SCIENTIFIC ABSTRACT

The primary objective of this study is the assessment of the safety of intramuscular administration of recombinant adeno-associated virus serotype 1 (rAAV1) carrying the FS344 alternatively-spliced cDNA of the human follistatin gene under control of the immediate early CMV promoter (rAAV1.CMV.FS344). FS344 translates a follistatin peptide (FS315) with reduced cell surface affinity; thus, FS315 is the primary soluble form of follistatin found in the serum. We have demonstrated the importance of this in our pre-clinical testing in mdx mice and non-human primates. Intramuscular delivery of rAAV1.CMV.FS344 results in highly-specific muscle hypertrophy without unwanted effects on ActRIIB binding sites at remote locations. In the mdx mouse, intramuscular injection of this construct not only increased muscle fiber size, but also increased muscle strength. In addition, we found reduction of muscle creatine kinase (CK), an indication of protection against the dystrophic process. In preparation for bringing this potential therapy forward to a clinical trial, we targeted the quadriceps muscle of non-human primates since weakness of this muscle in Becker muscular dystrophy (BMD) and sporadic inclusion body myositis (sIBM) results in frequent falls and loss of ambulation. Intramuscular injection of rAAV1.CMV.FS344 to the quadriceps of cynomolgus macaques increased muscle fiber size and strength. Complete necropsies in the non-human primate revealed no organ system pathology. There were no adverse effects on reproductive organs in particular. Female monkeys maintained normal menstrual cycles, and sperm counts and sperm motility of male monkeys were normal. Pituitary gonadal hormone profiles were unchanged from controls including levels of follicle stimulating hormone, luteinizing hormone, estradiol, and testosterone.

The proposed human trial will use an identical gene transfer paradigm to that used in the non-human primate study. rAAV1.CMV.FS344 will be injected into the quadriceps muscles of patients with BMD and sIBM. The goal of this project is to strengthen the quadriceps muscle by transfer the gene to this muscle. Dosing regimens have been chosen based on pre-clinical dose escalation studies and on-going toxicology studies showing no organ system pathology. The study will start with low dose gene transfer (1×10^{11} vg/kg) to each quadriceps muscle for the first cohort of sIBM (n =3) and BMD (n=3) patients. This dose will be diluted in 6ml and 0.5ml will be delivered per site (12 sites marked on an overlaid grid). If clinically meaningful results are accomplished with low dose, we will suggest to oversight committees that higher doses be avoided and the second cohort of six additional patients [sIBM (n =3) and BMD (n=3)] will receive the same low dose (1×10^{11} vg/kg). If a higher dose is necessary, we will increase the dose to 3.0×10^{11} vg/kg per quadriceps. As for other cohorts, if there is improvement, the third cohort will again receive 3.0×10^{11} vg/kg per quadriceps. If there is still no efficacy and adverse effects are not seen, we will increase the dose to 6.0×10^{11}vg/kg per quadriceps.

Objective, quantitative measures will be used as outcome measures for these disorders including a combination of functional (6 minute walk test), timed stair climbing, and direct muscle testing for strength (MVICT) of quadriceps muscles. These quantitative measures will be done at baseline, day 30, 60, 90, 180, and at the end of the 1st and 2nd years. MRI of quadriceps muscles (bilateral) will be done at day 180 to compare with

pre-treatment MRI. Patients will be evaluated at baseline, infusion visit (days 0-2), and return for follow-up visits on days 7, 14, 30, 60, 90, and 180. On Day 180, patients will undergo muscle biopsy to establish the size of muscle fibers and any potential toxicity from gene transfer. Safety endpoints will be assessed by changes in hematology, serum chemistry, urinalysis, immunologic response to AAV and FS344, and reported history and observations of symptoms. A muscle biopsy follow-up visit will be done 2 weeks post-biopsy, at which time stitches will be removed. Appropriate blood tests will continue at 9, 12, 18, and 24 months. Patients will be seen at the end of the 1st and 2nd years for a physical exam, strength testing, and immune studies.