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Pharmacokinetics, Biodistribution, and Exploration of the Mechanism of Central Nervous System Penetration of a PATrOL™- Enabled Investigational Genetic Therapy for Myotonic Dystrophy, Type 1 After a Single Subcutaneous or Intravenous Administration in the BALB/c Murine Model

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Disclosures

- I am a fulltime employee with NeuBase Therapeutics
- I have equity compensation from NeuBase Therapeutics

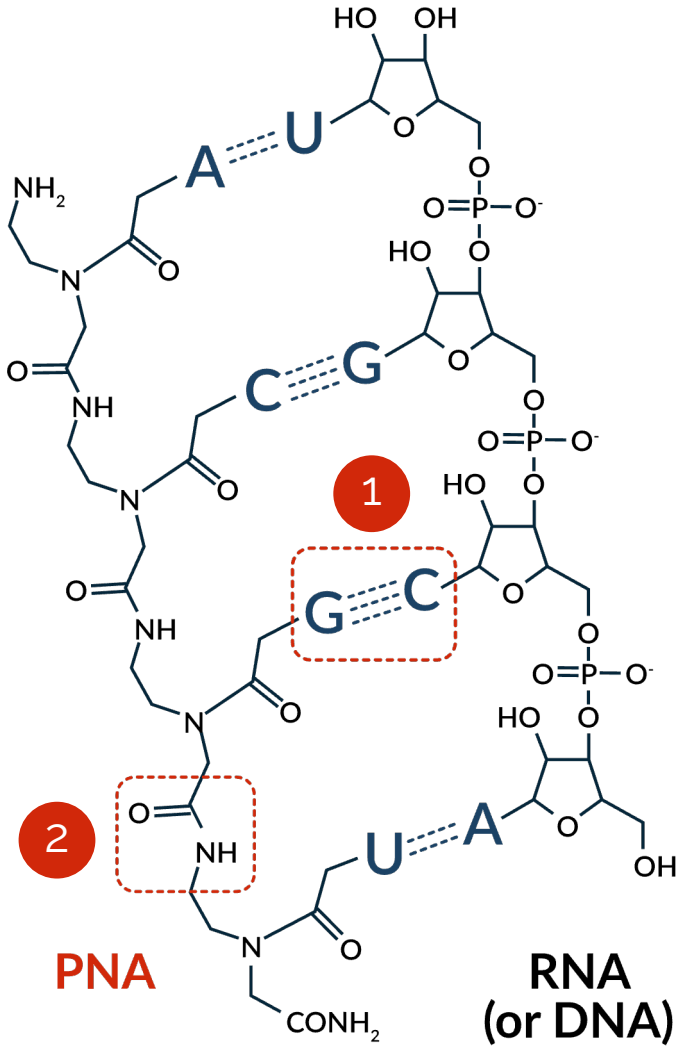
PATrOL™ : Peptide-Nucleic Acid Antisense Oligonucleobase Platform

1 **New Nucleobases: Highly Selective with Single Base Pair Precision**

NeuBase's A, G, T, C, or U are highly selective to binding the target DNA or RNA mutation while minimizing off-target effects

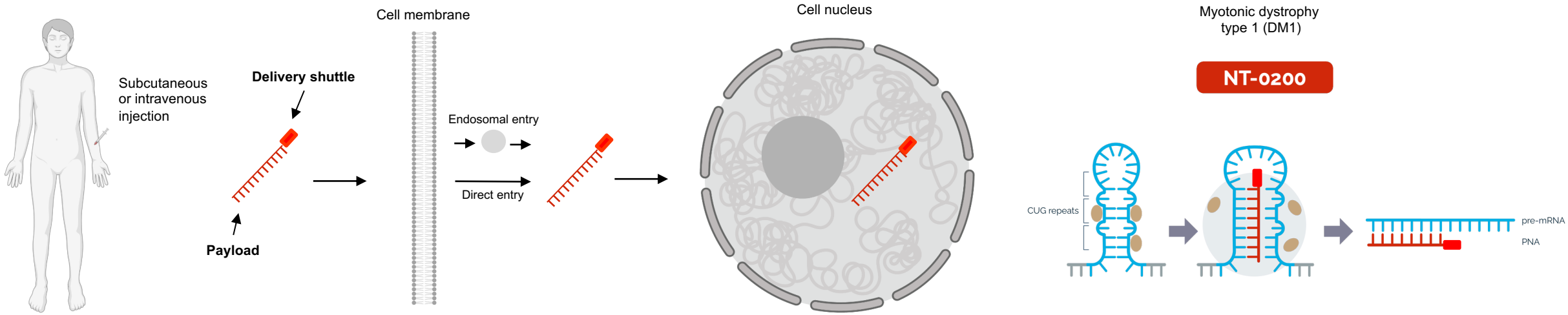
2 **Neutral Backbone: Well Tolerated with Enduring Treatment Potential**

Compared to negatively-charged sugar phosphate-based backbones, NeuBase's neutral backbone is non-immunogenic and confers high-binding affinity and key drug-like properties



PATrOL™ Shuttle Enables Broad Delivery after Systemic Administration

3 Novel Delivery Technology Achieves Broad Tissue Distribution Including into the Brain



Systemic route with broad and enduring biodistribution

Entry into cytoplasm of all cell types tested

Uniquely Drug the Genome™ (DNA or RNA)

Novel nucleobases engage the mutation to correct gene function and resolve disease

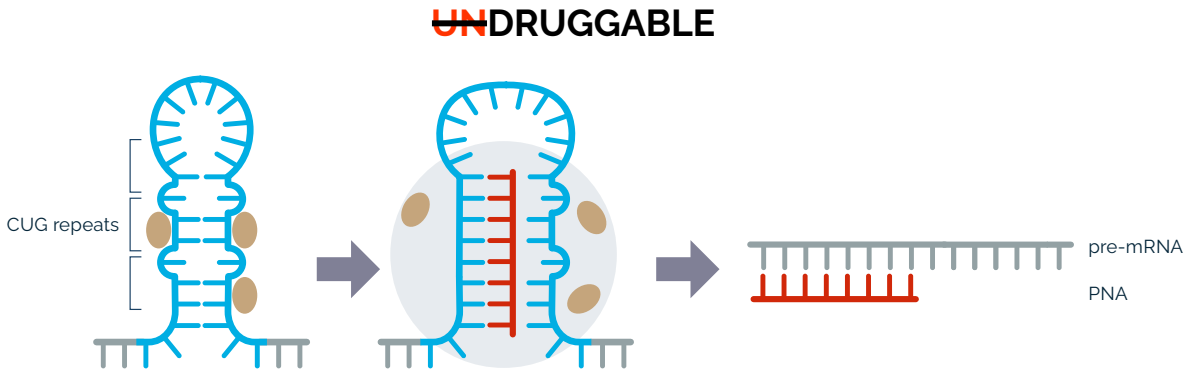
DM1: A Serious Genetic Disease with **High Medical Unmet Medical Need**

1 in 8,000 affected by DM1[1-4], with no effective therapies available

- Patients with DM1 suffer from cognitive deficits and muscle pathology (skeletal, smooth, and cardiac) caused by a trinucleotide expansion in the *DMPK* gene, forming toxic hairpins in the nucleus[1]
- Hairpins form nuclear aggregates together with MBNL splice proteins and result in widespread mis-splicing of pre-mRNAs
- Increases in severity from generation to generation
- Significant impact on quality of life with shortened life-expectancy

NT-0231.F targets pre-mRNA to release splicing factors and restore mRNA splicing

- Novel peptide-nucleic acid (PNA) investigational genetic therapy that targets the *DMPK* pre-mRNA
- Small molecule/naked oligo, non-biologic synthetic (no MAb/FAb)



[1]Mahadevan M et al. Science. 1992 Mar 6;255(5049):1253-5; [2]https://www.myotonic.org; [3]https://www.mda.org; [4]Pascual-Gilabert et. al., Drug Discovery Today, 2021, 26(7):1765-1772. Metrics are approximate.

PATrOL™ For DM1 : **Intramuscular Proof of Concept**

The HSA^{LR} Mouse Model:

The transgenic HSA^{LR} (human skeletal actin long repeat) mouse expresses the *ACTA$_1$* mutation, resulting in skeletal muscle pathology that mimics what is observed in human DM1

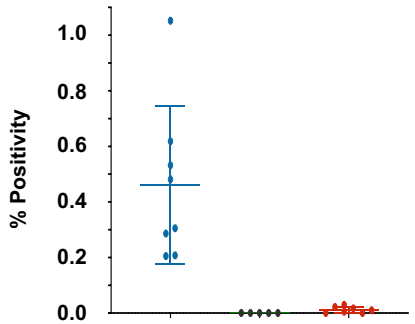
	Human	HSA^{LR}
Pathology	Muscle and brain	Skeletal muscle
Copy/cell	~20	~2000
Repeats/ transcript	100-1000	~ 220

Candidate molecules were screened in human DM1 fibroblast cell line. The lead candidate (NT-0231.F) was further evaluated in the HSA^{LR} model to evaluate, nuclear aggregates, splice defects, and muscle myotonia (slow muscle relaxation) response.

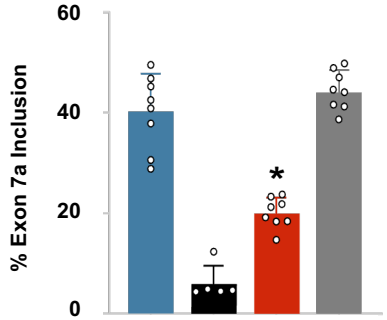
Evidence for Functional Rescue in HSA^{LR}

A single 21 mg/kg IM dose of NT-0231.F ameliorates nuclear aggregates, rescues normal gene splicing, and eliminates myotonia in HSA^{LR} mice

Nuclear Aggregates (RNA FISH) Day 22 Post-dose



Clcn1 Splice Rescue Day 21 Post-Dose

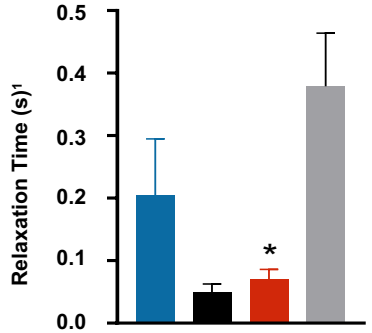


Restores functional chloride channel protein in myofiber membrane and eliminates myotonia

HSA^{LR} control
FVB control
NT-0231F, 21 mg/kg
HSA^{LR} no treatment

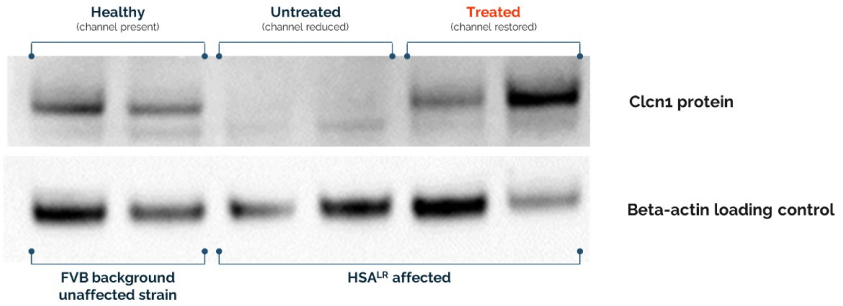
t test comparison to "HSA^{LR} Control"
* *P*<0.05

Muscle Relaxation Day 21 Post-Dose



1. Time to 60% relaxation

Restoration of soleus muscle chloride channel (*Clcn1*) protein levels 20 days after a single 29 mg/kg dose IV

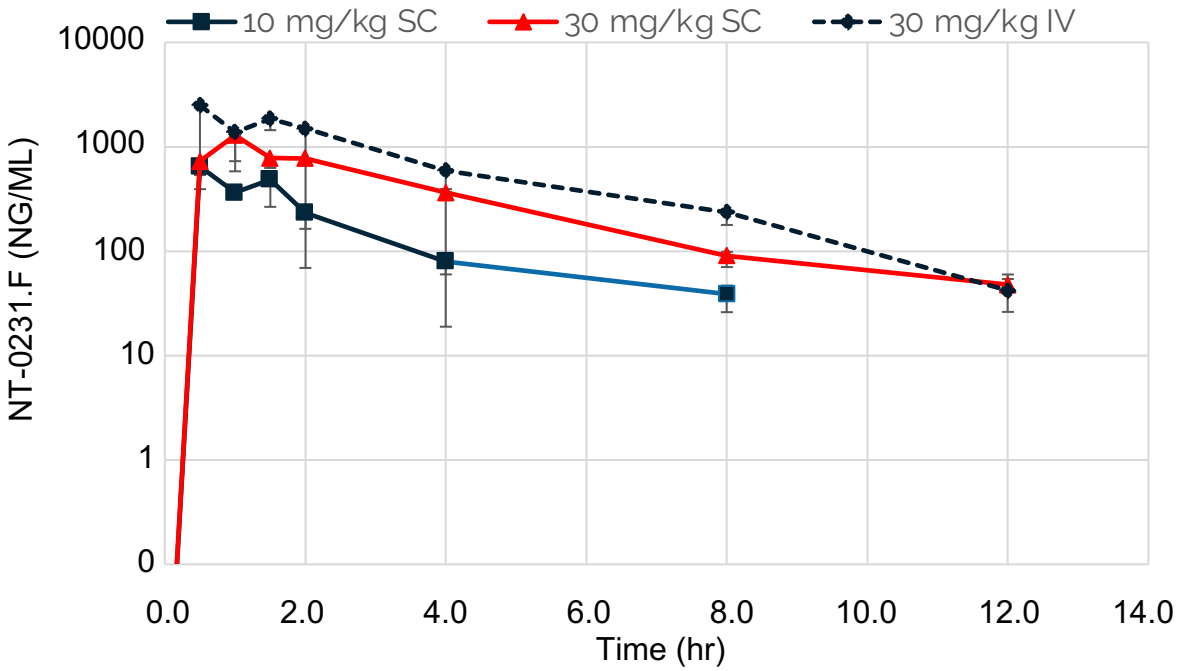


Pharmacokinetics (PK) and **Biodistribution** of NT-0231.F Following a **Subcutaneous** or **Intravenous** Injection in BALB/c mice

Study Design:

- 21 Male BALB/c mice were randomly assigned to 7 dose groups
- NT-0231.F was administered via a single dose SC (10 or 30 mg/kg) or IV (30 mg/kg); (n=3/time-points/ group)
- Blood, brain, kidney, heart, liver, quadricep femoris and tibialis anterior muscles 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
- The serum samples were analyzed for NT-0231.F using a LC-MS/MS assay with calibration curves ranging from 20 to 2000 ng/mL with an LLOQ of 20 ng/mL quantified by LC/MS/MS at Pyxant Laboratories
- Noncompartmental model-independent pharmacokinetic metrics were calculated from the mean plasma concentration-time data using Phoenix WinNonLin

PK Serum Profiles of NT-0231F* in wt BALB/C Mice Following **SC** or **IV Single-Dosing**

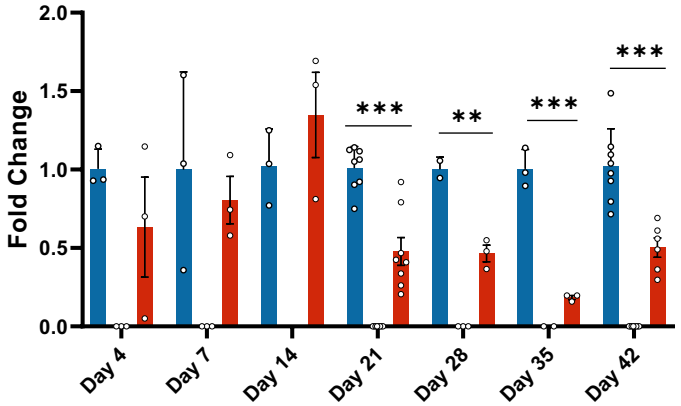


PK Parameter Estimates	Units	10 mg/kg SC	30 mg/kg SC	30 mg/kg IV
$T_{1/2}$	h	2.46	2.74	2.10
T_{max}	h	0.50	1.00	0.50
C_{max}	ng/mL	646.00	1,287.67	2,490.00
C_{last}	ng/mL	38.80	47.80	42.07
C0	ng/mL	NA	NA	4,501.54
T_{last}	h	8.00	12.00	12.00
$AUC_{0\text{-}last}$	h*ng/mL	1,354.43	3,909.96	8,655.12
$Vz/F_{(sc)}$ or $Vz_{(iv)}$	mL/kg	23,790.64	28,895.43	10,341.46
$Vss_{(iv)}$	mL/kg	NA	NA	9,332.67
CL/F or $CL_{(iv)}$	mL/h/kg	6,701.68	7,319.46	3,415.89
$MRT_{0\text{-}last}$	h	2.11	3.00	2.55
Bioavailability (F)	%	46.95	45.18	100.00

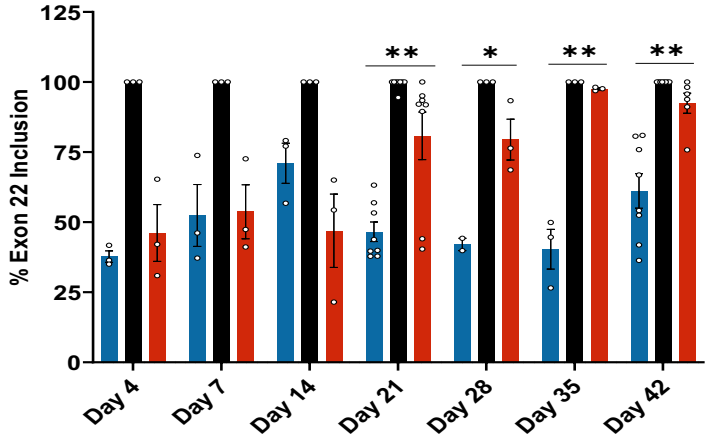
- Following SC administration, NT-0231.F was rapidly absorbed into the systemic compartment with T_{max} range from 0.5- to 1-hour post-dose, distributed, and declined in a monophasic manner. IV PK profile was similar
- NT-0231.F total body clearance following SC or IV administration were ≥4-fold greater than glomerular filtration rate (GFR), suggesting primary renal clearance
- Volume of distribution was ~110-fold greater than blood volume, suggesting wide tissue distribution

Single IV Dose Shows **Target Engagement** and Consequent **Splice Rescue** at Multiple Time Points in **Tibialis Anterior Muscle**

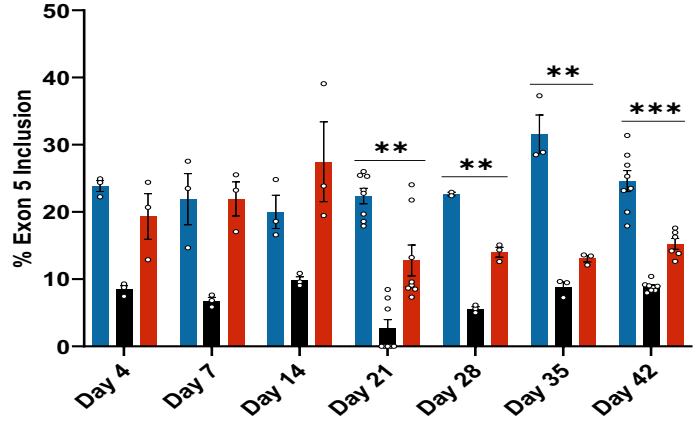
***ACTA1*-CUG^{exp} RNA Levels[1]**



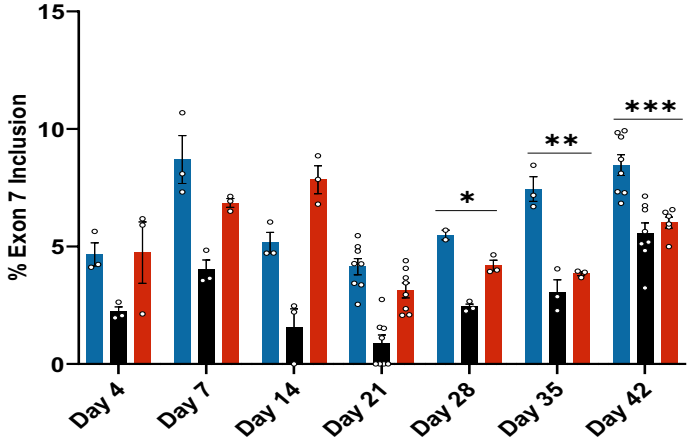
***Atp2a1* Splice Rescue[2]**



***Mbnl1* Splice Rescue[2]**



***Bin1* Splice Rescue[2]**

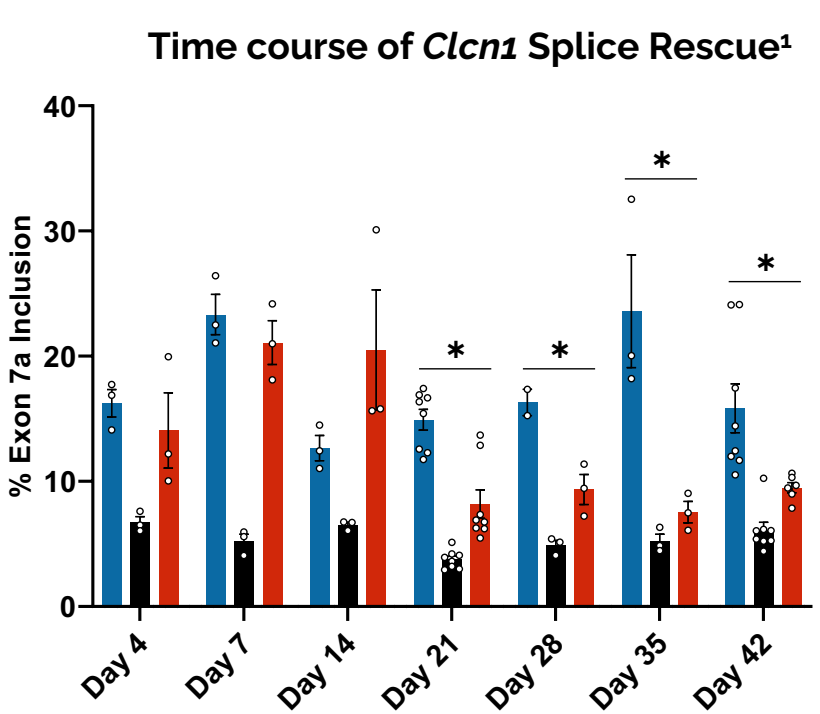


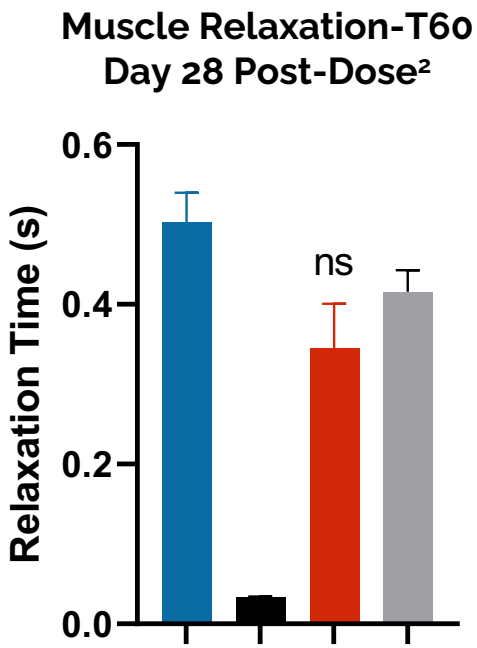
HSA^{LR} Control[3] FVB Control[3] NT-0231.F 40 mg/kg[4]

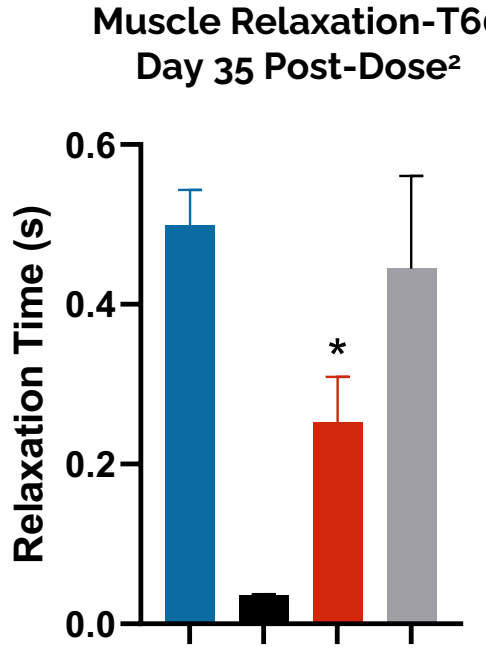
[1]Human skeletal muscle actin transgene with expanded CUG repeat mRNA measured by quantitative reverse transcription followed by polymerase chain reaction (RT-PCR) after random priming as per Tanner MK, Tang Z, Thornton CA. Nucleic Acids Res. 2021 Feb 26;49(4):2240-2254; [2]Murine transcript, relative usage by RT-PCR as per Wojtkowiak-Szlachcic A *et al.* Nucleic Acids Res. 2015 Mar 31;43(6):3318-31 and Klein AF *et al.* J Clin Invest. 2019 Nov 1;129(11):4739-4744; [3]Vehicle treated; [4]Oligo mass; t test comparisons to HSA^{LR} Control: *p < 0.05, **p < 0.01; ***p< 0.001.

Single IV Dose Illustrates **Splice Rescue of *Clnc1*** at Day 21 and Consequent **Reversal of Myotonia** at Day 35

Significant Induction of Splice Rescue > 21 Days → Chloride channel translation and membrane insertion → Significant Improvement of Relaxation > 28 Days







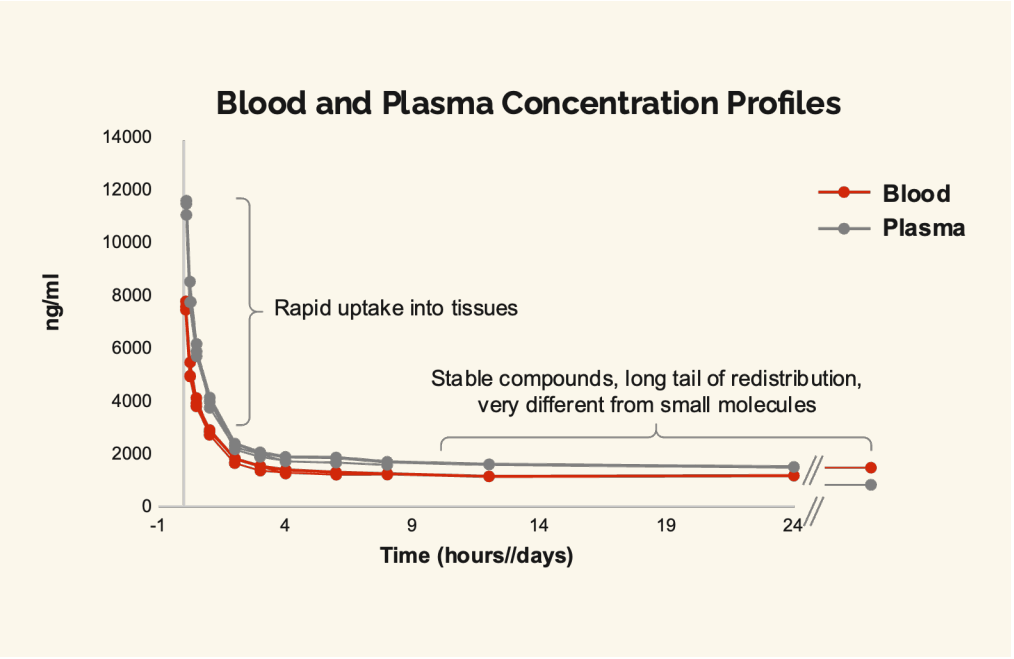
HSA^{LR} Control[3] FVB Control[3] NT-0231.F 40 mg/kg[4] HSA^{LR} No Treatment

[1] Murine transcript, relative usage by RT-PCR as per Wojtkowiak-Szlachcic A *et al.* Nucleic Acids Res. 2015 Mar 31;43(6):3318-31 and Klein AF *et al.* J Clin Invest. 2019 Nov 1;129(11):4739-4744; [2]Myotonia as measured by time to 60% relaxation of gastrocnemius muscle at 2nd electrically-stimulated maximal contraction, no changes to muscle force production; [3]Vehicle treated; [4]Oligo mass; t-test comparison to HSA^{LR} Control. *$p < 0.05$.

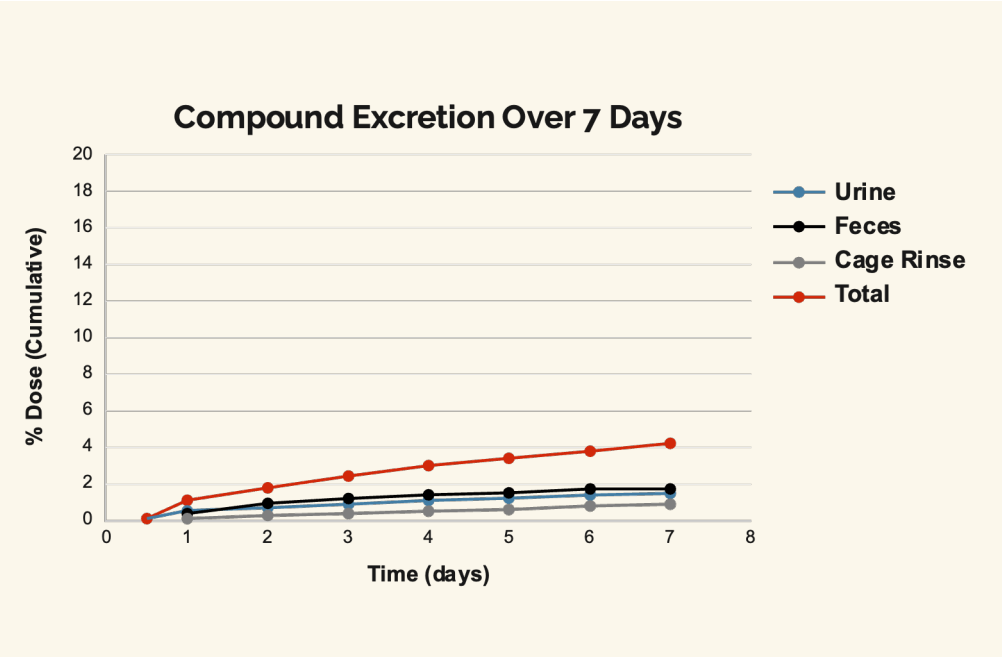
Pilot Study with **Radiolabeled Shuttle** Shows **Broad Biodistribution**

Single dose IV administration of 5 mg/kg of radiolabeled PATrOL™ shuttle end-labelled with 14C-Gly shows broad biodistribution in many tissues including skeletal muscle and brain tissues, with an in-circulation half-life of ~1.5 hours, and slow renal excretion[2]

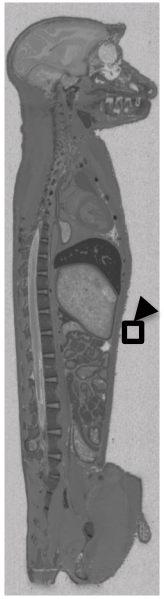
Rapid Uptake after Systemic Administration



Slow Renal Excretion



Broad and Therapeutically Relevant[1] Exposures



Any shading in animal darker than background (negative control) is compound[2]

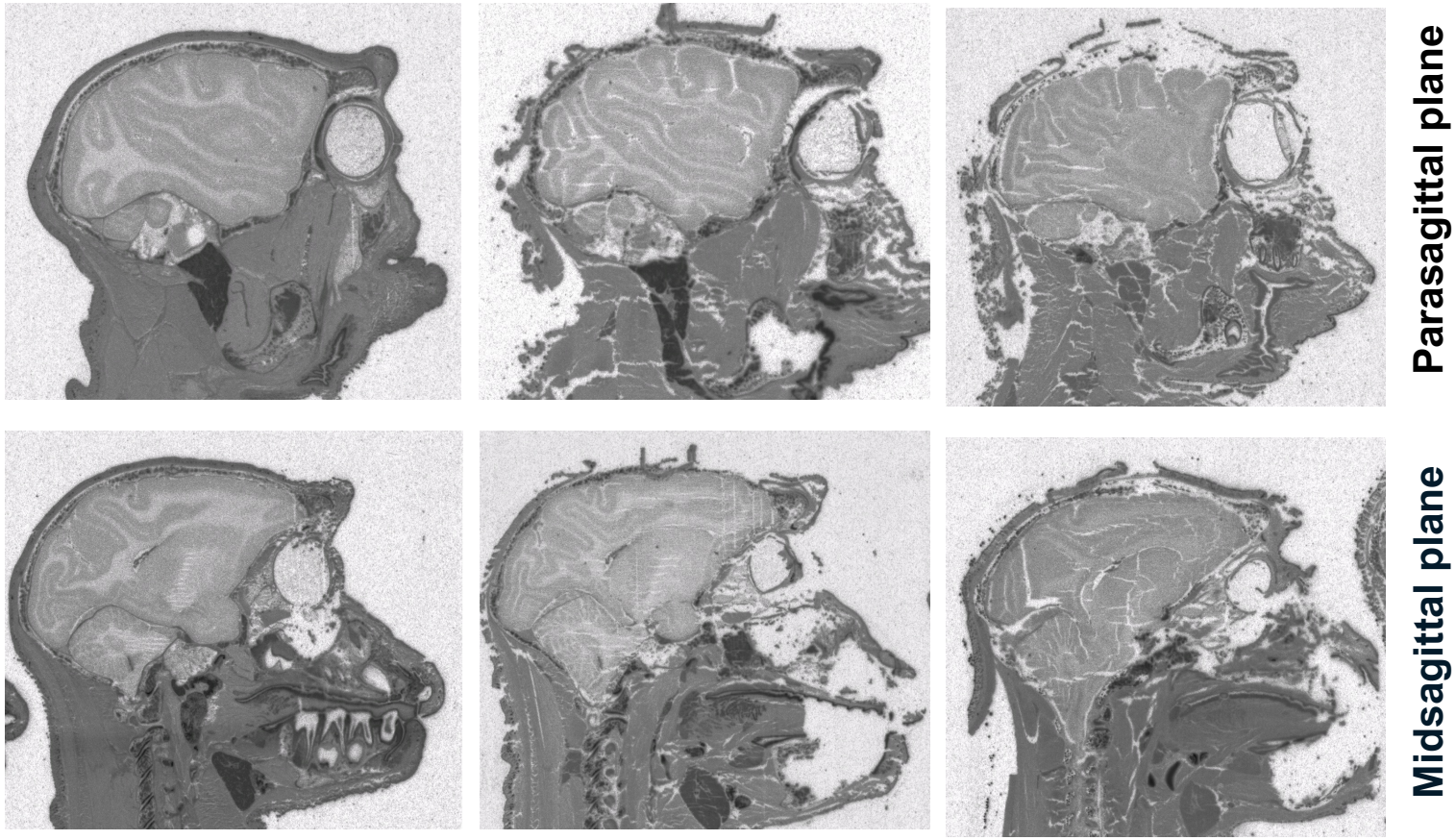
[1]Compound levels in tissues show activity in patient-derived cell lines; [2]PATrOL™ Shuttle (version 1) end-labelled with [14]C-Gly and assayed using Quantitative Whole-Body Autoradiography (QWBA) or using scintillation counting from biological fluids, all grey shading above background indicates presence of compound.

PATrOL Shuttle: **Concentrations in the Brain** After **IV** Administration

Single dose IV administration of 5 mg/kg PATrOL™ Shuttle end-labelled with ^{14}C-Gly and assayed using Quantitative Whole-Body Autoradiography (QWBA)

Through redistribution, concentrations of compound **increase by up to ~2x** in the CNS over 7 days[1]

Compound redistributes to **more homogeneous levels** across cortical and white matter structures



[1] 7 days vs. 4 hours; latest timepoint tested; All grey shading in images indicates presence of compound

Conclusions: Toward A Whole-Body Solution for DM1

- NT-0231.F shows molecular and functional rescue in the HSA^{LR} mouse following a single IM dose, and restoration of the chloride channel protein following IV dosing
- Feasibility studies with single IV and SC doses showed that NT-0231.F is rapidly absorbed in the systemic compartment declining in a monophasic manner. Additionally, NT-0231.F showed wide volume of distribution which suggests broad tissue distribution
- Molecular and functional rescue of disease in skeletal muscle of HSA^{LR} after single-dose IV supports systemic PK data and potential for whole-body distribution with NT-0231.F; DMSXL model in progress to test molecular and functional rescue in the brain
- Studies in NHPs with the radiolabeled PATrOL™ Shuttle showed rapid uptake after IV dosing and exposure in critical tissues for DM1, including skeletal muscle and the brain