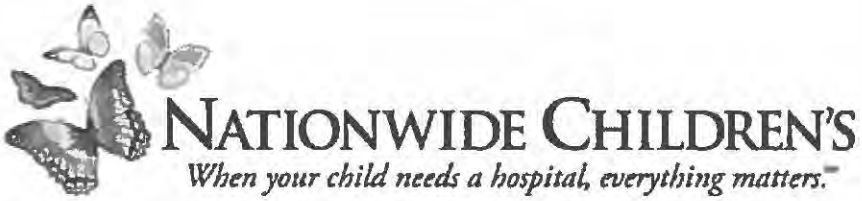
NCT #NCT01976091
[redacted]




**Phase I/IIa gene transfer clinical trial for LGMD2D (alpha-sarcoglycan deficiency) using**

**scAAVrh74.tMCK.hSGCA**

# CLINICAL PROTOCOL

**IND#: 15800**

**Version: 11.0 (10 Feb 2017)**

**Sponsor Investigator:** [redacted]

**Funded by:** NIH/NIAMS

**The Research Institute at Nationwide Children's Hospital**

**Center for Gene Therapy**

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# 1.0 PROTOCOL SYNOPSIS

| | |
|---|---|
| **Title** | Phase I/IIa gene transfer clinical trial for LGMD2D (alpha-sarcoglycan deficiency) using scAAVrh74.tMCK.hSGCA. |
| **Study Number** | N = 7: [Low dose single leg (n=1) and bilareral (n =3) and High dose bilateral (n=3)] |
| **Clinical Study Phase** | Phase I/IIa trial |
| **Number of Centers** | Single site (Nationwide Children's Hospital) |
| **Study Objectives** | Primary objective is safety |
| **Study Design** | Dose escalation study of self-complementary (sc) AAVrh74.tMCK.hSGCA delivered to single leg (low dose n=1) and bilaterally (n=6) via a major lower limb artery to the whole lower limb of LGMD2D (alpha-SG deficient) subjects. |
| **Patient Population** | **Inclusion Criteria**<br>• Subjects age 7 or older; cohort 1A must be adult and wheelchair-dependent<br>• Proven alpha-sarcoglycan deficiency by muscle biopsy or DNA testing.<br>• Subject enrolled in Cohort 1A must be adult and wheelchair dependent<br>• Subjects enrolled in Cohorts 1B or 2 must be able to walk independently, but must exhibit signs of lower extremity weakness (i.e. a Gowers' sign, use a handrail for climbing stairs) and walk ≤ 80% of predicted distance on the 6MWT based on normative data.<br>• Males and females of any ethnic group will be eligible<br>• Ability to cooperate with muscle testing.<br>• Willingness of sexually active subjects with reproductive capacity to practice reliable method of contraception (If appropriate), during the first six months after gene therapy (females) or until two negative sperm samples are obtained post gene transfer (males).<br><br>**Exclusion Criteria**<br>• Active viral infection based on clinical observations.<br>• The presence of SGCA mutations without weakness or loss of function.<br>• Symptoms or signs of cardiomyopathy, including:<br>• Dyspnea on exertion, pedal edema, shortness of breath upon lying flat, or rales at the base of the lungs<br>• Echocardiogram with ejection fraction below 40%<br>• Serological evidence of HIV infection, or Hepatitis B or C infection.<br>• Diagnosis of (or ongoing treatment for) an autoimmune disease.<br>• Abnormal laboratory values considered clinically significant (GGT > 3XULN, bilirubin ≥ 3.0 mg/dL , creatinine ≥ 1.8 mg/dL, Hgb < 8 or > 18 g/Dl; WBC > 15,000 per cmm).<br>• Concomitant illness or requirement for chronic drug treatment that in the opinion of the PI creates unnecessary risks for gene transfer.<br>• Pregnancy.<br>• Subjects with AAVrh74 or AAV8 binding antibody titers ≥ 1:50 as determined by ELISA immunoassay. |

| | |
|---|---|
| **Study Procedures** | The vector will be delivered via direct intravascular delivery to a major lower limb artery to single leg (cohort 1A) or to both legs sequentially (cohort 1B-2). Study subjects will receive general anesthesia during the procedure following Children's Hospital protocol. Detailed description of the vector administration procedure can be found in section 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.<br>1. The 6 minute walk test (6MWT) (primary variable to measure efficacy).<br>2. [redacted]<br>3. [redacted]<br>4. [redacted]<br>A statistically significant change in distance walked on the 6MWT will be considered evidence of a positive result. |
| **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, and 180 and at months 9 (physical therapy only), 12, 18, and 24. Immune studies will continue with blood samples collected locally and shipped to us at different time points up to two years. |
| **Sample Size** | Cohort 1A: One (n=1) adult LGMD2D wheelchair-dependent subject will receive single-limb perfusion at the low dose.<br>Cohort 1B: Three (n=3) LGMD2D subjects will receive bilateral whole limb perfusion at the low dose.<br>Cohort 2: Three (n=3) LGMD2D subjects will receive bilateral whole limb perfusion at the high dose.<br>• Cohort 1A and 1B receive low Dose: $1x10^{12}$ vg/kg per lower extremity (1A single lower limb; 1B both lower limbs)<br>• Cohort 2 will receive $3x10^{12}$ vg/kg per lower extremity in both lower limbs<br>If safety data is satisfactory at *low dose in single-limb (Cohort 1A)*, we will use the same dose for bilateral lower limb infusion (Cohort 1B). With satisfactory safety data we will increase to high dose for bilateral lower limb infustion (Cohort 2). |
| **Statistical Analysis** | This is a Phase I/IIa trial, with safety as the primary measure. The 6MWT will be the leading or first order variable for efficacy measures (secondary outcome). [redacted] The efficacy measures will be tested pre- and post-gene transfer at 60, 90 and 180 days and at months 9, 12, 18 and 24. [redacted] |
| **Long-term follow-up** | Subjects will be seen at the end of 1st and 2nd year for a physical exam, strength testing, and immune studies. 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. 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. |

## 2.0 ABSTRACT

The proposed clinical trial is a dose escalation study of self-complementary scAAVrh74.tMCK.hSGCA vector and transgene to LGMD2D (alpha-SG deficient) subjects delivered via a major lower limb artery of each leg sequentially by isolated limb infusion (ILI)[1]. Three cohorts (Cohorts 1A, 1B, and 2) will undergo gene transfer in a standard three-six dose escalation scheme to establish maximum tolerated dose (MTD) using toxicity. One (n=1) adult wheelchair-dependent patient will be enrolled in Cohort 1A, three (n =3) subjects will be enrolled in Cohort 1B, and three (n =3) subjects will be enrolled in Cohort 2. The first cohort (1A) will receive a vector dose of 1 x $10^{12}$vg/kg (optimized weight to height) in a single limb with delivery to the whole limb. This same dose will be delivered to both limbs in Cohort 1B. Cohort 2 will receive a total dose of 3 x $10^{12}$vg/kg per limb delivered to both lower extremities. The vector will be infused into an indwelling vascular sheath placed in the femoral artery. This will be a one-time vector infusion to an isolated limb with an approximately 10-minute dwell time. Cohort 2 will receive 6 x $10^{12}$ vg/kg total vector dose – split between the 2 lower extremities (3 x $10^{12}$ vg/kg per limb) delivered to the whole limb according to the same protocol. The total vector genome dose for each subject will be adjusted by rounding to the closest 5 kg (see section 6.2.7.3 Dose selection).

The primary objective of this study is the assessment of the safety of intravascular administration of scAAVrh.74.tMCK.hSGCA delivered via the femoral artery to lower extremities of LGMD2D (alpha-SG deficient) subjects. Safety monitoring during infusion will include: activated clotting times. Safety endpoints will be assessed by changes in hematology, serum chemistry, urinalysis, immunologic response to rAAVrh74 and hSGCA, and reported history and observations of symptoms.

The 6 minute walk test (6MWT) will be used as the secondary outcome for this study. [redacted] These quantitative physical therapy measures will be done at baseline, day 60, 90, 180, month 9, 18 and at the end of 1st and 2nd years.

Subjects will be evaluated at baseline, infusion visit (days 0-1), and return for follow up visits on days 2, 7, 14, 30, 60, 90, and 180 and at months 9, 12, 18, and 24. [redacted] This is the first vascular delivery clinical trial for muscular dystrophy, and should be considered a major milestone for the disease.

## 3.0 CLINICAL TRIAL AND PRINCIPAL INVESTIGATOR

The study will be carried out at Nationwide Children's Hospital. [redacted]

[redacted] will serve as Principal Investigator. [redacted]

This gene therapy trial is a follow up to the intramuscular trial where sustained gene expression was documented at 6 months in LGMD2D subjects without significant adverse events. Since that time, intravascular studies through lower limb arteries of non-human primates (rhesus

macaque) have demonstrated gene delivery using the same vector proposed in the clinical trial with the addition of a FLAG tag to differentiate endogenous α-SG from the protein expressed by scAAVrh.74.tMCK.SGCA. This vascular delivery clinical trial for LGMD2D (alpha-sarcoglycan deficiency) will be a dose escalation, Phase I/IIa study. The study is designed to assess safety, as well as clinical response to the scAAVrh.74.tMCK.hSGCA transgene. Seven subjects with alpha-sarcoglycan deficiency by muscle biopsy or DNA testing will be enrolled for this trial. The FDA has requested that the first subject be non-ambulatory and receive vector infusion to only one limb to assess safety.

# 4.0 SPECIFIC AIMS

## 4.1 PRIMARY OBJECTIVE

Determine the safety of intravascular administration of self-complementary scAAVrh74.tMCK.hSGCA delivered via a major lower limb artery targeting all of the lower limb muscles of LGMD2D (alpha-SG deficient) subjects.

## 4.2 SECONDARY OBJECTIVE

Assessment of the efficacy of the same vector measured by:

- The distance walked in 6 minutes (6MWT, leading or first order variable).

## 4.3 [redacted]

- [redacted]
- [redacted]
- [redacted]

## 5.0 BACKGROUND AND SIGNIFICANCE

The LGMDs include 21 disorders with known chromosomal linkage. Seven, designated LGMD1A-G, have autosomal dominant inheritance (3 of which have known gene defects). Fourteen disorders are inherited as recessive autosomal conditions, referred to as LGMD2A-N (all with known gene defects).

**LGMD2D is one of a family of disorders known as the limb-girdle dystrophies (Table 6.1 A-B).**

| AUTOSOMAL RECESSIVE-LGMD | | |
|---|---|---|
| DISEASE | PROTEIN | GENE |
| LGMD2A | Calpain3 | CAPN3 |
| LGMD2B | Dysferlin | DYSF |
| LGMD2C | γ – Sarcoglycan | SGCG |
| LGMD2D | α - Sarcoglycan | SGCA |
| LGMD2E | β - Sarcoglycan | SGCB |
| LGMD2F | δ - Sarcoglycan | SGCD |
| LGMD2G | Telethonin | TCAP |
| LGMD2H | TRIM32 | TRIM32 |
| LGMD2I | Fukutin-related protein | FKRP |
| LGMD2J | Titin | TTN |
| LGMD2K | O-Mannosyl transferase-1 | POMT1 |

| | | |
|---|---|---|
| LGMD2L* | Fukutin | FKTN |
| LGMD2M | O-Mannose β – 1, 2-N-acetylglucosaminyl transferase | POMGn1 |
| LGMD2N | O-Mannosyl transferases-2 | POMT2 |

| AUTOSOMAL DOMINANT-LGMD | | |
|---|---|---|
| DISEASE | PROTEIN | GENE |
| LGMD1A | Myotilin | Myotilin |
| LGMD1B | Lamin A/C | LMNA |
| LGMD1C | Caveolin-3 | CAV3 |
| LGMD1D | Desmin | Desmin |
| LGMD1E | DnaJ homolog subfamily B member 6 | DNAJB6 |
| LGMD1F | Transportin -3 | TNPO3 |
| LGMD1G | Heterogeneous nuclear ribonucleoprotein D-like | HNRPDL |

*No international agreement has been reached for the nomenclature of this LGMD. Recent publications assigned the LGMD type 2L to the ANO5 gene mutations.

The clinical trial pertains directly to LGMD2D subjects with α-SG deficiency, and may have implications for all sarcoglycan deficiencies (see Table 6.1A-B classification of LGMDs). Supportive care is the only treatment option available for LGMD2D subjects.

The clinical features of LGMD2D overlap with the dystrophinopathies.[5-8] The phenotypes range from severe to mild, similar to Duchenne (DMD) and Becker muscular dystrophies (BMD). The disease occurs worldwide. The usual age at onset ranges from 3 to 15 years, although some cases have delayed walking as in DMD. Subjects most commonly present with difficulty running, jumping and climbing stairs about age 3-5. Serum CK is markedly elevated (50 to 100 X normal is not unusual). Muscle weakness is generalized; biceps brachii are weaker compared to

DMD. Calf hypertrophy is common. Subjects become wheelchair dependent by age 13-15 years on average. Respiratory impairment occurs as the disease progresses leading to a need for ventilatory assistance. Scoliosis and joint contractures develop over time. Of particular importance regarding gene therapy as a potential treatment modality for LGMD2D is that cardiac muscle is spared in most cases (in contrast to the dystrophinopathies). In addition, no cognitive deficiencies are seen, again setting the stage for potential gene replacement of muscle without need to go to other body systems.

The other sarcoglycanopathies, LGMD2C (γ-SG), 2E (β-SG), and 2F (δ-SG), are similar in spectrum of presentation to LGMD2D. LGMD2C and 2F, tend to be more severe. Confusion with DMD is common except that both genders are equally affected in LGMD2D. In our experience, there is significant overlap in phenotype between the sarcoglycanopathies and other forms of autosomal recessive LGMDs, especially deficiencies of dysferlin (LGMD2B), calpain-3 (LGMD2A), and fukutin-related protein (FKRP, LGMD2I). In addition, many of the LGMDs have residual protein expression in muscle since most are the result of missense mutations. [redacted][2]

The sarcoglycanopathies have an estimated prevalence of 1 per 178,000 affected (MDA Website and ORD). If successful in the LGMD2D population, studies could be expanded to include other LGMDs that could potentially benefit from vascular gene delivery increasing the impact with benefit to 1 per 123,000. The findings in this study also have direct implications for the DMD population with an estimated annual incidence of newborn males of 1 in 5000

births.[9,10] In the USA, it is estimated that there are approximately 45,333 boys affected with DMD in anyone year (ORD). This may be an underestimate since we are doing so much better at prolonging the lives of these boys and young men. Furthermore, it has been estimated that the cost per year per subject with DMD is $126,000 (MDA website). Using this as a yardstick, the cost for the sarcoglycanopathies per subject based on the severity of the condition is approximately the same. For the DMD population, the cost estimates for the disease exceed $5 billion and $250M for the sarcoglycanopathies. The goal of this project is to promote ambulatory independence for subjects with LGMD2D. [redacted] The cascading effect to other neuromuscular disorders would also be very significant and would easily justify this initial investment. Apart from the overall cost for each subject, improving the quality of life for the muscular dystrophy subject could be significant by prolonged ambulation, enhancing self-esteem and overall feelings of well-being[11].

# 6.0 CLINICAL RESEARCH PLAN

## 6.1. STUDY POPULATION

Seven LGMD2D subjects with alpha-sarcoglycan deficiency by muscle biopsy or DNA testing, will be enrolled at Nationwide Children's Hospital for the gene transfer study. Subjects will encompass any ethnic, racial, or gender background. Patients must have proven muscle weakness to be enrolled.

## 6.2. PRE-TREATMENT ASSESSMENT:

A. Establish Subject Eligibility

### I. Inclusion Criteria

1. Cohort 1A must be adult and wheelchair-dependent; Cohorts 1B and 2 will be subjects age 7 or older.
2. Confirmed alpha-sarcoglycan deficiency or identified SGCA DNA mutation.
3. Subjects enrolled in Cohorts 1B or 2 must be able to walk independently, but must exhibit signs of lower extremity weakness (i.e. a Gowers' sign, use a handrail for climbing stairs) and walk ≤ 80% of predicted distance on the 6MWT based on normative data.
4. Males and females of any ethnic group will be eligible.
5. Ability to cooperate with muscle testing.
6. Willingness of sexually active subjects with reproductive capacity to practice reliable method of contraception (if appropriate), during the first six months after gene therapy (females) or until two negative sperm samples are obtained post gene transfer (males).

### II. Exclusion Criteria

1. Active viral infection based on clinical observations.

2. The presence of SGCA mutations without weakness or loss of function.

3. Symptoms or signs of cardiomyopathy, including:

   - 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%.

4. Serological evidence of HIV infection, or Hepatitis B or C infection.

5. Diagnosis of (or ongoing treatment for) an autoimmune disease.

6. Abnormal laboratory values considered clinically significant (GGT > 3XULN, bilirubin ≥ 3.0 mg/dL , creatinine ≥ 1.8 mg/dL, Hgb < 8 or > 18 g/Dl; WBC > 15,000 per cmm).

7. Concomitant illness or requirement for chronic drug treatment that in the opinion of the PI creates unnecessary risks for gene transfer.

8. Pregnancy.

9. Subjects with AAVrh74 or AAV8 binding antibody titers ≥ 1:50 as determined by ELISA immunoassay.

**B. 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.

**C. 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.

**D. Baseline Measures Prior to Injection (day -45 to day -1)**

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

<u>Day -45 to day -1 before gene transfer</u>

- Physical exam
- EKG
- Echocardiogram
- Antibody (IgG and IgM) testing for hepatitis B, and C, and for HIV
- Complete blood count (CBC) with differential and platelets, with smear
- Serum total protein
- Serum gamma-glutamyl transferase (GGT*)
- Serum total bilirubin
- Glucose
- Creatine kinase (CK)
- Creatinine/BUN
- Cystatin C
- Alkaline phosphatase
- ALT
- AST
- Amylase

- Electrolytes
- Fibrinogen
- Prothrombin time (PT), partial thromboplastin time (PTT), INR
- Urinalysis
- Serum binding antibody to rAAVrh74 and AAV8
- ELISpot assay to capsid proteins and alpha-sarcoglycan
- Pregnancy test (if appropriate)
- [redacted]
- [redacted]
- [redacted]
- Six minute walk test (6MWT).
- [redacted]
- [redacted]
- [redacted]

** Because GGT is very sensitive to alcohol consumption, subjects will be required to avoid alcohol the week before testing. 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.*

## 6.3 PROTOCOL FOR GENE TRANSFER

The clinical trial is a dose escalation study of scAAVrh74.tMCK.hSGCA delivered by isolated limb infusion via a sidearm sheath (REF CP-08403; Manufacturer: Arrow) placed percutaneously in the femoral artery and allowed to circulate through the limb vasculature. Prior to vector delivery the limb circulation is isolated from the central circulation by the careful placement of occlusive balloon catheters (Tyshak II Balloon Dilatation Catheter; Manufacturer: B. Braun Intervention Systems Inc., and NuMED, Inc.) one placed through the arterial sheath in a cephalad direction positioned above the profunda artery and a second occlusive balloon positioned via a similarly placed sidearm sheath placed in the femoral vein. Following placement of the arterial and venous sidearm sheaths and before the insertion of the occlusive balloons the subject is anticoagulated with unfractionated heparin. The balloons are then positioned under flouroscopy guidance and inflated to create stasis in the limb during the infusion procedure. [redacted]

[redacted]

For the subjects in cohort 1A the arterial and venous sheaths will be placed and procedure will continue as described above. For the subjects in cohorts 1B and 2—both limbs—the vascular sheaths will be placed on both sides (left and right) and the the subject will be anticoagulated and the occlusive balloon catheters positioned one side and the subject dosed. Upon completion of dosing of the first extremity, the other will be done. Three cohorts will undergo gene transfer in a dose escalation scheme to establish maximum tolerated dose (MTD) using toxicity. Cohort 1A (n=1) will receive a vector dose of $1 \times 10^{12}$ vg/kg in a single lower extremity. In Cohort 1B (n=3), three subjects will receive the same vector dose per limb delivered bilaterally to both lower extremities for a total dose of $2 \times 10^{12}$vg/kg. This will be a one-time vector infusion as described above. Subjects in Cohort 2 (n=3) will receive $3 \times 10^{12}$ vg/kg vector dose per extremity ($6 \times 10^{12}$ vg/kg total dose) delivered according to the identical protocol.

[redacted]

[redacted]

In an attempt to dampen the host immune response to our AAV-based therapy, subjects will be started on a prophylactic daily dose of prednisone (or alternative glucocorticoid in dose equivalency) at approximately 1mg/kg/day with a maximum dose of 60 mg/day. Post-treatment, we may increase this dose to approximately 2 mg/kg/day with a maximum dose of 60 mg/day, depending on T cell responses measured by ELISpot assay. Treatment will be initiated one day prior to the gene transfer and the subjects will remain on prednisone treatment for approximately one month. A tapering protocol for prednisone will be implemented based on individual subjects' immune response to the gene transfer, again assessed by the ELISpot assay. If GGT elevations are seen after 30 days; the prednisone treatment will be continued or restarted at an appropriate dose for duration necessary to quell the immune reaction with a maximum of 60 mg per day. The duration of treatment will be determined based on weekly levels of GGT and immune responses.

Subjects will be admitted to the Nationwide Children's Hospital the day of the gene transfer. Procedures will be performed under sterile conditions in the Cardiac Catheterization Suite. An intravenous catheter with heparin lock will be placed in the arm for delivery of sedation.

**I. Injection Preparation**

Preparation of the scAAVrh74.tMCK.hSGCA gene vector will be done by the Nationwide Children's Hospital research pharmacist according to the Manual of Operating Procedures

(MOOP). Immediately prior to transportation to the clinical setting, appropriate dilutions of the test article will be completed by the pharmacy. [redacted]

Documentation of the dilution will be completed by the pharmacy following standard pharmacy protocol.

The vector-containing syringes will be delivered to the designated Cardiac Catheterization Suite at Nationwide Children's Hospital. It will be delivered under ambient temperature (not frozen) and administered to the subject within 8 hours of preparation. Handling of scAAVrh74.tMCK.hSGCA gene will follow compliance standards for Biosafety Level 1 vectors. (http://www4.od.nih.gov/oba/RAC/guidelines_02/APPENDIX_G.htm#_Toc7246561)

**II. Vector Administration Protocol**

The overall procedure is such that the vector will be delivered via direct intravascular delivery into the femoral artery of the leg followed by an 8-10 minute dwell time.[1] Subjects will undergo appropriate anesthesia (general or conscious sedation as appropriate) for their age and muscle disease per Children's Hospital Department of Anesthesia protocol. Under sterile conditions, the following protocol will be performed:

a. The subject is transferred from a hospital bed to the Cardiac Catheterization Lab and positioned in the usual supine manner, attached to appropriate monitoring and placed under general anesthesia or conscious sedation as determined by the anesthesiologist

utilizing NCH anesthesia protocols that minimize the risk of anesthetic reaction in muscular dystrophy. The subject will be prepped and draped in the usual sterile fashion. A baseline activated clotting time (ACT) level will be obtained from a peripheral IV.

b. The femoral artery and femoral vein will be entered percutaneously using the Seldinger technique and guide wire advanced and positioned under fluoroscopy. A sidearm sheath will be advanced over the guide wire--one into the femoral artery and one into the femoral vein and both flushed with heparinized normal saline. Before the occlusion balloons are inserted through the sidearm sheaths, 200 IU/kg of heparin will be administered via a peripheral arm IV and allowed to circulate for 3-5 minutes, at which time a blood sample will be drawn from the peripheral IV and an ACT will be measured with the goal of > 200 seconds from an anticipated base line of 90-120 seconds drawn systemically (arm-PIV). If the post heparin ACT is less < 200 seconds, a second dose of heparin, 50-100 IU /Kg will be administered. An immediate follow up ACT will be obtained with the targeted ACT goal of > 200 seconds. This process will be repeated until ACT's are within the designated range and repeated every 30-60 minutes to assess ongoing anticoagulation levels.

c. The procedure for vascular delivery of scAAVrh.74tMCK.hSGCA to the isolated limb can be summarized as follows:

- The area near the inguinal ligament will be infused with several mLs of Marcaine or Lidocaine for local anesthetic effect. Percutaneous access of the femoral artery will be obtained using the Seldinger technique and advancing an introducer and dilator

over a guide wire. The dilator and wire are removed and sidearm sheath (REF CP-08403; Manufacturer: Arrow) placed and flushed appropriately with heparinized normal saline.

- Percutaneous access of the femoral vein is obtained in a similar fashion.
- Both sets of femoral vessels (right and left, artery and vein) will be cannulated before heparinization of the subject.
- Before the occlusion balloons are inserted through the sidearm sheaths, 200 IU/kg of heparin will be administered via a peripheral arm IV and allowed to circulate for 3-5 minutes, at which time a blood sample will be drawn from the peripheral IV and an ACT will be measured with the goal of > 200 seconds from an anticipated base line of 90-120 seconds drawn systemically (arm-PIV). If the post heparin ACT is less < 200 seconds, a second dose of heparin, 50-100 IU /Kg will be administered. An immediate follow up ACT will be obtained with the targeted ACT goal of > 200 seconds. This process will be repeated until ACT's are within the designated range.
- Using fluoroscopic guidance a Tyshak II Balloon Dilation catheter is advanced over a guide wire through the sheath into the femoral artery above the take-off of the femoral profunda artery and positioned to achieve arterial stasis when the occlusive balloon is inflated. Positioning the arterial balloon catheter above the femoral profunda artery take off enables perfusion and targeting of proximal muscles of the hip via the anterior circumflex artery.

- Again with fluoroscopic guidance a Tyshak II Balloon Dilation catheter is advanced over a guide wire through the sheath into the femoral vein to approximately the same level as the arterial balloon catheter and positioned to achieve venous stasis when the balloon is inflated.
- If needed a tourniquet may be placed over the region of the balloons to augment vascular stasis and isolation of the limb circulation.
- [redacted]
- [redacted]

- [redacted]
- [redacted]
- [redacted]
- At the conclusion of the post flush the balloon catheters and tourniquet are taken down.
- For subjects being dosed in both limbs the balloon catheters will be placed and positioned via fluoroscopic guidance and the treatment of the second limb undertaken as described above.
- At the end of the procedure all occlusive balloon catheters, and sheaths, will be removed.
- Direct pressure is used to control bleeding at the site and obtain hemostasis.
- Following gene transfer, subjects will return to a designated monitored bed for a minimum of 6 hours within Nationwide Children's Hospital. Vital signs will be obtained per Hospital post-op policy and hourly for four hours following the injection and then every 4 hours prior to discharge.

Subjects will be monitored for bleeding, perfusion to the extremity, compartment syndrome and neuromuscular integrity. Compartment syndrome will be assessed by direct limb palpation and Doppler ultrasound (as needed). Neuromuscular integrity will be determined by routine assessment of clinical pain and strength.

## 6.4 POST- GENE TRANSFER MONITORING

- Subjects will return to a designated bed following gene transfer with close monitoring of vital signs. CK levels will be checked after the procedure. Concomitant medications and all adverse events/serious adverse events will also be monitored and documented following injection. Subjects will be discharged the day after gene transfer (if no adverse side effects are observed).

Subjects will return for follow up visits on days 2, 7, 14, 30, 60, 90 and 180 and at months 9 (physical therapy and immunology only), 12, 18, and 24. Toxicity monitoring on each of these dates (except visit 9) (including day 1-2 post-gene transfer) will include:

- Physical Exam
- GGT* will be drawn as above and at 45 and 75 days post gene transfer to assess delayed sub-clinical hepatitis. GGT is assessed in preference to ALT/AST which are elevated in muscular dystrophy.
- Total bilirubin

- Glucose
- PT/PTT/INR
- CBC/Diff/Platelet with smear
- Serum Creatine kinase (CK)
- Creatinine/BUN
- Cystatin C
- Alkaline phosphatase
- AST
- Amylase
- ALT
- Electrolytes
- Total Protein
- Urinalysis
- Immune studies (day 7 visit and after)
- [redacted]
- [redacted]
- [redacted]

- Six minute walk test (6MWT). (60 day visit and after)
- [redacted]
- [redacted]

- Photograph of incision site and surrounding area to be completed until day 14

** Subjects will be required to avoid alcohol the week before testing. 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.*

Because of the possible concerns with viral shedding, all sexually active participants will be asked to refrain from sexual intercourse or practice barrier contraception for six months (females) or until two consecutive semen samples (adult males) are tested negative for the presence of vector. As part of this study we must ask for a semen sample from all sexually active males 18 years and older.

Semen samples will be requested on day 90 from all sexually active males age 18 years or older, for PCR analysis for vector genomes. This interval is based on selecting a date post-gene transfer that is longer than the 70 days required from the beginning of the first meiotic division to the completion of spermatogenesis. As mature sperm spend an additional 5-15 days in the epididymis, a date three months after gene transfer is therefore optimal to look for any possible germ line incorporation. Testing will continue until 2 consecutive negative samples are obtained.

Blood samples will be obtained for immune studies at days 7, 14, 30, 60, 90, 180 post gene transfer and 9, 12, 18, and 24 months; this will include testing for binding antibody to rAAVrh74, as well as ELISpot to detect T cell response to capsid antigens. GGT will be measured at 45 and 75 days post gene transfer to assess delayed sub-clinical hepatitis.

## 6.5 LONG-TERM MONITORING

Subjects will be seen at the end of 1st and 2nd years for a physical exam, strength testing, and immune studies. We will follow the most recent FDA guidelines with regard to long-term subject follow up following gene transfer. As discussed and based on prior 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. 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.

## 6.6 OUTCOME MEASURES

The study is a Phase I/IIa 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 any one Grade III or higher treatment-related toxicities, or two or more Grade II treatment-related toxicities.

B. Efficacy outcome measures include:

i. Strength and functional testing will be performed at baseline and at days 60, 90, 180, and at 9, 12, 18, and 24 months. This will include the 6MWT distance as the primary functional variable. [redacted] Pre- and post-treatment strength, distance, and time will be evaluated by using paired t-tests.

[redacted]

[redacted]

iii. Immune response will be assessed by IFN-γ ELISpots to alpha-sarcoglycan and AAV capsid. A rise in IFN-γ of 50 spot forming colonies per one million PBMCs towards virus or transgene will be considered significant. An additional measure of immune response will be the binding antibody assay to AAV.

## 6.7 STATISTICAL ANALYSIS

- [redacted]

Data will be shared with the DSMB as gathered for each study subject. Related serious adverse events will be discussed with the DSMB and reported to the FDA.

## 6.8. STUDY TIMELINE

**Table 2**

| STUDY TIMELINE | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ıdy Interval | Baseline / Screening | Vector Infusion (Inpatient) | | | Follow Up | | | | | | | | | | | |
| Visit # | 1 | 2 | | | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| Study rocedures | -45 to -1 | 0 day | 1 day | 2 day | 7 day | 14 day | 30 day | 45 day | 60 day | 75 day | 90 day | 180 day | 9 mo | 1 yr | 18 mo | 2 yr |
| sit Window | +/- 7 days | +/- 7 days | +/- 7 days | +/- 7 days | +/- 7 days | +/- 7 days | +/- 21 days | +/- 21 days | +/- 21 days | +/- 21 days | +/- 21 days | +/- 21 days | +/- 21 days | +/- 21 days | +/- 21 days | +/- 21 days |
| Informed Consent | X | | | | | | | | | | | | | | | |
| dical History | X | | | | | | | | | | | | | | | |
| ysical Exam | X | | X | X | X | X | X | | X | | X | X | | X | X | X |
| CHO/EKG | X | | | | | | | | | | | | | | | |
| hest X-Ray | X | | | | | | | | | | | | | | | |
| [redacted] | | | | | | | | | | | | | | | | |
| patitis , B & C, HIV | X | | | | | | | | | | | | | | | |
| afety labs* | X[E] | | X | X | X | X | X | X** | X | X** | X | X | | X | X | X |
| gnancy Test | X | | | | | | | | | | | | | | | |
| ımen Test[D] | | | | | | | | | | | X | | | | | |
| Physical Therapy sessments[C] | X[A] | | | | | | | | X | | X[A] | X[A] | X | X | X | X |
| ne Transfer | | X | | | | | | | | | | | | | | |
| ımunology studies[B] | X | | | | X | X | X | | X | | X | X | X | X | X | X |
| [redacted] | | | | | | | | | | | | | | | | |
| otograph of jection site | | X | X | X | X | X | | | | | | | | | | |
| oncomitant edications | To be collected from time of consent until final study visit | | | | | | | | | | | | | | | |

ıfety labs (CBC/Diff/Platelet with smear, PT/PTT/INR, Alkaline Phosphatase, ALT, Amylase, AST, BUN, CK, Creatinine, GGT, Glucose, Electrolytes, al Protein, Total Bilirubin, Cystin C, Urinalysis)

*GG1 only (day 45 and day 75)

**Table 2. Legend to annotations**

A. [redacted]

B. Immunology studies will be done pre-gene transfer (-45 to -1) and continue at post-treatment day 7, 14, 30, 60, 90, 180, and at months 9, 12, 18 and 24 post-gene transfer. Immunology studies consist of measuring binding antibodies to rAAVrh74, (ELISA), and detecting T cell responses to both rAAVrh74 and alpha-sarcoglycan peptides (ELISpot).

C. Physical therapy assessments ([redacted], 6MWT, [redacted] will be performed as appropriate based on clinical judgement, age and/or ambulatory status.

D. Vector identification by DNA-PCR in semen on male subjects, testing will continue every 7 days until obtaining 2 consecutive negative samples.

E. [redacted]

F. Fibrinogen level will be drawn

G. [redacted]

Note: There will be a flexibility of +/- 7 or +/- 21 working days as outlined for each of the planned study visits including screening to adjust the schedule to any unanticipated event. There will also be potential additions to care based on best medical management if the need arises including additional fluids to restore blood volume.

# 7.0 TEST MATERIAL AND ADMINISTRATION

## 7.1 DESCRIPTION OF BIOLOGICAL PRODUCT

The biological product is a non-replicating self complementary recombinant adeno-associated virus termed scAAVrh74.tMCK.hSGCA. The vector contains the human alpha-sarcoglycan gene under the control of a triple E-box muscle creatine kinase (tMCK) muscle specific promoter. This cGMP product was produced at the Clinical Manufacturing Facility (CMF) at Nationwide Children's Hospital using practices consistent with the US Food and Drug Administration's "Guidance for Industry – cGMP for Phase 1 Investigational Drugs", July 2008. The material produced will be used to support a vascular delivery phase I/IIa clinical trial. The manufacturing process includes procedures and practices to ensure the safety, identity, quality, purity and strength of the manufactured biologic. The HEK293 cell bank used in the production of the product meets specifications for product release as detailed in FDA and ICH guidelines. The production process is governed by over 100 standard operating procedures (SOP) and production documents. [redacted] The vector is supplied as a frozen liquid that is thawed before dilution and clinical administration. The vector DNA structure is illustrated below.

**Self-Complementary scAAVrh.74.tMCK.hSGCA**

[redacted]

## 7.1.1 PRODUCT DEFINITION

| | |
|---|---|
| Product Name: | scAAVrh74.tMCK.hSGCA |
| Gene Insert: | Human alpha-sarcoglycan cDNA |
| Control Elements: | triple E box mouse MCK promoter |
| AAV Serotype: | rh74 |

[redacted]

### 7.1.2. NAME AND ADDRESSES OF MANUFACTURER

The HEK293 Master Cell Bank and scAAVrh74.tMCK.hSGCA clinical grade vector was manufactured at:

Nationwide Children's Hospital

Clinical Manufacturing Facility

Center for Gene Therapy

700 Children's Dr.

Columbus, Oh 43205

## 7.2 STUDY DESIGN

Eligibility for subjects 7 years and older will be established by SGCA mutations, ability to cooperate for testing, willingness to practice contraception during the study (if appropriate), negative pregnancy test (for females, if appropriate), and no evidence of cardiomyopathy. HIV infection, hepatitis B, or C, or known autoimmune diseases are exclusion criteria.

IRB approved consent (18yrs. of age and older) and assent (ages 12 years up to 18 years old) forms will be obtained by the Principal Investigator [redacted] or Co-investigators and signed by parents and subjects. Pre-gene transfer immune studies include serum binding antibodies to

AAV and ELISpot assays for both AAV capsid and α-SG proteins. [redacted]

[redacted] This will be a vascular delivery trial through the femoral artery of a single leg (cohort 1A) and both lower extremities (cohorts 1B and 2) using scAAVrh74.tMCK.hSGCA. This will be a dose escalation study of scAAVrh74.tMCK.hSGCA delivered through the femoral artery to an isolated limb to maximize limb vector exposure. Three cohorts of LGMD2D subjects will be included. The lower limb muscles will be targeted to demonstrate increased muscle strength with the intent of promoting extended independent ambulation. Subjects in Cohort 1A will receive a dose of $1x10^{12}$ vg/kg per limb (Ideal weight to height). This first subject will receive a perfusion to a single lower extremity with a total dose of $1 \times 10^{12}$vg/kg. If safety is achieved, three subjects in Cohort 1B will receive the same dose per limb delivered to both lower extremities for a total dose of $2 \times 10^{12}$vg/kg. Subjects in Cohort 2 (n=3) will receive $6x10^{12}$ vg/kg total dose – split between the 2 extremities ($3 \times 10^{12}$ vg/kg per limb) delivered to the whole limb muscles according to the same protocol. Subjects will be appropriately sedated for the procedure with the assistance of an anesthesiologist. A detailed description of the infusion procedure is described in section 6.3 of this protocol. The goal for this study is to have efficient gene transfer, muscle fiber transduction, and gene expression levels sufficient to produce clinically meaningful results (improved ambulation= distance walked on 6MWT).

## 7.3 DOSE SELECTION

There will be three cohorts of LGMD2D subjects [Cohorts 1A (n=1), 1B (n=3), and 2 (n=3)] with dosing permitted by toxicology- biodistribution study and predicted by pre-clinical studies in non-human primates. The trial will start with low dose gene transfer (1 X $10^{12}$ vg/kg per limb); the first patient (Cohort 1A) enrolled will receive a single unilateral infusion to one lower extremity. The same dose will be delivered to three subjects (Cohort 1B) infused to both lower extremities. The total vector genomes dosed for each subject will be adjusted by rounding to the closest 5 kg per the discretion of the PI or designated co-investigator. The subjects in Cohort 2 (n=3) will receive a total dose of 3 x $10^{12}$vg/kg per limb delivered to both extremities. Table 6.3 below shows dosing for the clinical trial based on weights from clinic subjects eligible for the clinical gene transfer trial.

| | Patient wt (kg): | 20-29 | 30-39 | 40-49 | 50 - 59 | 60 - 69 | 70 - 79 | 80 - 89 | 90 - 99 | 100 - 109 | 110 - 119 | 120 129 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cohort | Dose/quad | Total Dose / Patient (total vector genomes) | | | | | | | | | | |
| (1) LD | 1e12vg/kg/limb | 4e13 | 6e13 | 8e13 | 1.0e14 | 1.2e14 | 1.4e14 | 1.6e14 | 1.8e14 | 2.0e14 | 2.2e14 | 2.4e14 |
| (2) HD | 3e12vg/kg/limb | 1.2e14 | 1.8e14 | 2.4e14 | 3.0e14 | 3.6e14 | 4.2e14 | 4.8e14 | 5.4e14 | 6e14 | 6.6e14 | 7.2e14 |

Reference Table: Total Dose (vg) per subject based on bodyweight (kg)

* LD = low dose; HD = high dose

If safety data is obtained at low dose, the third cohort of three additional subjects will receive the next escalating dose (6x$10^{12}$ vg/kg total dose) split between the two limbs.

Subjects within a cohort will be entered at intervals of approximately 4-6 weeks. Additional time will be allowed to review the biopsy results of the first subject in the first cohort prior to

dose escalation to the next cohort. Intervals within subjects in the second cohort can be reduced base on safety observed in first cohort.

## 7.4 DOSE LIMITING TOXICITY

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

**The classification for adverse events to be used is the following:**

1 Mild adverse event; did not require treatment

2 Moderate adverse event; resolved with treatment

3 Severe adverse event; inability to carry on normal activities; required professional medical attention

4 Life-threatening or permanently disabling adverse event

5 Fatal adverse event

In this grading system, **"severe"** is not equivalent to ***seriousness.***

The definitions to be employed will follow the final regulations issued by the FDA in September 2010 "Safety Reporting Requirements for INDs and BA/BE Studies", and described in section 7.4 of this protocol.

**Dose limiting toxicity (DLT)** is defined as any adverse event that is possibly, probably, or definitely related to the study agent. This would include any grade 3 event according to the classification given above.

Study enrollment will be halted by the investigators when any subject experiences a Grade 3, or higher adverse event toxicity that is possibly, probably, or definitely related to the study drug.

Laboratory tests with values within the clinically significant range will be repeated during the same visit whenever possible (refer to Table 6.4 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 as clinically indicated. For non-local residents, arrangements will be made to have the blood test redrawn in a laboratory close to home or by their primary care physician. To avoid any confusion for the primary care physician, they will be informed (with permission from the subject) of their participation in the study at the time of gene transfer. If the AE requires treatment, this will be carried out by the primary care physician or a doctor of choice selected by the subject. We will obtain copies of repeat laboratory tests and any relevant medical records for addition to the subject's research chart.

Only those adverse events requiring treatment will qualify as DLT. 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 enrollment.

**Table 6.4** Clinically Significant Laboratory Range

| Test | Units | Reference Range | | Clinically Significant Range |
|---|---|---|---|---|
| **Hemoglobin** | g/dL | 2Y – 5Y<br>6Y – 11Y<br>12Y – Adult | 11.5 – 13.5<br>11.5 – 15.5<br>12.0 – 16.0 | < 8.0 or > 18 |
| **WBC** | K/cu mm | 4Y – 5Y<br>6Y – 9Y<br>10Y – 20Y<br>21Y – Adult | 5.5 – 15.5<br>5.0 – 14.5<br>4.5 – 13.5<br>4.5 – 11.0 | ≤3.5 or ≥ 20 |
| **BUN** | mg/dL | 5 - 18 | | ≥35 |
| **PT** | sec | 12.4 – 14.7 | | ≤ 5.0 or ≥ 35.0 |
| **PTT** | sec | 24 - 36 | | ≥ 125 |
| **Platelet** | K/cu mm | 140 - 440 | | ≤ 90 or ≥ 800 |
| **Amylase** | U/L | 0 – 17Y<br>18Y – Adult | <110<br>30 - 110 | ≥ 200 |
| **Total Protein** | g/dL | 3Y – 15Y<br>16Y – Adult | 5.8 – 8.7<br>6.4 – 8.4 | ≤4.4 or ≥ 9.5 |
| **Total Bilirubin** | mg/dL | 0.1 – 1.0 mg/dL | | ≥ 3.0 |
| **Creatinine** | mg/dL | 4Y – 7Y<br>8Y – 10Y<br>11Y – 12Y<br>13Y – 17Y<br>18Y – Adult | 0.3 – 0.8<br>0.3 – 0.9<br>0.4 – 1.0<br>0.5 – 1.2<br>0.6 – 1.3 | ≥ 1.8 |
| GGT* | U/L | 8 – 78 | | 3XULN |
| ULN= upper limit normal | | | | |

* Subjects with history of alcohol abuse will be excluded from the study. Since GGT is very sensitive to alcohol consumption, subjects will be required to avoid alcohol the week before testing. 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.

## 7.5 STOPPING/DISCONTINUATION RULES

An independent Data Safety Monitoring Board (DSMB) will be established by the sponsoring 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 a Grade 3, or higher adverse event toxicity that is possibly, probably, or definitely related to the study drug. This will include:

- 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.

**Activities of Daily Living (ADL)**
*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

If after review by the DSMB the decision is made to continue, the study will proceed according to Section 6.2.7.6 (Dose escalation) of this protocol.

## 7.6 DOSE ESCALATION

There will be 4 weeks between dosing of subjects within a cohort. For the first cohort, the allowance of four weeks between dosing of subjects provides time for safety review 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.

This timeline will also provide time for review of any clinically significant improvement based on the 6MWT data. [redacted]

The investigators will confer with the IRB, DSMB, and FDA/CBER 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.

## 7.7 CONCOMITANT MEDICATIONS/THERAPIES

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 medical history. The PI will encourage participants to maintain the medication and supplements they are on at enrollment through the course of the study. Subjects on aspirin or drugs that could affect coagulation will continue their medication as indicated. Several investigations show that preoperative aspirin ingestion and intravenous heparin therapy can be administered safely without concerns about the risk of postoperative bleeding and should not lead to modification or cessation of such therapy.[13-15]

# 8.0 ADVERSE EVENT MONITORING AND REPORTING

## 8.1 DEFINITION OF AN ADVERSE EVENT

As stated above (6.2.7.4) 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:

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.

A single dash (-) indicates a grade is not available.

**Activities of Daily Living (ADL)**

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

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.

**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 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 graded 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.

**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 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).

## 8.2 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 serious 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.

## 8.3 SAFETY REPORTING

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

**NIAMS and DSMB:**

All Serious Adverse Events (SAEs) and Dose Limiting Toxicities (DLTs) will be reported to the **NIAMS** and the **DSMB** through KAI within **48 hours** of notification, regardless of relatedness to the clinical trial. KAI Research, Inc. serves as the NIAMS Executive Secretary.

**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 OBA/IRB** as soon as possible, but not later than **7 calendar days** after the sponsor's initial receipt of the information.

Serious adverse events that are unexpected and associated with the use of the gene transfer product, but are not fatal or life threatening, will be reported concurrently to the **FDA/NIH OBA/IRB** 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 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.

**FOLLOW-UP**

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/ NIAMS and the DSMB through KAI** 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/ NIAMS and the DSMB through KAI** 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/ NIAMS and the DSMB through KAI** 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.

### 8.3.1 SAFETY REPORTING: CONTENT AND FORMAT

The serious adverse event report will include, but need not be limited to: (1) the date of the event; (2) designation of the report as an initial report or a follow-up report, identification of all safety reports previously filed for the clinical protocol concerning a similar adverse event, and an analysis of the significance of the adverse event in light of previous similar reports; (3) clinical site; (4) the 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.

## 8.4 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.

## 8.5 FOLLOW-UP OF ADVERSE EVENTS

All adverse events will be followed until resolution or stabilization.

# 9.0 LONG-TERM FOLLOW-UP

We will follow the most recent FDA guidance with regard to long-term patient follow up following gene transfer. As indicated by the guidelines, our proposed vector has a very low probability of gene transfer-related delayed adverse events. 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.

The final results of the clinical trial will be shared with the participants at the completion of the study when all data has been collected and analyzed. 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.

## 9.1 ADVERSE EVENT REPORTING FROM PRIMARY CARE PHYSICIAN

Close communication will be established with the primary care physician of all study participants and will be maintained throughout the study. The important hallmarks of the study along with the proposed reporting plan will be explained. We will request that the primary care

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

During the consent process, the study investigator will emphasize the importance of subject communication with our study team. Any routine or non-routine doctor's visits or medical care received during the two years following gene transfer should be reported to the study team. The study doctor will explain to the participant that copies of any relevant medical records of those visits will be requested from their medical care provider.

# 10.0 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 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.

# 11.0 HUMAN SUBJECTS

## 11.1 HUMAN SUBJECTS INVOLVEMENT AND CHARACTERISTICS

The proposed study will involve seven subjects with alpha-sarcoglycan deficiency [LGMD2D] subjects. All subjects with this condition that are enrolled must have muscle weakness by clinical exam. LGMD2D is established on the basis of an alpha-sarcoglycan gene mutation with reduced or abnormally expressed alpha-sarcoglycan in the muscle. Subject selection will not exclude anyone on the basis of race, ethnic, or gender background. For complete details see the Inclusion and Exclusion Criteria Section.

## 11.2 SOURCES OF MATERIALS

Research material obtained from identifiable living human subjects includes reports of: history and physical assessments, blood, urine, chest X-ray, ECHO, EKG, [redacted], clinical assessments and admission/ discharge summaries for hospitalization occurring while the subject is on study, adverse events, and autopsies. Copies of case report forms, original test results, subject medical records, signed subject informed consent, correspondence, and any other documents of the subjects, relevant to the conduct of the study will be kept on

file by the Principal Investigator. All material or data collected as part of the study will be obtained specifically for research purposes.

## 11.3 POTENTIAL RISKS

Potential risks to study subjects include risks associated with administration of the study agent and with study procedures.

### 11.3.1 RISKS ASSOCIATED WITH SCAAVRH74.TMCK.HSGCA GENE TRANSFER

This is the first time that this viral serotype, scAAVrh74.tMCK.hSGCA, will be given to human subjects. Thus, the risks of participating in this study are not known. Studies on animals that received larger doses injected into muscle showed no side effects.

There is a very small chance that the study agent could damage the DNA in the cells of the subject's muscle. In the unlikely event that this occurred, it could put the subject at risk for developing cancer in the future. In animal studies to date, we have not seen the development of cancer. In addition, we and others have shown that the majority of vector DNA appears to persist as episomal rather than integrated DNA, which makes it very unlikely that mutagenesis will occur.[16-18] In summary, our data suggest that rAAV genomes persist in mouse muscle as transcriptionally active large and small concatameric episomes and when considering potential and theoretical integration events, rAAV vectors appear to possess a similar risk profile as plasmid DNA injected into muscle.[17] The majority of vector DNA appears to persist as episomal

rather than integrated DNA, which makes it very unlikely that mutagenesis will occur. [16] Therefore, we think the chance that cancer would develop is very small.

Liver tumors were reported in one study performed in newborn mice at another center with a different rAAV vector. There is no evidence to indicate that these tumors were caused by vector-induced DNA damage based on the number of AAV genomes per cell in tumor samples. [19,20] Also, other larger scale studies in newborn and adult mice have not shown this effect. [21] It remains difficult to ascertain the true risk of neoplasia, although it seems very small.

Subjects may also develop immune response to rAAVrh74. This could lead to immune rejection of virus precluding gene expression. We will measure both binding antibodies and T cell responses to the AAV capsid following gene transfer.

AAV injected by vascular delivery could also spread to other organs. The consequences are not known.

### 11.3.2 RISKS ASSOCIATED WITH PROCEDURES

#### 11.3.2.1 RISK RELATED TO GENERAL ANESTHESIA

Although rare, unexpected severe complications with anesthesia can occur and include the remote possibility of infection, bleeding, drug reactions, blood clots, loss of sensation, loss of limb function, paralysis, stroke, brain damage, heart attack or death.

#### 11.3.2.2 CANNULATION OF THE FEMORAL ARTERY AND FEMORAL VEIN

The risks of limb artery canulation include rarely permanent ischemic damage, sepsis, local infection, pseudoaneurysm, hematoma, and bleeding. Large artery canulation is used routinely

in the following clinical situations: extracorporal membrane oxygenation (in >10 kg patients) and isolated limb perfusion for the treatment of limb soft tissue sarcomas. Small artery canulation is used in acute care settings for invasive blood pressure monitoring and blood sampling. At the puncture site: bruising at the site where the catheter is inserted is common (usually in the groin). Occasionally, a larger bruise/swelling (hematoma) which may require transfusion, surgery or delayed discharge may occur. The risk of this is less than 4% (or 1 in 25). The risk of the vessel becoming blocked or being significantly damaged at the insertion site is less than 0.5% (or 1 in 200). To minimize risk to the limb and subject we are targeting the femoral vessels in the current protocol. In addition the subject will be heparinized (monitored by Activated Clotting Time measurements) to minimize the risk of thrombosis/emboli. In ILP/ILI procedures there is a small risk of compartment syndrome. This outcome is minimized in our protocol by utilizing pressure servo-regulating pump flows.

#### 11.3.2.3 ARTERIOGRAM

Fluoroscopy is used to assess vascular architecture, patency, and stasis prior to vector administration. Exposure to radiation and radio-opaque contrast material carry a small risk to the subject as the dose of radiation is limited to 8-16 minutes. . Therefore, a 8-16-minute study would give an effective dose of about 6,400-12,800 mrem. Per NIH Guidelines a dose range of 300 mrem to 5000 mrem is termed "minimal". Although contrast exposure can on extremely rare occurrences cause an allergic reaction that may be life threatening most cases of contrast related reactions are limited to localized erythema and sensation of warmth.

#### 11.3.2.4 PREDNISONE

The possible side effects of prednisolone (steroid) include acne, increased hair growth, thinning of the skin, glaucoma, roundness of the face, changes in behavior, disturbance of sleep, and increase of blood glucose level.

#### 11.3.2.5 LYMPHEDEMA

A possible long-term side effect of gene transfer procedure is a permanent swelling of the treated limbs, known as lymphedema that develops because of a build-up of fluid in the tissues surrounding the injected area. This can happen if the vessels are damaged or blocked during the gene transfer procedure.

#### 11.3.2.6 PERIPHERAL NEUROPATHY

You may get numbness or tingling after the procedure. This will be in your foot if you have ILP on your leg. It can be due to the inadvertent manipulation on your nerves around the area of procedure and is called peripheral neuropathy. Tell your doctor if this happens. It usually improves slowly over a few months but is sometimes permanent.

#### 11.3.2.7 ISCHEMIC INJURY

Due to manipulation of the blood vessels on the limb area during the procedure there is a chance that restriction of blood supply can damage tissues in the area. This occlusion if prolonged can cause permanent pain, ulcers, or infection of tissue area.

### 11.3.2.8 REPERFUSION INJURY

Your limb may become red and swollen. This usually starts about 48 hours after your treatment and is most noticeable after a week. The swelling gradually reduces over 2–3 months. The redness will gradually fade to brown and become lighter over the next few months. Your skin color should go back to normal after about six months, but some people are permanently left with a slight darkening of the skin.

### 11.3.2.9 BLOOD DRAWS

The risks of venous blood draws include discomfort at the site of puncture; possible bruising and swelling around the puncture site; rarely an infection; and, uncommonly, fainting, nausea, vomiting, and light-headedness from the procedure.

### 11.3.2.10 [redacted]

[redacted]

#### 11.3.2.11 MYOGLOBINURIA/RHABDOMYOLYSIS

There have been cases where myoglobinuria as a result of rhabdomyolysis has occurred after ILP infusion of scAAVrh74.tMCK.hSGCA. There is an increased risk of this due to the disease state of LGMD2D, but there have been cases seen in the subjects within this study. This is thought to possibly be caused by the increase in muscle strength after gene transfer that leads subjects to exercise more rigorously. Subjects will be asked to decrease exercise to a low to moderate level following the transfer and to visually monitor their urine for any brown discoloration. Subjects will also be warned to avoid over-exhaustion and to stay well hydrated. If brown urine discoloration or muscle pain were to occur, they should alert the PI as soon as possible or go to the nearest Emergency Room.

### 11.3.3 RISKS RELATED TO SUBJECT EVALUATIONS BEFORE AND AFTER GENE TRANSFER

Multiple blood draws could lead to pain at the site of venipuncture, as well as bruising that could persist for several days, and rarely, infection. Subjects may also feel light-headed or faint from the blood draw.

Muscle testing as a secondary outcome measure could result in soreness for 1 to 2 days after muscle testing.

## 11.4 ADEQUACY OF PROTECTION AGAINST RISKS

### 11.4.1 INFORMED OF RISKS

Volunteer subjects will be recruited from the Nationwide Children's Hospital, Columbus-MDA Clinic or self-referred in response to advertisement. This protocol will also be listed in the

Clinical Trials Data Bank of the NIH. Potential study subjects who demonstrate an interest in participating in the study will receive a copy of the informed consent form to review and an explanation of the nature, duration, and purpose of the study and possible consequences of the study from one of the investigators in a language they can understand. They will be informed that they may withdraw from the study at any time and for any reason without jeopardizing their future treatment. They will be asked to follow-up with necessary safety evaluations if they have received study agent prior to their desire to withdraw. They will be given full information regarding potential side effects of scAAVrh74.tMCK.hSGCA gene. Subsequently, an investigator will request the subject's permission to participate in the study. Volunteer participants who sign the study specific subject informed consent form approved by the Institutional Review Board of Nationwide Children's Hospital will be screened for eligibility to participate in the gene transfer study.

### 11.4.2 MINIMIZATION OF POTENTIAL RISK

Participants will be provided with contact numbers for any questions or concerns arising regarding the possible effects of the administration of scAAVrh74.tMCK.hSGCA gene. Results of all laboratory and safety exams regarding subject enrollment and following gene transfer will be reviewed with the subject. Since the effects on the reproductive system are not fully established and the possible concerns with viral shedding, subjects with reproductive capacity must be willing to refrain from sexual intercourse or use effective contraception for the active phase of the study (during the first six months after gene therapy (females) or until two negative sperm samples are obtained post gene transfer (males). As part of this study we must ask for a semen sample from all sexually active males 18 years and older.

Some of vector can be excreted from the body for at least a week after injection. This shedding of vector can be found in the blood, urine, saliva, and stool for up to a week following injection. For a period of two weeks after gene injection, people who may come into contact with participants' bodily fluids and waste must regularly wash their hands with soap and those who will have direct contact with their bodily fluids and waste must were protective gloves.

### 11.4.3 CONFIDENTIALITY

Access to the database will be limited to the key research personnel to ensure confidentiality. Case report forms, informed consent forms, laboratory study reports, and demographic profiles will be kept locked in a secure record storage and archiving under the responsibility of research staff. The subjects will be informed of what information is stored and of the secured access to their information by the following regulatory bodies: FDA-CBER, IRB, IBCSC, NCH internal monitors, Ohio Reproductive Medicine, and an outside contracted monitor of the study called a "CRO". Subjects will be alerted that others may have an interest in the innovative character of the protocol and in the status of the treated subjects. They will be informed that the institution and investigators will make efforts to provide protection from the media in an effort to protect the participants' privacy. They will be consulted as to whether, when, or how their identity is publicly disclosed.

### 11.4.4 PROVISIONS FOR INJURY

If a subject experiences an injury that is directly related to this study, the event will be followed up until its resolution. Nationwide Children's Hospital will provide medical treatment reasonably necessary for such injury or illness at no cost to the subject or his insurance company for [redacted]. In order for The Research Institute at Nationwide Children's Hospital to pay for these medical expenses the illness or injury must not be related to subject's underlying medical condition and subject must have followed the study directions.

If research subject seeks care for a research related injury or illness from a medical provider other than Nationwide Children's Hospital, then The Research Institute at Nationwide Children's Hospital will [redacted]

such injury or illness directly resulting from the study. Any claim for reimbursement must be supported by documentation such as a bill or invoice from a medical provider. In order for The Research Institute at Nationwide Children's Hospital to pay for these medical expenses the research subject's illness or injury must not be related to his/her underlying medical condition and research subject must have followed the study directions.

The Research Institute at Nationwide Children's Hospital's reimbursement for injury or illness as a result of research subject's participation in the study does not include any payment for lost wages, lost time, emotional distress or pain. This does not mean that the research subject gives up any of his legal rights to seek compensation for his illness or injury.

## 11.5 POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECTS AND OTHERS

This is primarily a safety study of transfer of scAAVrh74.tMCK.hSGCA with the potential to increase lower limbs muscle strength. If muscle strength is increased in knee extension/flexor it has the potential to make a difference for subjects in prolonging ambulation and preventing falls.

## 11.6 INCLUSION OF MINORITIES IN CLINICAL RESEARCH

### 11.6.1 SELECTION CRITERIA

Seven LGMD2D subjects will be enrolled in this trial. Eligibility criteria will include subjects with proven alpha-sarcoglycan deficiency by muscle biopsy or DNA testing. Participants will encompass any ethnic or racial background.

### 11.6.2 TARGETED/PLANNED ENROLLMENT

The number of subjects enrolled from each ethnic group will mirror that seen in the clinic population.

**Table 6.5. Local distribution of the disease (percent)**

| Gender | American Indian Alaskan Native | Asian or Pacific Islander | Hispanic | Black, Non-Hispanic | White, Non-Hispanic | Total |
|---|---|---|---|---|---|---|
| Female | 0 | 0 | 10 | 0 | 30 | 40 |
| Male | 0 | 0 | 40 | 0 | 20 | 60 |
| Total | 0 | 0 | 50 | 0 | 50 | 100 |

### 11.6.3 PROPOSED DATES OF ENROLLMENT AND RECRUITMENT

We anticipate beginning enrollment in January 2014, with completion of enrollment December 2017. This protocol is listed in the Clinical Trials Data Bank of the NIH.

# 12.0 DATA AND SAFETY MONITORING PLAN

## 12.1 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/IIa gene transfer clinical trial for LGMD2D (alpha-sarcoglycan deficiency) using scAAVrh74.tMCK.hSGCA" 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 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.

**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** through KAI at the end of each cohort, or at the DSMB's request.

An initial meeting (either by teleconference, webcast,or paper report) with the DSMB is scheduled prior to study initiation and at the DSMB's request. Reports will be submitted at least 2 weeks 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

**Stopping/Discontinuation Rules**

An independent Data Safety Monitoring Board (DSMB) has been approved by the study's sponsor (National Institute of Health). 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 and discussed with the DSMB by the investigators when any subject experiences a Grade 3 or higher adverse event toxicity that is 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, IRB, IBCSC, NIH-OBA and FDA,

the decision is made to continue, the study will proceed according to Dose Escalation plan.

**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 [redacted] 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

**Conflict of Interest**

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.

## 13.0 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.

## 13.1 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...) 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.

# 14.0 AGENT ACCOUNTABILITY

## 14.1 HANDLING OF INVESTIGATIONAL AGENT

"Investigator shall take adequate precautions, including storage of the investigational drug in a securely locked cabinet or other securely locked, substantially constructed enclosure, access to which is limited, to prevent theft or diversion of the substance into illegal channels of distribution." (Code of Federal Regulations, Title 21, Part 312 .69).

All materials used for injection, including sterile drapes, needles and syringes in contact with the vector will be sealed in leak proof primary and secondary containers. To ensure the highest safety precautions, all waste will be double bagged in autoclave bag bearing the biohazard symbol and sealed with autoclave tape bearing name and room number. The bag will then be autoclaved and disposed of in a biohazard waste container with name and room number.

All surfaces will be wiped with 10% bleach with a 70% ethanol rinse. Immediate decontamination procedures are performed whenever overt spills, splashes or other contaminating events occur. All absorbent material is placed onto a blue absorbent pad and transferred into a properly labeled biohazard bag.

Spills will be covered with absorbent paper towels and freshly prepared 10% bleach will be applied starting at the perimeter and working towards the center. At least 30 minutes will be allowed after contact time before clean up. In case of large spills, IBCSC and Environmental Services will be notified immediately.

If an accidental exposure occurs (such as accidental needle stick), the exposure site will be washed with soap and water.

Spills and accidents resulting in potential exposure of AAV to individuals will be reported to [redacted] IBCSC, and the Employee Health Services within 24 hours. Medical evaluations, surveillance, and treatment will be provided by the EHS. All information concerning the incident is to be documented and submitted to [redacted], IBCSC, and the EHS.

Only authorized personnel will be permitted in the surgery room during injection process. During the injection all personnel will be required to wear lab coat, gloves, respiratory mask, glasses, and toe covered shoes. All study staff must have documentation of taking the laboratory training safety module provided by the IBCSC. The link to the online training is the following:

http://rex/Training/Pages/IBCSC%20Training.aspx

## 14.2 DISPOSITION OF STUDY AGENT

"Investigator is required to maintain adequate records of the disposition of the drug, including dates, quantity, and use by subjects. If the investigation is terminated, suspended, discontinued, or completed, the investigator shall return the unused supplies to the sponsor, or otherwise provide for disposition of the unused supplies (as authorized by the sponsor)." (Code of Federal Regulations, Title 21, Part 312 .62, Section a).

## 15.0 BRIEF DESCRIPTIONS OF RESEARCH AREAS

**Coordinating Center**

The Nationwide Children's Neuromuscular Research Institute is the Coordinating Center for the entire study. Personnel consist of the following individuals:

Study Principal Investigator: [redacted]

Co-Investigator: [redacted]

Co-Investigator: [redacted]

Co-Investigator: [redacted]

Co-Investigator: [redacted]

Co-Investigator: [redacted]

Co-Investigator: [redacted]

Co-Investigator: [redacted]

Co-Investigator: [redacted]

Safety Monitor: [redacted]

Clinical Evaluators: [redacted]

[redacted]

Program Manager: [redacted]

Research Coordinators: [redacted]

[redacted]

**The Coordinating Center is responsible for the following:**

- Development and maintenance of the Manual of Operating Procedures
- Enrollment of participants
- Obtaining informed consent (by Principal Investigator only)
- Adverse event monitoring and reporting

**Overview of Investigator Responsibilities**

In conducting the clinical study, the Principal investigator is responsible:

1. For ensuring that a clinical investigation is conducted according to applicable regulations;

2. For protecting the rights, safety, and welfare of subjects under the investigator's care; and

3. For the control of the study drug under investigation

4. To conduct the study in accordance with the relevant, current protocol(s) and to only make changes in the protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of subjects;

5. To personally conduct or supervise the research study;

6. To inform the subjects, that the drugs are being used for investigational purposes and to ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met;

7. To report to the sponsor adverse experiences that occur in the course of the investigations(s) in accordance with 21 CFR 3 12.64;

8. To ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments;

9. To maintain adequate and accurate records and to make those records available to FDA for inspection in accordance with 21 CFR 3 12.68;

10. That IRB will be responsible for the initial and continuing review and approval of the clinical investigation;

11. To promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others;

12. To not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects;

13. To comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements in 2 1 CFR 3 12.

**Nationwide Children's Hospital**

Nationwide Children's Hospital is one of the nation's most progressive and sophisticated health care institutions. A multitude of comprehensive programs, integrating medical and surgical sub-specialties, is the core for tertiary care provided at Children's Hospital. Services are integrated between the inpatient and outpatient areas to enable a full continuum of subject care.

The Nationwide Children's Hospital's services for this study are

- Providing research beds and hospitals facilities for the inpatient phase of the research study

- Subject care services and nursing support during the inpatient phase of the research study

**Clinical Study Center/ Outpatient Neuromuscular Clinic**

- Providing facilities for the follow up visits after gene transfer.

## 16.0 REFERENCES

1 Seinen, J. M. & Hoekstra, H. J. Isolated limb perfusion of soft tissue sarcomas: A comprehensive review of literature. *Cancer treatment reviews*, doi:10.1016/j.ctrv.2012.10.005 (2012).

2 Mendell, J. R. *et al.* Dystrophin immunity in Duchenne's muscular dystrophy. *N Engl J Med* **363**, 1429-1437, doi:10.1056/NEJMoa1000228 (2010).

3 Mendell, J. R. *et al.* Limb-girdle muscular dystrophy type 2D gene therapy restores alpha-sarcoglycan and associated proteins. *Ann Neurol* **66**, 290-297, doi:10.1002/ana.21732 (2009).

4 Mendell, J. R. *et al.* Sustained alpha-sarcoglycan gene expression after gene transfer in limb-girdle muscular dystrophy, type 2D. *Ann Neurol* **68**, 629-638, doi:10.1002/ana.22251 (2010).

5 Laval, S. H. & Bushby, K. M. Limb-girdle muscular dystrophies--from genetics to molecular pathology. *Neuropathol Appl Neurobiol* **30**, 91-105, doi:10.1111/j.1365-2990.2004.00555.x (2004).

6 Klinge, L. *et al.* Sarcoglycanopathies: can muscle immunoanalysis predict the genotype? *Neuromuscul Disord* **18**, 934-941, doi:S0960-8966(08)00615-9 [pii]10.1016/j.nmd.2008.08.003 (2008).

7 Bueno, M. R. *et al.* A common missense mutation in the adhalin gene in three unrelated Brazilian families with a relatively mild form of autosomal recessive limb-girdle muscular dystrophy. *Hum Mol Genet* **4**, 1163-1167 (1995).

8 Carrie, A. *et al.* Mutational diversity and hot spots in the alpha-sarcoglycan gene in autosomal recessive muscular dystrophy (LGMD2D). *J Med Genet* **34**, 470-475 (1997).

9 Emery, A. E. Population frequencies of inherited neuromuscular diseases--a world survey. *Neuromuscul Disord* **1**, 19-29 (1991).

10 Emery, A. E. The muscular dystrophies. *Lancet* **359**, 687-695, doi:10.1016/S0140-6736(02)07815-7 (2002).

11 Grootenhuis, M. A., de Boone, J. & van der Kooi, A. J. Living with muscular dystrophy: health related quality of life consequences for children and adults. *Health and quality of life outcomes* **5**, 31, doi:10.1186/1477-7525-5-31 (2007).

12 Gao, G. *et al.* Clades of Adeno-associated viruses are widely disseminated in human tissues. *J Virol* **78**, 6381-6388, doi:10.1128/JVI.78.12.6381-6388.200478/12/6381 [pii] (2004).

13 Liu, G. *et al.* The Effect of Preoperative Aspirin and/or Heparin Therapy on Coagulation and Postoperative Blood Loss after Coronary Artery Bypass Surgery. *Crit Care Resusc* **1**, 139, doi:AACCM-JJune99.5 [pii] (1999).

14 Veikutiene, A. *et al.* [Influence of preoperative treatment with aspirin or heparin on platelet function and intensity of postoperative bleeding in early period after coronary artery bypass surgery]. *Medicina (Kaunas)* **41**, 577-583 (2005).

15 Sun, J. C., Crowther, M. A., Warkentin, T. E., Lamy, A. & Teoh, K. H. Should aspirin be discontinued before coronary artery bypass surgery? *Circulation* **112**, e85-90 (2005).

16 Song, S., Laipis, P. J., Berns, K. I. & Flotte, T. R. Effect of DNA-dependent protein kinase on the molecular fate of the rAAV2 genome in skeletal muscle. *Proc Natl Acad Sci U S A* **98**, 4084-4088, doi:10.1073/pnas.061014598061014598 [pii] (2001).

17 Schnepp, B. C., Clark, K. R., Klemanski, D. L., Pacak, C. A. & Johnson, P. R. Genetic fate of recombinant adeno-associated virus vector genomes in muscle. *J Virol* **77**, 3495-3504 (2003).

18 Duan, D. *et al.* Circular intermediates of recombinant adeno-associated virus have defined structural characteristics responsible for long-term episomal persistence in muscle tissue. *J Virol* **72**, 8568-8577 (1998).

19 Donsante, A. *et al.* Observed incidence of tumorigenesis in long-term rodent studies of rAAV vectors. *Gene Ther* **8**, 1343-1346, doi:10.1038/sj.gt.3301541 (2001).

20 Monahan, P. E., Jooss, K. & Sands, M. S. Safety of adeno-associated virus gene therapy vectors: a current evaluation. *Expert Opin Drug Saf* **1**, 79-91 (2002).

21 Song, S. *et al.* Stable therapeutic serum levels of human alpha-1 antitrypsin (AAT) after portal vein injection of recombinant adeno-associated virus (rAAV) vectors. *Gene Ther* **8**, 1299-1306, doi:10.1038/sj.gt.3301422 (2001).