Phase I/IIa Systemic Gene Delivery Clinical Trial of scAAV9.U7.ACCA for Exon 2 Duplication-Associated Duchenne muscular dystrophy

---

CLINICAL PROTOCOL

Version: V4.0 (18Nov2021)

Sponsor-Investigator: Megan Waldrop, M.D.

Co-Investigators: Kevin M. Flanigan, M.D.
Kim McBride, M.D.
Nicolas Wein, Ph.D.
Ramkumar Krishnamurthy, Ph.D.
Linda P. Lowes, Ph.D.
Maryann Kaler, FNP

The Research Institute at Nationwide Children's Hospital

Center for Gene Therapy

SIGNATURE PAGE

Study Title:	Phase I/IIa Systemic Gene Delivery Clinical Trial of scAAV9.U7.ACCA for Exon 2 Duplication-Associated Duchenne muscular dystrophy
Study Sponsor	Megan A. Waldrop, MD
Financial Sponsor	Audentes Therapeutics
Protocol Version	v4.0 18Nov2021

As Sponsor-Investigator, I agree to personally conduct this study in compliance with Principles of Good Clinical Practice as defined by federal, state, and local laws and regulations. I will abide by the current protocol (provided herein), including any protocol amendments that are approved by our institutional review board (IRB).

_____

Megan A. Waldrop, MD
Sponsor-Investigator
Nationwide Children's Hospital

TABLE OF CONTENTS

List of Tables

1 PROTOCOL SYNOPSIS

Name of Sponsor	Megan A. Waldrop, M.D.
Name of Investigational Product	scAAV9.U7.ACCA
Study Title	Phase I/IIa Systemic Gene Delivery Clinical Trial of scAAV9.U7.ACCA for Exon 2 Duplication-Associated Duchenne muscular dystrophy
Study Number	N = 1-3, 3.0 x 10^{13} vg/kg
Sponsor Investigator	Megan A. Waldrop, M.D.
Clinical Study Phase	Phase I/IIa
Number of Centers	Single site (Nationwide Children's Hospital)
Study Duration	We will evaluate short-term safety over a two-year period. Subjects will be evaluated at screening, during gene transfer, and for return follow up visits on days 7, 14, 30, 60, 90, 180 and months 12, 18 and 24. Additional immune studies will continue with blood samples collected locally and shipped to NCH at days 45, 75, 120, 150, and two weeks following steroid dose reduction.
Study Design	Open-label study of scAAV9.U7.ACCA
Subject Population	**Inclusion Criteria:** 1. Age greater than 6 months and less than 14 years 2. Confirmed duplication of exon 2 in the *DMD* gene using a clinically accepted technique that completely defines the mutation 3. Pre-ambulant (not yet walking) or ambulant (as defined by the ability to walk 10 meters without assistance) 4. Males of any ethnic group will be eligible 5. Ability to cooperate with muscle testing 6. In subjects age 4 and above, stable dose and regimen of corticosteroid therapy (prednisone, deflazacort, or their generic forms) for at least 12 weeks prior to gene transfer. **Exclusion Criteria:** 1. Active viral infection based on clinical observations 2. Symptoms or signs of cardiomyopathy, including: a. Dyspnea on exertion, pedal edema, shortness of breath upon lying flat, or rales at the base of the lungs b. Echocardiogram with ejection fraction below 40% 3. Serological evidence of HIV infection, or Hepatitis B or C infection 4. Diagnosis of (or ongoing treatment for) an autoimmune disease 5. Persistent leukopenia or leukocytosis (WBC ≤ 3.5 K/μL or ≥ 20.0 K/μL) or an absolute neutrophil count < 1.5K/μL

	6. Concomitant illness or requirement for chronic drug treatment that in the opinion of the SI creates unnecessary risks for gene transfer 7. AAV9 binding antibody titers ≥ 1:400 as determined by ELISA immunoassay 8. Abnormal laboratory values in the clinically significant range as listed in Table 7, based upon normal values in the Nationwide Children's Hospital Laboratory.
Study Procedures	The vector will be delivered via peripheral limb vein. Study subjects will receive sedation if deemed necessary for the procedure following Nationwide Children's Hospital dosing protocol.
Primary Outcome	Safety is the primary outcome for this clinical gene transfer trial.
Secondary Outcomes	We propose the following secondary efficacy outcome measures in leg muscles, each in comparison to baseline values: • Expression of dystrophin as demonstrated by immunofluorescent staining with a dystrophin C-terminus antibody and the exon-1 encoded epitope antibody (Manex 1A) in muscle biopsy sections. • Dystrophin protein expression quantified by western blot using a dystrophin C-terminus antibody and the exon-1 encoded epitope antibody (Manex 1A) and assessed by densitometry in muscle biopsy tissue. • Percentage of exon 2 exclusion from the dystrophin mRNA transcript as measured by RT-PCR analysis. • Transduction efficiency will be measured by qPCR of a U7-targeting sequence primer pair amplifying the DNA containing the U7snRNA, and expressed as vector genomes normalized to a single-copy genomic DNA control in each muscle sample.
Exploratory Outcomes	We propose the following exploratory clinical outcome measures, each in comparison to baseline values: 1) For Muscle Strength: • In subjects enrolling at 36 months of age or younger: improvement or stabilization of Bayley-IV scores (Bayley Scales of Infant and Toddler Development) • In subjects enrolling at greater than 36 months of age but younger than 5 years: improvement or stabilization of the North Star Ambulatory Assessment (NSAA) score, time to climb four stairs and amount of time to walk 100 meters (100MWT) • In subjects enrolling at age 5 years or greater: improvement or statistically significant stabilization in: o North Star Ambulatory Assessment (NSAA) score o Amount of time required to walk 100 meters (100MWT) o Time to climb four stairs

	○ Force generated in knee flexion and extension in maximum voluntary isometric contraction (MVICT). 2) As an exploratory efficacy measure, muscle MRI will be assessed using T2-weighted multi-slice spin echo (SE) axial images and single voxel 1H-MRS from both the lower leg and thigh to measure lipid fraction and (1)H_2O T2 signal. These will be performed at baseline and at 6, 12, 18 and 24 months post infusion and data will be assessed using published techniques[1] and compared to baseline in collaboration with Dr. Glenn Walter, University of Florida. *An MRI will not be performed if sedation is required. See Section 10.2 for more details. 3) Percentage of exon 2 exclusion from the dystrophin mRNA transcript as measured by RNA-Seq analysis.
Sample Size	DMD subjects will receive scAAV9.U7.ACCA at the minimal efficacious dose (MED). N = 1-3, 3.0×10^{13} vg/kg
Statistical Analysis	For the secondary outcome measure of dystrophin expression, quantification of the number of fibers expressing dystrophin (determined by quantification of the number of fibers), measurement of the intensity of dystrophin staining at the sarcolemma (based on quantitative image analysis), and the degree of exon 2 skipping (as determined by RT-PCR) will be performed for each patient at each time point in a blinded fashion, and intra-patient comparisons of pre- and post-treatment values will be analyzed using repeated measures ANOVA with a Dunnett correction for multiple comparisons vs. baseline. For detecting change, we will have 80% power to detect a very large effect size (SMD=1.4-3.3, for sample sizes from 3-6).
Long-term follow-up	Safety follow-up will continue over a two-year period that incorporates the active phase of the protocol. Subjects will then transfer to an annual monitoring program where data will be collected from annual standard of care visits for up to 5 years.

2 ADMINISTRATIVE INFORMATION

2.1 Document History

Table 1: **Document Record**

Document	Version and Date	Submission Purpose
U7.ACCA Clinical Protocol	DRAFT - v0.7 15Jan2019	FDA Initial Submission S/N 0000
U7.ACCA Clinical Protocol	v1.0 25Oct2019	Response to FDA hold S/N 0001
U7.ACCA Clinical Protocol	v1.0 13Dec2019	Changes requested by the DSMB, sent to FDA as S/N 0002
U7.ACCA Clinical Protocol	V2.0 20Jan2020	Protocol and Consent Changes
U7.ACCA Clinical Protocol	V3.0 25Mar2020	Protocol and Consent changes requested by the DSMB
U7.ACCA Clinical Protocol	V4.0 18Nov2021	Protocol and Consent Changes

2.2 Summary of Changes

The section below highlights content changes in this version of the protocol. Language deleted from the previous version of the protocol appears in ~~red/strikethrough~~. Language added to the previous version of the protocol appears in **bold**.

Table 2: **Revision Record**

Protocol Version	Description of Changes
V1.0 25Oct2019	• ~~Removed control group and dose escalation from trial.~~ • **Added RT-PCR measurement of exon 2 exclusion from dystrophin mRNA transcript to secondary outcomes.** • Moved RNASeq analysis of exon 2 exclusion from dystrophin mRNA transcript to exploratory outcomes. • **Changed inclusion criteria to greater than 6 months and less than 14 years.** • ~~Removed Western Blot analysis at days 90 and 180 as well as 12 months for dystrophin associated protein complex.~~ • ~~Removed Leukocyte marker analysis at days 90, 180, and 12 months.~~ • **Added language to indicate report of non-life threatening SAEs and DLTs to the IRB within 15 calendar days.** • **Added possibility of moving blood tests to other days of the visit if the child is under 10kg and may have too much blood drawn.**

	• **Added language to clarify the expected elevations in AST/ALT levels for the DMD population.** • **Clarified that Dose-Limiting Toxicity events must be unexpected to be considered as such.** • **Added Hepatology to the fields of expertise of the DSMB members.** • **Added language to allow for weekly blood tests past 30 days post-infusion if the subject's AST/ALT levels have not returned to baseline and the prednisone/prednisolone dose is maintained rather than decreased back to the standard clinical dose.** • ~~Removed specification of the antibody used for immunofluorescence staining and western blot.~~ • Updated CTCAE referenced to v5.0. • Changed Megan Waldrop from ~~Principal~~ Investigator to **Sponsor** Investigator. • Changed the N from N=3 to N=1-3.
V1.0 13Dec2019	• **Added labs for Total IgG, D-Dimer, Direct Bilirubin, SC5b-9, C3, and C4 at screening** • **Added Total IgG at Day 30, 60, 90, 120, 150, 180, Month 12, Month 18, Month 24** • **Added Direct Bilirubin to Day -1, 1, 7, 14, 30, 45, 60, 75, 90, 2 weeks post steroid, 120, 150, 180, Month 12, Month 18, Month 24** • ~~Removed redundancies in the Safety Reporting section.~~ • **Added Nicolas Abreu, Linda Lowes, Ramkumar Krishnamurthy, and Cassandra Karingada as Co-Investigators.**
V2.0 20Jan2020	• **Clarified Physical therapy testing schedule. Added climb 4 stairs test and 100MWT to 3 to 5 year olds. Added PFT testing to begin at age 5 years.** • ~~Removed PT functional testing assessment age related change~~ • **Clarified timing of screening blood work** • **Added language for addition and discontinuation of proton pump inhibitor (PPI) with increased prednisolone dosage.** • **Clarified timing of vital sign monitoring during gene transfer with and without sedation.** • **Added Maryann Kaler as Co-Investigator.**

V3.0 25Mar2020	• **Added CH50 lab test to Screening labs** • **Added C3, C4, Ch50, and Sc5b-9 to Day 7 labs** • Changed volume of BD Luer-Lok used for investigational product for gene transfer **to 50mL** ~~from 60mL~~ (60mL no longer available) • Changed vitals monitoring post gene transfer to **every 15 minutes for 4 hours post and then hourly until transfer to the Neurology floor, then every four hours until discharge**.
V4.0 18Nov2021	• ~~Removed Cassandra Karingada, Nicolas Abreu, and Johan Harris.~~ • **Added Sharmada Subramanian and Kristen Brown.** • Updated IQVIA monitor information. • Changed exploratory functional outcome for subjects under 36 months of age from ~~Bayley-III~~ to **Bayley-IV.** • **Added visit windows for follow up visits.** • **Clarified how the total vector genome dose will be rounded based on subject weight.**

2.3 Contact Information

Table 3: **Study Team**

Role in Study	Name	Address and Telephone Number
Sponsor Investigator	Megan Waldrop, MD	WA3013 700 Children's Dr Columbus, OH 43205 Ph: 614-722-2231 megan.waldrop@nationwidechildrens.org
Co-Investigator	Kevin Flanigan, MD	WA3011 700 Children's Dr Columbus, OH 43205 Ph: 614-722-2231 Kevin.flanigan@nationwidechildrens.org
Co-Investigator	Kim McBride, MD	700 Children's Dr Columbus, OH 43205 Ph: 614-355-5734 Kim.mcbride@nationwidechildrens.org
Co-Investigator	Ramkumar Krishnamurthy, PhD	700 Children's Dr Columbus, OH 43205 Ph: 614-722-2611 Ramkumar.Krishnamurthy@nationwidechildrens.org
Co-Investigator	Nicolas Wein, PhD	WA3020 700 Children's Dr Columbus, OH 43205 Ph: 614-722-2678

		Nicolas.wein@nationwidechildrens.org
Co-Investigator	Linda P. Lowes, Ph.D.	700 Children's Dr Columbus, OH 43205 Ph: 614-722-2849 Linda.lowes@nationwidechildrens.org
Co-Investigator	Maryann Kaler, FNP	WA2022 700 Children's Dr Columbus, OH 43205 Ph: 614-355-3572 Maryann.kaler@nationwidechildrens.org
Program Manager	Tabatha Simmons, PhD	WA2015 700 Children's Dr Columbus, OH 43205 Ph: 614-722-6921 Tabatha.simmons@nationwidechildrens.org
Clinical Research Coordinator	Sharmada Subramanian	700 Children's Drive Columbus, OH 43205 Ph: 614-722-2231 Sharmada.Subramanian@nationwidechildrens.org
Clinical Research Coordinator Team Lead	Kristen Brown	WA2014 700 Children's Dr Columbus, OH 43205 Ph: 614-722-6918 Kristen.Brown@nationwidechildrens.org
Regulatory Specialist	Rachel Manthe-Gross, PhD	W178 700 Children's Dr Columbus, OH 43204 Ph: 614-355-2681 Rachel.manthe-gross@nationwidechildrens.org

Table 4: **Data Safety Monitoring Board**

Name	**Address and Telephone Number**
Edward C. Smith, M.D.	Duke University Medical Center 3000 Erwin Road Durham, NC 27705 Ph: 919-668-0477 Edward.smith@duke.edu
Barry J. Byrne, M.D., Ph.D.	PO Box 100296 Gainesville, FL 32610-0296 Ph: 352-294-8968 Barry.byrne@ufl.edu
Arash Grakoui, Ph.D.	Emory University Room 3020 954 Gatewood Road NE Atlanta, GA 30322 Ph: 404-727-7822 Arash.grakoui@emory.edu

Robert Squires, M.D.	UPMC Children's Hospital Pittsburgh 4401 Penn Avenue Pittsburgh, PA 15224 Ph: 413-692-8648 Squiresr@upmc.edu
Volker Straub, M.D., Ph.D.	Newcastle University and Newcastle Hospitals NHS Foundation Trust Central Parkway Newcastle upon Tyne, NE1 3BZ, UK Ph: +44(0)1912418762/8655 Volker.straub@ncl.ac.uk

Table 5: **Study Vendor Listing**

Role in Study	**Name**	**Address and Telephone Number**
Monitor	IQVIA Biotech Heather Pindel	580 N. 4th Street, Suite 270 Columbus, OH 43215 Ph: 216-233-6184 Heather.Pindel@iqvia.com
EDC CRO	Medrio Jessica Dvotsky Ashton Choi	345 California St, Ste 325 San Francisco, CA 94104 Ph: 215-919-1368 jdvotsky@medrio.com Ph: 415-276-9223 achoi@medrio.com

3 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Table 6: **Abbreviations and Specialist Terms**

Abbreviation of Specialist Term	**Definition**
100MWT	100 Meter Walk Test
ABD1	Actin Binding Domain 1
ABD2	Actin Binding Domain 2
AE	Adverse Event
ALT	Alanine Transaminase
AST	Aspartate Aminotransferase
BA	Bioavailability
BE	Bioequivalence
BMD	Becker Muscular Dystrophy
BUN	Blood Urea Nitrogen
CBC	Complete Blood cell Count
CBER	Center for Biologics Evaluation and Research
CGH	Comparative Genomic Hybridization
CH1	Calponin Homology Domain 1
CK	Creatinine Kinase
CMP	Complete Metabolic Panel
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DMD	Duchenne Muscular Dystrophy
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
ECHO	Echocardiogram
eCRF	electronic Case Report Form
ELISA	Enzyme-Linked Immunosorbent Assay

Abbreviation of Specialist Term	Definition
ELISpot	Enzyme-Linked ImmunoSpot
FDA	Food and Drug Agency
GALGT2	beta-1,4-N-acetyl-galactosaminyltransferase 2
GCP	Good Clinical Practice
GeMCRIS	Genetic Modification Clinical Research Information Service
GGT	Gamma-Glutamyl Transpeptidase
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
IDS	Investigational Drug Services
IEC	Institutional Ethics Committee
IFN	Interferon
IND	Investigational New Drug
IRB	Institutional Review Board
IRES	Internal Ribosome Entry Site
IV	Intravascular
kg	Kilogram
L	Liter
MAPH	Multiplex Amplifiable Probe Hybridization
min	Minute
mL	Milliliter
MLPA	Multiplex Ligation-Dependent Probe Amplification
MOP	Manual of Operating Procedures
mOsmol	Milliosmols
MRI	Magnetic Resonance Imaging
mRNA	messenger RNA
MVICT	Maximum Voluntary Isometric Contraction
NAGLU	α-N-acetylglucosaminidase
NCH	Nationwide Children's Hospital
NIH	National Institutes of Health
NPO	Nothing by mouth
NSAA	North Start Ambulatory Assessment
OSU	Ohio State University
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
PFT	Pulmonary Function Test
PI	Principal Investigator
PT	Prothrombin Time
PTT	activated Partial Thromboplastin Time
qPCR	quantitative PCR
SAE	Serious Adverse Event
SFC	Spot Forming Colonies
SGSH	N-sulfoglucosamine sulfohydrolase
SI	Sponsor Investigator
snRNA	small nuclear RNA
vg	Vector Genomes

4 INTRODUCTION: KEY ROLES, BACKGROUND, AND SCIENTIFIC RATIONALE

4.1 Clinical Trial, Sponsor Investigator and Co-Investigators

The study will be carried out at The Abigail Wexner Research Institute at Nationwide Children's Hospital (NCH) in Columbus, Ohio, and Megan Waldrop, M.D., will serve as Sponsor Investigator. Dr. Waldrop is a board-certified Pediatric and Neuromuscular Neurologist and a faculty member of the NCH Center for Gene Therapy. She is also an Assistant Professor of Pediatrics and Neurology at The Ohio State University and an attending physician in the MDA and SMA Clinics at NCH. She completed fellowship training in Neuromuscular Medicine at Nationwide Children's Hospital/The Ohio State University and completed a fellowship in Neuromuscular Genetic Therapeutics (Gene Therapy) at Nationwide Children's Hospital. She is currently a co-investigator on three active gene therapy trials at NCH [redacted]

Dr. Kevin Flanigan, M.D., will serve as Co-Investigator. Dr. Flanigan, Professor of Pediatrics and Neurology at the Ohio State University (OSU), is the Director of the Center for Gene Therapy at The Abigail Wexner Research Institute of NCH, where he holds the Robert F. & Edgar T. Wolfe Foundation Endowed Chair in Neuromuscular Research. He trained in Neurology (residency) and Neuromuscular Medicine (fellowship) at the Johns Hopkins Hospital, followed by postdoctoral training in Human and Molecular Genetics at the University of Utah before joining the faculty at NCH. Dr. Flanigan has extensive experience as a clinical neuromuscular specialist and molecular geneticist. He has participated as an investigator in multiple clinical trials in muscular dystrophies, including trials of nonsense suppression, exon skipping, and myostatin inhibition. [redacted]. He is the principal investigator and original IND initiating investigator of two ongoing trials: [redacted]

Dr. Kim McBride, M.D., M.S., will be a Co-Investigator. He is an Associate Professor in the Department of Pediatrics at The Ohio State University College of Medicine, the chief of division of medical genetics, and an Investigator in the Center for Cardiovascular and Pulmonary Research at The Abigail Wexner Research Institute at Nationwide Children's Hospital. He received fellowship training in clinical genetics and biochemical genetics at Baylor College of Medicine, and is board certified in pediatrics, clinical genetics and clinical biochemical genetics. He has a special interest in inborn errors of metabolism and lysosomal storage diseases. He has participated in trials of enzyme replacement therapies, including Hunter[4] and Pompe,[5] and is active in natural history studies of Hunter syndrome,[6] phenylketonuria[7] and maternal

phenylketonuria[8]. More recently he has been a co-investigator on two gene transfer clinical trials: [redacted] Dr. McBride is also the Sponsor-Investigator of an additional gene transfer clinical trial: [redacted]

Dr. Wein, Co-Investigator, is an Assistant Professor of Pediatrics at OSU and a Principal Investigator within the Center for Gene Therapy at NCH. He has a long-standing interest in genetic therapies for muscular dystrophies. He was the first author on the *Nature Medicine* paper describing the function of the IRES, and played a critical role in the generation of the vector and in performing preclinical studies[2].

4.2 Abstract

The proposed clinical trial is a systemic (intravenous) delivery of scAAV9.U7.ACCA for DMD patients with a duplication of exon 2 in the *DMD* gene. Preclinical data shows that the small nuclear RNA (snRNA) construct delivered by the scAAV9.U7.ACCA vector causes significant skipping of exon 2, resulting in exclusion of the exon from the mature messenger RNA (mRNA) with a high degree of efficiency, leading to mRNA containing only a single exon 2 (wild type [WT] mRNA) or no copies of exon 2 (Del2 mRNA). Translation of the wild-type mRNA results in entirely normal dystrophin protein, whereas translation of the Del2 mRNA via translational initiation of an internal ribosome entry sequence, or IRES) results in a highly functional isoform expressed in patients known to walk into their eighth decade[2].

The study is designed as an open-label trial to assess safety and obtain preliminary efficacy data. scAAV9.U7.ACCA will be delivered to the systemic circulation via peripheral limb vein. The subjects will receive a gene transfer dose of 3×10^{13} vector genomes per kilogram (vg/kg).

This is a phase I/IIa safety and tolerability study. Muscle biopsies will be performed by open biopsy at baseline and three months post-injection in the quadriceps muscles, and (using the needle biopsy approach outlined below) at 6 and 12 months post-injection in different quadriceps muscles (details of the muscle biopsy protocols, sites, and muscles chosen are found below in Section 10.1).

4.3 Background and Significance

4.3.1 Disease Characteristics

DMD is the most common childhood form of severe muscular dystrophy. Inheritance is X-linked recessive with a birth prevalence estimate of 1 in 5000 live male births[9]. Approximately one-third of cases represent new mutations of the *DMD* gene while the remainder are inherited on the X chromosome from a carrier mother. Symptoms of gross motor delay may present around age 2 or 3 and prompt the need for diagnostic evaluation. DMD is progressive, with loss of ambulation occurring, in the pre-corticosteroid treatment era, by age 12[10]. Historically patients died from respiratory complications, but improved pulmonary and ventilatory measures (including nocturnal ventilatory support) have further improved survival[11] making dilated cardiomyopathy a more common cause of morbidity and mortality.

4.3.2 Disease Pathogenesis

The *DMD* gene was cloned over 30 years ago, which led to defining the molecular basis of the disease[12] and the identification of dystrophin as the deficient protein in muscles of DMD patients[13]. Dystrophin is a 427kDa cytoskeletal protein required for muscle fiber stability. Loss of this protein results in susceptibility to repeated cycles of necrosis and regeneration with satellite cell depletion and diminished regenerative capacity of the muscle which ends in fat and connective tissue replacement (fibrosis). The mutational spectrum within the DMD gene reveals that deletions of one or more exons are found in ~65% of cases clustered in two hotspot regions[14]. Originally, multiplex PCR kits were developed that were able to detect 95%-98% of all deletions[15, 16], but improved molecular methods of diagnosis – including multiplex ligation-dependent probe amplification (MLPA)[17], multiplex amplifiable probe hybridization (MAPH)[18], and comparative genomic hybridization (CGH) allow screening of all exons for copy number variation, facilitating detection of deletions and duplications. Methods of direct sequence analysis of the entire coding region are now in routine use, allowing detection of nearly all clinically relevant disease mutations[19].

4.3.3 Current Treatments for DMD

The only therapeutic approach that has clearly demonstrated unequivocal efficacy for treatment of DMD is glucocorticoids (prednisone or deflazacort). Treatment with one of these agents has been repeatedly shown to result in increased muscle strength and delayed loss of ambulation[20-22]. Deflazacort has recently gained FDA approval for treatment of boys with DMD, based largely on a slightly more favorable side effect profile[23]. However, treatment with either of these drugs can be associated with significant side effects, including weight gain, Cushingoid features, hypertension, cataract formation, loss of bone density, vertebral compression fractures, long bone fractures, and behavioral problems.

Molecular therapies, such a gene replacement, exon skipping, and nonsense mutation suppression provide a promising alternative to glucocorticoids. Exon skipping utilizes antisense oligomers to induce altered pre-mRNA splicing to restore an open reading frame, and eteplirsen has received FDA approval, based largely on a small but statistically significant increase in dystrophin expression[24, 25]. Nonsense suppression utilizes an oxadiazole to enable ribosomal readthrough of nonsense mutations to theoretically make full length dystrophin (PMID 28728956). However, both exon skipping and the as-yet unapproved nonsense suppression approach are only beneficial to small subsets of patients with specific mutations, 13% and 15% respectively[26-28].

4.4 Rationale

4.4.1 Rationale for Gene Therapy using U7snRNA exon skipping

Two exon splicing modification therapies have been shown to be effective and are FDA-approved in neuromuscular diseases, each using antisense oligomers: nusinersen in spinal muscular atrophy[29], and eteplirsen in DMD[30]. The U7 gene therapy approach uses a non-spliceosomal snRNA targeted to a specific exon via an antisense sequence. The U7snRNA interferes with spliceosome assembly with high efficiency, resulting in the exclusion of the target exon from the mRNA[31]. In contrast to antisense oligomer therapies, long term skipping can be achieved with a single dose, and this approach has been effective in improving muscle function in animal models of DMD[32, 33].

4.4.2 Rationale for therapeutic skipping of exon 2

Duplications of one or more exons account for 6% to 11% of all DMD-associated mutations[34, 35], and duplications of exon 2 are the most common, accounting for 10% of all duplication mutations. Dr. Flanigan's laboratory has developed an adeno-associated virus vector (scAAV9.U7.ACCA) in which four copies of U7snRNA are contained within the viral genome. Two copies are targeted toward the exon 2 splice donor site, and two are targeted toward the splice acceptor site.

In this study, scAAV9.U7.ACCA will be utilized to skip one or both of the duplicated exon 2 in the *DMD* gene. Viral-based skipping of exon 2 is a viable therapeutic approach because of an unusual feature of the *DMD* gene that provides an enormous therapeutic window. Specifically, if only one exon is skipped, wild-type dystrophin will be produced, but if both exons 2 are skipped, protein translation will utilize the IRES in exon 5 to produce a highly functional N-truncated dystrophin[2].

The most relevant preclinical studies supporting this have all been published by the Flanigan laboratory over the past eight years. The feasibility of this approach stems from the identification of the first North American founder allele in *DMD*, a predicted nonsense mutation within exon 1 (c.9G>A; p.Trp3X), which resulted in very mild Becker muscular dystrophy. It was first identified in several families in a cohort of 1,100 dystrophinopathy patients[35], who along with other families were then shown to share a 3.7 million base pair region extending across exon 1, confirming that it was a founder allele in this population [36]. Despite the fact that a nonsense mutation should result in expression of no dystrophin, symptoms were minimal. Most patients had only myalgias with exertion; the most severely affected individual stopped walking at age 62, while others had no symptoms into their eighth decade[36].

It was demonstrated that this mild phenotype was due to the expression of significant amounts of an isoform of dystrophin resulting from translational initiation from an AUG codon within exon 6 of *DMD*[37]. The resulting isoform is approximately 413 kiloDaltons (kD) in size, and lacks the calponin homology domain 1 (CH1) that makes up the first half of the actin binding domain 1 (ABD1) at the N-terminus of dystrophin. Despite the canonical view that an intact ABD1 is required for dystrophin functionality, the human "experiment" – the presence of the founder allele in humans without significant symptoms, and clearly no effect on reproductive fitness – argues that the dystrophin rod-domain-located actin binding domain 2 (ABD2) is sufficient for significant preservation of muscle function.

Using a dual-luciferase reporter approach, the IRES activity was mapped to exon 5, and showed that patients with other 5' mutations express the ΔCH1 isoform [37,2]. This work, published in *Nature Medicine*, established the presence of a glucocorticoid-responsive IRES consisting of nearly the entire exon. In order to establish the potential clinical utility of activation of this IRES, a new mouse model of DMD was made with a duplication of mouse exon 2 that essentially mimics the phenotypic features of the standard *mdx* model (which contains a nonsense mutation in exon 23) [3]. It was noted that duplications of exon 2 were nearly always associated with DMD, even though the duplication resulted in an altered reading frame and hence a premature termination codon in the second copy. At the same time, it was noted that deletions of exon 2, which are similarly out-of-frame, had never been reported in either Dr.

Flanigan's large cohort or in other exhaustive dystrophinopathy mutational databases available[34, 38]. Assuming that such a mutation had never been ascertained because of IRES activity, it was hypothesized that the IRES was active in the absence of exon 2 but not in the presence of a duplicated exon 2. Consistent with this hypothesis, studies in the dual luciferase reporter system showed this to be the case, and this was confirmed by the subsequent clinical identification of an essentially asymptomatic patient with a deletion of exon 2[2].

The immediate therapeutic implication of these results is that skipping of exon 2 would be expected to result in IRES activation. Furthermore, it is important to emphasize that for patients with DMD due to a duplication of exon 2, the presence of a functional IRES provides a wide margin of safety, as boys with exon 2 duplications cannot be "overtreated" to be made worse. Exon 2 skipping will result in either the wild-type transcript containing one copy of exon 2, or in the complete exclusion of exon 2, resulting in IRES activation and the expression of the ΔCH1 isoform which – as is demonstrated by patients who express it – is highly protective.

5 OBJECTIVES AND PURPOSE

5.1 Primary Outcome

Safety is the primary outcome for this clinical gene transfer trial. Stopping criteria are based on development of unacceptable toxicity defined as the occurrence of two or more unexpected Grade III or higher treatment-related toxicities.

5.2 Secondary Outcome

Secondary efficacy outcome measures: Muscle biopsy analysis for safety and dystrophin expression will be studied using multiple serial sections.

a) Expression of dystrophin as demonstrated by immunofluorescent in staining with a dystrophin C-terminus antibody and the exon 1-encoded epitope antibody (Manex 1A) in muscle biopsy sections.
b) Dystrophin protein expression quantified by western blot using a dystrophin C-terminus antibody and the exon 1-encoded epitope antibody (Manex 1A) and assessed by densitometry in muscle biopsy tissue.
c) Percentage of exon 2 exclusion from the dystrophin mRNA transcript as measured by RT-PCR analysis.
d) Transduction efficiency will be measured by qPCR of a U7-targeting sequence primer pair amplifying the DNA containing the U7snRNA, and expressed as vector genomes normalized to a single-copy genomic DNA control in each muscle sample.

For immunofluorescence, multiple serial sections will be studied. For each of these measures, statistical analysis based on differences between pre- and post-gene transfer muscle specimens will be analyzed using a paired t test ($p < 0.05$). Sections and western blots will be prepared by a single technician, who will blind the sample identifier; images will be obtained and expression assessed by another technician blinded to the sample time point. Quantification of immunofluorescent staining will be performed using a published protocol[39] that has been used for comparisons between multiple labs with highly reproducible results[40], with the results

reported as membrane-localized dystrophin staining intensity relative to normal muscle sections. The muscle analysis of gene expression will occur without breaking the blind.

Splicing of exon 2 will be assessed by RT-PCR analysis, which will be performed under the same blinding regimen as used for protein expression studies. RT-PCR and data analysis will be performed under standardized protocols, resulting in quantification of unskipped (Dup2), wild-type (WT), and exon 2 deleted (Del2) transcripts. Notably, both WT and Del2 transcripts are therapeutic.

Transduction efficiency will be measured by qPCR of a U7-targeting sequence primer pair amplifying the DNA containing the U7snRNA, and expressed as vector genomes normalized to a single-copy genomic DNA control in each muscle sample.

5.3 Exploratory Outcomes

Exploratory functional outcome measures will be performed on the schedule as described.

A) Muscle Function at 6 and 12 months post gene transfer in comparison to baseline values as follows[$]:
   a) In subjects enrolling under at 36 months of age or younger:
      - Improvement or statistically significant stabilization of Bayley-IV scores (Bayley Scales of Infant and Toddler Development)
   b) In subjects enrolling at greater than 36 months of age and younger than 5 years of age:
      - Improvement or statistically significant stabilization of the North Star Ambulatory Assessment (NSAA) score.
      - Time to climb 4 stairs
      - The amount of time required to walk 100 meters (100MWT)

   c) In subjects enrolling at 5 years or greater, improvement or statistically significant of the following:
      - North Star Ambulatory Assessment (NSAA) score.
      - Time to climb 4 stairs
      - The amount of time required to walk 100 meters (100MWT)
      - The force generated in knee flexion and extension in the maximum voluntary isometric contraction (MVICT)

      [$]*Subjects will be tested using age appropriate assessments.*

B) As an exploratory efficacy measure, muscle MRI will be assessed using T2-weighted multi-slice spin echo (SE) axial images and single voxel 1H-MRS from both the lower leg and thigh to measure lipid fraction and (1)H_2O T2 signal. These will be performed at baseline and at 6, 12, 18 and 24 months post infusion, but only if sedation is not required. Data will be assessed using published techniques[1] and compared to baseline values in collaboration with Dr. Glenn Walter, University of Florida (see Section 10.2 for more details).

C) As an exploratory efficacy measure, the percentage of exon 2 exclusion from the dystrophin mRNA transcript will be measured by RNA-Seq analysis.

D) Additional analyses performed on muscle biopsy tissue will include:

a) Immune staining at 90 and 180 days as well as 12 months will be performed on biopsy specimens for members of the dystrophin-associated protein complex, including the sarcoglycans and β-dystroglycan.
b) Muscle morphometrics will also be performed, including fiber size histograms and quantification of central nucleation at 90 and 180 days as well as 12 months.

6 STUDY DESIGN AND ENDPOINTS

6.1 Overall Study Design

The proposed phase I/IIa clinical trial is an open-label trial where subjects will take part in a single injection study of self-complementary AAV9 carrying the ACCA snRNA under the control of a U7 promoter (scAAV9.U7.ACCA) delivered one time through a peripheral vein of the DMD subjects.

The comparison of muscle tissue from baseline and 90 days post-gene transfer will be the basis of the secondary efficacy analysis. Upon completion of the 2-year study period, subjects will be monitored annually as per standard of care for up to 5 years.

There will be at least 6 weeks between dosing of the first and second subjects within the cohort. The allowance of 6 weeks between dosing of the first and second subjects in the cohort provides time for an DSMB review of the safety analysis from five time points (Days 1, 2, 7, 14 and 30) prior to dosing of the next subject.

Notably, we have not required a 30 day time point between subsequent subjects because we wish to accommodate the realistic possibility that siblings may both enroll in the study, and viral shedding might possibly expose the second of each sibling pair to the vector, generating antibodies that are exclusionary to the trial. We would consider the burden on families of living apart for six weeks to be excessive, and the prospect of making parents choose between one or another of their sons to enroll to be ethically untenable.

6.2 Study Population

All subjects will be enrolled at Nationwide Children's Hospital.

DMD subjects aged between 6 months and less than 14 years of age, with proven exon 2 duplication mutations of the DMD gene, will be eligible for enrollment in the gene transfer study. Subjects of any ethnic or racial background will be enrolled.

6.3 Number of Subjects

Up to 3 DMD subjects matching the inclusion/exclusion criteria will be enrolled. Enrollment will be proceed as outlined in Section 6.4.

6.4 Dosing plan

The proposed phase I/IIa clinical trial is an open-label single injection study of systemic (intravenous) delivery of scAAV9.U7.ACCA for DMD subjects with associated duplications of exon 2 in the DMD gene. Preclinical data shows that the small nuclear RNA (snRNA) construct delivered by the scAAV9.U7.ACCA vector causes significant skipping of exon 2, resulting in

exclusion of one (wild type) or both copies (Del2) of the exon from the mature messenger RNA (mRNA) with a high degree of efficiency. Translation of either the wild-type mRNA or N-truncated mRNA (via IRES translational initiation) results in a highly functional isoform. The dose selection is based on toxicology-biodistribution studies and predicted by pre-clinical studies in non-human primates.

This protocol is designed to deliver the vector via a one-time infusion through a peripheral vein of the DMD subjects. The subjects will receive the minimal efficacious dose (3×10^{13} vg/kg) as identified in murine studies. The total vector genome dose for subjects who weigh ≥ 20kg will be adjusted by rounding the subject body weight up to the closest whole kilogram. The total vector genome dose for subjects who weigh <20kg will be adjusted by rounding the subject body weight to the nearest tenth of a kilogram.

Dose (vg/kg)	N
3.0×10^{13}	1-3

6.5 Criteria for Study Termination

An independent Data Safety Monitoring Board (DSMB) will be established. Safety data will be monitored on a continual basis throughout the trial. The DSMB can recommend early termination of the trial for reasons of safety. Study enrollment will be halted by the investigators when any subject experiences two or more Grade 3, or higher adverse events that are **unanticipated** and **possibly, probably, or definitely related** to the study drug. This will include any subject death, important clinical laboratory finding, or any severe local complication in the injected area related to administration of the study agent. If after review by the DSMB, the decision is made to continue, the study will proceed according to Section 6.4 of this protocol.

6.6 Establish Subject Identification Number

All subjects will be given a unique sequentially assigned subject number. Subjects will be identified by code only to protect identity. This will be done prior to any research procedures when the subject is identified as a potential candidate.

The subject identification number will be generated as described below:

1) U7 (study identifier)
2) DUP2 (to indicate a DMD gene transfer trial)
3) The last two digits will represent the sequential number of candidates screened

Example: Subject ID: U7-DUP2-01

7 STUDY ENROLLMENT AND WITHDRAWAL

All subjects will be enrolled at Nationwide Children's Hospital. Subjects will be identified from among the muscular dystrophy clinic population, as well as among a large number of treatment-amenable subjects who have self-identified themselves to the investigators.

7.1 Inclusion/Exclusion

Inclusion Criteria

1. Age greater than 6 months and less than 14 years.
2. Confirmed duplication of exon 2 the DMD gene using a clinically accepted technique that completely defines the mutation[19, 35].
3. Pre-ambulant (not yet walking) or ambulant (as defined by the ability to walk 10 meters without assistance).
4. Males of any ethnic group will be eligible
5. Ability to cooperate with muscle testing.
6. In subjects age 4 and above, stable dose and regimen of corticosteroid therapy (including either prednisone or deflazacort and their generic forms) for at least 12 weeks prior to gene transfer.

Exclusion Criteria

1. Active viral infection based on clinical observations.
2. Symptoms or signs of cardiomyopathy, including:
    a. Dyspnea on exertion, pedal edema, shortness of breath upon lying flat, or rales at the base of the lungs
    b. Echocardiogram with ejection fraction below 40%
3. Serological evidence of HIV infection, or Hepatitis B or C infection
4. Diagnosis of (or ongoing treatment for) an autoimmune disease
5. Persistent leukopenia or leukocytosis (WBC ≤ 3.5 K/μL or ≥ 20.0 K/μL) or an absolute neutrophil count < 1.5K/μL
6. Concomitant illness or requirement for chronic drug treatment that in the opinion of the SI creates unnecessary risks for gene transfer
7. AAV9 binding antibody titers ≥ 1:400 as determined by ELISA immunoassay
8. Abnormal laboratory values in the clinically significant range in Table 7, based upon normal values in the Nationwide Children's Hospital Laboratory

Table 7: **Normal and abnormal laboratory values at Nationwide Children's Hospital Laboratory**

System	Assay	Normal Range	Abnormal (Clinically Significant)
Liver Function	GGT	8-80 U/L	>80 U/L
	Total Bilirubin	0.1-1 mg/dL	≥3 mg/dL
	Direct Bilirubin	<0.6 mg/dL	>2 mg/dL
Renal Function	Cystatin C	0.3-1.3 mg/dL	>1.8 mg/dL
Hematologic	Hemoglobin	(g/dL) 2 – 6 yrs: 11.5-13.5 6 – 12 yrs: 11.5-15.5 13-18 years: 13-15	For all ages: ≤ 8 or ≥ 18 g/dL

Hematologic	White Blood Cells	4 – 6 yrs: 5.5-15.5 6 – 10 yrs: 5-14.5 10 – 21 yrs: 4.5-13.5	For all ages: White blood cell count ≤ 3.5 or $\geq 20 \times 10^3$ cells/mL OR Absolute neutrophil count of $\leq 1.5 \times 10^3$ cells/mL

** For Hemoglobin, subjects will be excluded if they have a value less than the lower limit of normal for age at screening regardless of age. Abnormal but not clinically significant hemoglobin levels may be repeated during the screening period, and the subject accepted for enrollment if the results are not less than the lower limit of normal.*

7.2 Subject Withdrawal Criteria

If a subject chooses to withdraw at any point BEFORE gene transfer, no further follow up will be required, other than routine post-operative care if a muscle biopsy was completed.

If a subject chooses to withdraw at any point AFTER gene transfer, all safety laboratory studies will still need to be completed. The subject will not be required to undergo any further muscle biopsies, muscle MRIs (if applicable) or functional assessments.

8 STUDY AGENT

Preparation of the scAAV9.U7.ACCA gene vector will be done by the Nationwide Children's Hospital Investigational Drug Services (IDS) according to the Manual of Operating Procedures (MOP). The vector will be delivered undiluted to the gene transfer venue at Nationwide Children's Hospital in 50mL Becton Dickinson Luer-Lok polypropylene syringe(s). It will be delivered at room temperature (not frozen) and administered to the subject within 24 hours of preparation. Handling of scAAV9.U7.ACCA vector will follow compliance standards for Biosafety Level 1 vectors: (http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html)

The infusion rate will not be slower than the slowest rate at which the infusion set up compatibility testing was performed. The infusion rate will not approach or exceed 2 mL/kg/min (a rapid bolus rate) for any pediatric subject. The infusion will be given over approximately 60 to 90 minutes, incorporating competing concerns including maximizing uptake in the musculature and limiting sedation time versus limiting risk for IV infiltration and infusing slowly enough to observe for evidence of infusion reaction before entire dose is given. Depending on vector lot concentration, and other factors, infusion rate is expected to be around 0.03-0.08 mL/kg/min.

The vector will be flushed from the infusion tubing using 20 mL Lactated Ringer's/Hartmann's solution at the end of the infusion. The vector request, shipment, storage, preparation and management will be documented by the IDS.

8.1 Description of the Study Drug

	Investigational Product
Product Name	scAAV9.U7.ACCA
Dosage Form	The vector titer is based off linear qPCR and is stored in 2.0mL vials, filled with 1.0 mL per vial. All vials are 2.0mL screwcap polypropylene vials
Unit Dose	N = 1-3, 3.0 x 10^{13} vg/kg
Route of Administration	Intravenous injection of scAAV9.U7.ACCA via peripheral limb vein
Physical Description	scAAV9.U7.ACCA is provided frozen in vials. Once thawed, scAAV9.U7.ACCA is a clear liquid
Manufacturer	Nationwide Children's Hospital Clinical Manufacturing Facility 575 Children's Crossroads Columbus, OH 43215

9 STUDY PROCEDURES AND SCHEDULE

Subjects will complete 12 onsite visits and up to 5 local blood draws until 2 years post-transfer.

9.1 Study Visits

9.1.1 Screening (day -45 to day -1)

Informed consent will be obtained prior to the collection of any data and any study related procedures. Baseline measures from subjects will be obtained prior to gene transfer and will occur over two visits (up to 3 weeks apart). Because a pre-existing antibody response would be exclusionary, ELISA and ELISpot testing will be performed at the first visit, allowing the results to be reviewed prior to the open muscle biopsy and its subsequent risks (including sedation). The weight obtained at screening will be used to calculate the viral vector dose.

The following assessments will occur at these visits:

- Informed Consent
- Assent, if applicable
- Medical history
- Concomitant Medications
- Physical Exam
- Vital Signs (temperature, respiratory rate, heart rate, blood pressure, height (see Section 10.5 for more details), and weight)
- MRI of the lower legs (see Section 10.2 for more details)
- ECHO
- ECG
- Functional tests (variable depending on age of enrollment)
    - Enrollment age: ≤ 36 months
        - Bayley-IV scores (Bayley Scales of Infant and Toddler Development)
    - Enrollment age: > 36 months and < 5 years of age
        - North Star Ambulatory Assessment (NSAA)

        - Time to climb four stairs
        - Amount of time required to walk 100 meters (100MWT)

    - Enrollment age: ≥ 5 years old until Non-Ambulant
        - North Star Ambulatory Assessment (NSAA) score
        - Time to climb four stairs
        - Amount of time required to walk 100 meters (100MWT)
        - Force generated in knee flexion and extension in maximum voluntary isometric contraction (MVICT).
- Pulmonary functional tests (PFTs), including forced vital capacity. (See Section 10.3 for more details.)
- Open Muscle Biopsy (For details, see Section 10.2)

The following blood lab work will occur at this visit. (Lab work may be drawn over several days during visit if deemed necessary by study team except as noted above for ELISA and ELISpot.)

- Complete blood cell count (CBC) and differential, platelets
- Aspartate aminotransferase (AST)/alanine transaminase (ALT)
- Gamma-glutamyl transpeptidase (GGT)*
- A Comprehensive Metabolic Panel (CMP) that includes:
    - Albumin/Serum total protein
    - Serum total bilirubin
    - Electrolytes
    - Calcium
    - Creatinine/Blood Urea Nitrogen (BUN)
    - Serum glucose
    - Alkaline phosphatase
- Prothrombin time (PT)/activated partial thromboplastin time (PTT)/INR
- Amylase
- Creatine kinase (CK)
- Cystatin C
- Total IgG
- D-Dimer**
- Direct Bilirubin
- SC5b-9**
- C3, C4**
- CH50**
- Antibody testing for Hepatitis B, C and HIV
- ELISA for detection of total antibodies to AAV9
- ELISpots for T-cell responses to AAV9 and Dystrophin
- Blood for vector shedding studies

** For subjects taking drugs that potentially induce GGT synthesis through the cytochrome P450 system, the clinically significant range will be estimated starting at two times the levels obtained from the baseline screening test and up.*

*** These laboratory tests have been recommended by the DSMB to be obtained at screening. These will not necessarily be exclusionary but having a baseline value will be helpful for comparison if a subject were to have any platelet or renal abnormalities that may be immune mediated after gene transfer.*

The following urine lab work will occur at this visit:

- Urinalysis
- Urine for biobanking
- Urine for vector shedding studies

The following additional samples will be collected for vector shedding studies:

- Saliva
- Feces

For children under 10 kg in weight, the blood draw schedule will be reviewed to limit blood draw volumes to ≤ 3% of total body volume over a 24 hour period and ≤ 10% of total body volume over a 30 day period. The study team may deem it necessary to move or remove non-safety-related blood draws to other days if the volume drawn in one 24-hour period will be too near to 3% of total body volume.

Repeat testing of laboratory abnormalities within the screening period: Given the biologic variability inherent in some laboratory testing results, we will retest subjects with minimal and not clinically significant abnormalities resulting in values outside the NCH lab's normal range as defined by Table 7, such as minimal abnormalities of hemoglobin or CBC values. Similarly, subjects with anti-AAV9 endpoint titers at (but not above) the cutoff level of 1:400 will be re-tested for confirmation of titer. Normal values on the second of these tests will be considered to be acceptable for enrollment.

9.1.2 Gene Transfer Visit

9.1.2.1 Pre Infusion Visit (Day -1)

Subjects will arrive at the Nationwide Children's Hospital within 24 hours prior to gene transfer.

The following assessments will occur at this visit:

- Medical history
- Concomitant Medications
- Adverse Events
- Physical Exam
- Vital Signs (heart rate, respiratory rate, temperature, blood pressure, height (see Section 10.5 for more details) and weight)

The following blood lab work will occur at this visit:

- Complete blood cell count (CBC) and differential, platelets
- Aspartate aminotransferase (AST)/alanine transaminase (ALT)
- Gamma-glutamyl transpeptidase (GGT)*
- A Comprehensive Metabolic Panel (CMP) that includes:
    - Albumin/Serum total protein
    - Serum total bilirubin
    - Electrolytes
    - Calcium
    - Creatinine/Blood Urea Nitrogen (BUN)
    - Serum glucose
    - Alkaline phosphatase
- Prothrombin time (PT)/activated partial thromboplastin time (PTT)/INR
- Creatine kinase (CK)
- Direct Bilirubin
- ELISpots for T-cell responses to AAV9 and Dystrophin
- Whole blood for biobanking

** For subjects taking drugs that potentially induce GGT synthesis through the cytochrome P450 system, the clinically significant range will be estimated starting at two times the levels obtained from the baseline screening test and up.*

In previous gene therapy studies at NCH, there has been an observed antigen specific T-cell response to the AAV vectors. This is an expected response between 2 - 4 weeks following gene transfer. One possible consequence to such antigen specific T-cell responses is clearance of the transduced cells and loss of transgene expression. All subjects (ages 4 years and older) will already be on prednisone, prednisolone, or deflazacort for 12 weeks at time of enrollment as part of the inclusion criteria. The standard clinical dose of prednisone or prednisolone is 0.75 mg/kg/day, and the standard clinical dose of deflazacort is 0.9 mg/kg/day. In order to dampen the host immune response to our AAV-based therapy, for each subject the glucocorticoid dose will be increased to prednisone or prednisolone 1.0 mg/kg/day, not to exceed 60 mg/day; subjects on deflazacort at enrollment will be placed on prednisone or prednisolone for the immediate peri-injection period. For subjects younger than 4 years of age who may not be on a corticosteroid,

prednisolone will be prescribed for the purposes of dampening the host immune response. This treatment will begin one day prior to the gene transfer and only after passing all inclusion and exclusion criteria (in the event that a measure is repeated on Day -1). All subjects will also be prescribed a proton pump inhibitor (PPI) for the purposes of dampening potential gastrointestinal upset related to the increased corticosteroid dosage. This corticosteroid and PPI regimen will be continued after gene transfer as discussed in Section 10.6.1, followed by a return to the pre-treatment corticosteroid regimen.

9.1.2.2 Day of Gene Transfer (Day 0)

The venue for gene transfer will be determined during the screening visit. There is a range of subject ages, from 6 months through age 14 years. For the youngest subjects, sitting still during the gene transfer (up to 90 minutes) may be problematic. For these youngest subjects, gene transfer will be performed in the hospital's Procedure Center under sedation, and they will be monitored in the Pediatric Intensive Care Unit after the gene transfer. For older subjects – anticipated to be any subject above the age of 3 years – the gene transfer will be performed within the Pediatric Intensive Care Unit itself, followed by monitoring in the same unit. Transfer out of the PICU may be undertaken after the initial 24 hours of post-infusion monitoring, if the SI has no concerns.

Prior to Gene Transfer, the following assessments will occur at this visit:

- Medical history
- Concomitant Medications
- Adverse Events
- Physical Assessment (physical exam will consist of the anesthesia evaluation prior to sedation or by the study team if sedation is not required))
- Vital Signs (temperature, respiratory rate, heart rate, and blood pressure)

The following procedures work will occur at this visit:

- Picture of Injection Site, both prior to and after Gene Transfer
- Gene Transfer

The following samples will be collected for vector shedding studies 4 and 8 hours post gene transfer (see Section 10.4 for details):

- *Blood*
- *Urine*
- *Saliva*
- *Feces*

Prior to vector infusion, an assessment will be performed of vitals to be collected for clinical purposes by either anesthesia staff or the study team, depending on the planned use of anesthesia or not.

If sedation is required for subjects, they will continue on their usual diet until eight hours prior to gene transfer, after which they will have no solid food; clear liquids will be allowed up until two hours prior to gene transfer, after which they will be fully NPO. They will resume their usual diet after they have returned to pre-sedation baseline. The specifics of the gene transfer itself follow below. If no sedation is required, subjects will be allowed a normal diet until 2 hours prior to the gene transfer after which they will need to be fully NPO.

If the subject appears inadequately hydrated in the judgment of the SI, bolus(es) of 10-20 mL/kg normal saline may be given during the time between either admission or Procedure Center check-in and gene transfer.

If sedation is not required, the subject will become NPO 2 hours prior to gene transfer and will remain NPO for 2 hours after gene transfer is completed. D5 1/2NS will be infused in the second IV during the NPO period.

Gene transfer will be performed under sterile conditions within an appropriate inpatient facility. If deemed necessary by the SI, the gene transfer will occur under light to moderate sedation under the direction of a qualified anesthesiologist. If required, the subjects will be given sedation utilizing NCH anesthesia protocols that minimize the risk of anesthetic reaction in muscular dystrophy.

Sedation is being utilized in this instance to minimize anxiety or uncooperative behavior in the subjects, with an aim of maximizing potential study participation benefit to the pediatric subject by preventing study agent subcutaneous infiltration or IV loss with study agent spillage (the subjects will receive the *minimal efficacious dose* identified in the animal model). In those subjects who in the opinion of the SI (and in consultation with the anesthesiologist and parents) are determined to not need sedation in order to safely deliver the vector, sedation may be deferred, and the vector delivered in the PICU.

All subjects participating in this trial will receive an intravenous injection of scAAV9.U7.ACCA via peripheral limb vein. Dosing is the minimum efficacious dose as established in murine studies.

- n= 1-3 – 3.0 X 10^{13} vg/kg

The total vector genome dose for subjects who weigh ≥ 20kg will be adjusted by rounding up to the closest whole kilogram. The total vector genome dose for subjects who weigh < 20kg will be adjusted by rounding the subject body weight to the nearest tenth of a kilogram. Dosing is extrapolated from efficacy observed in preclinical studies as the minimal efficacious dose identified in the animal model.

Subjects will be closely monitored for side effects during the infusion, including continuous heart rate, respiratory rate, and pulse oximetry; and intermittent blood pressure monitoring. Heart rate, respiratory rate, pulse oximetry (only if sedation is being utilized), temperature, and blood pressure will be measured before and immediately after the infusion, and at least every five minutes during the infusion if sedated, and every 15 minutes if not sedated. Monitoring will be repeated at 15 minutes post-infusion, every 15 minutes for 4 hours following the infusion, and then every hour until transfer from the PICU to the Neurology floor. On the Neurology floor, vital signs will be measured every 4 hours until discharge.

Infusion reactions: Infusion will be terminated for evidence of an allergic reaction of **grade 2 or greater**, including anaphylaxis, based upon CTCAE v5.0 criteria, and reported using these criteria:

Grade 1: Transient flushing or rash, drug fever <38° C (<100.4° F); intervention not indicated
Grade 2: Rash, flushing, urticaria, dyspnea, drug fever >38° C:
Intervention or infusion interruption indicated; responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics); prophylactic medications indicated for ≤24 hrs
Grade 3: Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates)
Grade 4: Life-threatening consequences; urgent intervention indicated
Grade 5: Death

Under CTCAE v5.0 criteria, **anaphylaxis** is by definition grade 3 (Symptomatic bronchospasm with or without urticaria; allergy-related edema/angioedema, hypotension), and would result in infusion termination and systemic treatment.

Subjects will remain in an independent hospital room following gene transfer and remain hospitalized for up to 48 hours post gene transfer.

9.1.3 Post Gene Transfer Monitoring Plan

To facilitate these scheduled visits, all subjects will remain in the Columbus metropolitan area for a minimum of two weeks following gene transfer. If there is a systemic immune response that leads to acute respiratory distress syndrome, which implies a severe inflammation of the lung parenchyma, the subject would be hospitalized and treated aggressively with fluid replacement for hypotension if necessary and receive pulse methylprednisolone treatment (1 gram per day) over 3-5 days.

9.1.3.1 Day 1

The following assessments will occur at this visit:

- Medical history
- Concomitant Medications
- Adverse Events
- Physical Exam
- Vital Signs (blood pressure, heart rate, respiratory rate, and temperature)

The following blood lab work will occur at this visit:

- Complete blood cell count (CBC) and differential, platelets
- Aspartate aminotransferase (AST)/alanine transaminase (ALT)
- Gamma-glutamyl transpeptidase (GGT)*
- A Comprehensive Metabolic Panel (CMP) that includes:
    - Albumin/Serum total protein

  - Serum total bilirubin
  - Electrolytes
  - Calcium
  - Creatinine/Blood Urea Nitrogen (BUN)
  - Serum glucose
  - Alkaline phosphatase
- Prothrombin time (PT)/activated partial thromboplastin time (PTT)/INR
- Creatine kinase (CK)
- Direct Bilirubin
- Blood for vector shedding studies (see Section 10.4 for details)

** For subjects taking drugs that potentially induce GGT synthesis through the cytochrome P450 system, the clinically significant range will be estimated starting at two times the levels obtained from the baseline screening test and up.*

The following urine lab work will occur at this visit:

- Urinalysis
- Urine for biobanking
- Urine for vector shedding studies (see Section 10.4 for details)

The following additional samples will be collected for vector shedding studies (see Section 10.4 for details):

- Saliva
- Feces

The following procedures will occur at this visit:

- Picture of Injection Site

9.1.3.2 Day 2

On the second day after gene transfer (Day 2) the subject will be discharged from the hospital following approval from by a study team doctor.

The following assessments will occur at this visit:

- Medical history
- Concomitant Medications
- Adverse Events
- Physical Exam
- Vital Signs (blood pressure, heart rate, respiratory rate, and temperature)

Blood will be collected for vector shedding studies (see Section 10.4 for details):

The following urine lab work will occur at this visit:

- Urine for biobanking
- Urine for vector shedding studies (see Section 10.4 for details)

The following additional samples will be collected for vector shedding studies (see Section 10.4 for details):

- Saliva
- Feces

9.1.3.3 Days 7 (± 2 days), 14 (± 2 days), and 30 (± 3 days)

Subjects will return for follow up visits on **Days 7, 14, and 30** after treatment, with repeat examinations, blood and urine work as outlined below.

The following assessments will occur at this visit:

- Medical history
- Concomitant Medications
- Adverse Events
- Physical Exam
- Vital Signs (heart rate, respiratory rate, temperature, weight, height (see Section 10.5 for more details), and blood pressure)

The following blood lab work will occur at this visit:

- Complete blood cell count (CBC) and differential, platelets
- Aspartate aminotransferase (AST)/alanine transaminase (ALT)
- Gamma-glutamyl transpeptidase (GGT)*
- A Comprehensive Metabolic Panel (CMP) that includes:
    - Albumin/Serum total protein
    - Serum total bilirubin
    - Electrolytes
    - Calcium
    - Creatinine/Blood Urea Nitrogen (BUN)
    - Serum glucose
    - Alkaline phosphatase
- Prothrombin time (PT)/activated partial thromboplastin time (PTT)/INR
- Amylase
- Creatine kinase (CK)
- Total IgG (Day 30 only)
- Direct Bilirubin
- ELISpots for T-cell responses to AAV9 and Dystrophin
- Blood for vector shedding studies (see Section 10.4 for details)

** For subjects taking drugs that potentially induce GGT synthesis through the cytochrome P450 system, the clinically significant range will be estimated starting at two times the levels obtained from the baseline screening test and up.*

The following urine lab work will occur at this visit:

- Urinalysis

- Urine for biobanking
- Urine for vector shedding studies (see Section 10.4 for details)

The following additional samples will be collected for vector shedding studies (see Section 10.4 for details):

- Saliva
- Feces

On **Day 7**, additional tests will include complement studies, consisting of Sc5b-9, C3, C4, and CH50.

On **Day 30**, additional tests will include an ELISA for detection of total antibodies to AAV9, Cystatin C, and whole blood banking.

9.1.3.4 Days 45, 75, 120, 150, and 2 weeks after return of prednisone dose to baseline (± 3 days)

For convenience, for subjects outside of the Columbus area, these may be locally drawn and shipped to the NCH clinical laboratory.

The following blood lab work will occur at this visit:

- Aspartate aminotransferase (AST)/alanine transaminase (ALT)
- Gamma-glutamyl transpeptidase (GGT)*
- A Comprehensive Metabolic Panel (CMP) that includes:
    - Albumin/Serum total protein
    - Serum total bilirubin
    - Electrolytes
    - Calcium
    - Creatinine/Blood Urea Nitrogen (BUN)
    - Serum glucose
    - Alkaline phosphatase
- Prothrombin time (PT)/activated partial thromboplastin time (PTT)/INR
- Total IgG (Day 120, 150)
- Direct Bilirubin

** For subjects taking drugs that potentially induce GGT synthesis through the cytochrome P450 system, the clinically significant range will be estimated starting at two times the levels obtained from the baseline screening test and up.*

9.1.3.5 Day 60 (± 7 days)

The following assessments will occur at this visit:

- Medical history
- Concomitant Medications
- Adverse Events
- Physical Exam
- Vital Signs (heart rate, respiratory rate, temperature, weight, height (see Section 10.5 for

more details) and blood pressure)

The following blood lab work will occur at this visit:

- Complete blood cell count (CBC) and differential, platelets
- Aspartate aminotransferase (AST)/alanine transaminase (ALT)
- Gamma-glutamyl transpeptidase (GGT)*
- A Comprehensive Metabolic Panel (CMP) that includes:
    - Albumin/Serum total protein
    - Serum total bilirubin
    - Electrolytes
    - Calcium
    - Creatinine/Blood Urea Nitrogen (BUN)
    - Serum glucose
    - Alkaline phosphatase
- Prothrombin time (PT)/activated partial thromboplastin time (PTT)/INR
- Creatine kinase (CK)
- Total IgG
- Direct Bilirubin
- ELISA for detection of total antibodies to AAV9
- ELISpots for T-cell responses to AAV9 and Dystrophin
- Whole blood for biobanking
- Blood for vector shedding studies (see Section 10.4 for details)

** For subjects taking drugs that potentially induce GGT synthesis through the cytochrome P450 system, the clinically significant range will be estimated starting at two times the levels obtained from the baseline screening test and up.*

The following urine lab work will occur at this visit:

- Urinalysis
- Urine for biobanking
- Urine for vector shedding studies (see Section 10.4 for details)

The following additional samples will be collected for vector shedding studies (see Section 10.4 for details):

- Saliva
- Feces

9.1.3.6 Day 90 (± 7 days) and 180 (± 14 days), and Months 12 (± 14 days), 18 (± 14 days), and 24 (± 14 days) visits

The following assessments will occur at these visits:

- Medical history
- Concomitant Medications

- Adverse Events
- Physical Exam
- Vital Signs (heart rate, respiratory rate, temperature, weight, and blood pressure, height (see Section 10.5 for more details))
- ECHO
- ECG
- Functional tests (variable depending on age of enrollment)
    - Enrollment age: ≤ 36 months
        - Bayley-IV scores (Bayley Scales of Infant and Toddler Development)
    - Enrollment age: > 36 months and < 5 years of age
        - North Star Ambulatory Assessment (NSAA)
        - Time to climb 4 stairs
        - Amount of time required to walk 100 meters (100MWT)
        -
    - Enrollment age: ≥ 5 years old until Non-Ambulant
        - North Star Ambulatory Assessment (NSAA) score
        - Time to climb 4 stairs
        - Amount of time required to walk 100 meters (100MWT)
        - Force generated in knee flexion and extension in maximum voluntary isometric contraction (MVICT).
- Pulmonary functional test (PFT) (see Section 10.3 for more details)

The following blood lab work will occur at this visit:

- Complete blood cell count (CBC) and differential, platelets
- Aspartate aminotransferase (AST)/alanine transaminase (ALT)
- Gamma-glutamyl transpeptidase (GGT)*
- A Comprehensive Metabolic Panel (CMP) that includes:
    - Albumin/Serum total protein
    - Serum total bilirubin
    - Electrolytes
    - Calcium
    - Creatinine/Blood Urea Nitrogen (BUN)
    - Serum glucose
    - Alkaline phosphatase
- Prothrombin time (PT)/activated partial thromboplastin time (PTT)/INR
- Creatine kinase (CK)
- Cystatin C
- Total IgG
- Direct Bilirubin
- ELISA for detection of total antibodies to AAV9
- ELISpots for T-cell responses to AAV9 and Dystrophin
- Blood for vector shedding studies (see Section 10.4 for details)

** For subjects taking drugs that potentially induce GGT synthesis through the cytochrome P450 system, the clinically significant range will be estimated starting at two times the levels obtained from the baseline screening test and up.*

The following urine lab work will occur at this visit:

- Urinalysis
- Urine for biobanking
- Urine for vector shedding studies (see Section 10.4 for details)

The following additional samples will be collected for vector shedding studies (see Section 10.4 for details):

- Saliva
- Feces

At the **Day 90 and 180 and the Month 12**, either an open or needle muscle biopsy will occur (see Section 10.1 for more details). Additionally, at the **Day 180, and Months 12, 18 and 24 month visits**, MRI imaging will also be performed (*see Section 10.2 for more details)*

9.2 Schedule of Events

Table 8: **Schedule of Events**

U7.ACCA STUDY TIMELINE																			
Study Interval	Screening[1] s	Pre Infusion Visit	Hospital Admission Vector Injection	Inpatient		Follow-Up (Outpatient)													
Visit #	Visit 1[2] & 2	Visit 3[2]				Visit 4	Visit 5	Visit 6	Labs[8]	Visit 7	Labs[8]	Visit 8[2]	Labs[8]	Labs[8]	Labs[8]	Visit 9[2]	Visit 10[2]	Visit 11[2]	Visit 12[2]
Study Procedures	Days -45 to -1	Day -1	Day 0	Day 1	Day 2	Day 7	Day 14	Day 30	Day 45	Day 60	Day 75	Day 90	Day 120	2 wks post steroid return	Day 150	Day 180	Month 12	Month 18	Month 24
Informed consent[1]	X																		
Informed assent[1]	X																		
Medical History	X	X	X	X	X	X	X	X		X		X				X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X		X		X				X	X	X	X
Adverse events		X	X	X	X	X	X	X		X		X				X	X	X	X
Physical exam	X	X	X[6]	X	X	X	X	X		X		X				X	X	X	X
Vital signs	X	X	X[7]	X	X	X	X	X		X		X				X	X	X	X
Height	X	X				X	X	X		X		X				X	X	X	X
Weight	X	X				X	X	X		X		X				X	X	X	X
MRI[11]	X															X	X	X	X
ECHO	X											X				X	X	X	X
EKG	X											X				X	X	X	X
Bayley-IV[10]	X											X				X	X	X	X
NSAA/NSAA-Revised[10]	X											X				X	X	X	X
Time to climb 4 stairs[10]	X											X				X	X	X	X
100 m walk/run[10]	X											X				X	X	X	X
MVICT[10]	X											X				X	X	X	X
PFTs/Spirometry[14]	X											X				X	X	X	X
Muscle Biopsy[3, 4]	X											X				X	X		
Anaesthesia[9]	X		X									X				X	X		
CBC/Diff	X	X		X		X	X	X		X		X				X	X	X	X
AST/ALT	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum GGT	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
CMP	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
PT/PTT/INR	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Amylase	X					X	X	X											
CK	X	X		X		X	X	X		X		X				X	X	X	X
Cystatin C	X							X				X				X	X	X	X
Total IgG	X							X		X		X	X		X	X	X	X	X
D-Dimer	X																		
Direct Bilirubin	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
SC5b-9, C3, C4, CH50	X					X													
Antibody testing Hep. B, C and	X																		
ELISA	X							X		X		X				X	X	X	X
ELISpot	X	X				X	X	X		X		X				X	X	X	X
Whole Blood Banking		X						X		X									
Vector Shedding Studies[12]	X		X[13]	X	X	X	X	X		X		X				X	X	X	X
Urinalysis	X			X		X	X	X		X		X				X	X	X	X
Urine Banking	X			X	X	X	X	X		X		X				X	X	X	X
Steroid Administration[5]		X	X	X	X	X	X	X	X	X	X	X	X						
Admit to Hospital			X																
Study agent administration			X																

TABLE 8 FOOTNOTES	
	1. If there are changes to the study, parents or subjects will be re-consented or re-assented at their next visit.
	2. Visit will occur over multiple days.
	3. Muscle strength testing at baseline (days -45 to -1) and visits 8-10 will be performed at least 1 day preceding the biopsy.
	4. An open muscle biopsy will be peformed on one leg at the baseline, and on the other leg at 3 months post gene transfer. Needle muscle biopsy will be performed in either leg at 6 months and the contralateral at 12 months. Biopsies must be performed after the strength evaluation and lab tests. See section 12.1 for more details.
	5. Prophylactic prednisolone/prednisone as well as Proton Pump Inhibitor (PPI) begins on Day -1 and is tapered according to AST, ALT, and ELISpot results starting at Day 30. Anticipate that most subjects will be on prednisolone for up to 120 days.
	6. Day 0 physical exam will consist of the anesthesia evaluation prior to sedation or by study team if sedation is not required.
	7. Day 0 Vital Signs (Heart rate, respiratory rate, pulse oximetry (only if sedated), temperature, and blood pressure) will be measured as per Section 9.1.2.2.
	8. Samples scheduled in between f/u visits will be collected locally and shipped to us.
	9. Depending on the age of subject, there may be sedation required for gene therapy and needle biopsies. This determination will be at the discretion of the PI.
	10. The functional testing administered will be determind by the age of the subject.
	11. In order to avoid the risk of sedation for an exploratory efficacy measure, muscle MRI will not be performed in any subjects who are unable to remain still for a MRI without sedation. To evaluate if a subject will be able to remain still for a MRI, a mock MRI will be given at the screening visit.
	12. Vector shedding analysis post dosing will be performed on DNA isolated from different biological fluids (plasma, urine, saliva and feces) until three consecutive samples are negative for the presence of viral DNA for each specimen type.
	13. Vector Shedding on day 0 will be collected at 4 and 8 hours post gene transfer.
	14. If the subject is too young to reliably rest these values, it will not be performed and will not be recorded as a protocol deviation.
	15. For children under 10 kg in weight, the blood draw schedule will be reviewed to limit blood draw volumes to ≤ 3% of total body weight over a 24 hour period and ≤ 10% of total body weight over a 30 day period.

9.3 Muscle Strength Test Schedule

Table 9: **Muscle Strength Testing Schedule**

	6 mos - 3 yrs	3 - 4.9 yrs	5 - Non-Ambulant
Bayley IV			
Time to climb four stairs			
NSAA			
100 meter			
MVICT*			
PFTs			
Number of Assessments	1	3	5
The lowest age at which the MVICT will be administered will be based on the Clinical Evaluators' discretion in ability to cooperate.			

9.4 Long Term Follow up

We will follow the most recent FDA guidelines with regard to long-term subject follow-up following gene transfer. As discussed and based on prior experiences with rAAV or transgene, there is a very low probability of gene transfer-related adverse events. We will, however, evaluate short-term safety over a two-year period that incorporates the active phase of the protocol. Subjects will then transfer to an annual monitoring program where data will be collected from annual standard of care visits for up to 5 years.

If newly identified risks are associated with our product, or if the subjects suffer any adverse events during this period, we will initiate a long-term follow-up according to the FDA guidelines. We will, of course, notify CBER if there is any indication of need to extend follow-up period. All subjects will be provided with written instructions on how to contact the Sponsor Investigator or study coordinator if they experience any serious adverse event that they consider possibly related to study treatment or study participation. This information will also be included in the Informed Consent document. All subjects will be instructed to notify the Sponsor Investigator of a change of address or contact information.

The final results of the clinical trial will be shared with the participants at the completion of the study when all data has been collected, analyzed, and published. However, if significant findings become available that might increase the risk of the subjects or might affect their decision to remain in the study, then information will be made available as soon as it is available.

At the time of death, no matter the cause, permission for an autopsy will be requested of their families. Subjects will be asked to advise their families of this request and of its scientific and medical importance.

10 PROCEDURE CRITERIA

10.1 Muscle biopsy

The baseline open muscle biopsy will be performed in one quadriceps muscle, or other appropriate muscle as determined by the SI. Another open muscle biopsy of the quadriceps, or other muscle as determined by the SI (biopsy will be in the contralateral leg to the pre-treatment biopsy, unless otherwise determined by the SI), will be performed 3 months post-infusion.

All open muscle biopsies will be performed under anesthesia, as per NCH standard clinical protocols.

Needle muscle biopsies of the quadriceps muscles (using the Vacora device; C.R. Bard, Inc.) will be performed at 6 and 12 months. In order to avoid resampling of the previous biopsy site, the open biopsies are anticipated to be performed in the vastus lateralis muscles, and the needle biopsies in the vastus medialis muscles. Every effort will be made to sample these same muscles in all subjects; however, in subjects (particularly older ambulant subjects) with limited quadriceps musculature, alternate muscle biopsy sites may be selected at the SI's discretion (including biceps, gastrocnemius, tibialis anterior or deltoid muscles).

10.2 Magnetic Resonance Imaging (MRI)

In order to avoid the risk of sedation for an exploratory efficacy measure, muscle MRI will **not** be performed in any subjects who are unable to remain still for a MRI without sedation. To evaluate if a subject will be able to remain still, a mock-MRI will be performed in coordination with Child Life during the screening visit. If the mock-MRI is unable to be completed, the subject will not be required to provide a clinical MRI during the trial. If the MRI is unable to be performed without sedation, it will not be recorded as a protocol deviation.

10.3 Pulmonary functional tests (PFTs)

PFT testing will be performed in subjects 5 years or older. If the subject is too young to reliably test these values, it will not be performed and will not be recorded as a protocol deviation.

10.4 Vector Shedding Studies

Vector shedding analysis post dosing will be performed on DNA isolated from different biological fluids (plasma, urine, saliva and feces) until three consecutive samples are negative for the presence of viral DNA for each specimen type.

10.5 Height Measurements

Height will be measured with the patient standing when ambulatory. If the patient is non-ambulatory, ulna length measurement will be used to calculate height.

10.6 Concomitant Medications

Prior and concomitant medications will be captured in the eCRF form two weeks prior to study enrollment through the last study visit. The SI will encourage participants to maintain the medication and supplements they are on at enrollment through the course of the study. Subjects on aspirin or drugs that could affect coagulation will continue their medication as indicated.

Several investigations show that preoperative aspirin ingestion and intravenous heparin therapy can be administered safely without concerns about the risk of postoperative bleeding and should not lead to modification or cessation of such therapy.[41-43] Subjects 4 years old or greater must already be on a stable dose of prednisone, prednisolone, or deflazacort for 12 weeks at time of enrollment as part of the inclusion criteria.

10.6.1 Prophylactic Administration of Prednisone/Prednisolone

All subjects will be discharged from the hospital on oral prednisone or prednisolone at 1 mg/kg/day, which will be adjusted according to IFN-gamma T-cell studies in the following weeks. Subjects will also continue on a PPI until they return to their baseline corticosteroid dose or sooner based on the subject's symptoms and discretion of the sponsor investigator and/or parent.

If the **30 day** post-infusion IFN-gamma T-cell responses to AAV9 and dystrophin show ≤50 SFC per 1 x 10^6 PBMCs, the subject's prednisone or prednisolone dose will be decreased back to the standard clinical dose of 0.75 mg/kg/day. Those subjects who had been on deflazacort may be switched back to the standard clinical dose of 0.9 mg/kg day. For either regimen, approximations of these target doses may be allowed to account for pill formulations (for prednisone and deflazacort), as in clinical practice. If either the AST or ALT exceeds >2.5X the subject's baseline values, and results are confirmed on a follow-up blood test, the prednisone or prednisolone regimen will be maintained at 1 mg/kg/day until the enzyme levels fall within a range, 2.5X the baseline value. Subjects may be asked to undergo a weekly blood test outside of prescheduled visits should the SI deem it necessary to monitor the T-cell response or AST/ALT levels more closely.

If the IFN-gamma T-cell responses show ≥50 SFC per 1 x 10^6 PBMCs, we may increase the dose to approximately 2 mg/kg/day depending on T-cell response measured by ELISpot assay and prolong a subsequent tapering protocol based on the individual subject's immune response profile as assessed by subsequent ELISpot assays (as on the schedule above). Based upon other studies performed at NCH, we anticipate that oral prednisone or prednisolone may be administered for up to 120 days post gene transfer prior to the return to the standard clinical corticosteroid regimen of each subject.

11 ASSESSMENT OF SAFETY

The primary outcome for this clinical trial is safety.

11.1 Adverse and Serious Adverse Event

11.1.1 Definition of an Adverse Event

As stated above this protocol will follow the final regulations issued by the Food and Drug Administration addressing the safety reporting requirements for investigational new drug applications (INDs) found in 21 CFR part 312 and for bioavailability and bioequivalence studies found in 21 CFR part 320. "Safety Reporting Requirements for INDs and BA/BE Studies".

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM227351.pdf

Adverse Event (AE): Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Adverse events will be graded by the investigator accordingly (as indicated in Section 11.4):

1 = mild
2 = moderate
3 = severe
4 = life threatening or debilitating
5 = fatal

Association or relatedness to the study agent will be graded as follows:

5 = unrelated
4 = unlikely
3 = possibly
2 = probably
1 = definitely related.

Adverse reaction: An adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

Suspected adverse reaction (21 CFR 312.32(a)) Suspected adverse reaction (SAR) means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

11.1.2 Unexpected Adverse Events

Unexpected adverse events are those which are not previously reported with recombinant AAV vectors, commonly not seen in association with the subject's underlying disease or with the procedures to be used in this study, or are related to a known toxicity but differ because of greater severity or specificity.

Potential expected AEs include localized injection site reactions, which might also be related to the procedure and be independent of the AAV vector itself. Asymptomatic elevations in transaminases are a feature of Duchenne muscular dystrophy, as they are related to release of AST and ALT from muscle tissue and correlate with levels of creatine kinase. These are a feature of the disease itself. Levels of AST (up to 12.3X the upper limit of normal) and ALT (up to 22.6X the upper limit of normal) are expected to be seen at baseline, and in the setting of normal GGT function are not indicative of muscle injury[44]. They are thus not exclusionary for enrollment and will not be recorded as adverse events prior to gene transfer.

Systemic delivery of AAV vectors have resulted in AEs including asymptomatic transient transaminitis (up to levels of 2.5x the subject's baseline value and 3X the upper limit of normal for DMD subjects for AST and ALT) with preserved liver synthetic function and no significant elevation in GGT) following gene transfer (see Section 11.4.2).

11.1.3 Serious adverse event (SAE)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator/sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

To reiterate, an SAE is an event in categories 3, 4, and 5.

Category 3: Severe adverse event; inability to carry on normal activities; required professional medical attention
Category 4: Life-threatening or permanently disabling adverse event
Category 5: Fatal adverse event

11.1.4 Life-threatening (21 CFR 312.32(a))

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

The SI will fulfill the reporting responsibilities to FDA on behalf of Nationwide Children's Hospital using the web-based Adverse Event reporting system (GeMCRIS).

11.2 Relationship to Study Drug

Association or relatedness to the study agent, study procedures and the subject's pre-existing disease will be graded as follows:

1. Definitely related
2. Probably related
3. Possibly related
4. Unlikely related
5. Unrelated

If the relationship between the AE/SAE and the investigational product is determined to be "possible" or "probable", the event will be considered to be related to the investigational product for the purposes of expedited regulatory reporting.

11.3 Time Period and Frequency for Event Assessment and Follow-Up

11.3.1 Obligations of the Investigator

The Sponsor Investigator will submit an IND safety report to the FDA on any serious adverse event that is both unexpected and associated with the use of the gene transfer product (i.e., there is reasonable possibility that the event may have been caused by the use of the product; the investigator will not await definitive proof of association before reporting such events); as well as a written report on any finding from tests in laboratory animals that suggests a significant risk for human research participants including reports of mutagenicity, teratogenicity, or carcinogenicity. The report will be clearly labeled as a "Safety Report" and will be submitted to the FDA and to the local Institutional Review Board within the timeframes set forth in Section 11.3.2.

The Sponsor Investigator will adhere to any other serious adverse event reporting requirements in accordance with federal regulations, state laws, and the local institutional policies and procedures, as applicable.

The Sponsor Investigator will be responsible for ensuring that the reporting requirements are fulfilled and will be held accountable for any reporting lapses.

11.3.2 Safety Reporting

The investigator or his designee will report all serious and unexpected adverse events to the IRB, CBER/FDA and DSMB according to regulatory requirements described as follows:

IRB: All Serious Adverse Events (SAEs) and Dose Limiting Toxicities (DLTs), regardless of expectedness, relatedness, or if they meet the definition for unexpected problems in the clinical trial will be reported to the **IRB** as soon as possible, but not later than **15 calendar days** after the sponsor's initial receipt of the information.

DSMB: All Serious Adverse Events (SAEs) and Dose Limiting Toxicities (DLTs) that are life-threatening will be reported to the **DSMB** within **48 hours** of notification, regardless of expectedness, relatedness, or if they meet the definition for unexpected problems to the clinical trial.

FDA: Any serious adverse event that is fatal or life-threatening, that is unexpected, and associated with the use of the gene transfer product will be reported by the study investigator to the **FDA** as soon as possible, but not later than **7 calendar days** after the sponsor's initial receipt of the information.

Serious adverse events that are unexpected and associated with the use of the gene transfer product, but are not fatal or life-threatening, will be reported to the **FDA** as soon as possible, but not later than **15 calendar days** after the sponsor's initial receipt of the information.

Changes in this schedule will be permitted only where, under the **FDA IND** regulations [21 CFR 312(c) (3)], changes in this reporting schedule have been approved by the FDA and are reflected in the protocol.

If, after further evaluation, an adverse event initially considered not to be associated with the use of the gene transfer product is subsequently determined to be associated, then the event will be

reported to the **FDA** as soon as possible, but in no case later than **15 calendar** days after the determination is made.

Relevant additional clinical and laboratory data will become available following the initial serious adverse event report. Relevant follow-up information to an IND safety report will be submitted concurrently to the **FDA/IRB and the DSMB** as soon as the information is available and will be identified as such, i.e., "Follow-up IND Safety Report." If a serious adverse event occurs after the end of a clinical trial and is determined to be associated with the use of the gene transfer product, that event will be reported concurrently to the **FDA within 15 calendar** days of the determination.

Any finding from tests in laboratory animals that suggests a significant risk for human research participants including reports of mutagenicity, teratogenicity, or carcinogenicity will be reported to **FDA** and the **DSMB as soon as possible**, but not later than **15 calendar days** after the sponsor's initial receipt of the information.

Should a serious adverse event deemed possibly, probably or definitely related to the study agent occur during administration, the study agent will be discontinued, appropriate treatment will be given under medical supervision and the subject will be examined as frequently as necessary thereafter until symptoms cease or stabilize.

11.3.3 Safety Reporting: Content and Format

The serious adverse event report will include, but need not be limited to: (1) the date of the event; (2) designation of the report as an initial report or a follow-up report, identification of all safety reports previously filed for the clinical protocol concerning a similar adverse event, and an analysis of the significance of the adverse event in light of previous similar reports; (3) clinical site; (4) the Sponsor Investigator; (5) FDA's Investigational New Drug (IND) application number; (6) vector type , e.g., adeno-associated virus; (7) vector subtype, if relevant; (8) gene delivery method, e.g., *in vivo* transduction; (9) route of administration, e.g., intramuscular; (10) dosing schedule; (11) a complete description of the event; (12) relevant clinical observations; (13) relevant clinical history; (14) relevant tests that were or are planned to be conducted; (15) date of any treatment of the event; and (16) the suspected cause of the event. These items will be reported through an FDA safety report by using the recommended Adverse Event Reporting Template available on NIH's web site at:
http://osp.od.nih.gov/sites/default/files/resources/Adverse_Event_Template_.docx

11.3.4 Adverse Event Reporting from Primary Care Physician

Close communication will be established with the primary care physician of all study participants and will be maintained throughout the study. The important hallmarks of the study along with the proposed reporting plan will be explained. We will request that the primary care physician provide information regarding every routine visit and any intercurrent event taking place during the two years following gene transfer. Laboratory reports, hospitalizations, clinical notes and any other relevant medical records will be requested at the time of their occurrence. If non-routine visits are reported to us by the primary care physician, the study investigator will initiate an investigation to determine the possibility of an adverse event related to the gene transfer and will adhere to the adverse event reporting requirements in accordance with federal regulations, state laws, and the local institutional policies and procedures, as applicable.

During the consent process, the study investigator will emphasize the importance of subject communication with our study team. Any routine or non-routine doctor's visits or medical care

received during the two years following gene transfer should be reported to the study team. The study doctor will explain to the participant that copies of any relevant medical records of those visits will be requested from their medical care provider.

11.3.5 Follow-up of Adverse Events
All adverse events will be followed until resolution or stabilization.

11.4 Dose limiting toxicity (DLT)

Dose limiting toxicity is defined as any adverse event that is possibly, probably, or definitely related to the study agent, and is unexpected. This would include any grade 3 event, according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0; these classifications are outlined in **Table 10**.

Note: *Elevations in transaminases and transient alterations in WBCs, as outlined in Section 12.4.2, above, are expected, and will not be considered as dose limiting when within the ranges noted therein.*

Table 10: **Adverse Event Classification**

1	Mild adverse event; did not require treatment
2	Moderate adverse event; resolved with treatment
3	Severe adverse event; inability to carry on normal activities; required professional medical attention
4	Life-threatening or permanently disabling adverse event
5	Fatal adverse event

Study enrollment will be halted by the investigators when any subject experiences two or more **Grade 3, or higher** adverse event toxicity that are unexpected and **possibly, probably, or definitely related** to the study drug. The event will then be reviewed by the Data Safety Monitoring Board (DSMB) before making a determination to continue enrollment as described in Section 0.

Laboratory tests with values within the clinically significant range will be repeated during the same visit whenever possible (refer to CTCAE v5.0 for a complete list of clinically significant range values). If the test result returns after the subject leaves the clinic, they will be immediately contacted. For local residents they will be asked to return to the outpatient clinic for a repeat test. For non-local residents, arrangements will be made to have the blood test redrawn in a laboratory close to home or by their primary care physician. To avoid any confusion for the primary care physician, they will be informed (with permission from the subject) of their participation in the study at the time of gene transfer. If the AE requires treatment, this will be carried out by the primary care physician or a doctor of choice selected by the subject. We will obtain copies of repeat laboratory tests and any relevant medical records that will be added to the subject's research chart.

The SI will fulfill the reporting responsibilities under 21 CFR 312.32(c), to notify FDA in an IND safety report of potentially serious risks, as soon as possible, but no later than 15 calendar days after the investigator receives the safety information and determines that the information

qualifies for reporting. The investigator will confer with the DSMB and FDA before continuing to enroll.

11.4.1 Expected Adverse Events Related to Disease Progression

Subjects enrolled under this clinical protocol are expected to present clinically with adverse events related to natural progression of the disease. The draft guidance entitled "Duchenne Muscular Dystrophy Developing Drugs for Treatment over the Spectrum of Disease" (http://www.parentprojectmd.org/site/DocServer/Guidance_Document_Submission_-_Duchenne_Muscular_Dystrop.pdf?docID=15283) provides the basis of expected disease-related adverse events.

Adverse events determined to be due to the underlying disease progression will be recorded but will not be subject to the expedited reporting requirements outlined in Section 11.3.2. All AEs related to the disease and unrelated to the gene therapy administration will be reported annually to the FDA and IRB.

11.4.2 Anticipated Adverse Event Lab Findings related to Intervention

- Asymptomatic elevations in transaminases are a feature of Duchenne muscular dystrophy, as they are related to release of AST and ALT from muscle tissue and correlate with levels of creatine kinase. These are a feature of the disease itself. Levels of AST (up to 12.3X the upper limit of normal) and ALT (up to 22.6X the upper limit of normal) are expected to be seen at baseline, and in the setting of normal GGT function are not indicative of muscle injury[44]. They are thus not exclusionary for enrollment, and will not be recorded as adverse events prior to gene transfer.

  Following gene transfer, transient transaminases (up to levels of 2.5x the subject's baseline value) with preserved liver synthetic function and no significant elevation in GGT are anticipated, and will be recorded as Grade 1 adverse events, but not considered as adverse reaction in the presence of a normal GGT value. Mild decreases in leukocytes and lymphocyte counts within the first 30 days after gene transfer have been observed in other trials of AAV mediated gene transfer. Such transient decreases in lymphocytes or leukocytes will be recorded as adverse events but considered anticipated.

- Leukocytosis and neutrophilia during prednisone or prednisolone treatment:
  An elevation of up to 5000 cells/mm^3 above the subject's baseline value is expected in the neutrophil count (the primary granulocyte in circulation) and, consequently, in the leukocyte count within 5 hours of initiating prednisone or prednisolone therapy, based upon studies in healthy adult volunteers[45]. This occurs secondary to release of granulocytes from the marginated pool into the circulation accompanied by an increase in the size of the marginated granulocyte pool due to steroids.[46] This steroid effect may be seen with increased steroid dosing.

11.5 Discontinuation Rules

An independent Data Safety Monitoring Board (DSMB) will be established. Safety data will be monitored on a continual basis throughout the trial. The DSMB can recommend early termination of the trial for reasons of safety. Study enrollment will be halted by the investigators when any subject experiences two or more Grade 3, or higher adverse events that are **unexpected** and **possibly, probably, or definitely related** to the study drug. This will include any subject death, important clinical laboratory finding, or any severe local complication in the injected area related to administration of the study agent. If after review by the DSMB, the decision is made to continue, the study will proceed according to Section 6.4 of this protocol.

The investigators will confer with the DSMB on all Grade 3 or higher adverse events that are possibly, probably, or definitely related to the study agent before continuing enrollment.

12 CLINICAL MONITORING

12.1 Data and Safety Monitoring Plan

The Data and Safety Monitoring Board (DSMB) will act in an advisory capacity to review participant safety and study progress of Phase I/IIa Systemic Gene Delivery Clinical Trial of scAAV9.U7.ACCA for Exon 2 Duplication-Associated Duchenne muscular dystrophy.

12.1.1 DSMB Membership

The DSMB membership consists of persons completely independent of the investigator who have no financial, scientific, or other conflicts of interest with the trial. Current or past collaborators or associates of Dr. Waldrop must note any conflict of interest before their eligibility to serve on the DSMB is approved.

The DSMB will include experts in or representatives of the fields of:

- Pediatric Neurology and Neuromuscular Diseases
- Immunology
- Gene Therapy
- Hepatology
- Muscular Dystrophy Clinical Care
- Clinical Research and Clinical Trials

Individuals invited to serve on the DSMB as either voting or non-voting members must disclose any potential conflicts of interest, whether real or perceived. Conflicts of interest can include professional, proprietary, and miscellaneous interests as described in the NIH Grant Policy Statement and 45 CFR Part 94. Potential conflicts that develop during a member's tenure on a DSMB must also be disclosed. Written documentation attesting to an absence of conflict of interest is required annually.

12.1.2 DSMB Responsibilities

Responsibilities of the DSMB are to:

- Review the research protocol, informed consent documents and plans for data and safety monitoring;
- Evaluate the progress of the trial, including periodic assessments of data quality and timelines, participant recruitment, accrual and retention, participant risk versus benefit, trial site performance, and other factors that can affect study outcome;
- Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on participant safety or the ethics of the trials;
- Review study performance, make recommendations and assist in the resolution of problems reported by the Sponsor Investigator;
- Protect the safety of the study participants;
- Ensure the confidentiality of the trial data and the results of monitoring; and
- Assist by commenting on any problems with study conduct, enrollment, and sample size and/or data collection.

12.1.3 DSMB Reporting and Meetings

Reports describing the status of the study will be prepared by the Sponsor Investigator's staff and sent to the DSMB prior to a meeting, or at the DSMB's request.

A meeting (either by teleconference or webcast) with the DSMB will be scheduled prior to study initiation, after the Day 30 visit of the first subject and then approximately every 6 months, or at the DSMB's request. Reports will be submitted prior to a scheduled meeting for review by the DSMB.

Reports will include the following:

- A brief narrative of the study status, including the target enrollment, current and projected time to completing enrollment. Any significant events and/or difficulties should be briefly described in this narrative.
- A brief narrative for each participant describing gender, age, race and ethnicity and other relevant demographic characteristics. The narrative for each participant should briefly describe his/her study status (i.e., dose level, visit number, adverse event information);
- A timeline outlining the study progress relative to visit number for each participant, as well as time points for each SAE/Dose Limiting Toxicity. A total for Adverse Events (AEs) for each participant should be included.
- A summary of AEs by severity levels;
- A listing of AE details grouped by participant;
- A listing of SAE details grouped by participant;
- A listing of deaths
- A summary of clinically significant laboratory test results

12.2 Institutional Review Board Monitoring

The Sponsor Investigator must obtain IRB approval for the investigation. Initial IRB approval, and all materials approved by the IRB for this study including the patient consent form and recruitment materials must be maintained by the Investigator and made available for inspection. Amendments will be submitted to the Nationwide Children's Hospital IRB for their review and approval prior to implementation. When an amendment to a protocol substantially alters the study design or increases potential risk to the study subject, the Informed Consent form will be revised and if applicable, subject's consent to continue participation will again be obtained.

13 STATISTICAL CONSIDERATIONS

For the secondary outcome measure of dystrophin expression, quantification of the number of fibers expressing dystrophin (determined by quantification of the number of fibers), measurement of the intensity of dystrophin staining at the sarcolemma (based on quantitative image analysis), and the degree of exon 2 skipping (as determined by RT-PCR) will be performed for each patient at each time point in a blinded fashion, and intra-patient comparisons of pre- and post-treatment values will be analyzed using repeated measures ANOVA with a Dunnett correction for multiple comparisons vs. baseline. For detecting change, we will have 80% power to detect a very large effect size (SMD=1.4-3.3, for sample sizes from 3-6).

14 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

14.1 Data Management and Study Forms

An outside contracted monitor of the study called a "CRO" will also monitor the study on a regular basis to make sure the study is conducted in compliance with all regulatory aspects of the protocol.

All data and observations will be documented on electronic Case Report Forms (CRF) by source documentation using the Medrio Electronic Data Capture designed for the study. A Safety Monitor will have access to the data to monitor adherence to the protocol and to applicable FDA regulations, and the maintenance of adequate and accurate clinical records. An electronic Case Report Form will be completed for every subject that was registered for participation in the study. The Case Report Form will be reviewed in detail. Case Report Forms will be completed as information becomes available.

Case Report Forms will be reviewed in detail by the Safety Monitor in a regular basis for which the Safety Monitor will have access to subject medical records, laboratory data, and other source documentation. Safety monitor will make a decision as to the data acceptability. If errors or omissions are found in the course of a data audit, or if clarification of data is required, the electronic Case Report Form(s) in question will be corrected by the SI or their designee. Data Resolution may be generated on omissions or clarifications, to be completed, electronically signed and dated, and maintained as a part of the eCRF. The SI will sign and accept the indicated

electronic Case Report Form. This signature will indicate that thorough inspection of the data therein has been made and will thereby certify the contents of the form.

14.2 Electronic Case Report Forms

Adequate and accurate case records will be maintained and all relevant observations and data related to the study will be recorded. This will include medical history, physical examination, concomitant medications, adverse events, vitals, MRI, ECHO, ECG, muscle strength tests, PFTs, muscle biopsies, hematology, clinical chemistry, serology, coagulation, and immunologic results.

Electronic CRFs will be used in this study. The eCRF will be electronically signed and dated by the Sponsor Investigator or his/her designee after his/her review. After the completion of the study, completed eCRFs will be retained in the archives.

Completed eCRFs will be reviewed by the study monitor against the source documentation for accuracy and completeness.

14.3 Inspection of Records

The Investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, subject charts, study source documents, and other records relative to study conduct.

15 QUALITY ASSURANCE AND QUALITY CONTROL

15.1 Study Monitoring

The study will be monitored in compliance with the relevant parts of 21 CFR and according to the ICH GCP Guidelines.

The procedures outlined in the protocol and case report forms will be carefully reviewed by the SI and staff prior to study initiation to ensure appropriate interpretation and implementation. No deviations from the protocol shall be made except in emergency situations where alternative treatment is necessary for the protection, proper care and well-being of subjects.

During the study, a monitor will have regular contact with the investigational site for the following:

- Provide information and support to the investigator(s);
- Confirm that facilities remain acceptable;
- Confirm that the investigational team is adhering to the protocol and ICH/GCP guidelines, that data are being accurately recorded in the case report forms, and that investigational product accountability checks are being performed;
- Perform source data verification. This includes comparison of the data in the case report forms with the patient's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each patient

(e.g. clinic charts) including access to the site's Electronic Medical Record (EMR) and any videos captured during specified visit assessments [edit as applicable to study];
- Records and report any protocol deviations not previously reported;
- Confirms AEs and SAEs have been properly documents on eCRFs and confirm those SAEs that met criteria for reporting have been forwarded to the IRB.

The monitor will be available between visits if the investigator(s) or other staff needs information or advice.

16 ETHICS/PROTECTION OF HUMAN SUBJECTS

16.1 Ethics Review

The final study protocol, including the final version of the Informed Consent Form, must be approved or given a favorable opinion in writing by an IRB or IEC as appropriate.
The Sponsor Investigator is responsible for informing the IRB of any amendment to the protocol in accordance with Nationwide Children's Hospital requirements. The protocol must be re-approved by the IRB upon receipt of amendments and annually, as required by Nationwide Children's Hospital. When an amendment to a protocol substantially alters the study design or increases potential risk to the study subject, the Informed Consent form will be revised and if applicable, subject's consent to continue participation will again be obtained.

16.2 Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, and applicable regulatory requirements.

16.3 Written Informed Consent

Legally effective and properly executed written informed consent, in compliance with 21 CFR 50 and the International Conference on Harmonization (ICH) guidelines, will be obtained from each subject before the subject is entered into the trial or before any unusual or non-routine procedure is performed that involves risk to the subject. The Sponsor Investigator(s) will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

Attention will be directed to the basic elements that are required for incorporation into the informed consent under US Federal Regulations for Protection of Human Subjects [21CFR 50.25(a)]. If new information related to the study arises, subjects will be asked to sign a revised document. Signed consent forms will remain in each subject's research chart and be available for the verification by study monitors at any time. For this study, only the Sponsor Investigator will be obtaining initial consent. Reconsenting due to changes in the Informed Consent may be done by other study staff as delegated on the Delegation of Authority Log. The Sponsor Investigator(s) must maintain the original, signed Informed Consent Form. Subjects will be given a signed, dated copy of their consent form documents.

Per the National Institutes of Health's Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (https://osp.od.nih.gov), an autopsy must be requested should a study participant die following participation in a gene transfer trial, no matter what the cause of death. An autopsy is requested to obtain vital information about the safety and efficacy of gene transfer. Subjects' parent(s)/legal guardians will be asked to provide consent for an autopsy in advance of any death. Consent is requested at the beginning of the study to relieve the burden of making such a decision at the time of death should such an event occur.

17 DATA HANDLING AND RECORD KEEPING

17.1 Retention of Records

All primary data that are a result of the original observations and activities of the study and that are necessary for the reconstruction and evaluation of any study report will be retained in a secure archive at the study site for a period not less than 2 years after the last approval of a marketing application in an ICH region, and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have lapsed since the formal discontinuation of the clinical development of the investigational product.
The site will maintain a Regulatory Binder. In this binder, there will be tabbed sections for study documents including the following: study personnel identification and signature list, subject screening records, subject roster (names omitted), protocol and amendments or administrative changes, FDA Form 1572 (if required), study staff Curricula Vitae, IRB documentation, an approved sample ICF, correspondence, site monitoring reports, blank Data Documentation form, and lab accreditations and normal values. The site must keep this binder current and available for review by the IRB and/or FDA.

17.2 Retention of Samples

The identified storage laboratory will be responsible for arranging storage of any remaining or unused biological samples as well as properly documenting the storage procedures, once all study-required analyses are complete, as per sample processing requirements until such time that the IND holder provides external storage vendor transfer or destruction instructions.

17.3 Study Reports

17.3.1 Annual Study Reports

Within 60 days after the one-year anniversary of the date on which the investigational new drug (IND) application went into effect, and after each subsequent anniversary until the trial is completed, the Sponsor Investigator will submit information set forth as follows:

a) Clinical Trial Information. This will be a brief summary of the status of the trial in progress or completed during the previous year. The summary will include the following information for the trial: (1) the title and purpose of the trial; (2) clinical site; (3) the Sponsor Investigator; (4) clinical protocol identifiers, including the IRB and IBCSC protocol numbers, and the FDA IND application number; (5) participant population (such as disease indication and general age group); (6) the total number of participants planned for inclusion in the trial; the number entered into the trial to date; the number whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons; (7) the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed, and (8) if the trial has been completed, a brief description of any study results.

b) Progress Report and Data Analysis. Information obtained during the previous year's clinical and non-clinical investigations, including: (1) a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system; (2) a summary of all serious adverse events submitted during the past year; (3) a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications; (4) if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death; and (5) a brief description of any information obtained that is pertinent to an understanding of the gene transfer product’s action, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

c) A copy of the updated clinical protocol including a technical and non-technical abstract.

18 REFERENCES

1. Willcocks RJ, Rooney WD, Triplett WT, et al. Multicenter prospective longitudinal study of magnetic resonance biomarkers in a large duchenne muscular dystrophy cohort. Annals of neurology 2016;79:535-547.
2. Wein N, Vulin A, Falzarano MS, et al. Translation from a DMD exon 5 IRES results in a functional dystrophin isoform that attenuates dystrophinopathy in humans and mice. Nat Med 2014;20:992-1000.
3. Vulin A, Wein N, Simmons TR, et al. The first exon duplication mouse model of Duchenne muscular dystrophy: A tool for therapeutic development. Neuromuscular disorders : NMD 2015;25:827-834.
4. Muenzer J, Wraith JE, Beck M, et al. A phase II/III clinical study of enzyme replacement therapy with idursulfase in mucopolysaccharidosis II (Hunter syndrome). Genetics in medicine : official journal of the American College of Medical Genetics 2006;8:465-473.
5. Kishnani PS, Corzo D, Nicolino M, et al. Recombinant human acid [alpha]-glucosidase: major clinical benefits in infantile-onset Pompe disease. Neurology 2007;68:99-109.
6. Wraith JE, Beck M, Giugliani R, Clarke J, Martin R, Muenzer J. Initial report from the Hunter Outcome Survey. Genetics in medicine : official journal of the American College of Medical Genetics 2008;10:508-516.
7. Longo N, Arnold GL, Pridjian G, et al. Long-term safety and efficacy of sapropterin: the PKUDOS registry experience. Molecular genetics and metabolism 2015;114:557-563.
8. Grange DK, Hillman RE, Burton BK, et al. Sapropterin dihydrochloride use in pregnant women with phenylketonuria: an interim report of the PKU MOMS sub-registry. Molecular genetics and metabolism 2014;112:9-16.
9. Mendell JR, Shilling C, Leslie ND, et al. Evidence-based path to newborn screening for Duchenne muscular dystrophy. Ann Neurol 2012;71:304-313.
10. Flanigan K. Duchenne and Becker muscular dystrophies. Neurologic Clinics 2014;32:671-688.
11. Eagle M, Baudouin SV, Chandler C, Giddings DR, Bullock R, Bushby K. Survival in Duchenne muscular dystrophy: improvements in life expectancy since 1967 and the impact of home nocturnal ventilation. Neuromuscul Disord 2002;12:926-929.
12. Koenig M, Hoffman EP, Bertelson CJ, Monaco AP, Feener C, Kunkel LM. Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of the DMD gene in normal and affected individuals. Cell 1987;50:509-517.
13. Bonilla E, Samitt CE, Miranda AF, et al. Duchenne muscular dystrophy: deficiency of dystrophin at the muscle cell surface. Cell 1988;54:447-452.
14. Oudet C, Hanauer A, Clemens P, Caskey T, Mandel JL. Two hot spots of recombination in the DMD gene correlate with the deletion prone regions. Human molecular genetics 1992;1:599-603.
15. Beggs AH, Koenig M, Boyce FM, Kunkel LM. Detection of 98% of DMD/BMD gene deletions by polymerase chain reaction. Hum Genet 1990;86:45-48.
16. Chamberlain JS, Gibbs RA, Ranier JE, Nguyen PN, Caskey CT. Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. Nucleic acids research 1988;16:11141-11156.
17. Lalic T, Vossen RH, Coffa J, et al. Deletion and duplication screening in the DMD gene using MLPA. European journal of human genetics : EJHG 2005;13:1231-1234.
18. Dent KM, Dunn DM, von Niederhausern AC, et al. Improved molecular diagnosis of dystrophinopathies in an unselected clinical cohort. American journal of medical genetics Part A 2005;134:295-298.

19. Flanigan KM, von Niederhausern A, Dunn DM, Alder J, Mendell JR, Weiss RB. Rapid direct sequence analysis of the dystrophin gene. American journal of human genetics 2003;72:931-939.
20. Mendell JR, Moxley RT, Griggs RC, et al. Randomized, double-blind six-month trial of prednisone in Duchenne's muscular dystrophy. The New England journal of medicine 1989;320:1592-1597.
21. Balaban B, Matthews DJ, Clayton GH, Carry T. Corticosteroid treatment and functional improvement in Duchenne muscular dystrophy: long-term effect. American journal of physical medicine & rehabilitation 2005;84:843-850.
22. Griggs RC, Moxley RT, 3rd, Mendell JR, et al. Prednisone in Duchenne dystrophy. A randomized, controlled trial defining the time course and dose response. Clinical Investigation of Duchenne Dystrophy Group. Archives of neurology 1991;48:383-388.
23. Biggar WD, Harris VA, Eliasoph L, Alman B. Long-term benefits of deflazacort treatment for boys with Duchenne muscular dystrophy in their second decade. Neuromuscul Disord 2006;16:249-255.
24. Mendell JR, Goemans N, Lowes LP, et al. Longitudinal effect of eteplirsen versus historical control on ambulation in Duchenne muscular dystrophy. Annals of neurology 2016;79:257-271.
25. Mendell JR, Rodino-Klapac LR, Sahenk Z, et al. Eteplirsen for the treatment of Duchenne muscular dystrophy. Ann Neurol 2013;74:637-647.
26. Bushby K, Finkel R, Wong B, et al. Ataluren treatment of patients with nonsense mutation dystrophinopathy. Muscle & nerve 2014;50:477-487.
27. Finkel RS, Flanigan KM, Wong B, et al. Phase 2a study of ataluren-mediated dystrophin production in patients with nonsense mutation Duchenne muscular dystrophy. PloS one 2013;8:e81302.
28. Ryan NJ. Ataluren: first global approval. Drugs 2014;74:1709-1714.
29. SPINRAZA (R) [package insert]. Cambridge, MA: Biogen.
30. EXONDYS 51 (R) [package insert]. Cambridge, MA: Sarepta Therapeutics, Inc.
31. Gorman L, Suter D, Emerick V, Schumperli D, Kole R. Stable alteration of pre-mRNA splicing patterns by modified U7 small nuclear RNAs. Proceedings of the National Academy of Sciences of the United States of America 1998;95:4929-4934.
32. Goyenvalle A, Vulin A, Fougerousse F, et al. Rescue of dystrophic muscle through U7 snRNA-mediated exon skipping. Science 2004;306:1796-1799.
33. Vulin A, Barthelemy I, Goyenvalle A, et al. Muscle function recovery in golden retriever muscular dystrophy after AAV1-U7 exon skipping. Molecular therapy : the journal of the American Society of Gene Therapy 2012;20:2120-2133.
34. Aartsma-Rus A, Van Deutekom JC, Fokkema IF, Van Ommen GJ, Den Dunnen JT. Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. Muscle & nerve 2006;34:135-144.
35. Flanigan KM, Dunn DM, von Niederhausern A, et al. Mutational spectrum of DMD mutations in dystrophinopathy patients: application of modern diagnostic techniques to a large cohort. Human mutation 2009;30:1657-1666.
36. Flanigan KM, Dunn DM, von Niederhausern A, et al. DMD Trp3X nonsense mutation associated with a founder effect in North American families with mild Becker muscular dystrophy. Neuromuscul Disord 2009;19:743-748.
37. Gurvich OL, Maiti B, Weiss RB, Aggarwal G, Howard MT, Flanigan KM. DMD exon 1 truncating point mutations: amelioration of phenotype by alternative translation initiation in exon 6. Hum Mutat 2009;30:633-640.
38. Bladen CL, Salgado D, Monges S, et al. The TREAT-NMD DMD Global Database: analysis of more than 7,000 Duchenne muscular dystrophy mutations. Hum Mutat 2015;36:395-402.

39. Taylor L, Kaminoh Y, Rodesch C, Flanigan K. Quantification of dystrophin immunofluorescence in dystrophinopathy muscle specimens. Neuropathology and Applied Neurobiology 2012;38:591-601.
40. Anthony K, Arechavala-Gomeza V, Taylor LE, et al. Dystrophin quantification: Biological and translational research implications. Neurology 2014;83:2062-2069.
41. Liu G, McNicol PL, Macall P, et al. The Effect of Preoperative Aspirin and/or Heparin Therapy on Coagulation and Postoperative Blood Loss after Coronary Artery Bypass Surgery. Critical care and resuscitation : journal of the Australasian Academy of Critical Care Medicine 1999;1:139.
42. Veikutiene A, Sirvinskas E, Grybauskas P, Cimbolaityte J, Mongirdiene A, Veikutis V. [Influence of preoperative treatment with aspirin or heparin on platelet function and intensity of postoperative bleeding in early period after coronary artery bypass surgery]. Medicina 2005;41:577-583.
43. Sun JC, Crowther MA, Warkentin TE, Lamy A, Teoh KH. Should aspirin be discontinued before coronary artery bypass surgery? Circulation 2005;112:e85-90.
44. McMillan HJ, Gregas M, Darras BT, Kang PB. Serum transaminase levels in boys with Duchenne and Becker muscular dystrophy. Pediatrics 2011;127:e132-136.
45. Dale DC, Fauci AS, Guerry DI, Wolff SM. Comparison of agents producing a neutrophilic leukocytosis in man. Hydrocortisone, prednisone, endotoxin, and etiocholanolone. The Journal of clinical investigation 1975;56:808-813.
46. Summers C, Rankin SM, Condliffe AM, Singh N, Peters AM, Chilvers ER. Neutrophil kinetics in health and disease. Trends in immunology 2010;31:318-324.