Abstract #1049

PHARMACOLOGY, BIODISTRIBUTION, AND TOLERABILITY OF A PATrOL™-ENABLED INVESTIGATIONAL GENETIC THERAPY FOR MYOTONIC DYSTROPHY, TYPE 1

neubase

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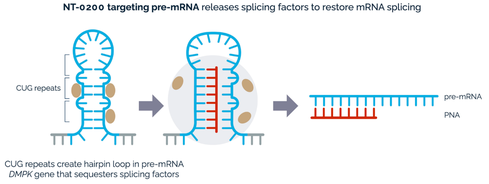
Abstract

Patients with myotonic dystrophy, type 1 (DM1) suffer from cognitive deficits and muscle pathology caused by a trinucleotide expansion in the *DMPK* gene. Initial studies of our novel delivery technology in transgenic animal models have demonstrated pharmacologic activity of the PATrOL™ platform-enabled peptide nucleic acid (PNA) pharmacophore in both brain and muscle after systemic administration. An exploratory radiolabeled biodistribution study of its delivery module administered intravenously in non-human primates showed distribution to brain, muscle, and heart— the major organs affected in DM1. To evaluate the capacity of our PATrOL™ DM1 lead candidate to correct the DM1 phenotype *in vitro*, we assessed knock down of the human *DMPK* transcript and correction of splicing defects in both fibroblasts and myoblast cultures derived from DM1 patients. Treatment with our DM1 lead candidate rapidly reduced the human *DMPK* transcript and corrected splicing defects at nanomolar concentrations. Molecular and functional pharmacologic activity, tissue distribution and tolerability were evaluated in the HSA^{LR} mouse model following single and repeated subcutaneous, intravenous, and intramuscular administration. As with the *in vitro* results in DM1 patient-derived cell lines, treatment with our lead candidate rapidly reduced mutant transcript levels, corrected splicing defects, and rescued myotonia in this mouse model. At well tolerated doses in rodents, the levels of our DM1 lead candidate in key target tissues were above those required to reverse splicing defects *in vitro* in patient cells. These encouraging preclinical data support the potential for our PATrOL™ DM1 lead candidate to be developed as a therapy for DM1 patients.

Introduction

Myotonic dystrophy type 1 (DM1) is an autosomal dominant, multisystem disease notable for prominent muscle weakness (skeletal, cardiac, respiratory), cataracts, insulin resistance, and central nervous system disorders. Prevalence is estimated to be ~1/8000. DM1 is caused by expanded CUG repeats in the 3' untranslated region (UTR) of the *DMPK* transcript that form hairpin loops that aggregate nuclear proteins, including muscleblind-like Splicing Regulator (MBNL 1), leading to widespread mis-splicing of mRNA. DM1 treatment strategies have focused on ways to disrupt the formation of toxic hairpins.

PATrOL™ is a peptide nucleic acid (PNA) antisense oligonucleobase platform comprised of highly selective nucleobases on a peptide backbone, allowing for the development of high-binding affinity PNA anti-gene drugs with low off-target effects. With our delivery technology, our PNAs display rapid tissue uptake (including into the brain) after intravenous (IV) administration with slow renal elimination.



Proof of Concept

Candidate molecules were screened in a human DM1 fibroblast cell line. The lead candidate, NT-0231.F, was further evaluated in a transgenic mouse model for DM1 (human skeletal actin long repeat [HSA^{LR}]), which replicates the nuclear aggregates, splice defects, and muscle myotonia (slow muscle relaxation) seen in human DM1. A single intramuscular dose of NT-0231.F reduces nuclear aggregates, splice defects, and skeletal muscle myotonia 21 days after administration.

Methods and Materials

This study was conducted to evaluate splice rescue and functional recovery (muscle relaxation) following NT-0231.F administration to HSA^{LR} mice. Male mice (12-16 weeks of age) were randomly assigned to weight matched dose groups (n=3/time points/group). Single and multiple doses (IV or subcutaneous [SC]) were evaluated.

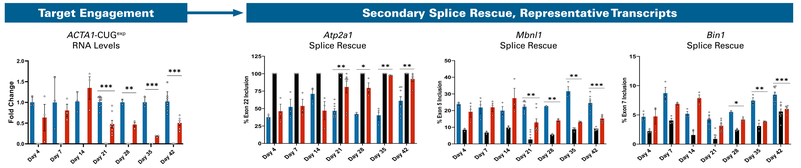
Single-dose study:

- NT-0231.F was administered IV or SC to HSA^{LR} mice (n=8/dose-time group)
- Blood and organs were collected at 0.5, 1, 1.5, 2, 4, 8, 12, and 24 hours, and 7, 14, 21, 28, 35, 42 days post-dose

Multi-dose study:

- NT-0231.F was administered weekly for 4 weeks SC to HSA^{LR} mice (n=8/dose-time group)
- Blood and organs were collected at the same timepoints described above

Target Engagement and Splice Rescue in Tibialis Anterior Muscle Following a Single 40-mg/kg IV dose of NT-0231.F

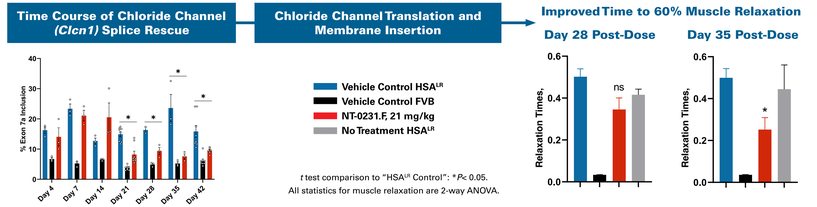


t test coto "HSA^{LR} Control": *P< 0.05; **P< 0.01; ***P< 0.001.

Conclusions

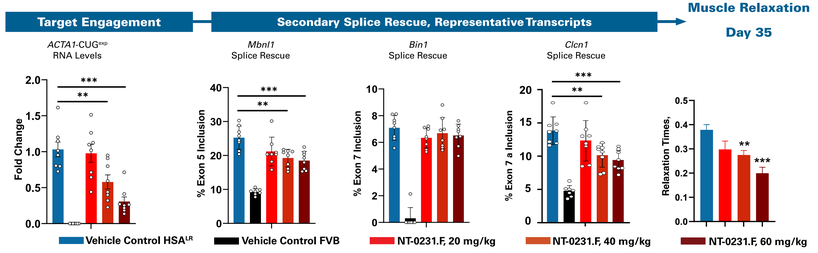
- Systemic administration (IV or SC) of NT-0231.F in the HSA^{LR} DM1 transgenic mouse model rapidly corrected splicing defects and rescued myotonia dysfunction in this animal model of DM1
- The HSA^{LR} transgenic model expresses >10-fold more CUG repeats per million transcripts, making it a higher bar therapeutic model than the human disease for our mechanism of action
- These encouraging preclinical data support the potential for PATrOL™ DM1 lead candidate to be developed as a therapy for DM1 patients

Chloride Channel Splice Rescue and Muscle Function Recovery Following a Single Dose of NT-0231.F



t test comparison to "HSA^{LR} Control": *P< 0.05.
All statistics for muscle relaxation are 2-way ANOVA.

Target Engagement, Splice Rescue, and Improved Muscle Function Following Multi-dose (weekly x 4 weeks) SC administration of NT-0231.F



t test comparison to "HSA^{LR} Control": *P< 0.05; **P< 0.01; ***P< 0.001.
All statistics for muscle relaxation are 2-way ANOVA.

Contact

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