baseline at Month 24 was 30.0 (95% CI 15.8, 44.2) in the 2 to <6-year age group and 13.4 (95% CI 3.4, 23.4) in the 6 to <12-year age group.

Analysis of clinical information relevant to dosing recommendations

Analysis of the 2.4×1011 vg Dose

In Studies AADC-010 and AADC-011, a dilution step was required to obtain the desired dose of 1.8×10^{11} vg. In Study AADC-011, patients younger than 3 years received undiluted vector (2.4×10^{11} vg, 5 patients). The dilution step was removed for logistical purposes. The removal of the dilution step did not cause an increase in dose that is expected to be clinically different than the 1.8×10^{11} dose. The

increase in dose after removal of the dilution step is approximately 30%, which is not a meaningful increase in a gene therapy product, where dose changes usually require log increases.

Overall, both doses of eladocagene exuparvovec provided clinical benefit to AADC-deficient patients. Improvements in patients who received the 2.4×10^{11} vg dose were similar to those patients of the same age who received the 1.8×10^{11} vg dose.

Efficacy at the 2.4×1011 vg Dose

Efficacy results for the 1.8×10^{11} and 2.4×10^{11} vg groups are similar, indicating that the dose change did not affect the efficacy of gene therapy. The proportion of patients who achieved motor milestones in the 2.4×10^{11} vg dose group was similar to the 1.8×10^{11} dose group.

Figure 29 Key Motor Milestone Acquisition at Month 12 at Time of Gene Therapy –

2.4×10^{11} vg Dose (ITT Population)

Key Motor Milestone[a]	ITT Population No. (%)	95% CI for Proportion
Head Control	3/5 (75.0)	(0.1941, 0.9937)
Sitting Unassisted	1/5 (25.0)	(0.0063, 0.8059)
Standing with Support	0/5	(0.0000, 0.6024)
Walking with Assistance	0/5	(0.0000, 0.6024)

Abbreviations: ITT, intent-to-treat
[a] Based on results of the PDMS-2.

In patients in the 2.4×10^{11} vg dose group, increases in both PDMS-2 and AIMS total scores (signifying improvement) were observed at the first assessment at 3 months post-infusion and scores continued to improve with each 3-month assessment. Improvements were similar to those observed in patients of similar age in the 1.8×10^{11} vg dose group.

Subgroup Analysis at the 2.4×10^{11} vg Dose

Although the number of patients in each age and gender subgroup who received 2.4×10^{11} vg eladocagene exuparvovec was too small to allow conclusions to be drawn, key motor milestone acquisition results appeared similar to those observed in the 1.8×10^{11} vg dose group.

No statistical analyses were performed on the 2.4×10^{11} vg dose group due to the small number of patients (n=4 for patients with 12-month data).

Persistence of efficacy and/or tolerance effects

Importantly, key motor milestone acquisition continued beyond the primary efficacy assessment at the 24-month time point, with a total of 10 patients (62.5%) achieving head control, 7 patients (43.8%) sitting unassisted, 2 patients (12.5%) standing with support, and 1 patient (6.25%) walking with assistance at the 36-month time point. Also, an additional patient was able to sit unassisted at Month 48 (patient 1002 from Study AADC-010), and one patient, who had achieved the 3 motor milestones of head control, sitting unassisted, and standing with support by Month 18, was able to walk with assistance at Month 36 (patient 1004 from Study AADC-010). These data support the persistence of efficacy for eladocagene exuparvovec, as acquisition of new motor milestones is dependent on the mastery and maintenance of previous milestones. Motor milestone acquisition not only continues throughout the ongoing follow-up, it is durable. At Month 24, 10 patients achieved head control and 7

patients achieved sitting unassisted; all milestones were sustained by each patient at each visit after the 24-month time point. To date, no eladocagene exuparvovec-treated patients have achieved milestones and subsequently lost the abilities they achieved.

Further to this, continued improvement of motor milestone acquisition has been observed over a 60-month (5 year) follow-up period. Of the 8 evaluable patients at the 60-monthtimepoint, 4 patients (50%) had achieved head control and sitting unassisted and 2 patients (25%) could stand with support.

2.6.5.4. Supportive study(ies)

Study AADC-011

The objective of this Phase 2b, open-label prospective study was to evaluate the safety and efficacy of intraputaminal infusion of eladocagene exuparvovec in children with AADC deficiency for a period of up to 1 year after study drug administration in order to:

- give those patients who were not enrolled in the Phase 1/2 trial (i.e. AADC-010) an opportunity for treatment
- increase experience in gene therapy for AADC deficiency
- increase the dosage slightly in patients younger than 3 years of age

Methods

Study participants

Eight (8) patients were enrolled in the study and received eladocagene exuparvovec infusion at the time of data cut-off (27 March 2019). All subjects were recruited at a single hospital centre in Taiwan, which specialises in the treatment of children with AADC deficiency.

Results

Disposition of Patients

Three (3) patients (37.5%) were enrolled in the 1.8×10^{11}vg dose group and 5 patients (62.5%) were enrolled in the 2.4×10^{11}vg dose group, for a total of 8 patients. One patient (Patient 302) was considered a screen failure due to the occurrence of an SAE of bronchopneumonia. This patient was subsequently rescreened and enrolled into the study as Patient 305.

Figure 30 Patient Completion Data

		Low Dose (N=3)[a]	High Dose (N=5)[a]	All Patients (N=8)[a]
Enrolled [b]				8
Completed		3 (100.0%)	4 (80.0%)	7 (87.5%)
Ongoing		0 (0.0%)	1 (20.0%)	1 (12.5%)
Duration of Follow Up (Months)	Mean (SD)	12.3 (0.19)	11.0 (2.78)	11.5 (2.21)
	Median	12.3	12.0	12.1
	Min, Max	(12.2, 12.6)	(6.1, 12.9)	(6.1, 12.9)

Abbreviations: SAE, serious adverse event, Max, maximum; Min, minimum; SD, standard deviation
[a] Percentages will be based on the number of patients in the database for the particular dose group and overall.
[b] One patient (302) was considered a screen failure due to the occurrence of an SAE. The same patient was subsequently rescreened and enrolled into the study as Patient 305.

Seven (7) of the 8 patients (87.5%) completed the study through Month 12. One patient (Patient 309, $2.4x10^{11}$ vg group) remains in follow-up, having completed 6 months of follow up at the time of data cut off. The mean (standard deviation [SD]) duration of follow-up was 11.5 (2.21) months.

Demographics

For all patients, the median age at baseline was 28.5 months (range 21.0 to 70.0 months). The median age at diagnosis was 11.0 months (range 1.0 to 28.0 months). Five (5) patients (62.5%) were male and 3 patients (37.5%) were female. All patients were Asian (6 patients were Asian-Chinese, and 2 patients were Asian-Other) and all patients carried the founder mutation (3 patients were homozygous and 5 patients were heterozygous). A listing of demographics by patient is provided.

Figure 31 Demographics and Baseline Data Summary Statistics (Safety Population)

Variable	Category	$1.8 x10^{11}$vg Dose (N=3)	$2.4 x10^{11}$vg Dose (N=5)	All Patients (N=8)
Age at Baseline (months)	Mean (SD)	55.00 (13.23)	24.80 (3.70)	36.13 (17.38)
	Median (min, max)	50.00 (45.0, 70.0)	24.00 (21.0, 30.0)	28.50 (21.0, 70.0)
Age at Diagnosis (months)	Mean (SD)	15.33 (10.97)	13.40 (9.81)	14.13 (9.51)
	Median (min, max)	9.00 (9.0, 28.0)	11.00 (1.0, 28.0)	11.00 (1.0, 28.0)
Gender	Male	2 (66.7%)	3 (60.0%)	5 (62.5%)
	Female	1 (33.3%)	2 (40.0%)	3 (37.5%)
Race	Asian-Chinese	2 (66.7%)	4 (80.0%)	6 (75.0%)
	Asian-Others	1(33.3%)	1 (20.0%)	2 (25.0%)
	Black	0 (0.0%)	0 (0.0%)	0 (0.0%)
	White	0 (0.0%)	0 (0.0%)	0 (0.0%)
Genotype	Homozygous Founder Mutation	1 (33.3%)	2 (40.0%)	3 (37.5%)
	Heterozygous Founder Mutation	2 (66.7%)	3 (60.0%)	5 (62.5%)
PDMS-2 total score at baseline	Mean (SD)	14.67 (10.02)	12.40 (3.36)	13.25 (6.04)
	Median (min, max)	11.00 (7.0, 26.0)	12.00 (8.0, 17.0)	11.50 (7.0, 26.0)
AIMS total score at baseline	Mean (SD)	4.00 (3.46)	1.80 (0.84)	2.63 (2.26)
	Median (min, max)	2.00 (2.0, 8.0)	2.00 (1.0, 3.0)	2.00 (1.0, 8.0)

Abbreviations: AIMS, Alberta Infant Motor Scale; Max, maximum; Min, minimum; PDMS-2, Peabody Developmental Motor Scale, second edition; SD, standard deviation

Prior and Concomitant Medications

Of note, use of dopaminergic agents for AADC deficiency was recorded for 6 patients, 75.0%. In all 6 patients, dopaminergic agents were taken prior to eladocagene exuparvovec infusion as standard of care for AADC and were not continued after the surgical procedure. Two patients continued taking Neupro for up to 3 months after the infusion.

Outcomes and estimation

Figure 32 Number and Proportion of Patients Achieving Key Motor Milestones

Motor Milestone[a]	$1.8x10^{11}$vg Dose (N=3)		$2.4x10^{11}$vg Dose (N=5)[b]	
	N (%)	95% CI	N (%)	95% CI
Head Control	1 (0.3333)	(0.0084, 0.9057)	3 (0.7500)	(0.1941, 0.9937)
Sitting Unassisted	0 (0.0000)	(0.0000, 0.7076)	1 (0.2500)	(0.0063, 0.8059)
Standing with Support	0 (0.0000)	(0.0000, 0.7076)	0 (0.0000)	(0.0000, 0.6024)
Walking with Assistance	0 (0.0000)	(0.0000, 0.7076)	0 (0.0000)	(0.0000, 0.6024)

Abbreviations: CI, confidence interval; PDMS-2, Peabody Developmental Motor Scales-Second Edition; vg, vector genome

[a] Based-on results of PDMS-2

[b] One patient had not reached the Month 12 visit and is not included in this summary.

Secondary Efficacy Endpoints

PDMS-2

All patients showed improvement in motor skills and developmental milestones as assessed by the PDMS-2 and shown for the PDMS-2 total score for each patient. An increase in total score (signifying improvement) was observed at the first assessment at 3 months post-infusion and scores continued to improve with each 3-month assessment.

AIMS

An increase in AIMS total score (signifying improvement) was observed at the first assessment at 3 months post-infusion and scores continued to improve with each 3-month assessment. Change from baseline data for AIMS total and subscale scores as assessed by fixed effect of visit, age (months) at gene therapy administration. Increases from baseline in all mean AIMS subscale scores were observed at Month 12.

Cognitive Language Development: Bayley-III

All patients showed improvement in cognitive, language, and expressive communication as assessed by the Bayley-III scales. An increase in total score (signifying improvement) was usually observed at the first assessment at 3 months post-infusion and scores continued to improve with each 3-month assessment. The increase from baseline in mean Bayley-III total score was statistically significant at Month 12 based on a repeated measures analysis that tested fixed effects. Increases from baseline in mean Bayley-III subscale scores were also observed at Month 12, with the largest improvements seen in the cognitive domain.

Body Weight

Body weight increased from baseline to Month 12 in both dose groups, with a transient decrease observed between Month 3 and Month 6 in the 1.8x1011vg dose group. Mean body weight increased by an average of 1.77 kg from baseline to Month 12 for patients in the 1.8x1011vg dose group and an average of 3.20 kg for patients in the 2.4x1011vg dose group

Neurologic Examination Findings

Following treatment with eladocagene exuparvovec, the number of patients with floppiness, OGC episodes, and limb dystonia decreased from baseline to Month 12. Immunogenicity

Anti-AAV2 antibody data

At 3 months post infusion was reported for 5 patients, 3 patients receiving 1.8×1011 vg and 2 patients receiving 2.4×1011 vg. The presence of anti-AAV2 antibodies was detected in all 5 patients at Month

3. At Month 6, data reported for 4 patients showed that antibody titres were generally decreasing. Only1patient had an evaluation at Month 9; their antibody titre remained stable from Month 6

Pharmacodynamic Endpoints

Change from Baseline in Neurotransmitter Metabolites The presence of the dopamine metabolite HVA was measured in CSF. For patients receiving a dose of 1.8×1011 vg, the concentration of HVA at Month 12 was approximately double the concentration at baseline. Individual patient data shows that HVA concentrations increased from baseline to Month 12 for all patients. For 2 of the 3 patients in the 1.8x1011vg dose group, a decrease in 5-HIAA levels was observed from baseline to Month 12, with the remaining patient in this group showing no change in 5-HIAA levels.

Putaminal-specific Uptake by PET Imaging

For patients receiving a dose of 1.8×1011 vg, mean putaminal-specific uptake on PET imaging was relatively unchanged during the study.

Figure 33

	Baseline n=3	Change from Baseline at Month 12 n=3
Mean (SD)	0.52 (0.05)	-0.03 (0.07)
Median (Min, Max)	0.54 (0.46, 0.55)	-0.06 (-0.08, 0.04)

Abbreviations: Max, maximum; Min, minimum; PET, positron emission tomography; SD, standard deviation; vg, vector genome

2.6.6. Discussion on clinical efficacy

Design and conduct of clinical studies

The aim of the therapy is to increase dopamine product in this area of the brain to improve motor development. Significant gross motor milestone at this age (from 0 to 2 years) include, head control, sitting unassisted, standing and walking. Achievement of any one of these milestones assessed by a standardised method (as described in the assessment of validated PDMS-2 scale) meets the primary efficacy endpoint. Secondary endpoints used include movement scales PDMS-2 & AIMS scores which are validated early motor development scales designed to assess gross and fine motor skills in children from birth to 5 years Peabody Developmental Motor Scale(PDMS-2) & 0-18 months or independent walking for the Alberta Infant Motor Scale (AIMS). Neither scale is validated for use in AADC but there are no paediatric scores validated to assess motor development in this population.

These endpoints & study design were the subject of scientific advice and paediatric investigation plan.

The applicant has not performed a dose finding study. It is accepted that a dose finding study in such an ultra-rare condition may not be possible. Due to the rare & severe nature of the disease, neurosurgical application of this active and single administration, the applicant wanted to administer a therapeutic dose in the phase 1/CU(compassionate use) trial. The applicant has based the dose for the phase 1 trial on preclinical studies with a similar active in non-human primates and on previous studies in human adults. This is in line with the Guideline on the quality, preclinical and clinical requirements of gene therapy medicinal products EMA/CAT/80183/2019. Therefore, the dose finding study and phase 1 clinical trial are performed as a single study.

The applicant based the dose for the phase 1 study on a proof of concept study in non-human primates where doses from 6×10 to 5×1011 vg were administered and in whom enzyme saturation occurred at a dose of 1.7×1011 vg. The dose was also based on data from human studies using a similar vector

for Parkinson's disease. Doses of an rAAV2 vector of up to 3x1011 vg via bilateral putamen dosing have been used in Parkinson's disease studies. The target population and disease pathology are different in subjects with Parkinson's disease. However, the studies do give information about the mode & volume of administration & the potential safety risks of the active. Based on the relative average mass of a child brain (1000 g) being approximately 60% that of an adult brain (1350 g), and using brain weight scaling to a child brain, the dose of 1.8×1011vg of eladocagene exuparvovec used in the AADC clinical trials in children is equal to approximately 60% of the highest dose used in Parkinson's disease clinical trials in adults, which again was well tolerated.

Response was observed in patients treated at the dose for the phase 1/CU trial, so this dose was chosen for the subsequent clinical development. Whilst dose finding and dose confirmation is not optimal for this active, the rationale for the chosen dose based on the POC study and data in human adults is accepted.

The applicant did treat 5 subjects with a higher dose in study AADC-011. The justification for this was the removal of a dilution step. The response in the group treated at the higher dose appears to be similar to that in subjects treated at the lower dose.

Of note, the dose finding study was performed with drug manufactured with process A and not the commercially manufactured process. There is no safety or efficacy data available for the commercially manufactured product & comparability has not been established against process A product to confirm dose equivalence of the commercial product. Differences in the manufacturing process are outlined in the quality sections of the report.

At the time of original submission, efficacy results were presented for 3 studies AADC-CU/1601, AADC-010 & AADC011. One study is completed AADC-CU/1601. Two of the studies are ongoing AAD-010 & AADC-011. Data from 21 patients treated at the proposed therapeutic dose was included in the original submission. A further 5 subjects were treated at a higher (30%) dose.

Updated efficacy and safety data was submitted throughout the two year procedure. Efficacy Data from an additional two subjects was included in the applicant's responses during the procedure therefore data on 28 subjects is presented in the updated integrated efficacy analysis at the time of marketing authorisation decision.

The database is small but this is a very rare orphan condition. Two of the studies are ongoing but data for up to 5 years is available for some subjects.

The population studied were children aged between 18 months and 8 years and 6 months. There were no adults or adolescents treated in the clinical trials. Only children with a severe phenotype were included in the clinical trials and there is no data to support a benefit in children with a mild or moderate phenotype who may achieve motor milestones spontaneously or respond to treatment with current standard of care.

All clinical studies were open label single-arm studies, using external historical control data as control.

There have been no GCP inspections conducted at the site. Each study report contains a statement that the study was performed in compliance with Good Clinical Practices including the archiving of essential documents. All studies were conducted at a single site outside Europe i.e. in Taiwan where the founder mutation for AADC was described & where the disease is more prevalent and there is expertise in its diagnosis and management.

A request for GCP inspection was made at the time of submission of the MAA. However due to the risk of Covid19 in the area, onsite GCP inspections in the affected areas could not be requested at this time. An onsite inspection was not considered possible due to the visa and quarantine restrictions. It

was considered that a virtual inspection may not be adequate to review the patient documentation at the trial site.

There is no experience from the CT programme on treating European patients with this product.

The historical control cohort used to contextualise the clinical trials results was based on the published literature review of all available reported AADC deficiency cases, which was performed by Wassenberg et al (Wassenberg 2017). This review identified 117 confirmed AADC deficiency cases, of which 103 had sufficient information to adjudicate severity. From these 103 cases, a total of 82 AADC-deficient patients were considered as having a severe phenotype, which was comparable to the patients enrolled in the 3 eladocagene exuparvovec clinical studies and therefore was utilized as the natural history control (Wassenberg 2017).

The limitation of a historic control based on literature is that it is difficult to match patients in the treated and control groups in terms of age, genotype & baseline scores on PDMS-2 & AIMS. It is not clear from the publication by Wassenberg et al whether the characteristics of the external control population i.e. age, genotype or phenotype match that of the subjects in the clinical trials treated with the active.

Hwu et al 2017 also published a study of the Natural history of a cohort of 37 subjects with AADC treated in Taiwan National University hospital. Some of these subjects went on to be treated in the Gene Therapy programme in Taiwan. More detail is available about this cohort of subjects in terms of age, genotype, phenotype, PDMS, AIMS & Bayley scores. This group may be a better match for the treated group but are taken from the same population as subjects treated in the clinical trial programme. A comparison with the group described by Wassenburg et al reflects worldwide experience and consensus about the clinical presentation and diagnosis of AADC.

In response to major objection relating to the natural history comparator group, the applicant established a natural history database from a review of all publications of patients treated with a confirmed diagnosis of AADC. From this database the applicant selected patients that matched the treatment group for phenotype. There were 49 subjects identified with a severe phenotype similar to those treated in the clinical development programme. Subjects described by Hwu et al in his publication who were not treated with gene therapy were included in this group. The applicant used data from this group as the comparator in the assessment of the efficacy endpoints. Of the 49 subjects identified with a similar phenotype 47 i.e. 95% did not achieve an improvement in milestones with standard of care. Two subjects achieved a response, 1 was able to roll from side to side, the other able to walk with assistance. Rolling from side to side was not a milestone agreed a priori for the primary efficacy endpoint analysis. While the applicant has provided more clinical & demographic data about this population in the NHDB, the data is taken from publications and while it can be used to contextualise the results the comparison was not considered robust enough for statistical comparison particularly with such a small sample size.

Protocol deviations were not explicitly collected in AADC-CU or in observational study AADC-CU/1601. For study AADC-010, all patients received treatment in accordance with the study protocol. No patient had any deviation that affected the validity of the data or the conclusions of the analysis. A total of 42 protocol deviations were reported; the primary reasons were missing data from postsurgical procedures (27) and time window deviations (7). A total of 17 protocol deviations were reported in Study AADC-011, 8 patients during the study. Five (5) protocol deviations were due to time window deviations. Five (5) deviations were due to drug storage conditions out of specified range. In addition, 1 patient (Patient 301) used prohibited medications more than 1 month after gene therapy; 1 patient missed the brain CT post-surgery due to the SAE of bradycardia (unlikely to be related to study drug); and 1 patient had an insufficient glucose sample.

Efficacy data and additional analyses

Data in support of efficacy was submitted from 3 clinical trials in children aged 18 moths to 8 years. On initial submission, the primary endpoint of reaching a motor milestone was reached in 9 (50%) of the ITT population. Seven (7)38.9% subjects were able to sit unassisted, and 2 (11.1%) were able to stand with support.

There was a significant benefit to treatment with Upstaza in some subjects compared to the natural history of the disease as described in the control group. The control group with severe phenotype are described as having 'poor head control' & achieved no other motor milestones such as sitting unassisted, standing or walking. Deterioration with loss of skills is described in some patients but in the majority of cases, there is no evident progressive clinical course with loss of function described.

Some clarifications were requested to confirm that the observed benefit was due to the active. Some subjects were started on dopaminergic agents after treatment with the IMP in the clinical trial. It was not clear initially whether these patients responded better to treatment i.e. achieved a motor milestone with the gene therapy or what the indication for restarting treatment with the dopaminergic agent was. According to Wassenberg et al positive responses have been reported with some Dopaminergic agents. The applicant provided updated data on milestone achievement in children who had been treated with dopamine agonists to control symptoms and there was no additional benefit in terms of milestone achievement to subjects treated with DA after gene therapy.

Some of the treatment group had symptoms of AADC after 12 months of age, The applicant has provided data in relation to baseline motor function in this group and no children had achieved a milestone prior to treatment with the gene therapy so would not have biased the results.

Patients continued to gain motor function after the time point of 24 months. For those who attained motor skills the gain was maintained up to 60 months. In some children additional milestones were achieved after the primary efficacy assessment. In the initial dataset presented three (3) patients obtained full head control, 2 achieved the ability to sit unassisted, 1 to stand with support & 1 patient was able to walk with assistance. At the data cut –off date in March 2019, eight (8) children who did not respond within the time point of the primary efficacy endpoint, showed a response. In study, AADC011 at 12 months, four subjects had achieved head control. On request the applicant presented an updated efficacy analysis including all patients treated in the three clinical trials who achieved milestones up to & after the time-point of the primary efficacy analysis to get an overview of the benefit to subjects after the 24-month time-point of the primary efficacy analysis**.**

The secondary efficacy endpoints showed a similar trend. All subjects showed an increase in PDMS score over time and this increase was greater for subjects treated at a younger age. The mean change in baseline PDMS-2 score at 24 months was 94.3 (95% CI 75.6-112.9). The scores continued to increase to 60 months. The change in score reflected increases in scores for grasping, locomotion, object manipulation and visual-motor integration. The AIMS score showed a similar trend with all subjects achieving an increase in score after treatment & a mean increase at 24 months of 21.9 (95% CI 16.1-27.6). Compared to baseline score the increases were statistically significant but were also clinically relevant.

Clinical benefit was also associated with improved neurological performance mainly in the area of floppiness, dystonia and stimulus provoked dystonia. Body weight increased from baseline in the majority of patients but it is unclear what the improvement was due to i.e. improved swallowing, increased appetite etc. There was no beneficial effect reported on symptoms related to serotonin deficiency or other autonomic symptoms of the disease.

Uncertainties regarding the dataset include the small size of the dataset. All studies were conducted at a centre in Taiwan outside the EEA. The founder mutation for the disease was discovered there. There

was only one Caucasian patient in the treated population. Although the underlying pathology of the disease, enzyme defect, clinical phenotype and natural course of the disease appears to be similar for the European population, the external validity of the results for a European population had not been discussed by the applicant at the time of the day 120 assessment report. In their responses the applicant provided data regarding the known prevalence of the disease in Europe and the genotypes and clinical phenotypes described in the European population. It is likely that the benefits observed in the European population would be similar to those observed in the clinical trial population. The AHEG agreed with this conclusion.

Other uncertainties with the data relate to the extrapolation of the indication to older children and adults. There were no children aged nine or over or adults treated in the clinical trials. All subjects treated in the clinical trials had the severe phenotype of disease. The initially proposed indication did not reflect the population studied in the clinical trial development programme. There is no explicit data to establish the benefit risk in these older populations. However, based on the expert feedback, CAT agreed that the indication can be extended to older age groups.

There is limited clinical data submitted to confirm efficacy with the commercially manufactured product. The quality comparability exercise conducted to determine whether there were any differences in quality of the products manufactured by process A, B and the commercial process C was limited by the amount of material available from product manufactured using process A and B. For this reason comparability cannot be concluded between process A and process B material. The comparability exercise conducted using process C material was also limited to 1 batch.

Additional expert consultation

The Ad Hoc expert group was convened to discuss the following questions:

1. Do you consider that the effects seen after treatment with Upstaza in the clinical studies are clinically meaningful?

Summary of AHEG response -The experts agreed that the current standard pharmacological treatment is not sufficient and there is high unmet medical need. The improvements seen in clinical trials varied between age and severity groups but were judged to be clinically meaningful. Some experts noted that the effects were only partial as the selected route of administration was not optimal and midbrain delivery would likely yield better results.

2. Do you think that the children who have achieved head control but have not achieved other motor milestones would benefit from treatment with Upstaza.

Summary of AHEG response-The experts noted that, from the clinical point of view, a patient who achieved head control and no other milestones would be still categorised as severely affected. There are no clinical arguments to exclude these patients from the treatment. Even small improvements can be meaningful from the point of view of patients and carers. Examples of improvements in mood control, oculogyric crisis or weight gain were given as important for the Quality of Life outcomes.

3. Do you consider that very severely affected children defined in terms of the presence of contractures, no voluntary movements or need for permanent ventilation should expect to benefit from treatment.

Summary of AHEG response- The experts notes that patients with these baseline characteristics were not included in the clinical trials. However, they could still benefit from the treatment and gain potential improvement in sleep or feeding, even if motor development remained unaffected. It was noted that surgery in these patients may be challenging and carry additional risks, especially with regards to intubation.

4. Do you think that there are any specific considerations or patient characteristics that will help to identify children that may benefit more from treatment.

Summary of AHEG response-The experts observed that the treatment effect tends to be more pronounced in children who are younger and in those who already experienced some improvement following dopaminergic treatment. Apart from those, there are no other identified characteristic which would act as predictor for response to treatment.

The experts noted that this type of data is of importance to be collected.

5. What is your view on the safety profile of the product, the risks of administration and potential longer term effects.

Summary of AHEG response-The experts were of the opinion that the safety profile seems to be generally acceptable. However, majority of the data were collected in one highly experienced centre. it is possible that the number of adverse events related to the surgical procedure could increase once more centres are allowed to administer Upstaza. The experts have recommended that comprehensive training and supervision program should be developed.

A close follow-up to characterise long term safety and efficacy (preferably integrated in the INTD registry) is also expected.

The frequent GI adverse events are likely not connected to the Upstaza administration but result from the underlaying disease

Additional efficacy data needed in the context of a MA under exceptional circumstances

To further characterise long-term efficacy and safety (as missing information) of the commercial product, 10-year follow-up data from study AADC-1602 and a registry-based study is requested, which should include the relevant efficacy endpoints such as motor behavioural development, as well as safety endpoints.

2.6.7. Conclusions on the clinical efficacy

The dataset is limited. The main benefit attained by 24 months in approximately half the population treated is the achievement of head control. Most subjects who gained head control went on to achieve other milestones after 24 months. There is no observed effect on autonomic symptoms of the disease and limited effects on serotonergic symptoms of the disease. The clinical relevance of the motor benefits of treatment compared to the risk of neurosurgery was discussed with an AHEG who supported that efficacy has been demonstrated and is clinically meaningful.

The applicant was asked to consider whether it is possible to identify subjects who may respond better to treatment & achieve unassisted sitting, standing or walking**.** Whilst there were no factors identified that would predict a better response, subjects treated at a younger age seemed to have a greater response indicated by a greater increase in PDMS score.

The additional benefits to treatment claimed by the applicant include a decrease in the annualized rate of upper respiratory tract infections and pneumonia. The percentage of subjects with episodes of RTI decreases from 90% approx. at year 1 to 27% at year 5. This is a clinically relevant change in rate of respiratory infections. However, the data is difficult to interpret without the baseline rate of URTI in the treated population. The applicant also presents data regarding improvements for all subjects in OGC events, body weight, language and cognition and fine motor movement that does not occur spontaneously in this patient population with severe disease. The clinical relevance of the described

benefits was not clear from the data submitted. The clinical relevance of the observed effects and how they relate to improved quality of life for patients also was not clear. These observed benefits must be viewed in the context of the limitations to the data, risks and potential risks of the active & the neurosurgery involved in the administration of the active.

In the opinion of the CAT the observed motor benefits could not be extrapolated to other populations such as subjects with a milder phenotype. In view of the existing medical need beyond the population covered by the granted indication, the applicant is strongly encouraged to pursue the clinical development of Upstaza in patients with less severe disease, to both enlarge the safety database and cover the critical medical need.

The CAT considers the following measures necessary to address the missing efficacy data in the context of a MA under exceptional circumstances:

To further characterise long-term efficacy and safety (as missing information) of the commercial product 10-year follow-up data from study AADC-1602 and a registry-based study is requested, which should include the relevant efficacy endpoints such as motor behavioural development, as well as safety endpoints.

The CHMP endorses the CAT conclusion on clinical efficacy as described above.

2.6.8. Clinical safety

2.6.8.1. Patient exposure

All patients received intraputaminal infusions of eladocagene exuparvovec during a single operative procedure, with infusions to 2 sites in the right putamen and 2 sites in the left putamen. Twenty-one patients received a total dose of $1.8x10^{11}$ vg. Five patients received a total dose of $2.4x10^{11}$ vg.

On initial submission, twenty-five patients completed 12 months of follow-up after the surgical procedure, and 17 patients completed 24 months of follow-up. Ten patients are still ongoing, 9 patients in Study AADC-010 (planned follow-up of 5 years) and 1 patient in Study AADC-011 (planned follow-up of 12 months). The ongoing patient in Study AADC-011 has been in follow-up for 6 months after receiving eladocagene exuparvovec $2.4x10^{11}$ vg. All patients in Study AADC-CU/1601 (n=8) have completed the planned 5-year follow-up.

Figure 34 Patient Completion Summary, Safety Population (N=26)

		1.8x10[11] vg Dose[a]	2.4x10[11] vg Dose[a]	Overall[a]
Screened		-	-	26
Screen Failures		-	-	1[b]
Enrolled		21	5	26
Treated		21	5	26
Discontinued		3	0	3
Reason for Discontinuation	Investigator's decision / patient died from encephalitis due to influenza B	1	0	1
	Patient and family could not return for 5-year follow-up examination	1	0	1
	Patient died 9 July 2015	1	0	1
Completed Through Month 12		21 (100.0%)	4 (80.0%)	25 (96.2%)
Completed Through Month 24		17 (81.0%)	0	17 (65.4%)
Completed Through Month 36		16 (76.2%)	0	16 (61.5%)
Completed Through Month 60		6 (28.6%)	0	6 (23.1%)
Ongoing at Data Cut-Off[c]		9 (42.9%)	1 (20.0%)	10 (38.5%)
Patients in Safety Population		21 (100.0%)	5 (100.0%)	26 (100.0%)
Duration of Follow-Up (Months)[d]	Mean (SD)	44.6 (19.06)	11.0 (2.78)	38.1 (21.77)
	Median	48.1	12.0	45.7
	Min, Max	(12.2, 68.3)	(6.1, 12.9)	(6.1, 68.3)

2.6.8.2. Adverse events

All patients experienced at least 1 adverse event. Dyskinesia was recorded for 24 patients (92.3%; Section 3.1.1.1) and was considered related to treatment for 23 patients (88.5%). Ten patients (38.5%) experienced an AE that was severe in intensity, and 23 patients (88.5%) experienced an SAE. Two patients died: 1 patient died 12 months after the surgical procedure due to bacterial encephalitis in Study 010 and 1 patient died after the completion of the 5-year follow-up period due to complications of AADC deficiency in Study 1601.

Figure 35 Summary of Treatment-Emergent Adverse Events by Dose Group and Overall,

Safety Population (n=26)

	$1.8x10^{11}$ vg Dose (N=21)	$2.4x10^{11}$ vg Dose (N=5)	Overall (N=26)
Number of TEAEs	425	88	513
Patients with ≥1 TEAE	21 (100.0%)	5 (100.0%)	26 (100.0%)
Patients Experiencing ≥1 Serious TEAE	18 (85.7%)	5 (100.0%)	23 (88.5%)
Highest TEAE Relationship to Treatment			
Unrelated	0	0	0
Unlikely/Remote	0	3 (60.0%)	3 (11.5%)
Possible	11 (52.4%)	0	11 (42.3%)
Probable	10 (47.6%)	2 (40.0%)	12 (46.2%)
Certain	0	0	0
Maximum TEAE Severity			
Mild	3 (14.3%)	3 (60.0%)	6 (23.1%)
Moderate	9 (42.9%)	1 (20.0%)	10 (38.5%)
Severe	9 (42.9%)	1 (20.0%)	10 (38.5%)
Deaths	2 (9.5%)	0	2 (7.7%)

Abbreviations: TEAE, treatment-emergent adverse event; vg, vector genome
Source: ISS, Table 2.2

2.6.8.3. Common Adverse Events

TEAEs by Incidence

Pyrexia and dyskinesia were the most commonly reported AEs, being reported by over 90% of patients. As dopamine levels are non-existent to low prior to gene therapy, the occurrence of dyskinesia is an expected event and can be attributed to hypersensitivity of dopamine receptors to newly available dopamine. All events of dyskinesia resolved within 7 months of clinical onset, and most resolved within 4 months of gene therapy. The majority of events were mild to moderate in severity; The majority of events were possibly/probably related to eladocagene exuparvovec.

Upper respiratory tract infection, gastroenteritis, and pneumonia were the next most commonly occurring events and were reported by 65% to 69% of patients.

The incidence of commonly reported AEs appeared to be similar between the $1.8x10^{11}$ and $2.4x10^{11}$ vg dose groups. Breath sounds were abnormal in all 5 patients (100%) in the $2.4x10^{11}$ vg dose group, which is not surprising given that respiratory complications are common in this patient population, but was reported for only 14.3% of patients in the $1.8x10^{11}$ vg dose group. Another difference between doses was in the occurrence of cyanosis, experienced by 33.3% of patients in the $1.8x10^{11}$ vg dose group and by 0% of patients in the $2.4x10^{11}$ vg dose group.

2.6.8.4. Serious adverse events and deaths

The majority of AEs were mild or moderate in intensity. Adverse events that were severe in intensity were experienced by 10 patients (38.5%). Gastroenteritis (n=3) and pneumonia (n=4) were the only AEs that were reported by more than 1 or 2 patients. Only 1 patient in the $2.4x10^{11}$ vg dose group experienced an event (respiratory failure) that was severe in intensity.

TEAEs by Relatedness

Dyskinesia was the most commonly occurring adverse event that was considered to be related to treatment and was experienced by nearly all patients (23 patients, 88.5%). Aside from dyskinesia, 4

other AEs were considered treatment-related: initial insomnia, salivary hypersecretion, feeding disorder, sleep disorder.

TEAEs by Time

The majority of AEs (380/513 events, 74.1%) occurred within the first 12 months following gene therapy treatment. All patients experienced AEs within this timeframe. The most frequently occurring events (pyrexia and dyskinesia) tended to occur in the first 12 months. All AEs considered to be related to treatment occurred within the first 12 months.

Twenty-four patients fell into the Month 12 to Month 24 follow-up period. Seventeen patients (70.8%) reported AEs between Month 12 and Month 24, but the total number of events reported was low (56/513 events, 10.9%). After Month 24, 17 patients remained in follow-up. Of these, 13 patients (76.5%) reported 77/513 (15.0%) AEs over the next 3 years.

Adverse events that were reported only between Month 12 to Month 24 or only after Month 24 were generally reported by 1 patient each, and are unlikely to be the result of latent, long-term effects of gene therapy. Adverse events that were reported only between Month 12 to Month 24 include polydactyly, allergic otitis media, hypermetropia, gastrointestinal motility disorder, hiatus hernia, croup infectious, irregular sleep phase, and peptic ulcer. Adverse events that were reported only after Month 24 include sinus bradycardia, functional gastrointestinal disorder, esophagitis, tooth loss, mass, herpangina, influenza, hyponatremia, foot deformity, knee deformity, scoliosis, aphasia, hypoglycemic seizure, genital swelling, asthma, sleep apnoea syndrome, contact dermatitis, pallor, acute osteomyelitis, inguinal hernia, and cholesteatoma.

Figure 36 Treatment-Emergent Adverse Events Reported to be Severe in Intensity, Safety Population (N=26)

Adverse Event Category[a]:	vg Dose (N=21)	vg Dose (N=5)	Overall[b] (N=26)
Total Number of Severe TEAEs	44	1	45
Patients with ≥1 Severe TEAE	9 (42.9%)	1 (20.0%)	10 (38.5%)
Respiratory, thoracic and mediastinal disorders	**4 (19.0%)**	**1 (20.0%)**	**5 (19.2%)**
Respiratory failure	1 (4.8%)	1 (20.0%)	2 (7.7%)
Apnoea	1 (4.8%)	0	1 (3.8%)
Choking	1 (4.8%)	0	1 (3.8%)
Pneumonia aspiration	1 (4.8%)	0	1 (3.8%)
Sleep apnoea syndrome	1 (4.8%)	0	1 (3.8%)
Infections and infestations	**4 (19.0%)**	**0**	**4 (15.4%)**
Pneumonia	4 (19.0%)	0	4 (15.4%)
Gastroenteritis	3 (14.3%)	0	3 (11.5%)
Bronchiolitis	1 (4.8%)	0	1 (3.8%)
Croup infectious	1 (4.8%)	0	1 (3.8%)
Urinary tract infection	1 (4.8%)	0	1 (3.8%)
Nervous system disorders	**3 (14.3%)**	**0**	**3 (11.5%)**
Dyskinesia	2 (9.5%)	0	2 (7.7%)
Encephalopathy	1 (4.8%)	0	1 (3.8%)
Loss of consciousness	1 (4.8%)	0	1 (3.8%)
Cardiac disorders	**2 (9.5%)**	**0**	**2 (7.7%)**
Cyanosis	2 (9.5%)	0	2 (7.7%)
Gastrointestinal disorders	**1 (4.8%)**	**0**	**1 (3.8%)**
Gastroesophageal reflux disease	1 (4.8%)	0	1 (3.8%)
Upper gastrointestinal haemorrhage	1 (4.8%)	0	1 (3.8%)
General disorders and administration site conditions	**1 (4.8%)**	**0**	**1 (3.8%)**
Death	1 (4.8%)	0	1 (3.8%)
Injury, poisoning and procedural complications	**1 (4.8%)**	**0**	1 (3.8%)
Endotracheal intubation complication	1 (4.8%)	0	1 (3.8%)
Vascular disorders	**1 (4.8%)**	0	**1 (3.8%)**
Hypovolaemic shock	1 (4.8%)	0	1 (3.8%)
Shock	1 (4.8%)	0	1 (3.8%)

Abbreviations: MedDRA, Medical Dictionary for Regulatory Activities; TEAE, treatment-emergent adverse event; vg, vector genome

2.6.8.5. Serious adverse event/deaths/other significant events

Two of the 26 patients in the integrated safety database have died:

One patient, a boy who was treated at 32 months of age, experienced a severe SAE of encephalopathy (encephalitis due to influenza B) approximately 11 months after study treatment in Study AADC-010 (ISS Listing 6). The patient died due to the encephalopathy after 12 months of follow-up. The infection and death were considered unrelated to eladocagene exuparvovec.

One patient, a boy who was treated at 53 months of age, died 4 months after the 5-year follow-up period in Study AADC-CU/1601 was completed. The cause of death, when the patient was nearly 10 years old, was considered to be related to underlying AADC disease and unlikely related to treatment with eladocagene exuparvovec.

Other Serious Adverse Events

Twenty-three patients (88.5%) experienced a SAE. Pneumonia (13 patients, 50.0%) and gastroenteritis (11 patients, 42.3%) were the most frequently occurring SAEs.

Other Significant Adverse Events

Surgical Related AEs

Ten patients experienced adverse events potentially related to the surgical procedure. Four events occurred on the same day as the surgical procedure: post-operative skull defect (mild), endotracheal intubation complication (severe), subcutaneous hematoma (mild), and transfusion reaction (mild). All events of hypotension (2 moderate, 4 mild) occurred on the day of or the day after the surgical procedure. Skin injury (verbatim term: left parietal scalp swelling, mild) started the day after surgery and wound complication (verbatim term: surgery wound swelling; moderate) occurred approximately 1 month afterwards. All of these events were considered unrelated or unlikely to be treatment-related and all events resolved.

Figure 37 Treatment-emergent Adverse Events Potentially Related to the Surgical Procedure, Safety Population (N=26)

Adverse Event Category[a]:	$1.8x10^{11}$ vg Dose (N=21)	$2.4x10^{11}$ vg Dose (N=5)	Overall (N=26)
Post-operative skull defect	1 (4.8%)	0	1 (3.8%)
Injury, poisoning and procedural complications			
Endotracheal intubation complication	1 (4.8%)	0	1 (3.8%)
Skin injury	1 (4.8%)	0	1 (3.8%)
Subcutaneous haematoma	1 (4.8%)	0	1 (3.8%)
Transfusion reaction	0	1 (20.0%)	1 (3.8%)
Wound complication	1 (4.8%)	0	1 (3.8%)
Nervous system disorders			
Cerebrospinal fluid leakage	3 (14.3%)	0	3 (11.5%)
Vascular Disorders			
Hypotension	3 (14.3%)	3 (60.0%)	6 (23.1%)

Abbreviations: MedDRA, Medical Dictionary for Regulatory Activities; vg, vector genome
[a] Adverse events were coded in MedDRA Version 19.1.
Source: ISS, Modified from Table 2.4

Cerebrospinal Fluid Leak

Cerebrospinal fluid leaks were rarely reported during clinical trials of eladocagene exuparvovec. Three patients experienced leakage of CSF:

Patient 1601-06 experienced 1 moderate SAE and 1 mild SAE of CSF leak approximately 1 month and 5 months after eladocagene exuparvovec infusion, respectively. Both events required hospitalization but were considered unlikely to be related to eladocagene exuparvovec and resolved.

Patient 010-1001 experienced a mild AE of CSF leak approximately 3 weeks following eladocagene exuparvovec infusion. The event was mild, considered unrelated to treatment, and resolved without intervention.

Patient 010-1004 experienced a mild AE of CSF leak approximately 5 months after eladocagene exuparvovec infusion. The event was mild, considered unrelated to treatment, and resolved without intervention.

2.6.8.6. Laboratory findings

No particular trends in clinical laboratory test results were observed. Cerebrospinal fluid cell counts and CSF protein were all within normal range at 12 months.

Vital signs, physical findings and other observations related to safety

Aside from shifts in temperature, vital signs were stable from baseline through Month 12 (Figure 38). Diastolic and systolic blood pressure that were abnormal at baseline remained abnormal at Month 12.

Figure 38 Vital Sign Shift Table, Safety Population (N=26)

	Baseline/Month 12 Result			
Parameter (unit)	**Normal/ Normal**	**Normal/ Abnormal**	**Abnormal /Normal**	**Abnormal/ Abnormal**
Diastolic Blood Pressure (mmHg), n=7	0	0	0	7 (100.0%)
Heart Rate (Beats/Min), n=20	19 (95.0%)	1 (5.0%)	0	0
Oxygen Saturation (%), n=2	2 (100%)	0	0	0
Respiratory Rate (Breaths/Min), n=20	19 (95.0%)	1 (5.0%)	0	0
Systolic Blood Pressure (mmHg), n=7	1 (14.3%)	1 (14.3%)	1 (14.3%)	4 (57.1%)
Temperature (ºC), n=22	8 (36.4%)	6 (27.3%)	7 (31.8%)	1 (4.5%)

Source: ISS Table 3.10

Physical Exam

The results of physical examinations were relatively stable from baseline to Month 12.

Electrocardiograms

Electrocardiogram results were normal at baseline through Month 12.

Neurological Exam

The results of MRI examinations appeared to remain stable from baseline to 12 months:

Four (4) patients with normal MRI results at baseline remained normal at 12 months, and 13 patients with abnormal results (structure changes and white matter changes) at baseline remained abnormal at 12 months.

2.6.8.7. Safety in special populations

Adverse Events by Sex

Adverse events in the most frequently reported System Organ Classes (SOCs) of Gastrointestinal Disorders, Infections and Infestations, Nervous System Disorders, and General Disorders and Administration Site Conditions occurred with a relatively similar incidence between males and females. Events within SOCs where there was at least a two-fold percentage difference in SOC occurrence between males and females and where there was more than 1 patient in the SOC are summarized in the Figure below. Despite the disparity in occurrence, these events are unlikely to indicate a true difference in safety of gene therapy based on gender.

Figure 39 Adverse Events with at Least Two-Fold Percentage Difference in SOC Occurrence Between Males and Females, Safety Population (n=26)

	Overall N=26	
	Males	Females
Adverse Event Category[a]:	N=13 (50.0%)	N=13 (50.0%)
Metabolism and Nutrition Disorders	9 (69.2%)	4 (30.8%)
Dehydration	4 (30.8%)	2 (15.4%)
Hypokalemia	3 (23.1%)	2 (15.4%)
Feeding disorder	2 (15.4%)	1 (7.7%)
Hypoglycemia	1 (7.7%)	1 (7.7%)
Hyponatraemia	1 (7.7%)	0
Injury, Poisoning and Procedural Complications	3 (23.1%)	6 (46.2%)
Endotracheal intubation complication	1 (7.7%)	0
Femur fracture	1 (7.7%)	0
Joint dislocation	1 (7.7%)	0
Radial head dislocation	0	1 (7.7%)
Skin injury	0	1 (7.7%)
Subcutaneous haematoma	0	1 (7.7%)
Thermal burn	1 (7.7%)	1 (7.7%)
Transfusion reaction	0	1 (7.7%)
Tooth avulsion	1 (7.7%)	0
Wound complication	0	1 (7.7%)
Surgical and Medical Procedures	1 (7.7%)	3 (23.1%)
Tooth extraction	1 (7.7%)	3 (23.1%)
Eye Disorders	2 (15.4%)	0
Hypermetropia	1 (7.7%)	0
Ocular hyperaemia	1 (7.7%)	0

Abbreviations: MedDRA, Medical Dictionary for Regulatory Activities; SOC, system organ class
[a] Adverse events were coded in MedDRA Version 19.1.
Source: ISS, Modified from Table 2.14 and Table 2.16

Adverse Events by Age

Adverse events in the most frequently reported SOCs of Gastrointestinal Disorders, Infections and Infestations, Nervous System Disorders, and General Disorders and Administration Site Conditions occurred with a relatively similar incidence between age groups. Events within SOCs where there was at least a two-fold percentage difference in SOC occurrence between the 2 highest age groups and more than 1 patient in the SOC are summarized in the Figure below. Despite the disparity in occurrence, these events are unlikely to indicate a true difference in safety of gene therapy based on age.

Figure 40 Adverse Events with at Least Two-Fold Percentage Difference in SOC Occurrence at the Higher Age Groups, Safety Population (n=26)

	Overall N=26		
	<2 years	2 to <6 years	6 to <12 years
Adverse Event Category[a]:	N=3	N=16	N=7
Injury, Poisoning and Procedural Complications	1 (33.3%)	4 (25.0%)	4 (57.1%)
Endotracheal intubation complication	0	1 (6.3%)	0
Radial head dislocation	0	1 (6.3%)	0
Femur fracture	0	0	1 (14.3%)
Joint dislocation	0	0	1 (14.3%)
Skin injury	0	0	1 (14.3%)
Subcutaneous haematoma	0	0	1 (14.3%)
Thermal burn	1 (33.3%)	0	1 (14.3%)
Tooth avulsion	0	1 (6.3%)	0
Transfusion reaction	0	1 (6.3%)	0
Wound complication	0	1 (6.3%)	0
Psychiatric Disorders	1 (33.3%)	3 (18.8%)	4 (57.1%)
Irritability	1 (33.3%)	2 (12.5%)	0
Initial insomnia	0	1 (6.3%)	3 (42.9%)
Sleep disorder	0	0	1 (14.3%)
Investigations	2 (66.7%)	6 (37.5%)	0
Breath sounds abnormal	2 (66.7%)	6 (37.5%)	0
Surgical and Medical Procedures	0	2 (12.5%)	2 (28.6%)
Tooth extraction	0	2 (12.5%)	2 (28.6%)

Abbreviations: MedDRA, Medical Dictionary for Regulatory Activities; SOC, system organ class
[a] Adverse events were coded in MedDRA Version 19.1.
Source: ISS, modified from Table 2.18, Table 2.20, and Table 2.22

Extrinsic Factors

All studies were performed at one site in Taiwan. Therefore, assessment of extrinsic factors such as geographical location was not performed. Other extrinsic factors likely to influence safety of the drug were not identified.

Safety in Other Clinical Settings

Vectors similar to eladocagene exuparvovec were originally developed for gene therapy in Parkinson's disease, and have also been used to treat AADC deficiency. Although these vectors are not eladocagene exuparvovec, they were similar in many ways. Intraputaminal infusion of these vectors has proven to be safe in several published studies, as summarized below.

Figure 41 Comparison of AAV-hAADC Vectors

Promoter	Intron	Poly A	Encoded Protein	Serotype	Vector Name
CMV-IEP	HBG2/3	Simian virus 40	hAADC	AAV2	eladocagene exuparvovec,
CMV-IEP	CMV/HBG	HGH	hAADC	AAV2	AAV-h*AADC* [a]
CMV-IEP	HGH	Simian virus 40	hAADC	AAV2	AAV-h*AADC*-2 [b]
CMV-IEP	HBG	HGH	hAADC	AAV2	AAV-h*AADC*-2 [c]
CMV-IEP	NS	NS	hAADC	AAV2	VY-AADC01 [d]

Abbreviations: AAV, adeno associate virus; AAV2, adeno associate virus (serotype 2); CMV-IEP, cytomegalovirus intermediate-early promoter; CMV/HBG, cytomegalovirus splice donor and human globin splice acceptor; hAADC, human aromatic L-amino acid decarboxylase; HBG2/3, human β-globin partial intron 2/partial exon 3; HGH, human growth hormone; NS, not specified; Poly A, polyadenylation sequence

[a] (Christine 2009)
[b] (Muramatsu 2010)
[c] (Kojima 2019)
[d] (Christine 2019)

In the first-in-human clinical trial, patients with Parkinson's disease received AAV-h*AADC* $9x10^{10}$ vg (n=5) and $3x10^{11}$ vg (n=5) via intraputaminal infusion of 100 uL per putamen (Christine 2009). Intracranial hemorrhage was observed in 3 patients along the trajectory of the catheter but not at the infusion site. The most common AEs were headaches and discomfort at the surgical site, all of which resolved. No AEs were attributed to the vector. In an effort to increase the percentage of transduced cells and cover more of the putamen, the concentration of vector was increased to $8.3x10^{11}$ vg/mL and infusion volume was increased to a maximum of either 450 uL/putamen or 900 uL/putamen for a maximum total dose of $1.5x10^{12}$ vg (Christine 2019). A third dose group in the Christine 2019 study received the highest dose of $2.6x10^{11}$ vg/mL in a volume up to 900 uL/putamen for a maximum total dose of $4.7x10^{12}$ vg. The surgical procedure and infusion of larger volumes was well tolerated; 14/15 patients returned home within 2 days after the surgery. The most frequently reported AE was headache, all of which resolved. Dyskinesia (n=4) was considered related to gene therapy and resolved with reductions in Parkinson's disease medications or addition of amantadine. Only 1 patient reported SAEs; these were deep vein thrombosis, pulmonary embolus, and atrial fibrillation which were attributed to immobility during the operation and resolved with routine clinical care.

In a second first-in-human clinical trial, 6 patients with Parkinson's disease were treated with $3x10^{11}$ vg via intraputaminal infusion (Muramatsu 2010). The surgical procedure was well tolerated. One patient had a venous haemorrhage, which was determined to be due to the infusion rather than to gene transduction; positron emission tomography imaging of this patient showed good AADC expression for up to 96 weeks. All patients had headaches around the burr hole for 2 days after surgery. No clinical laboratory abnormalities were reported. All patients completed all protocol specified visits. Additionally, patients with AADC deficiency were also treated (Kojima 2019). Six patients, ages 4 to 19 years, received AAV-h*AADC*-2 in a vector solution containing 0.0001% poloxamer-188 via intraputaminal infusion of 100 µL per putamen for a total dose of $2x10^{11}$ vg. The surgical procedure and infusion were well tolerated. One patient experienced a subdural hemorrhage. All patients exhibited choreic movements, which diminished 3 to 6 months after gene therapy. No vector-related AEs were observed. All 5 patients who entered the study with a severe phenotype were able to move their heads 2 to 8 months after gene therapy. The one patient who entered the study with a moderate phenotype was able to walk independently after 6 months, ride a bicycle after 10 months, and play on a swing after 18 months. Thus, treatment with AAV-h*AADC*-2 vector in 0.0001% poloxamer 188 was both safe and effective.

2.6.8.8. Immunological events

Anti-AAV2 Antibodies

Eighteen patients (69.2%) had at least 1 positive antibody titre within the first 12 months; 8 patients (30.8%) did not. Adverse events for patients with positive antibody titres generally occurred with a similar frequency as AEs for patients who did not have a positive antibody titre.

See PK/PD discussion on immunogenicity & efficacy discussion on clarification of level of antibodies that preclude treatment in the SmPC. The applicant states that the incidence of adverse events was similar in those that produced antibodies in response to treatment and those who did not. Subjects were excluded if their baseline titre was greater than a threshold level.

2.6.8.9. Safety related to drug-drug interactions and other interactions

There is no information presented on transgene in the systemic circulation. There is an immune response to the vector so the vector must enter the systemic circulation. The active is administered as a single dose and given the method and site of administration, there is unlikely to be a drug/drug interaction unless the concomitant drug is also administered intraputamen.

2.6.8.10. Post marketing experience

Eladocagene exuparvovec is not marketed in any country. No post-marketing information is available.

2.6.9. Discussion on clinical safety

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

There are several limitations to the initial safety data presented in support of the marketing authorisation. The safety database consists of 26 subjects between the ages of 18 months and 8 years and 6 months. An updated analysis provided safety data for 28 subjects. There is no safety data from adult or from adolescent subjects. There is no preclinical reproductive toxicology data presented. There is no safety data presented in the clinical trial programme for subjects treated with the commercially

manufactured product. The commercial product contains a novel excipient for which there is no preclinical toxicology data presented nor any clinical safety data presented on intraputaminal/intraputaminal use in the intended population**.**

During the course of the evaluation, the applicant has provided preclinical data in NHP relating to the excipient P188 and provides a risk assessment regarding its use in the central nervous system and in the paediatric population.

Twenty-six 26 (100%) of subjects reported an adverse event. Twenty-five 25(96%) reported a pyrexia after treatment, 15(57.7%) reported an upper GI haemorrhage & 18 subjects (69.2%) were diagnosed with an upper respiratory tract infection. Adverse events attributed to the active were dyskinesia, which was the most commonly occurring adverse event considered related to treatment and occurred in 23(88.5%) of subjects. All events of dyskinesia resolved within 7 months of clinical onset, and most resolved within 4 months of gene therapy. The majority of events were mild to moderate in severity; only2 events were severe.

Other treatment related adverse events included initial insomnia, which was reported in 4 subjects (19%), 2 (7.7%) salivary hypersecretion, 2(7.7%) a feeding disorder and 1 (3.8%) sleep disorder.

Adverse events related to the neurosurgery included cerebrospinal fluid leakage in 3 subjects (11.5%), hypotension 6(23.1%), and endotracheal intubation complication, post-operative skull defect, skin injury, subcutaneous haematoma 1 (3.8%) each.

Eighteen patients, (69.2%) of subjects, had at least 1 positive antibody titre within the first 12 months. There is no data presented relating to an immune response to the transgene itself nor to a cell mediated immune response.

A high percentage of subjects had a pneumonia or upper respiratory tract infection. The cause of these infections was not attributed to the treatment and presumably was considered to be due to the underlying disease. It is hoped that with improved feeding and head control the risk of respiratory infections would be decreased after treatment. Although the rate of infections decreased after treatment, without a baseline rate of URTI in the treated population it is not possible to interpret the clinical relevance of this finding.

The SmPC has been updated to adequately reflect the adverse event profile. Section 4.8 was updated in line with the treatment related adverse events in addition to the adverse events attributable to the method of administration of the product i.e. the surgical procedure. Nearly all children experienced an adverse event, many of which were serious adverse events. Many of the adverse events and reactions which occurred after treatment were attributed to the patient's underlying disease i.e. AADC. The SmPC was updated to state that children may experience exacerbations of symptoms of their underlying AADC deficiency. This could be due to the stress of treatment or the surgical procedure.

There is safety data available on some subjects for up to 5 years after treatment, which demonstrates that the risk of treatment related adverse events decreases with time. There is no longer term data presented. More data is needed on long-term safety and efficacy follow-up.

Additional expert consultations

Please refer to clinical efficacy discussion section for summary of AHEG consultation.

Additional safety data needed in the context of a MA under exceptional circumstances

In order to further characterise the long-term efficacy and safety of Upstaza in patients with aromatic L amino acid decarboxylase (AADC) deficiency and with a severe phenotype, the MAH shall conduct and submit the results of study PTC-AADC-MA-406, an observational, multicentre and longitudinal study of

patients treated globally with the commercial product, based on data from a registry, according to an agreed protocol.

2.6.10. Conclusions on the clinical safety

There is a limited safety database. Other treatment related adverse events are possible that have not been reported in the data set. There is limited preliminary safety data available from patients treated with the commercial manufactured product. There is limited safety data available on the intraparenchymal use of the novel excipient. The method of application involves a surgical procedure including anaesthesia of high risk and the risks may vary between centres and with the individual patient's underlying condition.

The CAT considers the following measures necessary to address the missing safety data in the context of a MA under exceptional circumstances:

In order to further characterise the long-term efficacy and safety of Upstaza in patients with aromatic L amino acid decarboxylase (AADC) deficiency and with a severe phenotype, the MAH shall conduct and submit the results of study PTC-AADC-MA-406, an observational, multicentre and longitudinal study of patients treated globally with the commercial product, based on data from a registry, according to an agreed protocol.

The CHMP endorses the CAT conclusion on clinical safety as described above.

2.7. Risk Management Plan

2.7.1. Safety concerns

Important identified risks

- Dyskinesia
- Procedural complications, including CSF leaks

Important potential risks

- Tumorigenicity
- Immunogenicity (including cellular and humoral immunogenicity)
- Third party transmission

Missing information

- Long-term safety (>10years)
- Use in children ≤18 months old

2.7.2. Pharmacovigilance plan

Studies PTC-AADC-MA-406 and AADC-1602 Long-term follow-up study are imposed as Specific Obligation(SO) in the context of Marketing Authorisation under exceptional circumstances for efficacy reasons and the details of the studies are described in Part IV of the RMP. They are imposed primarily for efficacy reasons but will also address secondary safety endpoints related to:

- Dyskinesia

- Procedural complications, including, CSF leaks
- Tumorigenicity
- Immunogenicity (including cellular and humoral immunogenicity)
- Third Party Transmission
- Long-term safety (>10years)
- Use in children ≤18 months.

2.7.3. Plans for post-authorisation efficacy studies

Studies PTC-AADC-MA-406 and AADC-1602 Long-term follow-up study are imposed as Specific Obligation(SO) in the context of Marketing Authorisation under exceptional circumstances for efficacy reasons.

Study Status	**Summary of objectives**	**Efficacy uncertainties addressed**	**Milestones**	**Due dates**
Efficacy studies which are conditions of the marketing authorisation				
None				
Efficacy studies which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
A Two-Part Registry of Participants Diagnosed with Aromatic L Amino Acid Decarboxylase Deficiency (AADC-d) With or Without Treatment with Upstaza (Eladocagene Exuparvovec) (PTC-AADC-MA-406) (AADCAchieve). **Part A** ONGOING **Part B** PLANNED	**Part A** • To describe the natural history of AADC-d in participants on standard of care **Part B** • To assess the long-term effectiveness and safety outcomes of treatment with Upstaza (eladocagene exuparvovec) in participants with AADC-d for a minimum of 10 years following gene therapy.	Long term efficacy & safety	Protocol Submission	31 July 2022
			Feasibility Assessment (For adding of iNTD as secondary data source)	31 December 2022
			Annual interim report	Progress reports will be provided with annual re-assessment (Part B).
			Final report	30 Jun 2036
AADC-1602 Long-term follow-up study for existing patient population enrolled in the clinical studies AADC-CU/1601, AADC-010 and AADC-011 ONGOING	To assess long-term durability of treatment and safety with eladocagene exuparvovec.	Long term efficacy & safety	Annual interim report	Progress reports will be provided with annual re-assessment
			Final report	30 Jun 2030

2.7.4. Risk minimisation measures

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Important identified risk: Dyskinesia	**Routine risk minimisation measures:** *SmPC section 4.4 and 4.8* *PIL section 2 and 4* *Information on time to recovery and use of dopamine antagonists to control symptoms* *Prescription only medicine* **Additional risk minimisation measures:** • *A surgical guide will be provided to the treatment centres performing the administration of eladocagene exuparvovec*	**Additional pharmacovigilance activities:** **Studies imposed as Specific Obligation under exceptional circumstances** • A Two-Part Registry of Participants Diagnosed with Aromatic L Amino Acid Decarboxylase Deficiency (AADC-d) With or Without Treatment with Upstaza (Eladocagene Exuparvovec) (PTC-AADC-MA-406) (AADCAchieve). • AADC-1602 Long-term follow-up study for existing patients
Important identified risk: Procedural complications, including CSF leaks	**Routine risk minimisation measures:** *SmPC* sections 4.4 & 4.2 *CT scanning post-surgery* PIL section 2 Information on monitoring patients for CSF leaks after administration *Prescription only medicine* **Additional risk minimisation measures:** • *A surgical guide will be provided to the treatment centres performing the administration of eladocagene exuparvovec* • *Controlled distribution through qualified treatment centres*	**Additional pharmacovigilance activities:** **Studies imposed as Specific Obligation under exceptional circumstances** • A Two-Part Registry of Participants Diagnosed with Aromatic L Amino Acid Decarboxylase Deficiency (AADC-d) With or Without Treatment with Upstaza (Eladocagene Exuparvovec) (PTC-AADC-MA-406) (AADCAchieve). • AADC-1602 Long-term follow-up study for existing patients
Important potential risk: Tumorigenicity	**Routine risk minimisation measures:** *Prescription only medicine* **Additional risk minimisation measures:** *None*	**Additional pharmacovigilance activities:** **Studies imposed as Specific Obligation under exceptional circumstances** • A Two-Part Registry of Participants Diagnosed with Aromatic L Amino Acid Decarboxylase Deficiency (AADC-d) With or Without Treatment with Upstaza (Eladocagene Exuparvovec) (PTC-AADC-MA-406) (AADCAchieve). • AADC-1602 Long-term follow-up study for existing patients
Important potential risk: Immunogenicity (Including cellular and humoral immunogenicity)	**Routine risk minimisation measures:** *SmPC section 4.2, 4.4 and 4.8* *Information on constituents of the immune response and on when elevation of anti-AAV2 antibodies occurred in clinical trials.* *Information on anti-capsid antibody levels in patients treated*	**Additional pharmacovigilance activities:** **Studies imposed as Specific Obligation under exceptional circumstances** • A Two-Part Registry of Participants Diagnosed with Aromatic L Amino Acid Decarboxylase Deficiency (AADC-d) With or Without Treatment

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	in the clinical trial programme are provided. *Prescription only medicine* **Additional risk minimisation measures:** *None.*	with Upstaza (Eladocagene Exuparvovec) (PTC-AADC-MA-406) (AADCAchieve). • AADC-1602 Long-term follow-up study for existing patients
Important potential risk: Third party transmission	**Routine risk minimisation measures:** *SmPC section 4.4 and 6.6* Information on preparation of Upstaza and handling of the medication and what to do in the case of accidental exposure **Additional risk minimisation measures:** • *Pharmacy manual and patient alert card will be provided.*	**Additional pharmacovigilance activities:** **Studies imposed as Specific Obligation under exceptional circumstances** • A Two-Part Registry of Participants Diagnosed with Aromatic L Amino Acid Decarboxylase Deficiency (AADC-d) With or Without Treatment with Upstaza (Eladocagene Exuparvovec) (PTC-AADC-MA-406) (AADCAchieve). • AADC-1602 Long-term follow-up study for existing patients
Missing information: Long-term safety (>10 years)	**Routine risk minimisation measures:** *Prescription only medicine* **Additional risk minimisation measures:** *None*	**Additional pharmacovigilance activities:** **Studies imposed as Specific Obligation under exceptional circumstances** • A Two-Part Registry of Participants Diagnosed with Aromatic L Amino Acid Decarboxylase Deficiency (AADC-d) With or Without Treatment with Upstaza (Eladocagene Exuparvovec) (PTC-AADC-MA-406) (AADCAchieve). • AADC-1602 Long-term follow-up study for existing patients
Missing information: Use in children ⩽18 months old	**Routine risk minimisation measures:** *SmPC sections 4.2* *Prescription only medicine* **Additional risk minimisation measures:** None	**Additional pharmacovigilance activities**: **Studies imposed as Specific Obligation under exceptional circumstances** • A Two-Part Registry of Participants Diagnosed with Aromatic L Amino Acid Decarboxylase Deficiency (AADC-d) With or Without Treatment with Upstaza (Eladocagene Exuparvovec) (PTC-AADC-MA-406) (AADCAchieve). • AADC-1602 Long-term follow-up study for existing patients

2.7.5. Conclusion

The CAT considers that the risk management plan version 1.0 is acceptable.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP and CAT considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Labelling exemptions

A request for a translation exemption of the labelling as per Art.63.1 of Directive 2001/83/EC has been submitted by the applicant proposing that the details listed in Article 54 appear in only one official language (English) on all packaging components (vial and outer carton). The main ground of the justification was the low estimated number of patients treated per country due to the low incidence/prevalence of the condition in the EU, and the fact that the medicinal product will not be delivered directly to the patient for self-administration

The QRD Group partially accepted the translation exemption for the use of English only labels on the immediate packaging (vial), but the Group requested that the outer carton have dual language English(EN)/Germany(DE) labelling as it was noted that a high proportion of the estimated patients to be treated, would be German speaking, therefore dual DE/EN would be preferable to EN only.

In addition, the applicant requested an exemption for omission of particulars on immediate packaging (vial label) under Art. 63(3) proposing that the expiry date only be included on the outer carton label, and not on the vial label. The main grounds of the justification were: individual packaging of the vial in carton providing sufficient information of the expiry period to the health care professionals, the distribution and shipment handled at patient-level, as well as limited amount of long-term stability data available at the time of the MAA submission.

The QRD Group conditionally approved the labelling exemption for the expiry date to only be included on the outer carton, and not on the vials, as long as the applicant accepts to include on the outer carton the warning 'Keep the vial in the carton until use'.

2.9.3. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Upstaza (eladocagene exuparvovec) is included in the additional monitoring list as it includes new active substance and is approved under exceptional circumstances.

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

AADC deficiency is a rare autosomal recessive disorder of dopaminergic and serotonergic pathways (Wassenberg 2017). AADC deficiency is due to the presence of pathological variants in the DDC gene that encodes for AADC, the enzyme responsible for the decarboxylation of L-DOPA and 5-HTP resulting in the production of dopamine and serotonin, respectively (Hyland 1990, Hyland 1992). AADC deficiency causes a marked or complete loss of dopamine production in the brain from birth. Consequently, patients with AADC deficiency have arrested motor development despite essentially preserved neurophysiology and neuroanatomy as determined by brain imaging. Most patients with AADC deficiency do not develop functional motor movement, fail to achieve motor milestones (e.g., full head control and the ability to sit, stand, and walk), and are at risk of an early death in the first decade of life (Hwu 2012). Consequently, patients with AADC deficiency require life- long care. The global incidence of AADC deficiency is not well described in the literature. The predicted birth rate of individuals with AADC deficiency is estimated to be between 1/42,000 to 1/90,000 in the US (Hyland 2018, Whitehead 2018, Himmelreich 2019), approximately 1/118,000 in the EU, and 1/182,000 in Japan (Himmelreich 2019). These birth rates translate into a current estimate of about 840 living patients with AADC deficiency in the US, 853 in the EU, and 125 in Japan (Himmelreich 2019).

The aim of treatment with Upstaza is to replace the enzyme aromatic L-amino acid decarboxylase in the putamen and allow production of dopamine and consequently motor development.

3.1.2. Available therapies and unmet medical need

No therapies are presently approved for the treatment of AADC deficiency. Existing therapies are primarily intended to treat symptoms and do not treat the underlying cause of the disease. Response to Dopaminergic agents has been described in some subjects (Wassenberg 2017). The majority of patients, particularly those with no motor development, do not respond to available treatments because these therapies cannot replace or increase dopamine production in the brain to adequately improve motor function and allow achievement of developmental milestones (Brun 2010, Wassenberg 2017). The lack of effective treatment clearly indicates that therapies to treat AADC deficiency are urgently needed to provide sustained and clinically meaningful improvement of motor development and function.

3.1.3. Main clinical studies

There were three trials submitted in support of the marketing authorisation application. All trials were unblinded single arm, single centre investigator led-studies. All studies took place in Taiwan. Historic control data from published studies was used as a comparator. The population studied were children between the ages of 18 months and 8 years and 6 months. All subjects had the severe phenotype of AADC deficiency and all subjects had a genetically confirmed diagnosis of AADC deficiency with at least

2 mutations in the AADC gene. The majority of subjects in all trials were homozygous for the founder mutation of the AADC gene.

Eladocagene exuparvovec was administered to 28 children with AADC deficiency, using an established stereotactic neurosurgical procedure:

- AADC-CU/1601 is completed. Eight patients were treated and followed for 5 years. All subjects in this trial were treated using process A material.
- AADC-010 is ongoing. Ten patients were treated and are being followed for 5 years. All patients were treated with process B material.
- AADC-011 is ongoing. Eight patients have been treated to date. All patients were treated with process B material
- An additional 2 patients received treatment by the end of 2019. These patients were included in the FAS for subjects treated with process B material.

3.2. Favourable effects

In the population studied, the favourable affects were achievement of motor milestones & improvement in motor function. At the time point of initial submission the primary efficacy analysis for all patients treated in the ITT population 9/18 patients achieved full head control and 7 of these were also able to sit unassisted. In contrast, no natural history control patients achieved these milestones. Two 2 (11.1%) were able to stand with support by 24 months.

At the data lock time point, which occurred after 24 months, 12/18 patients had full head control, 9 were able to sit unassisted, 3 could stand with support & 1 could walk with assistance.

The secondary efficacy endpoints showed a similar trend. All studies showed similar trends in improvement in motor milestones, PDMS-2 & AIMS scales.

All subjects showed an increase in PDMS-2 score over time and this increase was greater for subjects treated at a younger age. The mean change in baseline PDMS-2 score at 24 months was 94.3 (95% CI 75.6-112.9). The scores continued to increase to 60 months. The change in score reflected increases in scores for grasping, locomotion, object manipulation and visual-motor integration. The AIMS score showed a similar trend with all subjects achieving an increase in score after treatment & a mean increase at 24 months of 21.9 (95% CI 16.1-27.6).

There was an improvement in dystonia and stimulus provoked dystonia in all subjects.

Following gene therapy, all but 1 subject (95.8%) maintained or gained weight relative to age- and gender-matched control children at 12 months after receiving gene therapy.

In the updated analysis of efficacy (including all subjects treated from process A and process B material), using an updated historic comparator natural history database set up by the applicant to contextualise the results, the following results were reported.

At 24 months post treatment, of 22 subjects (14) 64% have achieved full head control (emerging or mastery of milestone), (11) 50%sitting without the need for additional support (emerging or mastery of milestone), (3) 14% had achieved crawling (emerging or mastery of milestone) with (4) 18% achieving standing with support (emerging or mastery of milestone) and no subject had mastered walking by 24 months. These percentages increase when emerging skills or subjects who have not fully but partially mastered the skill are counted.

At 60 months post treatment, there is data available for only 12 subjects: (9) 75% of subjects had full head control 5 years after treatment, with (8) 67% retaining the ability to sit unassisted, (3) 25% standing with support and (2) 18% walking with assistance. Again, the number of responders increases when children who partially mastered a skill are counted. The number of responders also increases when other milestones not included in the primary endpoint are assessed, including ability to crawl and take steps.

In a further update, 28 subjects treated in the CT programme, at 2 years, the time point of the primary efficacy analysis 64% (18) subjects demonstrated emerging or complete head control. Fifty per cent, 50% (14) were able to sit unassisted and 18% (5) had an emerging or complete mastery of standing with support.

Motor development continued after the 2 year time-point and at the last integrated efficacy assessment, by 60 months, 79% (22) subjects had acquired head control, 68% (19) were able to sit unassisted, 29% (8) could stand with support and 11% (3) could walk without assistance. Given the baseline motor function and age of these subjects, by 60 months the majority of children had achieved a significant milestone i.e. were able to sit unassisted.

The applicant also presented additional benefit observed in subjects treated. There was an observed decrease in the annualized rate of upper respiratory tract infections and pneumonia. However, the data is difficult to interpret without knowing the baseline rate of infection in this population.

3.3. Uncertainties and limitations about favourable effects

Uncertainties include the size of the dataset in the clinical trial programme.

There was no efficacy or safety data to directly support an indication in adolescents or in children less than 18 months of age. All studies were unblinded single arm studies. The comparator dataset was taken initially from a publication where all of the characteristics of the control population are not known. Acknowledging the limitations of the use of these publications and the lack of data provided about the subjects with AADC the applicant established a natural history database for AADC.

The applicant presented a comparison between 49 subjects with a similar phenotype from the NHDB and the study population. There may be inaccuracies and missing data inherent in the publications but this is a review of the best available data.

There is limited safety or efficacy data from patients treated with the commercial product. The comparability exercise conducted between product manufactured using process A and process B, was not conclusive. Therefore, the clinical data from patients treated with process B product is considered pivotal with data from the process A material as supportive.

There is a high number of empty capsids present in the commercial product - given the limited clinical data it is difficult to conclude or exclude that the presence of the high number of empty capsids in the product could have an effect on safety or efficacy. During the course of the evaluation, the applicant presents preliminary data on 2 subjects in Europe that have received the commercial product. From a safety perspective, the safety profile reported is similar to that presented in the clinical trials. There was evidence of improvement in neurological symptoms of dystonia and improved head control in subjects treated in Europe with the commercial product. However, uncertainties remain about the efficacy and safety of the commercial product or whether the quality differences observed in the commercial product could impact clinical outcomes.

At the 2-year post-treatment time point, the benefit observed in the majority of subjects was an improvement in head control. Approximately, 50% of subjects did achieve independent sitting at 2

years/24 months post-treatment. It is not possible to identify subjects who may respond better to treatment. Motor development continues after 2 years.

There was no beneficial effect observed with treatment on the autonomic or serotonergic symptoms of AADC.

The clinical relevance of the observed effects and how they relate to improved quality of life for patients is not entirely clear. The applicant cited recent literature and results of a survey of AADC caregivers that indicates that improved motor control would reduce the burden from their perspective. A summary of a limited retrospective survey carried out by the principle investigator of the clinical trials conducted also supports this view. These observed benefits must be viewed in the context of the limitations to the data, risks and potential risks of the active & the neurosurgery involved in the administration.

There is uncertainty regarding whether a different route of administration (i.e. into midbrain) would result in improved efficacy.

3.4. Unfavourable effects

Twenty-six 26 (100%) of subjects reported an adverse event.

Twenty-five 25(96%) reported a pyrexia after treatment. 15(57.7%) reported an upper GI haemorrhage & 18 subjects (69.2%) were diagnosed with an upper respiratory tract infection.

Adverse events attributed to the active were dyskinesia, which was the most commonly occurring adverse event considered related to treatment and occurred in 23(88.5%) of subjects.

Initial insomnia was reported in 4 subjects (19%), 2 (7.7%) salivary hypersecretion, 2(7.7%) a feeding disorder and 1 (3.8%) sleep disorder.

Adverse events related to the neurosurgery included cerebrospinal fluid leakage in 3 subjects (11.5%), hypotension 6(23.1%), endotracheal intubation complication, post-operative skull defect, skin injury, subcutaneous haematoma 1 (3.8%) each.

Eighteen patients (69.2%) of subjects had at least 1 positive antibody titre within the first 12 months.

There were adverse events and reactions related to the anaesthesia and surgical procedure identified in the response to the day 120 LOQ. Section 4.8 of the SmPC has been updated with these ADRs/AEs. Many patients experienced serious symptoms of their underlying disease after treatment. The SmPC has been updated to reflect this.

3.5. Uncertainties and limitations about unfavourable effects

The dataset is small and may not reflect all possible adverse reactions and events. There is limited data on long-term safety post treatment. There is very limited safety data available from patients treated with the commercial manufactured product. There is limited safety data available on the intraparenchymal use of the novel excipient in humans. There is no data available about immune response to the transgene itself nor the cell-mediated immune response to the virus. The method of application involves a surgical procedure of high risk and the risks may vary between centres, surgeons and patients. Many subjects experienced serious adverse events considered related to the underlying disease. These events may have been exacerbated by the anaesthetic and procedure required for administration of the active.

Effects Table for key motor milestones achieved (FAS-Subjects treated with Process B product) total population treatedData-lock 26th February 2020 N=20

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
Head Control Datalock point	Motor milestone assessment according to the Peabody Developmental Motor Scale second edition	N(%)	 14(70%)		Comparator population taken from published study of historic controls. Cannot be the basis for statistical claims due to the small population and historic comparator	
Sitting un-Assisted Datalock point			 13(65%)			

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Standing with Support Datalock point **Walking with support** Datalock point **Dystonia & Stimulus pro-voked dystonia** Month 12			 6(30%) 2(20%) 0(0%)		P unknown	
Unfavourable Effects						
Pyrexia **Dys-kinesia**			19(95%) 16(80%)		All events considered related & occurred within the first 12 months. All resolved within 7 months of onset	
Psy-chiatric Disorders-Sleep Disorder			4(20%)		Initial sleep disorder & insomnia	

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Surgery related Adverse events			5 subjects had 8 events		Endotracheal intubation failure Subcutaneous hematoma Brain oedema CSF leaks Potential intraparenchymal haemorrhage	

3.6. Benefit-risk assessment and discussion

3.6.1. Importance of favourable and unfavourable effects

The most important favourable effects were the achievement of motor milestones.

The most common motor milestone achieved was head control. Head control is considered an important developmental milestone in children. Achievement of head control improves postural control, visual orientation & may be the first step prior to the development of other milestones. Better head control may allow for improved ability to eat & reduce the risks of aspiration and respiratory infections by increasing the ability to clear secretions. Achievement of head control is of benefit to children with severe AADC but the benefit must be weighed against the risks of surgery.

The second most common milestone achieved was ability to sit unassisted. Sitting unassisted improves independence, quality of life, improves respiration, and reduces secretion accumulation and risk of respiratory infections. Ability to sit unassisted may also reduce the risk of gastro-oesophageal reflux.

The number of children who achieved milestones increased with time and when patients from all 3 trials had reached the time point of the primary efficacy endpoint were included. The applicant provides some publications, which support an improved quality of life for children treated. Caregivers were surveyed and expressed their view that achieving motor milestone would reduce the care burden for them and improve quality of life for both caregivers and children with the disease.

All subjects had an improvement in dystonia and stimulus provoked dystonia but few subjects had an improvement in OGC episodes. These neurological symptoms have a significant impact on the patient's quality of life. Further analysis of the time and frequency of OGC episodes in the updated analysis showed a modest reduction in time with an OGC episode and a modest reduction in frequency of OGC episodes in children treated. It is not clear how significant these improvements are to the patient's quality of life.

The safety & efficacy of the commercial product is not known as there is limited clinical data available from subjects treated with the commercial product. The quality comparability exercise conducted to determine whether there were any differences in quality of the products manufactured by process A, B and the commercial process C was limited by the amount of material available from product manufactured using process A and B. The main difference in quality characteristics of the commercial product appears to be an increase in the number of empty capsids. The applicant has presented comparable immunogenicity, safety and efficacy data from subjects treated with material from process A and Process B, where there is a greater difference in empty capsid number, to support an expected

comparable efficacy and safety profile for the commercial product. However, as the comparability exercise conducted between product manufactured using process A and process B, was not conclusive, clinical data from patients treated with process B product is considered pivotal with data from the process A material as supportive.

The most frequent adverse reactions reported related to the active was dyskinesia, which although troublesome is an indication that the active is working and that dopamine is being produced in the brain. However, dyskinesia can be prolonged in some subjects.

The long terms risks of intraputaminal administration of AAV are unknown.

The risks of surgery & anaesthesia, which occurred, were those that can be expected with this type of surgery. Adverse events and reactions associated with surgery and anaesthesia can be serious and are of concern particularly in patients with AADC. Surgery may also increase the risk of occurrence of adverse events associated with the disease itself. Other potential risks of surgery include intracranial haemorrhage, infection, and death. The risk of surgical complications can vary with operator experience and the active should only be administered in centres experienced with stereotactic neurosurgery and by appropriately trained staff.

3.6.2. Balance of benefits and risks

Given the severe nature of the disease and the absence of effective treatments, there are clinically significant benefits observed for some subjects treated with Upstaza.

However, the efficacy and safety data set available and population treated in the clinical trial programme is limited to 28 children between the ages of 18 months to 8 years. Uncertainties remain relating to the small size of the population and efficacy and safety data, the restricted impact of treatment on other symptoms of the disease, the paucity of data relating to the impact of treatment on quality of life, the limited data relating to the efficacy and safety of the commercial product, the lack of demonstration of comparability between process A and process B product and the clinical relevance, if any, of the high percentage of empty capsids in the commercial product.

The applicant argued that the indication could be extended to subjects with some motor milestones and to those with a more severe phenotype requiring ventilation, with contractures and with no motor movement. There was no new clinical data using the product presented to support the extension of indication. The applicant supported the extension of indication with publications using a different vector/active.

Considering the uncertainties and as this is the first adeno-associated viral vector gene therapy product that could be authorised for intraputaminal administration, an expert group has been convened to provide advice. Despite the small population studied in the clinical trials and the uncertainties as previously outlined in the assessment report, the experts were positive about the clinical relevance of the motor benefits received in patients. They considered that children with severe disease with head control could benefit from treatment. They considered that children with severe disease, no motor movements and requiring ventilation may benefit from treatment. They did not identify any specific patient characteristics to identify children that may benefit more from treatment. They considered that the safety profile was generally acceptable. However, it is possible that the number of adverse events related to the surgical procedure could increase once more centres are allowed to administer Upstaza. The experts have recommended that comprehensive training and supervision program should be developed. A close follow-up to characterise long term safety and efficacy (preferably integrated in the INTD registry) is also expected.

Benefits observed in children treated with Upstaza are considered clinically relevant by clinical experts involved in treating patients with AADC. The indication has been updated to reflect the expert opinion. Considering the unmet need, the motor benefits observed in children up to the age of 19 (treated with a similar gene therapy using a similar dose injected intra-putaminally), the CAT considered that the indication for treatment could be extended to adults.

3.6.3. Additional considerations on the benefit-risk balance

To evaluate long-term efficacy and safety (as missing information) of the commercial product a post-authorisation study is requested, which should include the relevant efficacy endpoints such as motor behavioural development, as well as safety endpoints.

The ongoing long-term follow-up PASS study of subject treated in the clinical trial programme should be completed and results submitted.

Marketing authorisation under exceptional circumstances

As comprehensive data on the product are not available, a marketing authorisation under exceptional circumstances was proposed by the CAT during the assessment, after having consulted the applicant.

The CAT considers that the applicant has sufficiently demonstrated that it is not possible to provide comprehensive data on the efficacy and safety under normal conditions of use, because the applied for indication is encountered so rarely that the applicant cannot reasonably be expected to provide comprehensive evidence. Therefore, recommending a marketing authorisation under exceptional circumstances is considered appropriate.

The CHMP endorses the CAT conclusion on marketing authorisation under exceptional circumstances as described above.

3.7. Conclusions

The overall benefit/risk balance of Upstaza is positive, subject to the conditions stated in section 'Recommendations'.

The CHMP endorse the CAT conclusion on Benefit Risk balance as described above

4. Recommendations

Outcome

Based on the CAT review of data on quality, safety and efficacy, the CAT considers by consensus that the benefit- risk balance of Upstaza is favourable in the following indication(s):

Upstaza is indicated for the treatment of patients aged 18 months and older with a clinical, molecular, and genetically confirmed diagnosis of aromatic L-amino acid decarboxylase (AADC) deficiency with a severe phenotype (see section 5.1).

The CAT therefore recommends the granting of the marketing authorisation under exceptional circumstances subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (see Annex I: Summary of Product Characteristics, section 4.2).

Based on the draft CHMP opinion adopted by the CAT and the review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit- risk balance of Upstaza in the treatment of aromatic L-amino acid decarboxylase (AADC) deficiency is favourable and therefore recommends the granting of the marketing authorisation under exceptional circumstances subject to the following conditions:

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

- **Additional risk minimisation measures**

Prior to the launch of Upstaza in each Member State, the MAH must agree about the content and format of the educational material (ie, Surgical Guide and Pharmacy manual), including communication media, distribution modalities, and any other aspects of the programme, with the National Competent Authority.

The MAH should ensure that Upstaza is distributed to selected treatment centres performing the administration of the product where qualified staff will have been delivered with educational materials, including the Upstaza Surgical Guide and the Pharmacy manual.

The treatment centres will be selected based on the following criteria:

- Presence of or affiliation with a neurosurgeon experienced in stereotactic neurosurgeries and capable of administrating Upstaza;
- Presence of a clinical pharmacy capable of handling and preparing adeno-associated virus vector-based gene therapy products;
- Ultra-low temperature freezers ($\leqslant$ -65 ºC) available within the treatment centre pharmacy for treatment storage.

Training and instructions for safe handling and disposal of affected materials for 14 days following product administration should also be provided along with information regarding exclusion from donation of blood, organs, tissues, and cells for transplantation after Upstaza administration.

The qualified staff (ie, neurologists, neurosurgeons, and pharmacists) at the treatment centres should be provided with educational materials including:

- Approved Summary of Product Characteristics.
- Surgical education for Upstaza administration, including description of required equipment, and materials and procedures needed to perform stereotactic administration of Upstaza. The Upstaza Surgical Guide aims at ensuring correct use of the product in order to minimise the risks associated with the administration procedure including cerebrospinal fluid leak.
- Pharmacy education including information on Upstaza receipt, storage, dispensing, preparation, return and/or destruction, and accountability of product.

Prior to scheduling the procedure, a PTC Therapeutics representative will review the Upstaza Surgical Guide with the neurosurgeon and the Pharmacy manual with the pharmacist.

Patients and their caregivers should be provided with the following materials, including:

- Patient Information Leaflet, which should also be available in alternative formats (including large print and as audio file).
- A patient alert card to
  - Highlight the precautionary measures to minimise the risk of shedding.
  - Highlight importance of follow-up visits and reporting side effects to the patient's physician.
  - Inform healthcare professionals that the patient has received gene therapy, and the importance of reporting adverse events.
  - Provide contact information for adverse event reporting.

The CHMP does endorse the CAT conclusion on the additional risk minimisation measures.

- **Obligation to conduct post-authorisation measures**

The MAH shall complete, within the stated timeframe, the below measures:

Description	**Due date**
In order to further assess process consistency and maintain patient's safety, the applicant shall provide the results of the next active substance and next finished product concurrent process validation batches, including hold time data for the finished product batch. This data should be provided by March 2023.	March 2023

The CHMP endorses the CAT conclusion on the obligation to conduct post-authorisation measures as described above.

Specific Obligation to complete post-authorisation measures for the marketing authorisation under exceptional circumstances

This being an approval under exceptional circumstances and pursuant to Article 14(8) of Regulation

(EC) No 726/2004, the MAH shall conduct, within the stated timeframe, the following measures:

Description	Due date
Study AADC-1602 (Follow-up of clinical trials): In order to further characterise the long-term efficacy and safety of Upstaza in patients with aromatic L amino acid decarboxylase (AADC) deficiency and with a severe phenotype, the MAH shall submit the results of study AADC-1602, a 10-year follow-up of the patient population enrolled in the clinical studies AADC-CU/1601, AADC-010 and AADC-011.	Annual submission at each annual renewal Final report: 30 June 2030
Study PTC-AADC-MA-406 (Registry-based study) In order to further characterise the long-term efficacy and safety of Upstaza in patients with aromatic L amino acid decarboxylase (AADC) deficiency and with a severe phenotype, the MAH shall conduct and submit the results of study PTC-AADC-MA-406, an observational, multicentre and longitudinal study of patients treated globally with the commercial product, based on data from a registry, according to an agreed protocol.	Annual submission at each annual renewal

The CHMP endorses the CAT conclusion on the specific obligation to complete post-authorisation measures for the conditional marketing authorisation under exceptional circumstances as described above.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

New Active Substance Status

Based on the review of available data on the active substance, the CAT considers that eladocagene exuparvovec is to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union.

The CHMP endorses the CAT conclusion on the new active substance status claim.

5. Appendix

5.1. CAT/CHMP AR on new active substance dated 12 May 2022