Phase I/IIa gene transfer clinical trial for Duchenne Muscular Dystrophy using rAAVrh74.MCK.GALGT2

CLINICAL PROTOCOL

[redacted]

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1.0 PROTOCOL SYNOPSIS

Title	Phase I/IIa gene transfer clinical trial for Duchenne Muscular Dystrophy using rAAVrh74.MCK.GALGT2
Study Number	Cohort 1: N = 1 [Low dose: 2.5 x 10^{13} vg/kg per leg, delivered bilaterally (total 5.0 x 10^{13} vg/kg)] Cohort 2: N=1 [High dose: 5x10^{13} vg/kg per leg, delivered bilaterally (total 1.0 x 10^{14} vg/kg)]
Clinical Study Phase	Phase I/IIa trial
Number of Centers	Single site (Nationwide Children's Hospital)
Study Objectives	The primary objective is assessment of safety.
Study Design	Dose escalation study rAAVrh74.MCK.GALGT2: - Intravascular limb infusion for gene transfer delivered bilaterally (two cohorts) via a major lower limb artery to the whole lower limb of DMD subjects.
Patient Population	**Inclusion Criteria** • Ambulant patients age 4 years or older • Confirmed mutations in the *DMD* gene using a clinical accepted technique that completely defines the mutation • Measurably impaired muscle function (defined as less than 80% of the predicted value for age 100 MWT), but with sufficient muscle preservation to ensure assessment of muscle transfection based on clinical evaluation by the PI and expert colleagues. This degree of preservation will include: o Ability to extend the knee fully against gravity o Preserved ambulation with ability to walk ≥ 350 meters during the 6MWT o A magnetic resonance image of the quadriceps showing preservation of sufficient muscle mass to permit transfection • Males of any ethnic group will be eligible • Ability to cooperate with muscle testing • Stable dose of corticosteroid therapy (including either prednisone, prednisolone, deflazacort or their generic forms) for at least 12 weeks prior to gene transfer **Exclusion Criteria** • Active viral infection based on clinical observations • The presence of a *DMD* mutation without weakness or loss of function • Subject is amenable to or is currently being treated with eteplirsen • Symptoms or signs of cardiomyopathy, including: o Dyspnea on exertion, pedal edema, shortness of breath upon lying flat, or rales at the base of the lungs

	○ Echocardiogram with ejection fraction below 40% • Serological evidence of HIV infection, or Hepatitis B or C infection • Diagnosis of (or ongoing treatment for) an autoimmune disease • Persistent leukopenia or leukocytosis (WBC ≤ 3.5 K/μL or ≥ 20.0 K/μL) or an absolute neutrophil count < 1.5K/μL • Concomitant illness or requirement for chronic drug treatment that in the opinion of the PI creates unnecessary risks for gene transfer • Subjects with rAAVrh74 binding antibody titers ≥ 1:50 as determined by ELISA immunoassay • Presence of circulating anti-Sda antibodies as determined by study approved laboratory • Abnormal laboratory values in the clinically significant range, based upon normal values in the Nationwide Children's Hospital Laboratory
Study Procedures	• The vector will be delivered by intravascular limb infusion (ILI-GT) to [redacted] each lower limb. Study subjects will receive general anesthesia during the procedure following Children's Hospital protocol. • Detailed description of vector administration procedures can be found in section 3.6.3 of this clinical protocol.
Primary Outcome	Safety is a primary outcome for this clinical gene transfer trial.
Secondary Outcomes	We propose the following efficacy outcome measures in leg muscles, each in comparison to baseline values: • Expression of GALGT2 as demonstrated by immunofluorescent staining with anti-CT epitope antibodies or WFA lectin in muscle biopsy sections at 90 and 180 days post-injection • GALGT2 protein expression quantified by western blot and assessed by densitometry in muscle biopsy tissue at 90 and 180 days post-injection We propose the following exploratory clinical outcome measures, each in comparison to baseline values: • The 6 minute walk test (6MWT), with either stabilization or a statistically significant change in distance walked on the 6MWT at 90 and 180 days after injection to be considered evidence of a positive result. • Strength and functional testing will be performed at baseline and at days 90,180, and at months 12, 18 and 24. The maximum voluntary isometric contraction testing (MVICT), which will provide the force generated by the muscles of knee extension and knee flexion, will be an exploratory measure, as will the 100 meter walk time and the North Star Ambulatory Assessment. • [redacted]

	[redacted]
Study Duration	We will evaluate short-term safety over a two year period. Subjects will be tested at baseline and return for 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 us at days 45, 75 and two weeks following steroid dose reduction.
Sample Size	Two cohorts of DMD subjects will receive whole isolated limb infusion (ILI) at a standard dose escalation scheme to establish maximum tolerated dose (MTD) using toxicity. Cohort 1: N = 1 [Low dose: 2.5 x 10^{13} vg/kg per leg, delivered bilaterally (total 5.0 x 10^{13} vg/kg)] Cohort 2: N=1 [High dose: 5x10^{13} vg/kg per leg, delivered bilaterally (total 1.0 x 10^{14} vg/kg)]
Statistical Analysis	This is a phase I/IIa trial, with safety as the primary measure. Quantification of the number of fibers expressing GALTG2 as determined by quantification of the number of fibers and the intensity of anti-CT antibody or WFA lectin staining at the sarcolemma based on quantitative image analysis will be analyzed using a paired t-test. The 6MWT will be the leading exploratory efficacy measure (secondary outcome). [redacted] Strength measures, an exploratory outcome, will also be performed by the 100 meter walk/run and MVICT of limb muscles and analyzed by paired t-tests with a significance level of $p = 0.05$. For each of these measures, statistical analysis based on differences between pre- and post-gene transfer examinations will be analyzed using a paired t-test, with a p value of < 0.05 indicating significance.
Long-term follow-up	We will follow the most recent FDA guidance with regard to long-term subject follow up post gene transfer. As indicated by the guidelines, our proposed vector has a very low probability of gene transfer-related delayed adverse events. We will, however, evaluate short-term safety over a two-year period that incorporates the active phase of the protocol. [redacted]

2.0 INTRODUCTION

2.1 Abstract

The proposed clinical trial is a dose escalation study of rAAVrh74.MCK.GALGT2 for DMD patients. There will be a modified intravascular limb infusion(ILI); [redacted][3] henceforth designated as intravascular limb infusion for gene therapy (ILI-GT) delivered sequentially to the whole each lower limb of DMD subjects via a major lower limb artery.

ILI-GT will be delivered [redacted] to the muscles of both legs of DMD subjects. This approach to vascular gene transfer builds upon previously published methods used in mice and non-human primates. [2-4] [redacted]

Two cohorts will undergo gene transfer in a standard dose escalation scheme to establish maximum tolerated dose (MTD) using toxicity. One subject will be enrolled into the first cohort and one subject will be enrolled in the second cohort. The first cohort will receive the minimally efficacious dose as established by primate studies, consisting of 2.5×10^{13} vg/kg per leg, delivered bilaterally (to total 5.0×10^{13} vg/kg per subject). Once safety is established in the low-dose cohort, the second cohort will receive 5×10^{13} vg/kg per leg, delivered bilaterally (to total 1.0×10^{14} vg/kg per subject). [redacted]

The baseline open muscle biopsy will be performed in one quadriceps muscle. Open muscle biopsy of the quadriceps (in the leg contralateral to the pre-treatment biopsy) will be performed at 90 days post ILI-GT procedure. [redacted] Every effort will be made to sample these same muscles in all patients; however, in subjects (particularly older ambulant subjects) with limited quadriceps musculature, alternate muscle biopsy sites may be selected at the PIs discretion (including gastrocnemius or tibialis anterior muscles).

The primary objective of this study is the assessment of the safety of intravascular administration of rAAVrh74.MCK.GALGT2 to DMD patients. Safety endpoints will be assessed by changes in hematology, serum chemistry, urinalysis, immunologic response to

rAAVrh74 and GALGT2, and reported history and observations of symptoms. Efficacy measures will be used as secondary outcome for this disorder including a combination of functional 6 minute walk test (6MWT) and direct muscle testing for strength (MVICT) of lower limb muscles.

Subjects will be evaluated at baseline, infusion visit (days 0-2), and return for follow up visits on days 7, 14, 30, 60, 90, and 180 and months 12, 18 and 24.

2.2 Clinical Trial and Principal Investigator

The study will be carried out at The Research Institute at Nationwide Children's Hospital (NCH) in Columbus, Ohio, with Dr. Kevin Flanigan as the Principal 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 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 in Columbus. Dr. Flanigan has extensive experience as a clinical neuromuscular specialist and molecular geneticist. He has participated as an investigator in multiple clinical trials in the muscular dystrophies, including trials of nonsense suppression, exon skipping, and myostatin inhibition. [redacted]

Dr. Paul Martin, co-Investigator, is a Professor of Pediatrics and of Physiology and Cell Biology, and is an investigator at the NCH Center for gene therapy. A specialist in glycobiology, neuromuscular development and muscular dystrophy, he defined the presence of synaptic βGalNAc-containing carbohydrates (the CT carbohydrate antigens) at the mammalian neuromuscular junction, defined the local synthesis of those glycans by Galgt2, and showed that transgenic overexpression of Galgt2 stimulated both the glycosylation of α-dystroglycan with the CT carbohydrate and ectopic expression of normally synaptic dystroglycan binding partners, including synaptic laminins and utrophin, resulting in inhibition of the onset of muscular dystrophy in dystrophin-deficient mdx mice. Dr. Martin developed the AAV gene therapy vector used in this trial, and delineated mechanistic insights into Galgt2's function.

Dr. Louis Chicoine, co-Investigator, [redacted] is neonatal focus has been in pulmonary vascular development and disease. Utilizing this clinical and research experience and knowledge of vascular physiology he led the team that developed the isolated limb infusion technique for gene therapy in non-human primates and showed that robust transgene expression results from an arterial approach of vector delivery. He has also worked to transition the technique into the clinic. [redacted]

2.3 Background and Significance

2.3.1 Disease 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[5]. 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 occurring, in the pre-corticosteroid treatment era, by age 12[6]. Historically patients died from respiratory complications, but improved pulmonary and ventilatory measures (including nocturnal ventilatory support) have further improved survival[7]. Prolonged survival from in turn unmasks a decline in cardiac function, marked by dilated cardiomyopathy, raising additional treatment challenges in the late-stage patient.

2.3.2 Disease Pathogenesis

Nearly 30 years ago, the *DMD* gene was cloned, defining the molecular basis of the disease[8] and leading to the identification of dystrophin as the deficient protein in muscle from DMD patients[9]. 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[10]. Originally multiplex PCR kits were developed that were able to detect 95%-98% of all deletions[11, 12], but improved molecular methods of diagnosis – including multiplex ligation-dependent probe amplification (MLPA)[13], multiplex amplifiable probe hybridization (MAPH)[14], 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[15], allowing detection of nearly all clinically relevant disease mutations[15].

2.3.3 Current Treatment for DMD

The only therapeutic approach that has clearly demonstrated unequivocal efficacy for treatment of DMD is treatment with the glucocorticoids prednisone or deflazacort. Treatment with one or another of these agents has been repeatedly shown to result in increased strength and delay loss of ambulation[16-18]. Deflazacort has recently gained FDA approval for treatment of boys with DMD, based largely on a slightly more favorable side effect profile[19], and although it is not yet commercially available in the US many patients order it from overseas. However, treatment with these drugs is 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 provide a promising alternative to glucocorticoids. One approach is direct gene replacement. Other approaches include exon skipping and nonsense mutation suppression (or "readthrough"). Exon skipping utilizes antisense oligomers to induce altered pre-mRNA splicing to restore an open reading frame, and a single such agent (eteplirsen) has received FDA approval, based largely on a small but statistically significant increase in dystrophin expression[20, 21]. However, both exon skipping and the as-yet unapproved nonsense suppression approach[22-24] would only be beneficial to small subsets of patients with appropriate mutations. For example, eteplirsen would be beneficial to only 13% of patients, and the nonsense suppression agent ataluren to only to 15%.

2.3.4 Rationale for Gene Therapy using GALTGT2

Gene replacement with GALGT2 competes favorably with any of these alternative molecular strategies that are on the horizon. None of the molecular-based therapies are proven to work clinically to provide life-altering treatments. Surrogate gene therapy with GALGT2 has advantages over other molecular approaches because of the potential for one-time dosing; others would require life-long treatment ultimately be far more expensive and impractical than gene replacement therapy. [redacted]. This provides reasonable assurance for avoiding off target effects. This project builds upon expertise of our group in developing gene replacement therapy with micro-dystrophin by IM[26] or vascular delivery[27]. Overexpression of GALGT2 results in correction of pathology not only in mouse models of DMD[28], but also models of limb-girdle muscular dystrophy type 2D (due to mutations in alpha-sarcoglycan)[28] and congenital muscular dystrophy type 1A (due to mutations in laminin alpha2)[29]. In boys with DMD, expectations from this form of treatment include protection against eccentric contraction related to membrane fragility from loss of dystrophin at the sarcolemma and increase force generation.

3.0 CLINICAL RESEARCH PLAN

3.1 Study Population

[redacted] subjects age 4 years and older, and with proven mutations of the *DMD* gene will be enrolled at Nationwide Children's Hospital for the gene transfer study. Subjects will encompass any ethnic or racial background.

3.2 Inclusion/Exclusion Criteria

Inclusion Criteria

1. Ambulant patients age 4 years or older
2. Confirmed mutations in the *DMD* gene using a clinically accepted technique that completely defines the mutation
3. Measurably impaired muscle function, (defined as less than 80% of the predicted value for age 100 MWT), but with sufficient muscle preservation to ensure assessment of muscle transfection based on clinical evaluation by the PI and expert colleagues. This degree of preservation will include:
   a. Ability to extend the knee fully against gravity
   b. Preserved ambulation with ability to walk $\geq$ 350 meters during the 6MWT
   c. A magnetic resonance image of the quadriceps showing preservation of sufficient muscle mass to permit transfection
4. Males of any ethnic or racial group will be eligible
5. Ability to cooperate with muscle testing
6. Stable dose of corticosteroid therapy (including prednisone, prednisolone, 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. The presence of a DMD mutation without weakness or loss of function
3. Subject is amenable to or is currently being treated with eteplirsen.
4. 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%

5. Serological evidence of HIV infection, or Hepatitis B or C infection
6. Diagnosis of (or ongoing treatment for) an autoimmune disease
7. Persistent leukopenia or leukocytosis (WBC $\leq$ 3.5 K/µL or $\geq$ 20.0 K/µL) or an absolute neutrophil count < 1.5K/µL
8. Concomitant illness or requirement for chronic drug treatment that in the opinion of the PI creates unnecessary risks for gene transfer
9. AAVrh74 binding antibody titers $\geq$ 1:50 as determined by ELISA immunoassay
10. Presence of circulating anti-Sda antibodies as determined by study approved laboratory.*
11. Abnormal laboratory values in the clinically significant range in the Table 1 below, based upon normal values in the Nationwide Children's Hospital Laboratory:

[redacted]

Table 1.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
Renal Function	Creatinine	0.3-1.mg/dL	>1.8 mg/dL
Hematologic	Platelet Count	140-440x10^3 platelets/mm^3	≤90 or ≥ 800 x 10^3 platelets/mm^3
	Hemoglobin*	(g/dL) 2 – 6 yrs: 11.5-13.5 6 – 12 yrs: 11.5-15.5 12 – 18 yrs: Male: 13-15 Female: 12-16	For all ages: ≤ 8 or ≥ 18 g/dL
	White Blood Cell Count	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 ≥ 20 x 10^3 cells/mL Or Absolute neutrophil count of ≤ 1.5 x 10^3 cells/mL

[redacted]

3.3 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.

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 NIH/OBA 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 be available for the verification by study monitors at any time. Subjects will be given a signed, dated copy of their consent form documents.

3.4 Establish Subject Identification Number

Every research participant will be assigned a Subject Identification code. This will be done prior to any research procedures being scheduled, as they will require a Subject ID.

The Subject Identification code is generated as follows:

1) GAL (study identifier)
2) The letters DMD (for the DMD gene transfer trial)
3) The last two digits represent a sequential number of all candidates being screened

Example: Subject ID: GAL-DMD-01

3.5 Dosing Plan

The proposed phase I/IIa clinical trial is an open-label, single injection ascending dose [redacted] delivered sequentially to both whole lower limbs of DMD subjects [redacted]. The dose selection is based on toxicology-biodistribution studies and predicted by pre-clinical studies in non-human primates.

The ILI-GT protocol will deliver the vector [redacted] to the muscles of both legs of DMD subjects. Two cohorts will undergo gene transfer in a standard dose escalation scheme to establish maximum tolerated dose (MTD) using toxicity as an outcome measure. One subject will be enrolled in the first cohort and a one subject will be enrolled into the second cohort. The first cohort will receive the minimally efficacious dose as established by primate studies, consisting of 2.5×10^{13} vg/kg per leg, delivered bilaterally (to total 5.0×10^{13} vg/kg per subject). Once safety is established in the low-dose cohort, the second cohort will receive 5×10^{13} vg/kg per leg, delivered bilaterally (to total 1.0×10^{14} vg/kg per subject). The total vector genome dose for each subject will be adjusted by rounding up the subject body weight to the closest kilogram.

Cohort	Treatment	Dose per leg (vg/kg)	Total Dose (vg/kg)	N
1	Low Dose	2.5×10^{13}	5.0×10^{13}	1
2	High Dose	5.0×10^{13}	1.0×10^{14}	1

There will be at least 4-6 weeks between dosing of subjects within a cohort. The allowance of four weeks between dosing of subjects provides time for an internal review of the safety analysis from five time points (days 1, 2, 7, 14 and 30) prior to dosing of the next subject. The 30-day post-injection data from one subject in the first cohort will be reviewed and discussed with the DSMB prior to dose escalation to the higher dose cohort. Dose escalation will be based on dose-limiting toxicity (DLT).

3.6 Gene Transfer Protocol

3.6.1 Baseline Measures Prior to Vector Injection (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. A complete physical examination, medical history and concomitant medication history will be obtained. Vitals will be obtained, including temperature, respiratory rate, heart rate, blood pressure, height, and weight. Pre-treatment studies will also include [redacted] echocardiogram, ECG, the North Star Ambulatory Assessment (NSAA), a video recorded six minute walk test (6MWT), the 100 meter walk/run test, the maximum voluntary isometric contraction testing (MVICT) of knee extension and knee flexors bilaterally, pulmonary function testing (PFTs) including spirometry, and an open muscle biopsy.

Lab work will include the following:

- 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)
- Amylase
- Creatine kinase (CK)
- Cystatin C
- Antibody testing for Hepatitis B,C and HIV1 and 2.
- Urinalysis
- Whole blood for biobanking
- Urine for biobanking

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

Additionally, ELISA will be performed for detection of total antibodies to AAVrh74 and anti-Sda, and ELISpots for T-cell responses to AAVrh74 and GALGT2.

A baseline open muscle biopsy will be performed in one quadriceps muscle. (Note that the rationale and methods of muscle biopsy site selection and performance for this and subsequent biopsies are discussed in Section 3.8)

The baseline evaluation will take place over two visits (one to two 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 muscle biopsy with its attendant risks (including sedation).

Prescribed and over the counter medications used in the prior two weeks will be recorded at the baseline visit and changes in these medications will be recorded during each subsequent visit. The PI will encourage participants to maintain the medication and supplements they are on at enrollment through the course of the study. [redacted] Subjects must already be on prednisone, prednisolone, or deflazacort for at least 12 weeks at time of enrollment as part of the inclusion criteria.

3.6.2 Pre Infusion Visit (Day -1)

Subjects will arrive to the Nationwide Children's Hospital within 24 hours prior to gene transfer. They will undergo a physical exam, a review of concomitant medications and updates to the medical history. Vital signs (heart rate, respiratory rate, temperature, and blood pressure) and weight will be obtained. [redacted] The labs obtained on Day -1 will include:

- Complete blood cell count (CBC) and differential, platelets
- Aspartate aminotransferase (AST)/alanine transaminase (ALT)
- Gamma-glutamyl transpeptidase (GGT)*
- Prothrombin time (PT)/activated partial thromboplastin time (PTT)
- Creatine kinase (CK)

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

An ELISpot for T-cell responses to AAVrh74 and GALGT2 will also be performed.

In previous gene therapy studies at NCH, there has been observed an 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 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. This prednisone dose will be delivered beginning 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). This corticosteroid regiment will be continued after gene transfer as discussed in Section 3.7.1, followed by a return to the pre-treatment corticosteroid regimen.

3.6.3 Day of Gene Transfer (Day 0)

[redacted]

[redacted]

[redacted]

[redacted]

3.6.4 Vascular delivery via intravascular limb infusion for gene therapy (ILI-GT)

All subjects participating in this trial will receive an intravascular limb infusion of rAAVrh74.MCK.GALGT2 delivered [redacted] to both legs. This will be a dose escalation study, involving 2 cohorts. Dosing for the first cohort is the minimum efficacious dose as established in primate studies. Once safety is established in the low-dose cohort, the second cohort will receive a 2-fold dose escalation.

- Cohort 1 (n=1), Low Dose: 2.5 x 10^{13} vg/kg per leg, delivered bilaterally (to total 5.0 x 10^{13} vg/kg per subject)
- Cohort 2 (n=1), High Dose: 5x10^{13} vg/kg per leg, delivered bilaterally (to total 1.0 x 10^{14} vg/kg per subject)

[redacted]

The intravascular limb infusion approach to vascular gene transfer builds upon previously published methods in mice and non-human primates[2-4]. The modified approach has been previously approved by the FDA [redacted]

The subjects will be given sedation utilizing NCH anesthesia protocols that minimize the risk of anesthetic reaction in muscular dystrophy. Peripheral vascular access will be obtained with IV and or arterial approaches. [redacted]

[redacted]

[redacted]

[redacted]

Infusion reactions:

[redacted]

Infusion will be terminated for evidence of an allergic reaction of **grade 2 or greater**, including anaphylaxis, based upon CTCAE v.4 criteria, and reported using these criteria:

Grade 1: Transient flushing or rash, drug fever <38 degrees C (<100.4 degrees 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 v.4 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.

[redacted]

3.7 Post Gene Transfer Monitoring Plan

3.7.1 Up to 48-Hour Inpatient Monitoring (Days 1 and 2)

Patients will be closely monitored for blood vessel rupture, compartment syndrome, and neuromuscular integrity, including by Doppler ultrasound (as needed). Neuromuscular integrity will be assessed by routine assessment of clinical pain and strength.

The subject will see the principal investigator or designee on the morning of **Day 1** for a physical examination as well as review and documentation of concomitant medications and all adverse events/serious adverse events following injection. Vitals including blood pressure, heart rate, respiratory rate, and temperature will be performed as well as the following lab values:

- Complete blood cell count (CBC) and differential, platelet
- 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
- Creatine kinase (CK)Urinalysis
- [redacted]

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

A photograph will be taken of each injection site.

On the second day after gene transfer (**Day 2**) the subject will be discharged from the hospital after a physical examination by the principal investigator or designee, as well as review and documentation of concomitant medications, medical history and all adverse events/serious adverse events following injection. [redacted]

3.7.1.1 Post-injection immune suppression

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.

If the **30 day** post-infusion IFN-gamma T-cell response shows ≤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 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 and 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.

If the IFN-gamma T-cell response shows ≥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 discussed below). Based upon other gene transfer trials performed at NCH, we anticipate that oral prednisone or prednisolone may be administered for up to 120 days post gene transfer prior to return to the standard clinical corticosteroid regimen of each subject.

3.7.2 Post-Gene Transfer Monitoring (Days 7, 14, 30)

Subjects will return for follow up visits on **Days 7, 14, and 30** after treatment, with repeat physical examination, review of concomitant medications, updates to the medical history, adverse events, vital signs (heart rate, respiratory rate, temperature, weight, and blood pressure) and blood and urine studies at each visit.

Lab work will include the following:

- 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)
- Amylase
- Urinalysis
- [redacted]
- ELISpots for T-cell responses to AAVrh74 and GALGT2
- Creatine kinase (CK)[#]
- [redacted]

** For subjects taking drugs that potentially induce GGT synthesis through the cytochrome P450 system, the clinically significant range will be estimated at two times the levels obtained from the baseline screening test.*
[#] CK will not occur on Day 14

On **Day 30**, additional tests will include an ELISA for detection of total antibodies to AAVrh74, Cystatin C, and whole blood banking to investigate vector shedding.

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 patient 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.

3.7.3 Post-Gene Transfer Monitoring after Day 30 (Days 45, 60, 75, 90, 180; Months 12, 18, and 24)

After the follow-up visit at Day 30 post treatment, subjects will continue outpatient active monitoring through 2 years post-gene transfer. Subjects will return for follow-up visits at Days 60, 90, and 180 and at Months 12, 18 and 24 after treatment. Please refer to the graphical Study Timeline of Events below for a summary of studies and procedures at each visit.

At **Days 45 and 75**, targeted safety labs will be drawn, assessing liver function (AST, ALT, and GGT). For convenience, for subjects outside of the Columbus area, these may be locally drawn and shipped to the NCH clinical laboratory.

The **Day 60 visit** safety assessments include updates to the medical history, physical examination, concomitant medications, vital signs (heart rate, respiratory rate, temperature, weight, and blood pressure) and blood work, including: AST, ALT, GGT, ELISA for detection of serum total antibodies to AAVrh74 and ELISpots for T-cell responses to AAVrh74 and GALGT2. Urine and whole blood will be banked for possible vector shedding studies.

The **Day 90, Day 180, 12 month, 18 month, and 24 month visits** include updates to the medical history, physical examination, concomitant medications, vital signs (heart rate, respiratory rate, temperature, weight, and blood pressure, height), ECG, echocardiogram, and the following blood and urine studies:

- 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)
- Creatine kinase (CK)
- Cystatin C
- Urinalysis
- ELISpots for T-cell responses to AAVrh74 and GALGT2
- ELISAs for detection of serum total antibodies to AAVrh74

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

At each of these time points, functional testing will be performed, including the video-recorded 6 MWT, the 100 m walk/run, the NSAA, and MVICT. In addition, at the **Day 180, 12 month, 18 month, and 24 month visits**, [redacted]

3.8 Muscle biopsy

The baseline open muscle biopsy will be performed in one quadriceps muscle, or other appropriate muscle as determined by the PI. Open muscle biopsy of the quadriceps, or other muscle as determined by the PI (biopsy will be in the contralateral leg to the pre-treatment biopsy, unless otherwise determined by the PI), will be performed at 3 months post-infusion. [redacted] Every effort will be made to sample these same muscles in all patients; however, in subjects (particularly older ambulant subjects) with limited quadriceps musculature, alternate muscle biopsy sites may be selected at the PIs discretion (including gastrocnemius or tibialis anterior muscles).

3.9 Study Timeline

Table 2. Schedule of Events

(ILI-GT PROTOCOL) STUDY TIMELINE																		
Study Interval	**Screening**		**Pre Infusion Visit**	**Hospital Admission Vector Injection**	**Inpatient**		**Follow-Up (Outpatient)**											
Visit #	**Visit 1[2]**	**Visit 2**	**Visit 3[2]**				**Visit 4**	**Visit 5**	**Visit 6**	**Labs[8]**	**Visit 7**	**Labs[8]**	**Visit 8[2]**	**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**	**2 weeks post steroids**	**Day 180**	**Month 12**	**Month 18**	**Month 24**
Informed consent[1]	X																	
Medical History	X		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
Adverse events				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
Vital signs	X		X	X[7]	X	X	X	X	X		X		X		X	X	X	X
Height	X												X		X	X	X	X
Weight	X		X						X		X		X		X	X	X	X
[redacted]	[redacted]														[redacted]	[redacted]	[redacted]	[redacted]
ECHO	X												X		X	X	X	X
EKG	X												X		X	X	X	X
North Star Ambulatory Assessment	X												X		X	X	X	X
Video recorded 6MWT	X												X		X	X	X	X
100 m walk/run	X												X		X	X	X	X
MVICT	X												X		X	X	X	X
PFTs/Spirometry	X														X	X	X	X
[redacted]		[redacted]											[redacted]		[redacted]	[redacted]		
Anaesthesia		X		X									X		X	X		
CBC/Diff	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
Serum GGT	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X
Serum total protein/albumin	X				X		X	X	X				X		X	X	X	X
Serum Total bilirubin	X				X		X	X	X				X		X	X	X	X
Electrolytes	X				X		X	X	X				X		X	X	X	X
Calcium	X				X		X	X	X				X		X	X	X	X
Creatinine/BUN	X				X		X	X	X				X		X	X	X	X
Random Glucose	X				X		X	X	X				X		X	X	X	X
Alkaline Phosphatase	X				X		X	X	X				X		X	X	X	X
PT/PTT/INR	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
Cystatin C	X								X				X		X	X	X	X
Antibody testing Hep. B, C and HIV	X																	
Urinalysis	X				X		X	X	X		X		X		X	X	X	X
ELISA	X								X		X		X		X	X	X	X
ELISpot	X		X				X	X	X		X		X		X	X	X	X
Steroid Taper[5]			X	X	X	X	X	X	X	X	X	X	X					
Admit to Hospital				X														
Photograph injection site				X	X													
Ultrasound (Injection needle guide)				X														
Study agent administration				X														
Whole Blood Banking		X							X		X							
Urine Banking		X			X	X	X	X	X		X							

1. If there are changes to the study, parents will be re-consented 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. [redacted].
5. Prophylactic prednisolone/prednisone taper begins on Day -1 and is tapered according to AST, ALT, and ELISpot results. 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.
7. Day 0 Vital Signs (Heart rate, respiratory rate, pulse oximetry, temperature, and blood pressure) will be measured before and immediately after the infusion, and at least every five minutes during the infusion, and repeated at 15 minutes post-infusion. VS will be obtained hourly for 4 hours following the injection and then every 4 hours until discharge.
8. The immunology studies will continue at post-treatment day 7, 14, 30, 45, 75, and 9, 12, 18, and 24 months post-gene transfer. Samples scheduled in between f/u visits will be collected at locally and ship to us. Immunology studies include ALT, AST and GGT.

Note: There will be a flexibility of working days for each of the planned study visits including screening to adjust the schedule to any unanticipated event. The windows for each appointment are as follows:

- Days 7 and 14 ± 2 days
- Day 30 ± 3 days
- Days 60 and 90 ± 7 days
- Day 180, and Months 12, 18 and 24 ± 14 days

3.10 Long-Term Monitoring

We will follow the most recent FDA guidelines with regard to long-term subject follow up after gene transfer. As discussed and based on prior experience with rAAV or transgene, there is a very low probability of gene transfer-related delayed adverse events. We will, however, evaluate short-term safety over a two-year period that incorporates the active phase of the protocol. Following the active two-year follow-up phase of the study, subjects will then be asked to transfer to a monitoring program where data will continue to be collected from annual visits with their standard care physician.

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 Principal 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 Principal Investigator of a change of address or contact information.

[redacted] 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 what 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.

3.11 Outcome Measures

The intravascular limb infusion for gene therapy (ILI-GT) stage of the study is proposed as a phase I/II clinical trial inclusive of safety and efficacy outcome measures.

A. 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** unanticipated Grade III or higher treatment-related toxicities.

B. Efficacy outcome measures: Muscle biopsy analysis for safety and transgene expression will be studied using multiple serial sections.

   i. Expression of GALGT2 as demonstrated by immunofluorescent staining with anti-CT epitope antibodies or WFA lectin in muscle biopsy sections
   ii. GALGT2 protein expression quantified by western blot and assessed by densitometry in muscle biopsy tissue

   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. [redacted]

[redacted]

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

i. The 6 minute walk test (6MWT), with either stabilization or a statistically significant change in distance walked on the 6MWT at 6 or 12 months after injection to be considered evidence of a positive result.
ii. Strength and functional testing will be performed at baseline and at days 90,180, and at months 12, 18 and 24. This will include the 6MWT distance as the primary functional variable. The maximum voluntary isometric contraction testing (MVICT), which will provide the force generated by the muscles of knee extension and knee flexion, will be an exploratory measure, as will the 100 meter walk time and the NSAA.
iii. [redacted]

[redacted]

3.12 Statistical Analysis

[redacted] Measure of the 100 meter walk/run and MVICT of lower limb muscles will be analyzed by paired t-tests with a significance level of $p = 0.05$.

ALL data will be shared with the DSMB as gathered for each study subject. Any adverse events will be fully discussed with the DSMB and reported to the FDA.

4.0 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".

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

4.1 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.

4.2 Dose limiting toxicity (DLT)

Study enrollment will be halted by the investigators when any subject experiences two 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) before making a determination to continue enrollment as described in Section 4.5 Dose Escalation.

Laboratory tests with values within the clinically significant range will be repeated during the same visit whenever possible (refer to Table 1 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. [redacted] 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 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.

4.3 Stopping/Discontinuation Rules

An independent Data Safety Monitoring Board (DSMB) will be established by the funding agency including a safety monitor for the study. 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 4.5 (Dose Escalation) of this protocol.

4.4 Expected Adverse Events Related to Disease Progression

Subjects enrolled in 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 5.0. All AEs related to the disease and unrelated to the gene therapy administration will be reported annually to the FDA, NIH/OBA and IRB.

4.5 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 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. 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 patient'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[35]. 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.[36] This steroid effect may be seen with increased steroid dosing.

4.6 Dose Escalation

There will be at least 4-6 weeks between dosing of subjects within a cohort. The allowance of four weeks between dosing of subjects provides time for an internal review of the safety analysis from five time points (days 1, 2, 7, 14 and 30) prior to dosing of the next subject and time for review of the safety analysis of all the subjects within a cohort by the investigators and the DSMB. The 30-day post-injection data from one subject in the first cohort will be reviewed and discussed with the DSMB prior to dose escalation to the higher dose cohort. Dose escalation will be based on dose-limiting toxicity (DLT).

The investigators will confer with the IRB, and DSMB on all Grade 3 or higher adverse events that are possibly, probably, or definitely related to the study agent before continuing enrollment. Based on the outcome of the safety and efficacy analysis at the end of each cohort decisions will be made to proceed with dose escalation for the following cohort.

5.0 ADVERSE EVENT MONITORING

5.1 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 Events will be collected throughout the study from enrollment to last follow up visit.

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 7.5. 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.

5.2 Serious adverse event (SAE)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or funding agency, 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 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

5.3 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 funding agency, its occurrence places the 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/ OBA on behalf of Nationwide Children's Hospital using the web-based Adverse Event reporting system (GeMCRIS).

5.4 Obligations of the Investigator

The Principal Investigator will submit an electronic report to NIH Office of Biotechnology Activities (NIH OBA) through the GeMCRIS web-based reporting system 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, NIH Office of Biotechnology Activities (NIH OBA) and to the local Institutional Biosafety Committee within the timeframes set forth in section Safety Reporting.

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

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

5.5 Safety Reporting

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

DSMB and OBA/IRB: All Serious Adverse Events (SAEs) and Dose Limiting Toxicities (DLTs) will be reported to the **DSMB and NIAMS,** through **KAI,** and the **IRB** within **48 hours** of notification, regardless of expectedness, relatedness, or if they meet the definition for unanticipated problems to the clinical trial

FDA/NIH OBA/IRB: 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 concurrently to the **FDA/NIH** and the **OBA/IRB** as soon as possible, but not later than **7 calendar days** after the funding agency'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 concurrently to the **FDA/NIH OBA/IRB** as soon as possible, but not later than **15 calendar days** after the funding agency'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 concurrently to the **FDA/NIH OBA/IRB** 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/NIH OBA/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/NIH OBA/IRB and the DSMB 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/NIH OBA/IRB** and the **DSMB as soon as possible** to the , but not later than **15 calendar days** after 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.

5.5.1 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 Principal 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) by using the recommended Adverse Event Reporting Template available on NIH OBA's web site at:
http://osp.od.nih.gov/sites/default/files/resources/Adverse_Event_Template_.docx
A copy of this report will also be sent to the IRB, IBCSC, FDA, and DSMB according to regulatory requirements described in section Safety Reporting.

5.6 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.

Potential expected AEs include localized injection site reactions, which might also be related to the procedure and be independent of the AAV vector itself. Systemic delivery of AAV vectors have resulted in AEs including asymptomatic transient transaminitis (up to levels of 2.5x the patient's baseline value and 3X the upper limit of normal for AST and ALT) with preserved liver synthetic function and no significant elevation in GGT) following gene transfer (see Section 4.5 for a listing of anticipated adverse event lab findings related to the intervention).

5.7 Follow-up of Adverse Events

All adverse events will be followed until resolution or stabilization.

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.

6.0 STUDY REPORTS

6.1 Final Study Report

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

6.2 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 Principal 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 Principal Investigator; (4) clinical protocol identifiers, including the NIH OBA protocol number, CCH 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.

6.3 Data and Safety Monitoring Plan

6.3.1 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 "Phase I/II gene transfer clinical trial for Duchenne Muscular Dystrophy using rAAVrh74.MCK.GALGT2 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 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 Principal 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.

6.3.2 DSMB Reporting and Meetings

Reports describing the status of the study will be prepared by the Principal Investigator's staff and sent to the DSMB at the end of each cohort, or at the DSMB's request.

An initial meeting (either by teleconference or webcast) with the DSMB will be scheduled prior to study initiation and after Day 30 visit of the last subject in each cohort, which will be 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

6.3.3 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. Flanigan 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:

- Neurology and Neuromuscular Diseases
- Immunology

- Gene Therapy
- 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.

6.4 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 well-being 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.

The Safety Monitor will be a contracted Clinical Monitor through Novella Clinical every two months. The Monitor will be responsible for source verification against the Electronic Database Capture System, ensure adverse events are reported and review the regulatory binder.

6.4.1 Data Management and Study Forms

All data and observations will be documented on electronic Case Report Forms (CRF) by source documentation using the Open Clinica 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 PI or his 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 PI 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.

In collaboration with the study team, the Research Informatics Core designed a data collection system (Open Clinica) for managing the clinical trial. A web-based database was created and it will be managed by authorized users. CRFs will be transcribed to this web-based database. Data will be extracted from source documents (lab reports, echo reports, etc) 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 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.

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