Phase I/IIa Clinical intramuscular gene transfer of rAAV1.CMV.huFollistatin344 trial to patients with Duchenne muscular dystrophy

CLINICAL PROTOCOL

IND#: 14845

Follistatin gene therapy trial to include boys with Duchenne muscular dystrophy (DMD).

Version: 5.0 (16 January 2015)

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1.0 PROTOCOL SYNOPSIS

Title	Phase I/IIa Clinical intramuscular gene transfer of rAAV1.CMV.huFollistatin344 trial to patients with Duchenne muscular dystrophy.
Study Number	N = 6 DMD boys
Clinical Study Phase	Phase I/IIa trial
Number of Centers	Single site (Nationwide Children's Hospital)
Study Objectives	Safety and increase distance in 6 MWT
Study Design	rAAV1.CMV.huFollistatin344 injections to gluteus muscles, quadriceps, tibialis anterior
Patient Population	**Inclusion Criteria** 1. Age 7 or older 2. Confirmed DMD gene mutations 3. Impaired muscle function based on clinical evidence including difficulty climbing stairs, getting from the floor (Gowers' sign), and weakness of individual muscles of extremities 4. Males of any ethnic group will be eligible 5. Ability to cooperate with study procedures including muscle testing. 6. Willingness of sexually active subjects with reproductive capacity to practice reliable method of contraception 7. Subjects must be on stable dose of prednisone or deflazacort for three months at time of enrollment. Subjects will continue the steroid dose post gene transfer as part of the standard of care for DMD patients. Dose adjustment could be considered if there are adverse effects. **Exclusion Criteria** 8. Active viral infection based on clinical observations. 9. The presence of a DMD gene mutation without weakness or loss of function 10. Symptoms or signs of cardiomyopathy, including: o Dyspnea on exertion, pedal edema, shortness of breath upon lying flat, or rales at the base of the lungs o Echocardiogram with ejection fraction below 40% 11. Serological evidence of HIV infection, or Hepatitis A, B or C infection 12. Diagnosis of (or ongoing treatment for) an autoimmune disease 13. Concomitant illness or requirement for chronic drug treatment that in the opinion of the PI creates unnecessary risks for gene transfer 14. Subjects with rAAV1 binding antibody titers > 1:50 as determined by ELISA immunoassay 15. Abnormal laboratory values for liver, kidney, CBC, in the clinically significant range, based upon normal values in the Nationwide Children's Hospital Laboratory
Study Procedures	The vector will be delivered to both limbs via multiple, direct <u>intramuscular injections</u> of rAAV1.CMV.huFollistin344; the number of injections per muscle will depend on the size of the patient. A total dose of 2.4 X 10^{12} vg/kg (1.2 X 10^{12} vg/kg/limb) will be delivered to the lower limbs of 6 DMD subjects. Patients will also receive conscious sedation by an anesthesiologist at Nationwide Children's Hospital for the injections.
Primary Outcome	Safety is a primary outcome for this clinical gene transfer trial.

Secondary Outcomes	• The distance walked on the 6MWT is the major functional outcome to be evaluated. Other tests include time to walk and run, strength test, functional test, and reaching ability. • MRI studies of leg muscles will be done pre and post treatment (6, 12 and 24 months) to study muscle size, fibrosis and fat content. • EIM measurement will quantify the pathological status of muscles. • Muscle biopsies on quadriceps muscles (a muscle biopsy on one leg at baseline screening visit and the post gene transfer biopsy on the opposite leg at day 180), will include general histological evaluation. Assessment will consist of morphometric analyses for quantification of fiber size, fibrosis, inflammatory process, satellite cells and regeneration. Muscle tissue obtained at biopsy will also be assessed for viral DNA (qPCR), and follistatin transgene expression. .
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, 45, 60, 90, 180, and 9, 12, 18 and 24 months post-gene transfer.
Sample Size	Six DMD patients will receive rAAV1.CMV.huFollistatin344 to both limbs by multiple injections to gluteal muscles, quadriceps and tibialis anterior muscles.
Statistical Analysis	This is a phase I/IIA trial, with safety as the primary measure. For each measure based on differences between pre- and post-gene transfer examinations (clinical) evaluated by paired t test, with a p value of < 0.05 indicating significance.
Long-term follow-up	We will follow the most recent FDA guidance with regard to long-term subject follow up post gene transfer. As indicated by the guidelines, our proposed vector has a very low probability of gene transfer-related delayed adverse events. We will, however, evaluate short-term safety over a two-year period that incorporates the active phase of the protocol. 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 an outgrowth of the safety record and functional improvement seen in the BMD follistatin gene therapy trial. In this study we propose to inject AAV1.CMV.huFS344 at a total dose of 2.4 X 10^{12} vg/kg to six DMD patients. This dose will be divided between gluteal muscles, quadriceps and tibialis anterior. This is a wider distribution of vector than given to BMD patients, who overall improved the distance walked on the 6MWT without adverse events related to viral transduction into a single muscle.

The primary objective of this study is safety and endpoints will include hematology, serum chemistry, urinalysis, immunologic response to rAAV1 and follistatin, and reported history and observations of symptoms. Efficacy measures will be used as secondary outcomes and include the distance walked on the 6MWT, functional tests by PT, life quality questionnaire, MRI, EIM, and muscle biopsy. Subject will have follow up visits on days 7, 14, 30, 45, 60, 90, 180 and 9, 12, 18 and 24 months post-gene transfer.

3.0 CLINICAL TRIAL AND PRINCIPAL INVESTIGATOR

The study will be carried out at the Research Institute at Nationwide Children's Hospital. Dr Mendell, Professor of Pediatrics and Neurology, Director of the Center for Gene Therapy at the Research Institute at Nationwide Children's Hospital, Columbus, OH and Director of the Neuromuscular Disease Center, will serve as Principal Investigator. Dr Mendell brings to this trial more than forty years of clinical trial experience in neuromuscular disorders. With regard to gene therapy, he served as principal investigator for the first viral mediated gene transfer for muscular dystrophy (Duchenne muscular dystrophy) (DMD, IND BB-IND 12936 Serial # 004 using a hybrid AAV2.5 consisting of AAV2 with 5 amino acid substitutions from AAV1, CMV promoter, and mini-dystrophin).[1] In DMD, rAAV.2.5.CMV.mini-dystrophin was delivered by intramuscular injection to the biceps muscle. In a subsequent gene transfer study for limb girdle muscular dystrophy, type 2D subjects with α-SG deficiency received intramuscular injections to the extensor digitorum brevis muscle using rAAV1.tMCK.α-SG (IND BB-IND 13434 Serial # 002)[2, 3]. He also holds an approved IND (#14845) for AAV1.CMV.follistatin gene therapy in Becker muscular dystrophy (BMD) and sporadic inclusion body myositis (sIBM), a study that is currently underway with intramuscular injections to the quadriceps muscles bilaterally. No adverse events have been encountered in any of these trials.

4.0 SPECIFIC AIMS

4.1 PRIMARY OBJECTIVE

Determine the safety of intramuscular administration of rAAV1.CMV.huFS344 for DMD patients via direct intramuscular injection (IM protocol) to the gluteal muscles, quadriceps and tibialis anterior.

4.2 SECONDARY OBJECTIVE

Efficacy measures will be used as secondary outcomes and include the distance walked on the 6MWT, functional tests by PT, life quality questionnaire, MRI, EIM, and muscle biopsy. Subject will have follow up visits on days 7, 14, 30, 45, 60, 90, 180 and 9, 12, 18 and 24 months post-gene transfer.

5.0 BACKGROUND AND SIGNIFICANCE

DMD is the most common, severe childhood form of muscular dystrophy. Inheritance follows an X-linked recessive pattern. Birth prevalence has been estimated at 1 in 5000 live male births.[4] Approximately one-third of cases represent new mutations of the *DMD* gene with the remaining inherited on the X chromosome from a carrier mother. Questions usually begin to surface between ages 3 to 5 regarding reduced motor skills that alert a need for diagnostic evaluation. DMD is relentlessly progressive with loss of ambulation by age 12.[5] Historically patients died from respiratory complications. Now, a variety of factors protect the respiratory system related to improved supportive equipment, antibiotics, vaccines, and other ancillary methods.[6] Prolonging life unmasks decline in cardiac function with complications of dilated cardiomyopathy. This poses further clinical challenges and a need for recognition and medical intervention that did not previously exist. Non-progressive cognitive dysfunction might be present in DMD and BMD.

Disease Pathogenesis

More than 20 years ago the DMD gene was cloned defining the molecular basis of the disease.[7] The identification of the dystrophin as the deficient protein followed closely on the heels of this discovery.[8] Dystrophin is a 427kDa cytoskeletal protein required for muscle fiber stability. Loss of this protein results in susceptibility to repeated cycles of necrosis and regeneration with satellite cell depletion, diminished regenerative capacity of the muscle, ending in fat and connective tissue replacement (fibrosis). The mutation spectrum within the DMD gene reveals that deletions of one or more exons are found in ~65% of cases clustered in two hotspot regions.[9] Originally multiplex PCR kits were developed that were able to detect 95%-98% of all deletions[10, 11]. Detection of duplications, representing about 6% of the DMD mutations, initially required Southern blots to detect. Overtime the demand for more rapid, less expensive detection methods have encouraged the introduction of additional tools to identify the full spectrum of mutations (deletions, duplications, splice-site and point mutations). Multiplex ligation-dependent probe amplification (MLPA)[12] or multiplex amplifiable probe hybridization (MAPH)[13] will screen all exons providing detection of most deletions and duplications. If MLPA or MAPH are negative, the gene should be scanned for subexonic rearrangements or point mutations using DNA sequence analysis.[14] This has become more than an academic

exercise because of treatment paradigms that depend on the full characterization of the mutation endpoints that help establish if patients are candidates for molecular therapies.

Treatment for DMD

Despite virtually hundreds of clinical trials in DMD, only one treatment has consistently demonstrated efficacy. Unequivocal evidence for glucocorticoid-induced improvement was established through a double-blind, randomized controlled trial in a large cohort of subjects (n=103).[15] Patients received 0.75 mg /kg/day or 1.5 mg/kg/day versus placebo. At six months there was approximately equal efficacy for the high and low dose prednisone groups compared to placebo in muscle strength (manual muscle testing score), the time needed to rise from supine to a standing (prednisone 3.4 vs. placebo 6.2 seconds), to walk 9 m (prednisone 7.0 vs. placebo 9.7 seconds), to climb four stairs (prednisone 4.0 vs. placebo 7.1 seconds), and in forced vital capacity (prednisone 1.7 vs. placebo 1.5 liters) ($P<0.001$ for all comparisons). Similar results were later reported with deflazacort (0.9 mg/kg/day), an alternative, sodium-sparing corticosteroid that was also shown to prolong ambulation (untreated DMD patients stopped walking at 9.8 ± 1.8 years compared to deflazacort-treated at 12.3 ± 2.7 years, $P<.005$).[16] A dose-related effect was observed in two follow up studies that demonstrated lesser but similar benefits using prednisone 0.30 and 0.35 mg/kg/day[17, 18] A weekend dosing regimen (10 mg/kg/wk: half on Saturday and half on Sunday) showed equal efficacy to daily steroids.[19] Additional follow up studies reported that the number of boys having scoliosis surgery in treated groups was significantly less than untreated boys ($P < 0.05$);[20, 21] and treatment extended independent ambulation by 3.3 years compared to untreated (9.2 ± 1.48 years vs. 12.5 ± 3.02 ($p < 0.0001$).[21]

The side-effect profile of glucocorticoids consistently demonstrates weight gain with a cushingoid appearance. DMD boys on steroids are also at risk for hypertension, cataract formation, loss of bone density, vertebral compression fractures, and long bone fractures. Long-term administration may be limited in some cases by steroid-induced behavioral problems frequently observed with this class of drugs.

Molecular therapies provide a promising alternative to glucocorticoids. In addition to direct gene replacement, three molecular pharmacologic approaches have demonstrated promising findings representing current research aspirations. These include exon skipping, readthrough of stop codon mutations, and replacement of dystrophin using utrophin, a dystrophin-like cytoskeletal protein. The presence of revertants fibers encouraged scientists to develop a pharmacologic path to reproduce and expand this spontaneous natural achievement in order to generate dystrophin expression levels that would result in clinically meaningful outcomes.

Exon skipping is well underway in clinical trials but is still early in demonstration of efficacy. Limitations include development of products related to specific exons. The target of exon skipping is at the pre-mRNA level allowing one or more exons to be omitted to restore the dystrophin reading frame. This is accomplished with splice-switching oligomers, typically 20-30 nucleotides in length and complementary in sequence to regions of the pre-mRNA transcript. Pre-clinical efficacy has been demonstrated in the mdx mouse, dystrophin/utrophin knock-out

mouse, and CXMD dog[22-24] using both 2'O-methyl-ribooligonucleoside-phoshophorothioate (2OMe) and phosphorodiamidate morpholino oligomers (PMOs). Two proof-of-principle clinical trials in DMD used a 2OMe oligomer (PRO051, ProsensaTherapeutics) or a PMO (Sarepta Therapeutics) delivered directly to muscle targeting exon 51.[25, 26] Evidence favoring exon skipping was validated by RT-PCR and a newly synthesized dystrophin protein correctly localized to the sarcolemma. Phase I/II extension studies were performed with both oligomers following systemic delivery. In the PRO051 trial, four doses were tested in the initial 5 week phase (0.5, 2.0, 4.0, and 6.0 mg/kg) prior to an open-label 12-week extension phase.[27] Dose-related efficacy was achieved with evidence of new dystrophin expression in approximately 60-100% of muscle fibers in 10 of 12 patients and modest improvement in the 6-minute walk test. In the AVI phase II open-label study with AVI-4658 (Eteplirsen®) conducted in the UK, 7 of 19 patients saw a modest response with a mean increase of sarcolemmal dystrophin from 8.9% to 16.4%.[28] The results were variable with one patient responding following treatment with 2 mg/kg, while all other responders received the higher dosing levels of 10 and 20 mg/kg. The PMO has now been tested in a phase I/II randomized, double-blind, placebo-controlled, multiple dose efficacy trial at Nationwide Children's Hospital, Columbus, OH, USA to assess 30 and 50 mg/kg dosing over 24 weeks (http://www.clinicaltrials.gov). At the 24-week time point, dystrophin was increased and there was stability in the distance walked on the 6MWT. This was followed by an open label extension study with all data reported at the 1 year time point showing increase dystrophin and stability in the 6MWT (Mendell et al Ann Neurol 2013;74:637-47).

A second molecular approach involves suppression of stop codon mutations of the *DMD* gene that comprise approximately 15% of DMD cases. Two pharmacologic tactics have shown pre-clinical efficacy. In mdx mice, mutation suppression was shown with the aminoglycoside antibiotic, gentamicin.[29] In a follow up clinical study, treatment of four DMD/BMD subjects failed to show a benefit.[30] A subsequent clinical trial, also in a small cohort, challenged the findings suggesting that readthrough had occurred, demonstrating full-length dystrophin as the product of gentamicin treatment.[31] In a more definitive trial, done by Dr. Mendell at Nationwide Children's Hospital, DMD patients (n = 16) with stop codons, treated weekly or twice weekly for six months (7.5 mg kg IV), showed a significant increase in dystrophin levels with the highest levels reaching 13 and 15% of normal.[32] Muscle strength was stabilized and a modest increase in forced vital capacity was achieved. Although this study demonstrates the therapeutic potential of gentamicin, higher doses might be necessary to improve functional outcomes. The known renal toxicity of aminoglycoside antibiotics and the inconvenience of intravenous administration pushed the field in the direction of identifying an orally administered agent.

Ataluren, formerly referred to as PTC124 (PTC therapeutics), fulfilled the requirement of an orally administered pharmacologic readthrough agent for stop codon mutations.[33] Pre-clinical studies in the *mdx* mouse demonstrated dystrophin expression in skeletal, cardiac, and diaphragm muscle and protected skeletal muscle from eccentric contraction-induced injury. A phase I study in healthy volunteers established safety and tolerability at doses exceeding what was required for pre-clinical efficacy.[34] In a phase IIa proof-of-concept 28-day study in DMD/BMD patients, dystrophin appeared to increase post treatment. A randomized, double-blind, placebo-controlled phase IIb trial followed, evaluating safety and efficacy over a 48 week treatment period. PTC, Inc. released preliminary results indicating a very strong safety profile; however, the primary endpoint of the 6 minute walk test did not reach statistical significance

[http://clinicaltrials.gov/ct2/show/NCT00592553]. Continued subgroup analysis to look at efficacy is underway. Influence for further pursuit of treatment in DMD may come from on-going clinical trials in cystic fibrosis[35] and hemophilia[[http://clinicaltrials.gov/ct2/show/results/NCT00947193], presuming results are favorable. Upregulation of utrophin is another therapeutic strategy for DMD that has shown promise in the mdx mouse. Utrophin shares 80% sequence homology with dystrophin and has been shown to partially restore function as a dystrophin surrogate in pre-clinical transgenic mice [36] and gene replacement studies.[37] In normal muscle utrophin expression is limited to the neuromuscular and myotendinous junctions but in mdx mice and DMD patients it is overexpressed throughout the sarcolemma of all muscle fibers, putatively compensating for the absence of dystrophin. Upregulation of utrophin holds a particular advantage because of the unlikely occurrence of an immune response as seen following mini-dystrophin gene replacement.[1]

Several small molecules demonstrate upregulation of the utrophin gene through the utrophin-A promoter. SMT C1100 is a novel small molecule that was identified through an exhaustive high-throughput small molecule screen. When used in the mdx mouse, utrophin staining is increased at the sarcolemma and dystrophic muscle pathology is reversed.[38] Summit PLC, a UK biotechnology company, has advocated for its use in DMD clinical trials. A similar small molecule transcriptionally upregulating utrophin, BMN195 (Biomarin Pharmaceuticals), was tried in a phase I safety trial in healthy volunteers but disappointingly failed to achieve adequate concentrations in the blood. The non-steroidal anti-inflammatory drug nabumetone[39] (also known as Relafen®) was shown to upregulate endogenous utrophin mRNA and protein in C2C12 cells.[39] It remains a candidate agent considering its current use in the treatment of juvenile rheumatoid arthritis, although its side effect profile as an NSAID with warnings regarding life-threatening heart or circulation problems, including heart attack or stroke may be a limiting factor as a life-long therapy.

Heregulin represents an additional molecule capable of transactivating the utrophin-A promoter. When delivered to the mdx mouse for 3 months by intraperitoneal injections of a small peptide encoding the epidermal growth factor-like region of heregulin ectodomain, utrophin was upregulated, accompanied by increased resistance to eccentric contraction, and a reduction of muscle pathology.[40] The amelioration of dystrophic phenotype by heregulin-mediated utrophin up-regulation offers a pharmacological therapeutic modality that deserves consideration in future planning to alleviate DMD.

Another approach to upregulate utrophin employs a recombinant biglycan (rhBGN) that can be delivered systemically in the mdx mouse and ameliorate pathology and improve function at the diaphragm.[41] There is clear evidence that biglycan can recruit utrophin to the sarcolemma. rhBGN has shown promise when used in cell culture and in vivo without changing mRNA levels of utrophin indicating that functional benefits arise from posttranscriptional effects. rhBGN is being evaluated for clinical trials in DMD.

Rationale for Follistatin (FS344) Gene Therapy

The planned approach for gene therapy for DMD is an extension of our current follistatin (FS) gene therapy trial in BMD where we have found that gene transfer to the quadriceps muscles bilaterally improved the distance walked on the 6 minute walk test in 4 of 6 patients. The results were robust in 2 patients with improvement over 100 meters at 1 year and 58 m and 30 m in two other patients. We believe that these results are also favorable for treating DMD patients and we presented these findings to the FDA along with our safety data as an amendment to our IND 14845. The FDA permitted us to treat this younger population without additional experiments. The paper describing the treatment of Becker patients has just been published in Molecular Therapy: Mendell, JR, et al. A Phase I/IIa Follistatin Gene Therapy Trial for Becker Muscular Dystrophy, available on-line October 2014.

Myostatin is a transforming growth factor-beta family member that normally acts to limit skeletal muscle growth. Mice genetically engineered to lack myostatin have about twice the amount of muscle mass, and similar effects are seen in cattle, sheep, dogs, and human. [42-45] FS is a potent myostatin antagonist with the added advantage of controlling muscle mass through pathways independent of the myostatin signaling cascade. [42] Myostatin null mice carrying a follistatin transgene demonstrate four times the muscle mass of wild-type. There are two isoforms of follistatin generated by alternative splicing and initially translated to isoforms FS317 and FS344. [46] Post translational modification of each cleaves a 29 amino acid signal peptide giving rise to FS288 and FS315. FS288 functions collaboratively in reproductive physiology with activin and inhibins of the hypothalamic pituitary-gonadal axis. [47] FS315 more reliably targets skeletal muscle, has no known cardiotoxicity or other adverse effects and is ideal for gene delivery to muscle. AAV1.CMV.FS344 delivered by direct intramuscular injection to quadriceps and tibialis anterior muscles of the mdx mouse increased muscle mass and strength throughout the lower extremities with a demonstrable remote effect on these same parameters in the upper limbs and increased muscle mass in the paraspinal muscles. [48] This we attributed to the muscle acting as a secretory site for follistatin with the circulating isoform reaching remote sites [49] AAV1.CMV.FS344 was further tested in the non-human primate to explore a paradigm applicable to clinical trial. In the cynomolgus macaque we injected AAV1.FS344 directly into the quadriceps muscle resulting in an increase in size and strength of this muscle. [50] It should be noted that when we injected the non-human primate in one muscle (as opposed to multiple muscles in the mouse) we did not see remote effects. These pre-clinical studies in the absence of toxicity enabled a Phase I/IIa clinical trial in patients with BMD (IND 14845). The BMD clinical results are the first ever gene therapy to muscle showing a functional benefit (increase distance walked on the 6MWT). We are now ready to move ahead to treat boys with DMD.

6.0 CLINICAL RESEARCH PLAN

6.1. STUDY POPULATION

Six DMD subjects age 7 years and older with confirmed DMD gene mutations based on mutation will be enrolled at Nationwide Children's Hospital for the gene transfer study. Subjects will encompass any ethnic or racial background.

6.2. PRE-TREATMENT ASSESSMENT

A. Establish Subject Eligibility

I. Inclusion Criteria

1. Age 7 or older
2. Confirmed DMD gene mutations
3. Impaired muscle function based on clinical evidence including difficulty climbing stairs, getting from the floor (Gowers' sign), and weakness of individual muscles of extremities
4. Males of any ethnic group will be eligible
5. Ability to cooperate with study procedures including muscle testing.
6. Willingness of sexually active subjects with reproductive capacity to practice reliable method of contraception
7. Subjects must be on stable dose of prednisone or deflazacort for three months at time of enrollment. Subjects will continue the steroid dose post gene transfer as part of the standard of care for DMD patients. Dose adjustment could be considered if there are adverse effects.

II. Exclusion Criteria

1. Active viral infection based on clinical observations.
2. The presence of a DMD gene mutation 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 A, B or C infection
5. Diagnosis of (or ongoing treatment for) an autoimmune disease

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

7. Subjects with rAAV1 binding antibody titers > 1:50 as determined by ELISA immunoassay

8. Abnormal laboratory values for liver, kidney, CBC, in the clinically significant range, based upon normal values in the Nationwide Children's Hospital Laboratory

System	Assay	Normal Range	Abnormal (Clinically Significant)
Liver Function	GGT	8-80 U/L	>3 times upper limit of normal
	Total Bilirubin	0.1-1 mg/dL	≥3 mg/dL
Renal Function	Creatinine	0.3-1.3 mg/dL	>1.8 mg/dL
Hematologic	Hemoglobin	Age dependent	For all ages: ≤ 8 or ≥ 18 g/dL
Hematologic	White Blood Cells	Age dependent	For all ages: ≤ 3.5 or ≥ 20 x 10^3 cells/mL Or Absolute neutrophil count of ≤ 1.5 x 10^3 cells/mL

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 correspondent agencies for regulatory purposes. If new information related to the study arises, subjects will be asked to sign a revised document. Signed consent forms will remain in each subject's research chart and 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/Screening Assessment (day-45 to day -1)

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

Day -45 to day -1 before gene transfer

- Medical history
- Physical exam
- EKG
- ECHO
- Chest X-ray
- MRI of the gluteal muscles, quadriceps and TA (should not be repeated if the patient had the MRI test done within the previous year)
- Hepatitis A,B, &C Screen
- HIV screening
- CBC/Diff/Platelet with smear
- Total Protein
- Alanine transaminase (ALT)
- Aspartate Aminotransferase (AST)
- Serum gamma-glutamyl transferase (GGT)
- Total bilirubin
- Glucose
- Creatine kinase (CK)
- Creatinine
- BUN
- Cystatin C
- Alkaline phosphatase
- Amylase
- Prothrombin time (PT), partial thromboplastin time (PTT)
- Urinalysis
- Neutralizing antibody and/or binding antibody to AAV1
- ELISA assay to detect antibody to follistatin
- ELISPOT assay to detect T cell response to AAV1 capsid proteins and follistatin
- Quantification of blood circulating follistatin
- Six minute walk test (6MWT), functional and strength measures by PT
- Life quality questionnaire
- Pulmonary Function Test (PFT)
- Electrical Impedance Myography (EIM) to quantify pathological status of muscles: Skulpt, Inc. has developed a handheld device specifically for performing EIM measurements. This device will be used to measure EIM in this study. The device is not yet approved by FDA, but has been tested in over 30 other individuals with no adverse events (AEs) (serious or otherwise) reported to date and currently being tested in SMA infants as part of the Network for Excellence in Neuroscience Clinical Trials

(NeuroNEXT) trials, and SMA gene therapy trial. The device has undergone independent electrical safety testing by Intertek (www.intertek.com) to the requirements contained in the following standards:

IEC 60601-1 Issue 1998/12/01 Ed: 2 Medical Electrical Equipment Part 1: General Requirements and Safety; (Amd. 1-1991(CENELEC EN 60601-1: 1990) (Amd. 2-1995) (Corrigendum-1995))

- Baseline muscle biopsy on quadriceps muscles of one leg
- Subjects will be instructed to perform strengthening/endurance exercises at home 3 times per week, and asked to report the frequency of exercise. They will be incentivized through a video game and it will be emphasized that this should be relatively mild effort. The subjects will be incentivized to complete the exercise program by offering them $10 per week for completion of log showing that exercise has been done.

* Study participants will continue prednisone post gene transfer as part of the standard management of DMD boys. Glucocorticoids are considered standard of care for DMD and most boys will be on a steroid regimen at the time they are recruited.

6.3 PROTOCOL FOR GENE TRANSFER

I. Injection preparation

Preparation of the rAAV1.CMV.huFS344 will be in the investigational drug service based on the drug order from and following pharmacy standard protocol. **The vector shall be diluted and manipulated in polypropylene syringes only. Polystyrene and polycarbonate materials should never come in contact with the vector**.

Prior to transportation to the clinical setting appropriate dilutions of the test article will be completed by the pharmacy. The dose will be diluted in normal saline, divided in 9 ml per leg with 0.5 ml delivered per site on each leg. Documentation of the dilution will be completed by the pharmacy following standard pharmacy protocol. The formulated syringes will be maintained refrigerated at 2-8C until transport to the patient procedure room where it will be delivered on wet ice in the appropriately labeled cooler.

The final volume delivered to the PI will be 18 ml total volume distributed in 18 polypropylene syringes of 1 ml each, nine syringes being delivered to the right leg and nine to the left leg. The vector-containing syringe will be delivered to the designated procedure room at Nationwide Children's Hospital. It will be delivered on wet ice (not frozen) and administered to the subject within 8 hours of of thaw. Handling of rAAV1.CMV.huFS344 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

Conscious sedation will be given to all DMD participants by an anesthesiologist who will be with patient at the time of injection. In addition, the skin over the gene transfer site will be pre-treated with a lidocaine/prilocaine eutectic mixture incorporated in a cream base (EMLA cream) or a cellulose disk (EMLA patch). Comparable cream-based anesthesia such as xylocaine cream might be used. Procedures will be performed under sterile conditions. The injection site will be cleansed with three successive applications of non-iodine containing surgical prep swabs and draped with disposable sterile drapes. A standard clinical Doppler ultrasound will be used with a sterile sheath around the transducer to maintain asepsis of the injection field. This will increase the precision of gene transfer to be sure that we inject muscle and not connective tissue or fat.

The total dose of vector per extremity, 1.2 X 10^{12} vg/kg will be distributed between the targeted muscles that include gluteals, quadriceps, and tibialis anterior. The initial sites of injection, at least three injections per muscle, will be targeted based on MRI showing preserved muscle. The sites will be confirmed at the time of injection by ultrasound guidance for the needle injections. The procedure should take approximately 30 minutes to inject muscles of both extremities.

6.4 POST GENE TRANSFER MONITORING

6.4.1 IMMEDIATELY FOLLOWING GENE TRANSFER

Vital signs including blood pressure, heart rate, respiratory rate and temperature will be performed post-administration. Concomitant medications and all adverse events/serious adverse events will also be monitored and documented following injection. Patients will be discharged the day after gene therapy (if no side effects are observed).

6.4.2 FOLLOW UP

Subjects will return for follow up visits on days 7, 14, 30, 45, 60, 90,180, and 9, 12, 18, and 24 months post-gene transfer. Toxicity monitoring on each of these dates (including day 1 post-gene transfer) are described in the following sections of the protocol.

We will follow the most recent FDA guidelines with regard to long-term patient follow up following gene transfer. 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 (see assessment of endpoints below). If newly

identified risks are associated with our product, or if the patients suffer any adverse events during this period, we will initiate a long-term follow-up according to the FDA guidelines.

6.5 ASSESSMENT OF ENDPOINTS

A. Safety monitoring post-gene transfer

Monitoring will occur on Study Days 1, 7, 14, 30, 45, 60, 90,180, and continue at 9, 12, 18, and 24 months post gene transfer. The following parameters will be included at different time points during the monitoring evaluations (see study timeline section 6.7):

- Physical Exam
- ALT
- AST
- GGT
- Total bilirubin
- Glucose
- PT/PTT
- CBC/Diff/Platelet with smear
- Serum Creatine kinase (CK)
- Creatinine/BUN
- Cystatin C
- Alkaline phosphatase
- Amylase
- Total Protein
- Urinalysis
- Blood antibody to AAV1 and follistatin
- ELISPOT assay to detect T cell response to AAV1 capsid proteins and follistatin
- Blood circulating follistatin
- Photograph of injection site/ surrounding area
- Adverse events
- Pulmonary Function Test (PFT)

B. Efficacy Monitoring

I. Primary Outcome

Safety is a primary outcome for this clinical gene transfer trial.

II. Secondary Outcomes:

- The distance walked on the 6MWT is the primary functional outcome to be evaluated. Time to walk and run, strength test, functional test, and reaching ability will be included in PT testing. Functional testing will be performed at baseline and at days 30, 60, 90,180, and 9, 12, 18 and 24 months post-gene transfer.
- Life quality questionnaire will be completed at baseline and 6, 12, 18 and 24 months post-gene transfer.
- In order to maximize potential benefit of follistatin gene therapy, subject will be instructed to continue strengthening exercise at home at least 3 times per week as instructed by physical therapists (PT), and asked to report the frequency of exercise. The subjects who follow the exercise instruction will be incentivized by video display and offered $10 per week if they fill out their log showing bike riding complete.
- MRI studies of leg muscles will be done pre and post gene transfer (6, 12 and 24 months) to study muscle size, fibrosis and fat content.
- EIM measurement will quantify the pathological status of muscles.
- Muscle biopsies on quadriceps muscles (a muscle biopsy on one leg at baseline screening visit and the post gene transfer biopsy on the opposite leg at day 180), will include general histological evaluation. Assessment will consist of morphometric analyses for quantification of fiber size, fibrosis, inflammatory process, satellite cells and regeneration. Muscle tissue obtained at biopsy will also be assessed for viral DNA (qPCR), and follistatin transgene expression

6.6 STATISTICAL ANALYSIS

This is a phase I/IIA trial, with safety as the primary measure. For each measure based on differences between pre- and post-gene transfer examinations (clinical) analyses will be based on a paired t test, with a p value of < 0.05 indicating significance.

6.7 STUDY TIMELINE

STUDY TIMELINE																
Study Interval	**Baseline Screening**	**Vector Injection (Inpatient)**			**Follow Up (Outpatient)**											
Visit	**1**	**2**			**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Days in Study	**-45 to -1**	**-1**	**0**	**1**	**7**	**14**	**30**	**45**	**60**	**90**	**180**	**194***	**9 mo**	**1 yr**	**18 mo**	**2 yr**
Informed Consent	X															
Medical History	X															
Physical Exam & vitals	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
ECHO/EKG	X															
Chest X-Ray	X															
MRI	X										X			X		X
Hepatitis A, B & C HIV	X															
Safety labs	X			X	X	X	X	X	X	X	X					
Urinalysis	X			X	X	X	X	X	X	X	X					
Pulmonary function test	X						X			X	X		X	X		
PT evaluation	X						X		X	X	X		X	X	X	X
Life quality questionnaire	X										X			X	X	X
EIM	X									X	X			X		
Immunology studies	X				X	X	X	X	X	X	X		X	X	X	X
Circulating follistatin	X						X			X	X		X	X	X	X
Gene Transfer			X													
Muscle Biopsy	X										X					
Photograph of injection site			X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events			X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	**To be collected from time of consent until final study visit, recorded on separate CRF**															

Note: There will be a flexibility of a +/- 7 working days 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.

*: The follow up post-biopsy visit scheduled 2 weeks post-biopsy is made optional to give subjects the choice to have the stiches removed at their local doctor's office.

7.0 TEST MATERIAL AND ADMINISTRATION

7.1 DESCRIPTION OF BIOLOGICAL PRODUCT

The biological drug product is a non-replicating, adeno-associated virus termed rAAV1.CMV.huFS344. The product will follow the IND 14845 CMC section, identical to what has been produced for the BMD study that is now completed. The product will be produced at the Clinical Manufacturing Facility (CMF) at Nationwide Children's Hospital according to current Good Manufacturing Practices (cGMP). The manufacturing process includes procedures to ensure the safety, identity, quality, purity and strength of the manufactured biologic. The HEK 293 cell bank used in the production of the product will meet specifications for product release as detailed in FDA and ICH guidelines. The production process is governed by over 90 standard operating procedures (SOP), Master Batch Records, Methods and registered Forms. The vector will be formulated in 20 mM Tris (pH 8.0), 1 mM $MgCl_2$ and 200 mM NaCl containing 0.001% pluronic F68. The vector will be supplied as a frozen solution that is thawed before clinical administration.

7.1.1 PRODUCT DEFINITION

Product Name: rAAV1.CMV.huFS344

Gene Insert: Human cDNA for FS344 (alternatively spliced follistatin)

Control Elements: CMV promoter

AAV Serotype: Serotype AAV1

7.2 DOSE SELECTION

The dose is based on our BMD gene therapy trial using the same vector. In that study we used two doses without safety concerns. No dose limiting toxicity was found. For the low dose rAAV1.CMV.huFS344 trial (n=3) we injected 12 sites in each extremity (quadriceps only). The total dose per patient was 6 X 10^{11} vg/kg (3e11 vg/kg/quadriceps) and the high dose was 1.2 X 10^{12} vg/kg per patient (6e11vg/kg/quadriceps). There did not seem to be a significant dose response effect. In the low dose cohort patients improved in the distance walked in the 6MWT as follows: 58m, 125m, and 9 m. In the high dose cohort, patients improved in the 6MWT by 108m and 29m. One patient in the high dose group did not improve. Based on our toxicology studies prepared for IND 14845 that included a high dose cohort in C57Bl mice at 2.0 x 10^{13} vg/kg without any organ system toxicity and the clinical trial done at 1.2 X 10^{12} vg/kg, we are proposing that all six DMD boys receive 2.4 X 10^{12} vg/kg per patient.

7.3 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".

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.

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

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, IRB, IBCSC, CBER, FDA, OBA, and NIH before continuing enrollment.

Table of Clinically Significant Laboratory Ranges

Test	Units	Reference Range		Clinically Significant Range
Hemoglobin	g/dL	2Y – 5Y 6Y – 11Y 12Y – Adult	11.5 – 13.5 11.5 – 15.5 12.0 – 16.0	< 8.0 or > 18
WBC	K/cu mm	4Y – 5Y 6Y – 9Y 10Y – 20Y 21Y – Adult	5.5 – 15.5 5.0 – 14.5 4.5 – 13.5 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 18Y – Adult	<110 30 - 110	≥ 200
Total Protein	g/dL	3Y – 15Y 16Y – Adult	5.8 – 8.7 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 8Y – 10Y 11Y – 12Y 13Y – 17Y 18Y – Adult	0.3 – 0.8 0.3 – 0.9 0.4 – 1.0 0.5 – 1.2 0.6 – 1.3	≥ 1.8
GGT*	U/L	8 – 78		3XULN
ULN= upper limit normal				

7.4 STOPPING AND 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, IRB, IBCSC, NIH-OBA and FDA, the decision is made to continue according to this study protocol.

7.5 CONCOMITANT MEDICATIONS AND 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 follow up visits. 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.[51-53] Subjects must already be on prednisone for three months at time of enrollment as part of the inclusion criteria.

8.0 ADVERSE EVENT MONITORING AND REPORTING

8.1 DEFINITION OF AN ADVERSE EVENT

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

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

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

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

Adverse events will be graded by the investigator accordingly:

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: All Serious Adverse Events (SAEs) and Dose Limiting Toxicities (DLTs) will be reported to the DSMB within 48 hours of notification, regardless of relatedness to the clinical trial.

Any serious adverse event that is fatal or life-threatening, that is unexpected, and associated with the use of the gene transfer product will be reported to the FDA as soon as possible, but not later than 7 calendar days after the sponsor's initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the gene transfer product, but are not fatal or life-threatening, will be reported to the FDA as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information. Changes in this schedule will be permitted only where, under the **FDA IND** regulations [21 CFR 312(c) (3)], changes in this reporting schedule have been approved by the FDA and are reflected in the protocol.

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/ DSMB** as soon as the information is available and will be identified as such, i.e., "Follow-up IND Safety Report." If a serious adverse event occurs after the end of a clinical trial and is determined to be associated with the use of the gene transfer product, that event will be reported concurrently to the **FDA/NIH OBA/IRB/ DSMB** within 15 calendar days of the determination.

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://www.google.com/url?sa=t&rct=j&q=adverse%20event%20reporting%20template&source=web&cd=1&cad=rja&ved=0CD0QFjAA&url=http%3A%2F%2Foba.od.nih.gov%2Foba%2Frac%2FAdverse_Event_Template.pdf&ei=cEoJUvjaC-amygHxuYGgDA&usg=AFQjCNEkxJ-bsheco4BAh62i2kJ1VJh3Yw&bvm=bv.50500085,d.aWc

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 WITH 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 non-routine visits during the two years following gene transfer. 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 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

The proposed study will involve a total of 6 Duchenne Muscular Dystrophy (DMD) subjects. All subjects with this condition that are enrolled must have muscle weakness by clinical exam. DMD is established on the basis of confirmed DMD gene mutations. Subject selection will not exclude anyone on the basis of race or ethnic background. For complete details see the Inclusion and Exclusion Criteria Section.

11.1 SOURCES OF MATERIAL

Research material obtained from identifiable living human subjects includes reports of: history and physical assessments, blood, urine, chest X-ray, ECHO, EKG, EIM, pulmonary function tests, and MRI, 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.

11.2 POTENTIAL RISKS

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

11.2.1 RISKS ASSOCIATED WITH rAAV.CMV.HUFS344 GENE TRANSFER

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.[54-56] In summary, our data suggest that AAV genomes persist in mouse muscle as transcriptionally active large and small concatameric episomes and when considering potential and theoretical integration events, AAV vectors appear to possess a similar risk profile as plasmid DNA injected into muscle.[55] The majority of vector DNA appears to persist as episomal rather than integrated DNA, which makes it very unlikely that mutagenesis will occur. [54] 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. [57, 58] Also, other larger scale studies in newborn and adult mice have not shown this effect. [59] It remains difficult to ascertain the true risk of neoplasia, although it seems very small.

Subjects may also develop immune response to AAV1. 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.

11.2.2 RISKS ASSOCIATED WITH PROCEDURES

11.2.2.1 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.

11.2.2.2 GENE TRANSFER TO MUSCLES

In this trial, patients will receive at least 18 injections per extremity. This will be done under sterile conditions by the PI accompanied by the ultrasonographer. Both will be gowned and gloved. Patients will also receive conscious sedation that will be directed by the anesthesiologist at Nationwide Children's Hospital. There is a low risk of complications that could include hypotension, respiratory compromise, and complications from low blood pressure such as stroke or myocardial infarction. These would be rare and unlikely complications of the procedure.

11.2.2.3 MUSCLE BIOPSY

The muscle biopsy has potential complications from procedure that include infection at the biopsy site, bleeding and/or pain at the site of the biopsy, and psychological trauma from the scar at the site of the incision; in some cases because of keloid formation this can be quite large.

11.2.2.4 STEROIDS

The possible side effects of prednisolone or Deflazacort (steroids) 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 ADEQUACY OF PROTECTION AGAINST RISKS

11.3.1 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 rAAV1 and follistatin 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.

11.3.2 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 rAAV1.CMV.huFS344. 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 until two negative sperm samples are obtained post gene transfer (males).

Some of the vector can be excreted from the body for a 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.

11.3.3 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, and an outside contracted monitor designated for 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.

11.3.4 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 medical expenses up to $10,000. 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 pay up to $10,000 to reimburse the research subject for treatment of 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.

11.4 POTENTIAL BENEFITS OF RESEARCH TO SUBJECTS AND OTHERS

This is primarily a safety study of transfer of rAAV1.CMV.huFS344 **.** Based on the results of the BMD clinical trial using the same vector, subjects participating in this trial could improve their walking ability. There is also anticipated benefit to determine the safety of this vector system that may provide efficient and sustained efficacy.

11.5 INCLUSION OF MINORITIES

11.5.1 SELECTION CRITERIA

Six DMD subjects will be enrolled in this trial. Eligibility criteria will include subjects (age 7 and older) with proven DMD mutations. Participants will encompass any ethnic or racial background.

11.5.2 TARGETED / PLANNED ENROLLMENT

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

Local Distribution of Disease (Percent)

Gender	American Indian Alaskan Native	Asian or Pacific Islander	Hispanic	Black, Non-Hispanic	White, Non-Hispanic	Total
Female	0	0	0	0	0	0
Male	0	0.7	1.4	0.7	97.2	100
Total	0	0.7	1.4	0.7	97.2	100

11.5.3 PROPOSED DATES OF ENROLLMENT AND RECRUITMENT

We anticipate beginning enrollment in Winter 2014, with completion of enrollment December 2015. This protocol will be 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/II gene transfer clinical trial for Duchenne Muscular Dystrophy using rAAV1.CMV.huFS344."

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

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

Reports will include the following:

A brief narrative of the study status, including the target enrollment, current and projected time to completing enrollment. Any significant events and/or difficulties should be briefly described in this narrative.

- A brief narrative for each participant describing age, race and ethnicity and other relevant demographic characteristics. The narrative for each participant should briefly describe his 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) will be designated by the study's sponsor. 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 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 Dr. Mendell 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.

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).

The pharmacy will maintain records of all products received, compounded, dispensed, returned and destroyed.

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 rAAV to individuals will be reported to Dr. Mendell, 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 Dr. Mendell, 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.

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. The NCH Pharmacy Disposal Policy 3-F-6 will be followed. 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: Jerry R. Mendell, M.D.

Program Manager: Kathleen Church, MSW

Clinical Evaluators: Linda Lowes. PT, PhD, Katherine Berry, Lindsay Alfano, PT.

Safety Monitor: Gloria Galloway, M.D.

Co-Investigator: Kevin Flanigan, M.D.

Co-Investigator: Zarife Sahenk, M.D., PhD

Co-Investigator: Louise Rodino-Klapac, PhD

Co-Investigator: Mark Hogan, M.D.

Co-Investigator: David Arnold, M.D.

Co-Investigator: Samiah Al-Zaidy, M.D.

Co-Investigator: Glenn A. Walter, PhD

Research Coordinators: Brent Yetter, Ana Maria Gomez, MD; Sohyun McElroy, PhD.

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.

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