Pharmacology, Biodistribution, and Tolerability of a PATrOL™-Enabled Investigational Genetic Therapy for Myotonic Dystrophy Type 1

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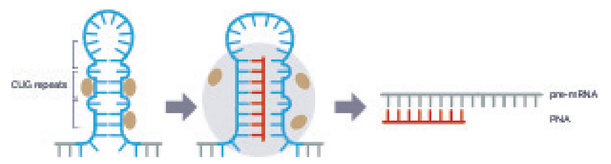
Myotonic dystrophy type 1 (DM1) is an autosomal dominant, multisystem disease notable for prominent muscle weakness (skeletal, cardiac, respiratory), cataracts, insulin resistance, and central nervous system disorders. Prevalence is estimated to be ~1/8000. DM1 is caused by expanded CUG repeats in the 3′ untranslated region (UTR) of the *DMPK* transcript that form hairpin loops that aggregate nuclear proteins, including muscleblind-like (MBNL) protein, 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 development of high-binding affinity PNA anti-gene drugs with low off-target activity. By avoiding the use of a sugar-phosphate backbone, PNAs are non-immunogenic and avoid liver scavenger receptor clearance. With our delivery technology, our PNAs display rapid tissue uptake (including into the brain) after intravenous (IV) administration with slow renal elimination.

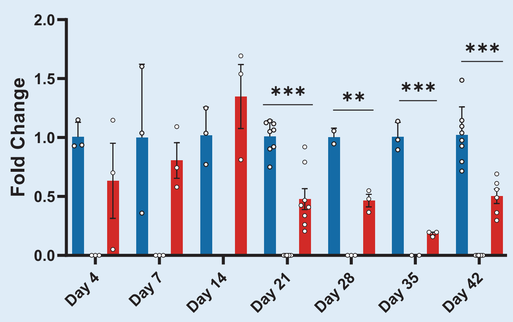


Initial constructs were screened in human DM1 fibroblasts for activity and potency for correcting splice defects. The lead candidate (NT-0231.F) has been evaluated in male HSA^{LR} mice 12 to 16 weeks of age with 8 animals in each vehicle/treatment group. Intramuscular administration of NT-0231.F provided proof of concept data for muscle relaxation and splice rescue (see poster 121). Additional single- and multi-dose studies have been completed.

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

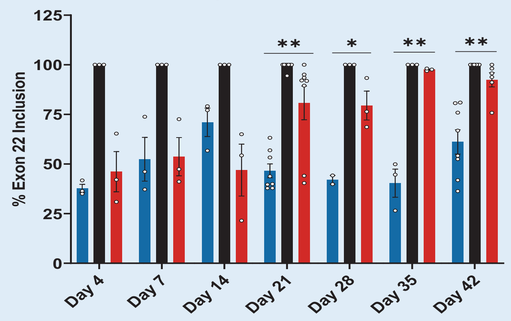
Target Engagement

ACTA1-CUG^{exp} RNA Levels

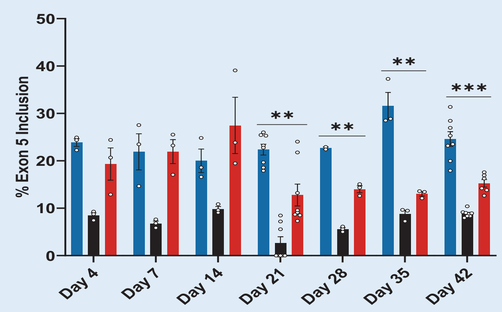


Secondary Splice Rescue, Representative Transcripts

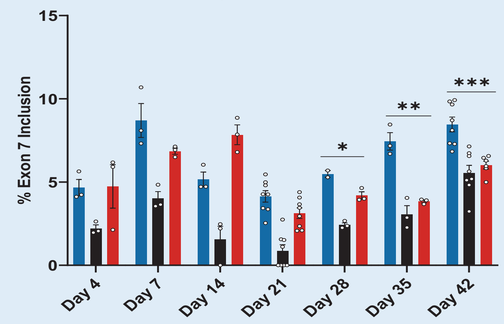
Atp2a1 Splice Rescue



Mbnl1 Splice Rescue

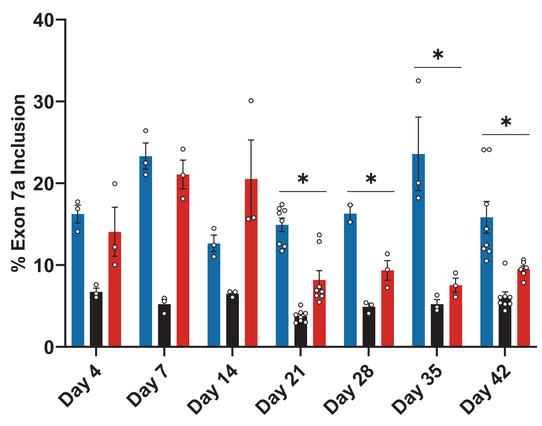


Bin1 Splice Rescue



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

Time Course of Chloride Channel (*Clcn1*) Splice Rescue[2]

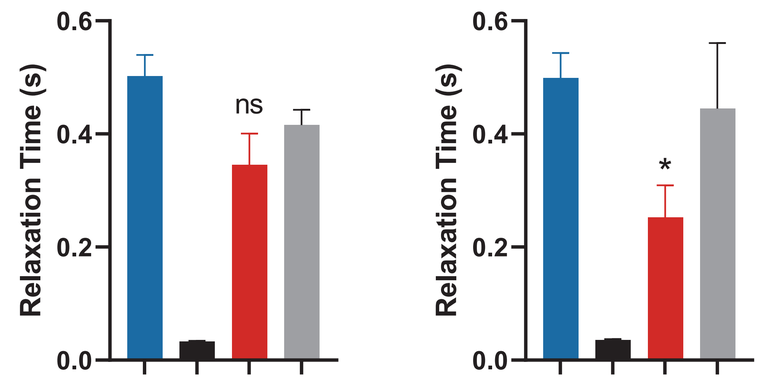


Chloride Channel Translation and Membrane Insertion

Vehicle Control HSA^{LR}
Vehicle Control FVB
NT-0231.F, 21 mg/kg
No Treatment HSA^{LR}

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

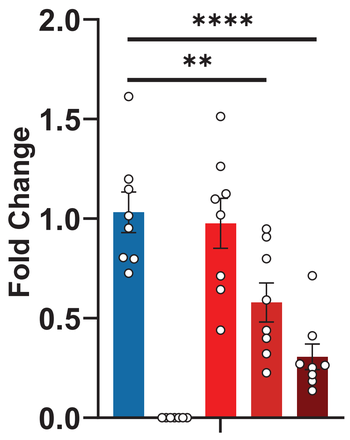
Improved Time to 60% Muscle Relaxation



Target Engagement, Splice Rescue, and Improved Myotonia Following Multi-Dose (weekly x 4 weeks) SC Administration of NT-0231.F

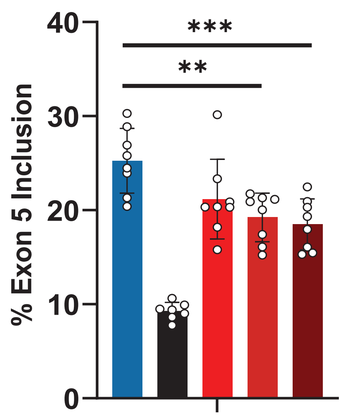
Target Engagement

ACTA1-CUG^{exp} RNA Levels

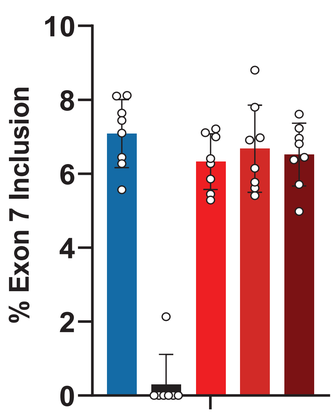


Secondary Splice Rescue, Representative Transcripts

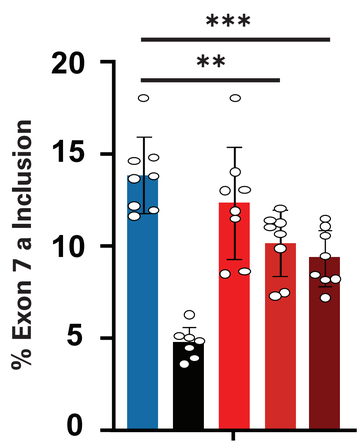
Mbnl1 Splice Rescue



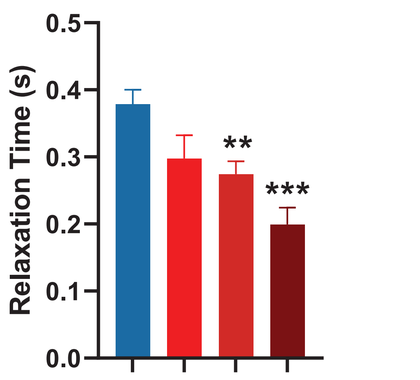
Bin1 Splice Rescue



Clcn1 Splice Rescue



Muscle Relaxation Day 35



Vehicle Control HSA^{LR} | Vehicle Control FVB | NT-0231.F, 20 mg/kg | NT-0231.F, 40 mg/kg | NT-0231.F, 60 mg/kg

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.

Conclusions

- Systemic administration (IV and SC) of NT-0231.F in the HSA^{LR} mouse rapidly corrected splicing defects and rescued myotonia dysfunction in this animal model of DM1
- These encouraging preclinical data support the potential for PATrOL™ DM1 lead candidate to be developed as a potential therapy for DM1 patients

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Poster 120

A PATrOL™ - Enabled Investigational Genetic Therapy for DM1: Mouse Pharmacokinetics, Biodistribution, and CNS Penetration After Systemic Administration

Sandra Rojas-Caro, Renta Hutabarat, Eunah Cho, Barry Badeau, Dani M. Stoltzfus, Noel R. Monks, Anthony Rossomando, William Mann, Dietrich A. Stephan

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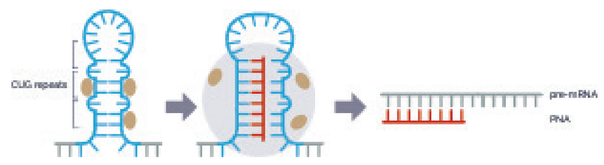


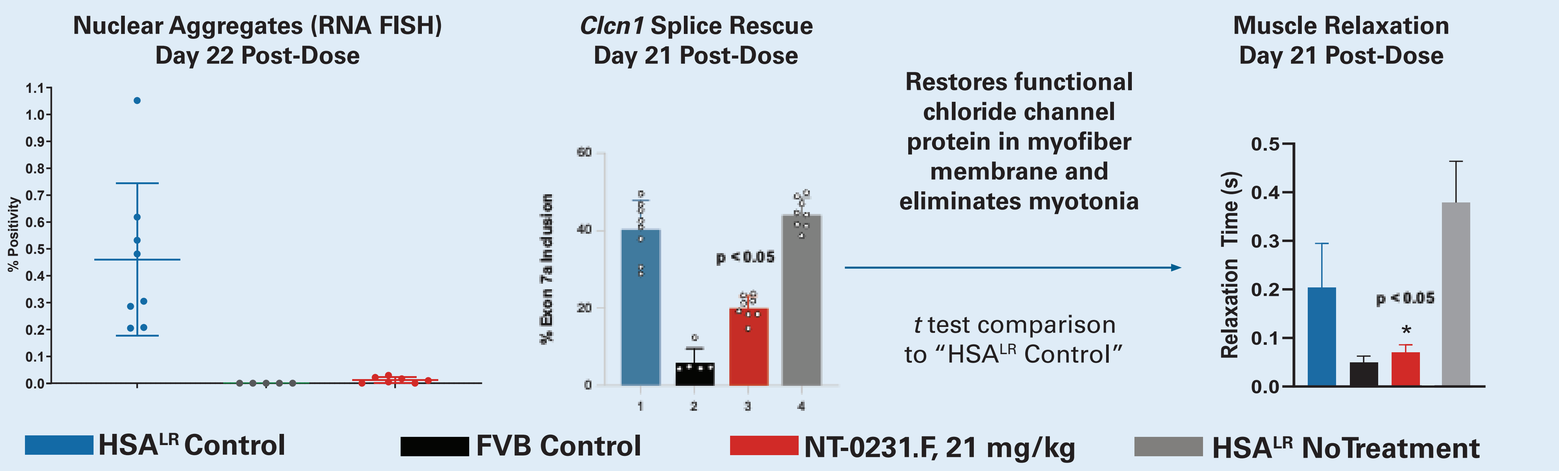
Proof of Concept

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. Additional data from IV and SC administration is reported on poster 120.

Single 21 mg/kg Intramuscular Dose

A single dose of NT-0231.F ameliorates nuclear aggregates, rescues normal mRNA splicing, including the chloride channel gene *(Clcn1)*, and eliminates myotonia in HSA^{LR} mice

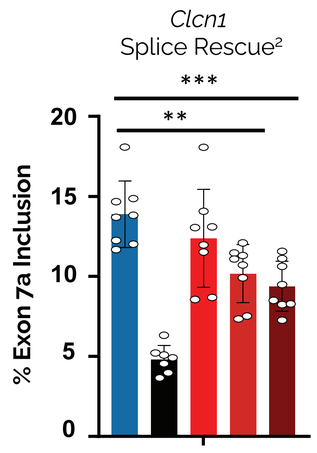


Study Objectives

This study was conducted to evaluate pharmacokinetics (PK) and biodistribution of NT-0231.F in BALB/c mice following a single subcutaneous (SC) or intravenous (IV) injection. Male mice were randomly assigned to 7 dose groups (n=3 per group).

- NT-0231.F was administered via a single SC (10 or 30 mg/kg) or IV (30 mg/kg) dose to BALB/c mice (n=3/time points/group)
- Blood and organs were collected at 0.5, 1, 1.5, 2, 4, 8, 12, and 24 hours post-dose, and at 7, 14, 21, and 28 days post-dose
- NT-0231.F was measured by liquid chromatography–mass spectrometry assay with calibration curves ranging from 20 to 2,000 ng/mL with an lower limit of quantification of 20 ng/mL
- Noncompartmental model-independent pharmacokinetic metrics were calculated from the mean plasma concentration-time data

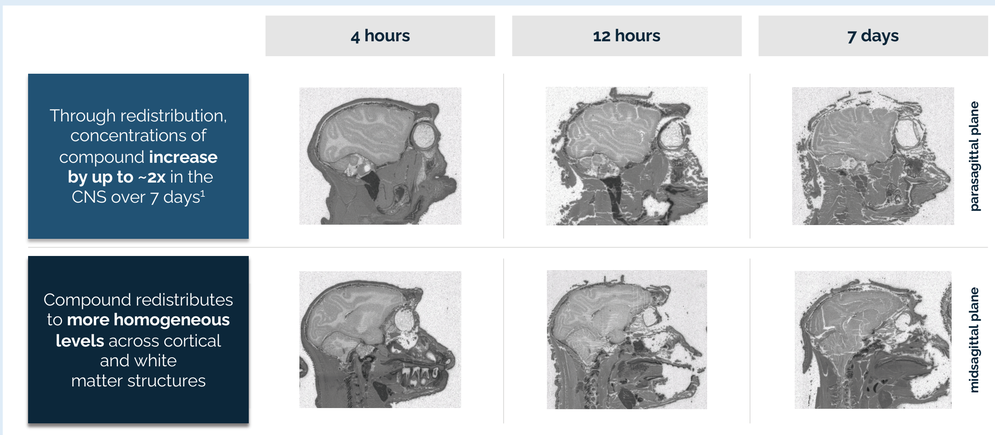
PK Serum Profiles of NT-0231.F in WT BALB/C Mice Following SC or IV Dosing Suggests Wide Tissue Distribution



PK Parameter Estimates	Units	10 mg/kg SC	30 mg/kg SC	30 mg/kg IV
$T_{1/2}$	hr	2.46	2.74	2.10
T_{max}	hr	0.50	1.00	0.50
C_{max}	ng/mL	646.00	1287.67	2490.00
C_{last}	ng/mL	38.80	47.80	42.07
C0	ng/mL	NA	NA	4501.54
T_{last}	hr	8.00	12.00	12.00
$AUC_{0\text{-}last}$	hr*ng/mL	1354.43	3909.96	8655.12
$Vz/F_{(sc)}$ or $Vz_{(iv)}$	mL/kg	23790.64	28895.43	10341.46
$Vss_{(iv)}$	mL/kg	NA	NA	9332.67
CL/F or $CL_{(iv)}$	mL/hr/kg	6701.68	7319.46	3415.89
$MRT_{0\text{-}last}$	hr	2.11	3.00	2.55
Bioavailability (F)	%	46.95	45.18	100.00

Following SC administration, NT-0231.F was rapidly absorbed into the systemic compartment with T_{max} range from 0.5- to 1-hour post-dose, and declined in a monophasic manner. Following IV administration, the T_{max} for NT-0231.F was 0.5-hour post-dose (first sampling time point) and declined in a monophasic manner.

Single-dose IV administration of 5 mg/kg PATrOL™ Shuttle end-labeled with ^{14}C-Gly and assayed using Quantitative Whole Body Autoradiography (QWBA). All grey shading above background indicates presence of compound.



[1] 7 days vs 4 hours; latest time point tested; all gray shading in images indicates presence of compound.

Conclusions

- Following administration, NT-0231.F maximal plasma concentration (C_{max}) and area under the plasma concentration-time curve ($AUC_{0\text{-}t}$) were approximately dose-proportional; SC bioavailability was ~46% of IV
- Serum $T_{1/2}$ was ~ 2.5 hours following SC or IV administration
- NT-0231.F total body clearance following SC or IV administration were ≥4-fold greater than glomerular filtration rate (GFR), suggesting primary renal clearance
- Volume of distribution was ~110-fold greater than blood volume, suggesting wide tissue distribution
- QWBA in non-human primates showed that drug-derived radioactivity distributed quite uniformly in relevant brain sub-regions (cerebellum, olfactory bulb, thalamus, caudate putamen, cerebral cortex and substantia nigra)

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Poster 121