

SCIENTIFIC ABSTRACT

The primary objective of this study is the assessment of the safety of intramuscular administration of recombinant adeno-associated virus-2.5 (rAAV2.5)-minidystrophin gene vector using a cytomegalovirus (CMV) promoter in dystrophin deficient Duchenne muscular dystrophy subjects. The secondary objective is to determine the dose of rAAV2.5-CMV-minidystrophin vector required to achieve a detectable level of dystrophin in muscle of subjects with this disorder.

A recombinant virus vector constructed from AAV has been altered to carry the human mini-dystrophin gene expressed from a CMV promoter. The construct has been shown to initiate the production of an attenuated functional dystrophin in laboratory animals. This construct can reverse the dystrophic phenotype in the mdx mouse, a laboratory animal model for DMD exhibiting dystrophin deficiency. Intramuscular injection of AAV2.5 restores muscle histology to normal and increases muscle strength to levels exceeding control mdx mice but not to the same degree as wild-type mice.

The proposed human clinical trial is a phase I, double-blind randomized protocol with injection of rAAV2.5-CMV-minidystrophin gene vector into muscle. Two cohorts of subjects with DMD (each with null mutations) will undergo gene transfer in a standard three-six-dose escalation scheme to establish maximum tolerated dose (MTD) using toxicity. A minimum of three subjects will be enrolled into each cohort. The first cohort will receive a total of 3.0 ml volume of study agent in three separate 1.0 ml injections into the selected muscle (preferably biceps brachii) with a total dose of 3×10^{12} genome copies (1×10^{12} genome copies per injection). The three injections will be 0.5 cm apart. The first injection will be in the anatomical midline of the muscle with vector delivered over a length of 1 cm. The other two injections will be 0.5 cm on either side, at parallel delivery sites. The second cohort will receive 3×10^{13} total number of genome copies delivered to muscle according to the same paradigm. In each cohort, only one extremity will receive vector with transgene while the opposite extremity will be injected with placebo. The first three subjects receive a saline placebo and in the second cohort, the placebo consists of empty capsids. Patients currently on prednisone or comparable glucocorticoids may continue the medication with no change in dose 3 months prior to gene transfer. On the day of the vector infusion, 4 hours before gene transfer, patients will receive intravenous methylprednisolone 2.0 mg/kg (not to exceed 1 gm total), with repeat doses on two consecutive mornings. The methylprednisolone is specifically given to diminish the immediate inflammation from the needle injection, which is known to arouse an inflammatory reaction.

Safety endpoints to be assessed include inflammatory reaction to the vector assessed by muscle biopsy, and changes in hematology, serum chemistry,

Neuromuscular Research Institute/ Mendell
Recombinant DNA Advisory Committee Submission

urinalysis, immunologic response to AAV and minidystrophin, and reported history and observations of symptoms.