



**NATIONWIDE CHILDREN'S**

*When your child needs a hospital, everything matters.™*

**Study Title: Systemic gene delivery Phase I/IIa clinical trial for Duchenne muscular dystrophy using rAAVrh74.MHCK7.micro-dystrophin**

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## **CLINICAL STUDY PROTOCOL**

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Center for Gene Therapy

Study Title: Systemic gene delivery Phase I/IIa clinical trial for Duchenne muscular dystrophy using rAAVrh74.MHCK7.micro-dystrophin

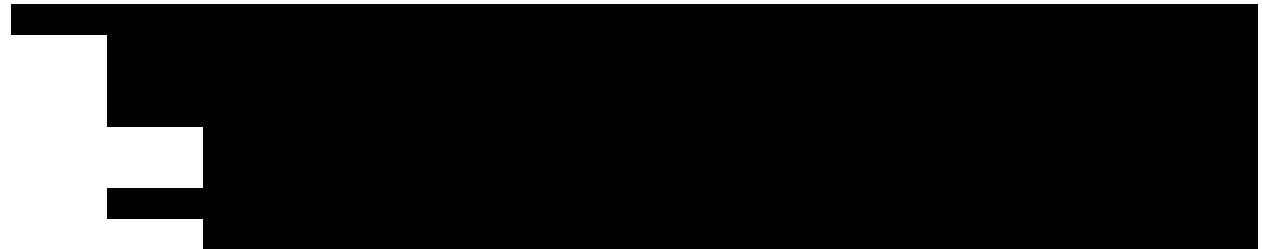
Version: 0.12 October 26, 2017

I have read the protocol described above. I agree to conduct this trial according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the trial in accordance with all applicable regulations, and guidelines as stated in the protocol and other information supplied to me.

Signed: \_\_\_\_\_  
Anne Connolly, M.D.

Date: \_\_\_\_\_

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## 1. Protocol Synopsis

Title	Phase I/IIa gene transfer clinical trial for DMD using AAVrh74.MHCK7.micro-dys administered through peripheral arm vein
Study Number	DMD n = 12
Clinical Study Phase	Phase I/IIa trial
Number of Centers	Primary Center: Nationwide Children's Hospital and Collaborative Center: Washington University (St. Louis Children's Hospital)
Study Objectives	Primary objective is safety
Study Design	Peripheral infusion of gene over 1 hour
Patient Population	<p>Inclusion Criteria</p> <ol style="list-style-type: none"> <li>1. Age of enrollment: Cohort A (n=6) is between 3 months to 3 years of age, inclusive; Cohort B (n=6) is between 4 to 7 years of age, inclusive.</li> <li>2. Molecular characterization of the DMD gene with frameshift (deletion or duplication), or premature stop codon mutation between exons 18 to 58.</li> <li>3. Indication of symptomatic muscular dystrophy: <ul style="list-style-type: none"> <li>• CK elevation &gt;1000 U/L <b>and</b></li> <li>• Cohort A: below average on the Bayley-III motor assessment for gross motor defined as a scaled score of ≤9. Any subject that is 43-47 months of age, inclusive, at time of screening will have the scaled score calculated compared to normative data for 42 month old children. The Bayley-III provides normative data for children 1-42 months of age.</li> <li>• Cohort B: below average on the 100 Meter Timed Test defined as ≤ 80% predicted<sup>1</sup>.</li> </ul> </li> <li>4. Males of any ethnic group will be eligible.</li> <li>5. Ability to cooperate with motor assessment testing.</li> <li>6. For Cohort A subjects: No previous treatment with corticosteroids. For Cohort B subjects: Stable dose equivalent of oral corticosteroids for at least 12 weeks prior to screening and the dose is expected to remain constant (except for potential modifications to accommodate changes in weight) throughout the study.</li> </ol> <p>Exclusion Criteria</p> <ol style="list-style-type: none"> <li>1. Active viral infection based on clinical observations.</li> <li>2. Signs of cardiomyopathy, including echocardiogram with ejection fraction below 40%.</li> <li>3. Serological evidence of HIV infection, or Hepatitis B or C infection.</li> </ol>

	<ol style="list-style-type: none"> <li>4. Diagnosis of (or ongoing treatment for) an autoimmune disease.</li> <li>5. Abnormal laboratory values considered clinically significant (GGT &gt; 3XULN, bilirubin ≥ 3.0 mg/dL, creatinine ≥ 1.8 mg/dL, Hgb &lt; 8 or &gt; 18 g/dL; WBC &gt; 18,500 per cmm).</li> <li>6. Concomitant illness or requirement for chronic drug treatment that in the opinion of the PI creates unnecessary risks for gene transfer.</li> <li>7. Subjects with AAVrh74 [REDACTED] antibody titers &gt; 1:400 as determined by ELISA immunoassay.             <ul style="list-style-type: none"> <li>• If endpoint titer is positive at screening, testing may be repeated prior to exclusion.</li> <li>• If present in infant and mother is positive for same antibody titers, mother will be asked not to breast feed and infant can be enrolled if antibodies drop ≤1:400 within 12 weeks.</li> </ul> </li> <li>8. Has a medical condition or extenuating circumstance that, in the opinion of the investigator, might compromise the subject's ability to comply with the protocol required testing or procedures or compromise the subject's wellbeing, safety, or clinical interpretability.</li> <li>9. Severe infection (e.g., pneumonia, pyelonephritis, or meningitis) within 4 weeks before gene transfer visit (enrollment may be postponed).</li> <li>10. Has received any investigational medication (other than corticosteroids) or exon skipping medications (including ExonDys 51), experimental or otherwise, in the last 6 months prior to screening for this study.</li> <li>11. Has had any type of gene therapy, cell based therapy (e.g. stem cell transplantation), or CRISPR/Cas9.</li> <li>12. Family does not want to disclose patient's study participation with primary care physician and other medical providers.</li> </ol>
<p>Vector administration</p>	<p>The vector will be delivered via peripheral limb vein. Study subjects will receive sedation if deemed necessary for the procedure following Children's Hospital dosing protocol. Six (Cohort A) and six (Cohort B) will receive intravenous micro-dystrophin vector (2X10<sup>14</sup> vg/kg [REDACTED]).</p> <p>One-day prior to gene transfer subjects in Cohort A will be started on prednisolone (prednisone or deflazacort acceptable) 1 mg/kg and maintained for 30 days while monitoring immune response using IFN-γ ELISPOT Assay to AAV or micro-dystrophin. If the liver enzyme gamma-glutamyl transferase (GGT) is elevated at day 30, steroids will be maintained until levels drop below 50 U/L.</p>

	<p>Cohort B subjects receiving daily glucocorticoid medication will be maintained on stable dose of corticosteroids throughout trial but may be increased for short time if GGT is elevated &gt;50 U/L. Those on weekend dosing will receive an added daily dose equivalent to 1 mg/kg for 30 days.</p>
<p>Primary Outcome</p>	<p>Safety is the primary outcome for this clinical gene transfer trial.</p>
<p>Secondary Outcomes</p>	<p>The following efficacy outcome measures will be done at designated time-points:</p> <ul style="list-style-type: none"> <li>• Time points for secondary outcomes: day 30, day 60, day 90, day 180-months 9, 12, 18, 24, 30 and 36. (Visit window permitted for visits 3-5 is <math>\pm 7</math> days. Visit window for visits 6-10 is <math>\pm 14</math> days. Visit window for visits 11-14 is <math>\pm 21</math> days.)</li> <li>• The Bayley Scales of Infant and Toddler Development Third Edition Gross Motor Subtest (Bayley-III) scores will serve as the secondary outcome measure for this trial for Cohort A. Control data for DMD infants at this age using the Bayley-III Gross Motor Scale has been obtained and published from data collected in the MDA Network Clinical Centers<sup>5</sup>. Ongoing data is being collected in DMD boys up to 5 years of age in our MDA clinic at Nationwide Children's Hospital. The Bayley-III is designed to assess the developmental functioning of infants and young children 1-42 months of age (Bayley N. Bayley Scales of Infant and Toddler Development: Third Edition 2006 published by PsychCorp).</li> <li>• Physical Therapy Assessments: The 100 Meter Timed Test (100m) will be a primary motor outcome and the North Star Ambulatory Assessment (NSAA) will be an exploratory outcome initiated for Cohort A as soon as the child is four years of age. The 100m will be the primary motor outcome for Cohort B. Exploratory outcomes for Cohort B will include the NSAA, Timed Up and Go modified for children (TUG)<sup>1,2</sup>, ascend and descend of 4 steps, and hand-held dynamometry (HHD) for knee extensors and knee flexors, and elbow flexors and elbow extensors.</li> <li>• Baseline muscle biopsies to assess dystrophin expression will be done days -30 to -7 pre-treatment on all subjects. All subjects will have a post-treatment biopsy at Day 180. Micro-dystrophin gene expression will be quantified (immunofluorescence and western blot analysis) and compared in pre and post muscle biopsies. Dystrophin will also serve as a secondary outcome measure for both cohorts.</li> <li>• A decrease in CK following gene therapy will serve as an exploratory outcome. CK levels will only be drawn on study</li> </ul>



	visits that occur over a two day period to allow for coordination with the physical therapy assessments.
Study Duration	We will evaluate short-term safety over a three year period. Subjects will be tested at baseline and return for follow up visits on days 1, 7, 14, 30, 60 and months 3, 6, 9, 12, 18, 24, 30, and 36. Immune studies will continue with blood samples collected at time points up to three years.
Sample Size	Twelve DMD subjects (Cohort A, n=6; Cohort B, n=6) will be enrolled at Nationwide Children's Hospital for the gene transfer study. Subjects will encompass males of any ethnic or racial background. Cohort A will include subjects 3 months to 3 years, inclusive at the time of enrollment. Cohort B will include subjects ages 4 to 7 years old, inclusive, at the time of enrollment and will preferentially be enrolled to receive gene delivery prior Cohort A.
Statistical Analysis	<p>This is a Phase I/IIa trial, with safety as the primary measure. Bayley-III pre- and post-gene transfer will be the primary motor outcome for Cohort A. The 100 Meter Timed Test (100m) will be a primary motor outcome and the North Star Ambulatory Assessment (NSAA) will be an exploratory outcome initiated for Cohort A as soon as the child is four years of age. The 100m will be the primary motor outcome for Cohort B. Exploratory outcomes for Cohort B will include the NSAA, Timed Up and Go modified for children (TUG)<sup>1,2</sup>, ascend and descend of 4 steps, and hand-held dynamometry (HHD) for knee extensors and knee flexors, and elbow flexors and elbow extensors. As the sample size is small and the motor outcomes will differ between cohorts as well as over time for some patients within Cohort A, all analyses will be descriptive in nature and will not employ inferential statistics. Both raw scores as well as z-scores will be presented for each individual patient on all outcomes. Data will be summarized using means and standard deviations for all patients combined and by cohort, at each follow-up time. Moreover, based on a preliminary definition of efficacy as an increase of 15%, the number and percentage of patients overall and within each cohort who experience an efficacious change from baseline will be reported.</p> <p>Frozen sections will be stained for dystrophin using direct immunofluorescence (IF). Stained slides will be shipped to Sarepta Therapeutics for micro-dystrophin quantification. Full slide scanning will be performed and quantification of micro-dystrophin intensity and percent positive fibers will be achieved using validated image scanning and MuscleMap™ analysis algorithm. Blinded frozen muscle biopsy shavings will also be shipped to Sarepta Therapeutics to perform quantitative protein analysis for micro-dystrophin using a validated western blot method. Muscle morphometrics will also be performed blinded by Sarepta</p>

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	Therapeutics, including fiber size histograms and quantification of central nucleation. 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 following unblinding ( $p < 0.05$ ).
Long-term follow-up	We will follow the most recent FDA guidance with regard to long-term patient follow up following gene transfer.

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### **2. Introduction**

#### **2.1. Clinical Trial and Co-PI and Sponsor-Investigator**

The study will be a two center study carried out at The Research Institute at Nationwide Children's Hospital in Columbus, Ohio and Washington University, St. Louis Children's Hospital in St. Louis, Missouri. The two centers have worked together for many years and most recently in two infant studies (natural history of infants and toddlers in DMD and a corticosteroid study in infants/young boys with DMD)<sup>1</sup>. The two groups working together will reduce the travel burden significantly for participants. The gene delivery will be performed only at The Research Institute at Nationwide Children's Hospital, but subjects will have the option of being followed at both Centers after the Day 30 visit per PI discretion. There is already in place inter-rater reliability outcome studies for the two teams of physical therapists who will be performing outcome measures. Dr. Jerry Mendell, Professor of Pediatrics and Neurology, will serve as both Sponsor of the IND and Principal Investigator (i.e. Sponsor-Investigator). Dr. Mendell brings to this trial more than forty years of clinical trial experience in neuromuscular disorders. With regard to gene therapy, he was the first to do viral-mediated gene transfer for muscular dystrophy as the principal investigator in two viral-mediated gene transfer trials, one in Duchenne muscular dystrophy (DMD, IND 12936 using a hybrid AAV2.5 consisting of AAV2 with 5 amino acid substitutions from AAV1, CMV promoter, and mini-dystrophin)<sup>2</sup>, and the other in Limb Girdle Muscular Dystrophy, type 2D (LGMD2D, IND 13434, AAV1, tMCK promoter, and alpha-sarcoglycan gene)<sup>3</sup>. He is currently conducting a gene therapy study using rAAV1.CMV.FS344 (IND # 14845) in two groups, sporadic inclusion body myositis and Becker muscular dystrophy. The results of the BMD and sIBM trials have been published<sup>4,5</sup>. He is also conducting a gene therapy trial in infants with SMA type 1 and showing significant efficacy (IND 15699). The results in this same group using the same dose for infusion have been safe (presented at the Presidential Symposium of the ASGCT 2016, and Late-breaking abstracts WMS 2017).<sup>6</sup> Efficacy has been documented given that this dose of AAV delivering the SMN gene to 15 subjects has saved the lives of all participants. 13 have survived past 20 months (compared to 8% in natural history studies, 15 have reached 13.6 months (25% survival) and 15/15 have reached 10.5 months (50% survival). Furthermore, 7 are feeding themselves, 2 subjects are crawling, 4 are standing with support, and 2 subjects are walking independently.

Dr. Anne Connolly, who will be a Co-PI on this study (PI at the Washington University site), is Professor of Neurology and Pediatrics. She serves as Co-Director of the Washington University MDA Clinic. She has made significant contributions in establishing the standard of care guidelines for DMD, SMA and Congenital muscular dystrophies. She has also made significant contributions toward efforts for newborn screening for DMD in the USA and China.

#### **2.2. Clinical Trial Design**

The proposed clinical trial is an open-label single-dose trial using rAAVrh74.MHCK7.micro-dystrophin for DMD subjects. Cohort A will include six subjects ages 3 months to 3 years, and Cohort B will include six subjects ages 4 to 7 years old. All subjects will receive intravenous micro-dystrophin vector ( $2 \times 10^{14}$  vg/kg [REDACTED]). Cohort B subjects will be enrolled first before Cohort A. Upon completion of Cohort B the safety data will be presented to FDA and DSMB requesting permission to enroll Cohort A. [REDACTED]

[REDACTED] The Bayley-III Gross Motor Subtest will serve as the secondary outcome measure for all children in Cohort A (Bayley Scales of Infant and Toddler Development: Third Edition 2006 published by PsychCorp). The 100 Meter Timed Test (100m) will be a primary motor outcome and the North Star Ambulatory Assessment (NSAA) will be an exploratory outcome initiated for Cohort A as soon as the child is four years of age. The 100m will be the primary motor outcome for Cohort B. Exploratory outcomes for Cohort B will include the NSAA, Timed Up and Go modified for children (TUG)<sup>7,8</sup>, ascend and descend of 4 steps, and hand-held dynamometry (HHD) for knee extensors and knee flexors, and elbow flexors and elbow extensors. Pre and post-treatment (180 Day) needle muscle biopsies<sup>9</sup> will be done on quadriceps or gastrocnemius muscles with appropriate conscious sedation under advisement of anesthesiologist (or anesthetist). EMLA Cream (or comparable lidocaine/prilocaine emulsion) will be used over site of biopsy. Dystrophin gene expression will serve as a secondary outcome measure. Quantification will be done using validated immunofluorescence and immunoblot assays. A decrease in CK following gene therapy will serve as an exploratory outcome. CK levels will only be drawn on study visits that occur over a two day period to allow for coordination with the physical therapy assessments.

**Table 1. Treatment Cohorts**

Cohort	Age	N	Vector Dose (vg/kg)	Volume (ml/kg)	Corticoid Steroids
A	3 – 48 mos	6	$2 \times 10^{14}$	[REDACTED]	1 mg/kg glucocorticoid maintained for 30 days post infusion unless GGT remains elevated
B	4 – 7 yrs	6	$2 \times 10^{14}$	[REDACTED]	Stable dose of oral corticosteroids 12 weeks prior to, and throughout, study

The primary objective of this study is the assessment of the safety of intravenous administration of rAAVrh74.MHCK7.micro-dystrophin for DMD subjects via peripheral limb vein. Safety endpoints will be assessed by changes in hematology, serum chemistry<sup>10,11</sup>, urinalysis, immunologic response to rAAVrh74 and micro-dystrophin, and reported history and observations of symptoms. Efficacy assessed by gene expression is a critical secondary outcome measure judged by micro-dystrophin gene expression on muscle biopsies. In addition, efficacy will be measured by the Bayley-III Gross Motor Subtest (Cohort A only). The 100 Meter Timed Test (100m) will be a primary motor outcome and the North Star Ambulatory Assessment (NSAA) will be an exploratory

outcome initiated for Cohort A as soon as the child is four years of age. The 100m will be the primary motor outcome for Cohort B. Exploratory outcomes for Cohort B will include the NSAA, Timed Up and Go modified for children (TUG)<sup>7,8</sup>, ascend and descend of 4 steps, and hand-held dynamometry (HHD) for knee extensors and knee flexors, and elbow flexors and elbow extensors.

### **2.3. Collaboration with Washington University**

All gene transfers and muscle biopsy procedures will be conducted at Nationwide Children's Hospital for every enrolled subject. Per the discretion of the PI, subjects may be seen for follow-up visits after the Day 30 visit with the study team at Washington University. All study team members including physicians, physical therapists, clinical research coordinator, etc. at Washington University will be trained on the approved protocol prior to first scheduled follow-up visit. Blood work for immunology studies will be shipped to Nationwide Children's for testing. It is expected that several subjects will be referred directly by Washington University, because of this, allowing subjects to be seen for follow-up appointments at this site will alleviate unnecessary patient travel. Subjects will not be seen at Washington University until a reliance agreement has been put in place.

## **3. Background and Significance**

### **3.1. Disease and Characteristics**

DMD is the most common, severe childhood form of muscular dystrophy. Inheritance follows an X-linked recessive pattern. Birth prevalence has been estimated at 1 in 5000 live male births.<sup>12</sup> Approximately one-third of cases represent new mutations of the *DMD* gene with the remaining inherited on the X chromosome from a carrier mother. Questions usually begin to surface between ages 3 to 5 regarding reduced motor skills that alert a need for diagnostic evaluation. DMD is relentlessly progressive with loss of ambulation by age 12.<sup>13</sup> Historically, patients died from respiratory complications. Now, a variety of factors protect the respiratory system related to improved supportive equipment, antibiotics, vaccines, and other ancillary methods.<sup>14</sup> Prolonging life un masks decline in cardiac function with complications of dilated cardiomyopathy. This poses further clinical challenges and a need for recognition and medical intervention that did not previously exist. Non-progressive cognitive dysfunction might be present in DMD and BMD.

### **3.2. Disease Pathogenesis**

More than 20 years ago the DMD gene was cloned defining the molecular basis of the disease.<sup>15</sup> The identification of dystrophin as the deficient protein followed closely on the heels of this discovery.<sup>16</sup> 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, diminished regenerative capacity of the muscle, ending in fat and connective tissue replacement (fibrosis). The mutation spectrum within the DMD gene reveals that deletions of one or more exons are found in ~65% of cases clustered in two hotspot regions.<sup>17</sup> Detection of duplications represents

6% of the DMD mutations. Additional tools identify the full spectrum of mutations (deletions, duplications, splice-site and point mutations).

### **3.3. Treatment for DMD**

Despite virtually hundreds of clinical trials in DMD, only one treatment has consistently demonstrated efficacy. Unequivocal evidence for glucocorticoid-induced improvement was established through a double-blind, randomized controlled trial in a large cohort of subjects (n=103). Corticosteroids are now considered the standard of care; however, the side effect profile may preclude treatment for some boys. The only other treatment that seems to show an effect is exon skipping which is only available to patients amenable to Exon 51 skipping.<sup>18</sup> The treatment does not increase strength but slows the progression of the disease. This was demonstrated in a study done at Nationwide Children's Hospital and is under further investigation at present.<sup>18</sup>

Gene replacement therapy has been studied for the past 10-15 years and shows very favorable results in pre-clinical studies in mice and canine species deficient in dystrophin. In 2006 the only clinical trial in DMD was done at Nationwide Children's Hospital and the results were published in the New England Journal of Medicine because of the potential impact of the findings.<sup>2</sup> Importantly, the dystrophin cDNA is 11kB and our vehicle for gene transfer, adeno-associated virus (AAV) can only hold 5 kB. Thus, the dystrophin gene must be scaled down in order to fit within the packaging constraints of AAV particles. This miniaturized transgene was termed mini-dystrophin and was delivered by intramuscular injection to DMD patients (IND 12936, closed). As this was a clinical trial, subjects' immune systems were sensitive to the slight changes that occurred when the gene was expressed in their muscle. When this was done in 2006 we discovered that if you inject the gene into a patient with a deletion of the DMD gene as the cause of the disease, it could cause an immune response and reject the gene. This happened in two subjects with deletions in regions of dystrophin that were present in the Mini-Dystrophin gene. From this trial, a new version of the truncated dystrophin gene was created termed micro-dystrophin, which retains different region of the native gene than mini-dystrophin. The micro-dystrophin transgene used in this trial is the same that was used in a clinical trial evaluating safety and expression of intramuscular injections of the product, rAAVrh74.MCK.micro-dystrophin (IND 16144). This construct demonstrated preliminary safety in the clinical trial.

Our current design is relatively simple but carefully planned. We will ensure that the micro-dystrophin and the patient's mutation are compatible so that we can achieve expression of our transgene without inducing immune rejection. We will also pre-test subjects for pre-existing immunity to AAV, so that we can avoid rejection when we transfer the gene.

## **4. Clinical Study Plan**

### **4.1. Study population**

#### **4.1.1. Subject Eligibility**

##### **4.1.1.1. Inclusion Criteria**

1. Age of enrollment: Cohort A (n=6) is between 3 months to 3 years of age, inclusive; Cohort B (n=6) is between 4 to 7 years of age, inclusive.
2. Molecular characterization of the DMD gene with frameshift (deletion or duplication), or premature stop codon mutation between exons 18 to 58.
3. Indication of symptomatic muscular dystrophy:
  - CK elevation >1000 U/L **and**
  - Cohort A: below average on the Bayley-III motor assessment for gross motor defined as a scaled score of  $\leq 9$ . Any subject that is 43-47 months of age, inclusive, at time of screening will have the scaled score calculated compared to normative data for 42 month old children. The Bayley-III provides normative data for children 1-42 months of age.
  - Cohort B: below average on the 100 Meter Timed Test defined as  $\leq 80\%$  predicted<sup>1</sup>.
4. Males of any ethnic group will be eligible.
5. Ability to cooperate with motor assessment testing.
6. For Cohort A subjects: No previous treatment with corticosteroids. For Cohort B subjects: Stable dose equivalent of oral corticosteroids for at least 12 weeks prior to screening and the dose is expected to remain constant (except for modifications to accommodate changes in weight) throughout the study.

#### 4.1.1.2. Exclusion Criteria

1. Active viral infection based on clinical observations.
2. Signs of cardiomyopathy, including 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. Abnormal laboratory values considered clinically significant (GGT > 3XULN, bilirubin  $\geq 3.0$  mg/dL, creatinine  $\geq 1.8$  mg/dL, Hgb < 8 or > 18 g/Dl; WBC > 18,500 per cmm).
6. Concomitant illness or requirement for chronic drug treatment that in the opinion of the PI creates unnecessary risks for gene transfer.
7. Subjects with AAVrh74 XXXXXXXXXX antibody titers > 1:400 as determined by ELISA immunoassay.
  - If endpoint titer is positive at 1:100 testing may be repeated prior to exclusion.
  - If present in infant and mother is positive for same antibody titers, mother will be asked not to breast feed and infant can be enrolled if antibodies drop  $\leq 1:400$  within 12 weeks.
8. Has a medical condition or extenuating circumstance that, in the opinion of the investigator, might compromise the subject's ability to comply with the protocol required testing or procedures or compromise the subject's wellbeing, safety, or clinical interpretability.
9. Severe infection (e.g., pneumonia, pyelonephritis, or meningitis) within 4 weeks before gene transfer visit (enrollment may be postponed).
10. Has received any investigational medication (other than corticosteroids) or exon skipping medications (including ExonDys 51), experimental or otherwise, in the last 6 months prior to screening for this study.

11. Has had any type of gene therapy, cell based therapy (e.g. stem cell transplantation), or CRISPR/Cas9.
12. Family does not want to disclose patient's study participation with primary care physician and other medical providers.

**Table 2:** Abnormal Laboratory value Range

System	Assay	Normal Range	Abnormal (Clinically Significant)
Liver Function	GGT	8-80 U/L	> 3 times upper limit of normal
Liver Function	Total Bilirubin	0.1-1 mg/dL	≥ 3 mg/dL
Renal Function	Creatinine	0.3 -1.3 mg/dL	> 1.8 mg/dL
Hematologic	Hemoglobin	Age dependent	For all ages: ≤ 3.5 or ≥ 20 x 10 <sup>3</sup> cells/mL <b>Or</b> Absolute neutrophil count ≤ 1.5 x 10 <sup>3</sup> cells/mL
Hematologic	White Blood Cells	Age dependent	

#### 4.2. 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 informed consent will be signed prior to study procedures and will require IRB approval at both participating centers. 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)]. The final IRB-approved document as well as any subsequent approved modified consent document(s) must be provided to correspondent agencies for regulatory purposes. 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 will be available for verification by study monitors at any time. Subjects will be given a signed, dated copy of their consent form.

#### 4.3. Establish Subject Identification Number



All subjects will be given a unique sequentially assigned subject number. Subjects will be identified by number only to protect identity.

#### **4.4. Pre-Treatment Assessments (Day -60 to Day -2):**

After obtaining informed consent and completing the registration procedures, a baseline patient history will be collected, including records of all medications and supplements that the patient is taking. The following assessments will be performed to confirm subject eligibility for this study. Baseline tests which must be completed prior to treatment administration include those listed below.

##### **4.4.1. Day -60 to Day -2 Before Gene Transfer**

- Physical Exam
  - Medical History
  - Vitals including pulse oximetry
  - Cardiac MRI (value of MRI of heart has been extensively studied in our MDA Clinic showing increased ability to detect fibrosis and more precise measures of ejection fraction)
  - EKG
  - ECHO
  - Chest X-ray
  - Hepatitis B & C Screen
  - HIV Screen
  - CBC/Diff/Platelet with Smear
  - Total Protein
  - Serum Gamma-Glutamyl Transferase (GGT)\*
  - ALT
  - AST
  - Total Bilirubin
  - Glucose
  - Electrolytes (CO<sub>2</sub>, Chloride, Potassium, and Sodium)
  - Creatinine Kinase (CK) (only on 2 day visits)
  - Creatinine/BUN
  - Cystatin C
  - Alkaline Phosphatase
  - Amylase
  - Prothrombin Time (PT), Partial Thromboplastin Time (PTT), INR
  - Urinalysis
  - Serum Antibody to AAVrh74
  - ELISPOT Assay to Capsid Proteins and Micro-Dystrophin
  - Physical Therapy Assessments
  - Adverse Event Reporting
  - Photograph of injection site
  - Concomitant Medications
- Note: use of ExonDys 51 will disqualify participants from inclusion in this trial
- Muscle Biopsy (to be completed Day-30 to -7)

\* GGT will be used to monitor liver enzymes rather than ALT or AST because of the source of these enzymes from damaged muscle in DMD where levels can reach 9-10X ULN. ALT and AST can vary by 30-40% from day to day making interpretation difficult. GGT is not affected by muscle disease<sup>11</sup>.

#### **4.4.2. Pre-Treatment Muscle Biopsy (Gastrocnemius or Quadriceps Muscle)**

Pre-gene transfer muscle biopsy will be done after establishing eligibility including appropriate laboratory testing. If a muscle biopsy is available for later comparison it will not be repeated. Muscle biopsies will be used to quantify transgene expression comparing baseline to day 180. The biopsies will be done on the same leg as the original biopsies. However, in some cases at the discretion of the PI, the opposite extremity may be preferred if it will be less traumatic to the subject. The muscles to be biopsied at both time points will either be the gastrocnemius or quadriceps muscle, at the discretion of the sponsor-investigator, Dr. Mendell. It is also important to emphasize that the biopsies will be read blinded and will not be identified as pre or post treatment. The biopsies will be done by the appropriate staff at NCH.

The muscle biopsies will be processed in the Neuromuscular Pathology Lab at Nationwide Children's Hospital which operates under a CAP/CLIA license for their diagnostic neuromuscular testing. There will be an assigned blinded identifier for each subject. Frozen sections will be stained for dystrophin using direct immunofluorescence (IF). Stained slides will be shipped to Sarepta Therapeutics for micro-dystrophin quantification. Full slide scanning will be performed and quantification of micro-dystrophin intensity and percent positive fibers will be achieved using validated image scanning and MuscleMap™ analysis algorithm. Blinded frozen muscle biopsy shavings will also be shipped to Sarepta Therapeutics to perform quantitative protein analysis for micro-dystrophin using a validated western blot method. Muscle morphometrics will also be performed blinded by Sarepta Therapeutics, including fiber size histograms and quantification of central nucleation. 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 following unblinding ( $p < 0.05$ ). Blinded analysis will also include PCR analysis for viral DNA.

#### **4.4.3. Pre-Treatment Immunosuppressives**

Subjects in Cohort A (not taking glucocorticoids upon enrollment per inclusion criteria) will be started on an oral dose of prophylactic prednisolone/prednisone (glucocorticoid) 1 day prior to gene transfer. In most cases this will be prednisolone, 1 mg/kg/day, but prednisone at the same dose is acceptable as well as a comparable glucocorticoid administered via IV if it were to be required. It is the intent for this to serve as an immunosuppressive to dampen host immune response to AAV or transgene. If GGT below 50 U/L at day 30, steroids will be weaned over 1 week.

Subjects in Cohort B will remain on their stable dose of corticosteroids throughout trial but may be increased for short time if GGT level is > 50 U/L. Those on weekend dosing will receive a daily dose of 1 mg/kg of glucocorticoid for 30 days.

#### **4.4.4. Cardiac MRI**

Cardiac MRIs will only be performed on subjects greater than 3 years of age (Cohort B) at the time of enrollment. Only conscious sedation will be utilized during the cardiac MRI. If the subject requires general anesthesia, the cardiac MRI will not be performed. The cardiac MRI will occur at baseline and Year 1 post gene transfer.

### **5. Gene Transfer Protocol**

Subjects will be admitted to Nationwide Children's Hospital for gene transfer, either PICU or Pulmonary PICU, the night before gene transfer and will be examined by either the PI or Co-Is (DAY -1). On the day of gene transfer (DAY0), the pharmacy will prepare rAAVrh74.MHCK7.micro-dystrophin gene vector according to the Manual of Operating Procedures (MOOP). The research pharmacist will transport the vector to the clinical setting in a vector-containing syringe at room temperature, and the vector needs administered to the subject within 24 hours of preparation. The final volume will be administered to the subject by the PI or designee in the subject's hospital room. All subjects will receive intravenous micro-dystrophin vector ( $2 \times 10^{14}$  vg/kg [REDACTED]). This is the same volume that proved safe in the SMA infant gene therapy study. Documentation of the dilution will be completed by the pharmacy following standard pharmacy protocol, and handling of the rAAVrh74.MHCK7.micro-dystrophin gene will follow compliance standards for Biosafety Level 1 vectors.

#### **5.1. Vector Administration Protocol**

Vector administration will be through a peripheral limb vein. It is unlikely that conscious sedation or a sedative (like lorazepam) will be required, but this will be determined based on PI observation and consultation with parents. The skin over the injection site for gene transfer will be pre-treated with a lidocaine/prilocaine eutectic mixture incorporated in a cream base (EMLA cream) or a cellulose disk (EMLA patch). Comparable cream-based anesthesia such as xylocaine cream might be used. Intravenous catheter placement will be performed following NCH policy, and two IVs will be placed. One intravenous catheter will be for infusion and one for a secondary site in the event that there are complications with the first site. The total dose of vector in [REDACTED] to be infused will be  $2 \times 10^{14}$  vg/kg [REDACTED]. Monitoring of subjects during viral administration will include blood pressure, heart rate, respiratory rate and temperature.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

STUDY TIMELINE																	
Study Interval	Baseline Screening	Vector Infusion (Inpatient)			Follow Up												
Visit #	1	2			3	4	5	6	7	8	optional	9	10	11	12	13	14
Study Interval	-60 to -2	-1 day	0 day	1 day	7 day	14 day	30 day	60 day	90 day	180 day	1-2 week post biopsy	9 mo	1 yr	18 mo	2 yr	30 mo	3 yr
Informed Consent	x																
Medical History	x																
Vitals	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Physical Exam	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x
ECHO/EKG	x												x		x		
Chest X-Ray	x																
MRI <sup>3</sup>	x												x				
Hepatitis B & C, HIV	x																
Safety labs <sup>1</sup>	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x
Urinalysis	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x
Physical Therapy Assessments	x						x	x	x	x	x	x	x	x	x	x	x
Gene Transfer			x														
Immunology studies	x				x	x	x	x	x	x		x	x	x	x	x	x
Muscle Biopsy <sup>2</sup>	x									x							
Photograph of injection site	x	x	x	x	x	x	x										
Adverse Events	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Concomitant Medications	To be collected from time of consent until final study visit, recorded on separate CRF																

<sup>1</sup>Safety labs (CBC/Diff/Platelet with smear, PT/PTT/INR/Fibrinogen, Electrolytes, Alk Phosphatase, Amylase, BUN, CK (only on 2 day visits), Creatinine, Cystatin C, GGT, Glucose, Total Protein, Total Bilirubin, Urinalysis) (CK will only be collected when the subject has a 2 day visit)

<sup>2</sup>All subjects will have biopsy at Screening. All subjects will have second biopsy Day 180.

<sup>3</sup> MRIs will only be performed on subjects greater than 3 years of age (Cohort B) at the time of enrollment. Only conscious sedation will be used during the cardiac MRI. If general anesethia is required, the cardiac MRI will not be performed.



## 7. Adverse Event Reporting

In reporting adverse events we 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".

<https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM227351.pdf>

### 7.1. Classification of Adverse Events:

The classification for adverse events will follow NIH guidelines outlined in Common Terminology Criteria for Adverse Events v4.0 (CTCAE; published May 28, 2009), which includes:

**Grade 1** Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

**Grade 2** Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.

**Grade 3** Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.

**Grade 4** Life-threatening consequences; urgent intervention indicated.

**Grade 5** Death related to AE.

*A Semi-colon indicates 'or' within the description of the grade.*

### Dose Limiting Toxicity (DLT)

## 8. Adverse Event Monitoring

### 8.1. Definition of an Adverse Event

**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: 1 = mild, 2 = moderate, 3 = severe, 4 = life threatening or debilitating, and 5 = fatal as indicated above in section 6.0.

Association or relatedness to the study agent, study procedures and the subject's pre-existing disease will be graded as follows: 5 = unrelated, 4 = unlikely, 3 = possibly, 2 = probably, and 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 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.

## 8.2. Serious adverse event (SAE)

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or 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.

**Grade 3** Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.

**Grade 4** Life-threatening consequences; urgent intervention indicated.

**Grade 5** Death related to AE.

## 8.3. 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 PI will fulfill the reporting responsibilities to FDA and the NIH Office of Biotechnology Activities (OBA) on behalf of Nationwide Children's Hospital using the web-based Adverse Event reporting system (GeMCRIS).

## 8.4. Obligations of the Investigators

The investigator or site staff is responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE. All AE (serious and not serious) occurring during the observation period established in this protocol must be documented on the CRF, in the AE corresponding section.

The Investigator will adhere to any other serious adverse event reporting requirements in accordance with national regulations, local laws, and the national institutional policies and procedures, as applicable. The Investigator will be responsible for ensuring that the reporting requirements are fulfilled and will be held accountable for any reporting lapses.

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

## **8.5. Safety Reporting**

The Sponsor-Investigator, or their designee, will report all serious and unexpected adverse events to the IRB, CBER/FDA, OBA/NIH, and DSMB according to regulatory requirements described as follows:

Any Grade 3 or higher adverse event that is unexpected and related/probably related to the gene transfer product will be reported to the Sponsor-Investigator within 24 hours. The Sponsor-Investigator will then inform the DSMB within 48 hours from notification and before enrollment of additional subjects. All Serious Adverse Events (SAEs) (regardless of expectedness, relatedness, or if they meet the definition for unanticipated problems) must be reported to the DSMB Chair within 48 hours of the Sponsor-Investigator receiving notification of the event.

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 to the FDA and NIH OBA as soon as possible, but not later than 7 calendar days after the Sponsor-Investigator'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 and NIH OBA 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 NIH OBA within 15 days of the determination.

Relevant additional clinical and laboratory data will become available following the initial serious adverse event report. Any follow-up information relevant to a serious adverse event will be reported within 15 calendar days of the sponsor's receipt of the information. 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 to the FDA and NIH OBA 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 as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information (i.e., at the same time the event must be reported to the FDA).

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.

### **8.5.1. Serious Adverse Event 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 Investigator; (5) NIH OBA protocol number; (6) FDA's Investigational New Drug (IND) application number; (7) vector type, e.g., adeno-associated virus; (8) vector subtype, if relevant; (9) gene delivery method, e.g., *in vivo* transduction; (10) route of administration, e.g., intramuscular; (11) dosing schedule; (12) a complete description of the event; (13) relevant clinical observations; (14) relevant clinical history; (15) relevant tests that were or are planned to be conducted; (16) date of any treatment of the event; and (17) the suspected cause of the event. These items will be reported electronically through the GeMCRIS reporting system (E-mail address for Reporting Adverse Events: [GeMCRIS@od.nih.gov](mailto:GeMCRIS@od.nih.gov)) by using the recommended Adverse Event Reporting Template available on NIH OBA's web site at:

[https://osp.od.nih.gov/wp-content/uploads/Incident-Reporting-Template-2016\\_2.docx](https://osp.od.nih.gov/wp-content/uploads/Incident-Reporting-Template-2016_2.docx)<http://www.gemcris.od.nih.gov/>

A copy of this report will also be sent to the IRB, FDA, and DSMB according to regulatory requirements described in section Safety Reporting.

## **8.6. 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.

## **8.7. Follow-up of Adverse Events**

All adverse events will be followed until resolution or stabilization. In the case of death, the Sponsor will request permission from the family to perform an autopsy.

## **8.8. 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 at study initiation that the subject's family provide information regarding non-routine visits during the study. 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. The study team will emphasize that any non-routine doctor visit or hospitalization post gene transfer and up to 30 days after their

last study visit 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. In the case of death, the Sponsor will request permission from the family to perform an autopsy.

## 9. Dose Limiting Plan

### 9.1. Dose Limiting Toxicity (DLT)

DLT is defined as any unanticipated serious adverse event that is possibly, probably, or definitely related to the study agent. This would include any grade 3 AE according to the classification given above.

Study enrollment will be halted by the investigators when any subject experiences one or more **Grade 3, or higher** adverse event toxicity that are **unanticipated** and **possibly, probably, or definitely related** to the study drug. The event will then be reviewed by the Data Safety Monitoring Board (DSMB) and evaluated if the trial should be terminated early following the Stopping/Discontinuation Rules.

Laboratory tests with values within the clinically significant range (Table 2) will be repeated during the same visit whenever possible. 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 adverse effect (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 PI will fulfill the reporting responsibilities under 21 CFR 312.32(c), to notify the 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, IRB, and CBER/FDA before continuing enrollment.

### 9.2. Stopping/Discontinuation Rules

An independent Data Safety Monitoring Board (DSMB) and safety monitor will monitor safety data 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 **one or more Grade 3, or higher** adverse event toxicity that are **unanticipated** and **possibly, probably, or definitely related** to the study drug. This will include any subject death not related to underlying disease condition, 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, IRB, and FDA, the decision is made to continue, the study will proceed. If the liver enzyme gamma-glutamyl transferase (GGT) is elevated at day 30, steroids will

be maintained until levels drop below 50 U/L. If elevation continues for 90 days, we will halt enrollment until further discussion with the DSMB and FDA.

### **9.3. Dosing Schedule**

The safety analyses from three time points (days 1, 7, and 14,) will be reviewed prior to dosing of the next subject. There will be at least a four-week dosing interval between subjects. This will allow time for review of the safety data from the previous subjects. Dose escalation will be considered in collaboration with FDA and DSMB.

The investigators will confer with the IRB and DSMB on all Grade 3 or higher adverse events with 48 hours that are unanticipated and possibly, probably, or definitely related to the study agent before continuing enrollment. Based on the outcome of the safety and efficacy analysis of the event, decision will be made to proceed with additional subjects.

## **10. Study Reports**

### **10.1. Final Study Report**

The final study report will include data through the final study visit but will not include long-term follow-up information.

### **10.2. 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 sites; (3) the Investigators; (4) clinical protocol identifiers, including the NIH OBA protocol number, NCH 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.

### **10.3. Data Safety Monitoring Plan**

#### **10.3.1. The Data Safety Monitoring Board**

The Data and Safety Monitoring Board (DSMB) will act in an advisory capacity to review participant safety and study progress for the clinical trial. 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 timeliness, 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 trial;
- review study performance, make recommendations and assist in the resolution of problems reported by the Sponsor-Investigator;
- protect the safety of the study participants;
- review safety data to determine whether to recommend dose escalation;
- 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.

#### **10.3.2. DSMB Reporting and Meetings**

Reports describing the status of the study will be prepared by the Sponsor-Investigator's staff and sent at the DSMB's request. The DSMB will meet prior to dosing the first subject. The DSMB will be provided with a report after patient 1 and a teleconference will occur after patient 3.

A meeting (either by teleconference or webcast) with the DSMB will be scheduled after Day 30 visit of the third patient, 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
- A listing of protocol deviations

### **10.3.3. Membership**

The DSMB membership will consist 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. Mendell must note any conflict of interest before their eligibility to serve on the DSMB is approved. The DSMB may include experts in or representatives of the fields of:

- Neurology
- Immunology
- Virology / Gene Therapy
- 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.

## **10.4. Clinical Monitoring of the Study**

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 PI 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 wellbeing of subjects.

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.

### **10.4.1. Data Management and Study Forms**

All data and observations will be documented on Case Report Forms (CRF) by source documentation. A Study 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. A CRF will be completed for every subject that was registered for participation in the study. CRF will be completed as information becomes available or within three days of a Study Visit.



Case Report Forms will be reviewed in detail by the Study Monitor on a regular basis for which the Study Monitor will have access to subject medical records, laboratory data, and other source documentation. Study 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 Case Report Form(s) in question will be corrected by the PI or his designee. Data Resolution may be generated on omissions or clarifications, to be completed, signed and dated, and maintained as a part of the CRF.

The appropriate study team member will sign off Case Report Forms. This signature will indicate that thorough inspection of the data therein has been made and will thereby certify the contents of the form.

In collaboration with the study team, the Research Informatics Core will design a data collection system for managing the clinical trial. A web-based database will be created and managed by authorized users. CRFs will be transcribed to this web-based database. Data will be extracted from source documents (lab reports, echo reports...) and transferred to the database as well. All source documents will be kept in the Subject Research Chart. The secured portal will feature view and edit capability with field validations for quality controls, change history attribute and reporting.

An external Contract Research Organization (CRO) will also monitor the study in a regular basis to make sure the study is conducted in compliance with all regulatory aspects of the protocol.

## 11. References

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2. Mendell JR, Campbell K, Rodino-Klapac L, Sahenk Z, Shilling C, Lewis S et al. Dystrophin immunity in Duchenne's muscular dystrophy. *N Engl J Med* 2010; 363(15): 1429-37
3. Mendell JR, Rodino-Klapac LR, Rosales-Quintero X, Kota J, Coley BD, Galloway G, et al. Limb-girdle muscular dystrophy type 2D gene therapy restores alpha-sarcoglycan and associated proteins. *Ann Neurol* 2009;66:290-297.
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