Abstract #585

PHARMACOKINETICS, BIODISTRIBUTION, AND CNS PENETRATION OF A PATrOL™-ENABLED INVESTIGATIONAL GENETIC THERAPY FOR MYOTONIC DYSTROPHY, TYPE 1 FOLLOWING SYSTEMIC ADMINISTRATION IN MICE

neubase

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

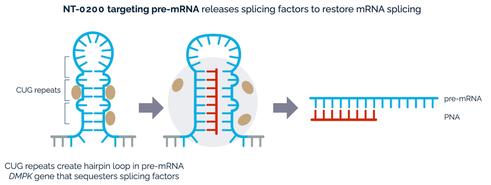
Abstract

Initial studies using a PATrOL™ platform-enabled peptide nucleic acid (PNA) pharmacophore combined with a novel delivery technology in transgenic animal models demonstrated pharmacologic activity in both brain and muscle following systemic administration. Patients with DM1 suffer from cognitive deficits and muscle pathology caused by a trinucleotide expansion in the *DMPK* gene. An exploratory radiolabeled biodistribution study of the delivery module administered intravenously in nonhuman primates showed distribution to brain, muscle, and heart, the major organs affected in DM1. A single dose of our PATrOL™ DM1 selected candidate was administered subcutaneously (10 or 30 mg/kg total mass; 7 or 20 mg/kg oligo mass) or intravenously (30 mg/kg total mass, 20 mg/kg oligo mass) in BALB/c mice to evaluate the pharmacokinetics and biodistribution and to examine central nervous system (CNS) penetration. Blood and tissues were collected over a time course ranging from 0.5 hours to 28 days. Serum and tissue compound concentrations were quantified by liquid chromatography with tandem mass spectrometry. Pharmacokinetic parameters were estimated using Phoenix WinNonLin. After subcutaneous administration, compound maximal plasma concentration (C_{max}) and area under the plasma concentration-time curve ($AUC_{0\text{-}t}$) were approximately dose-proportional; bioavailability was ~46%. Plasma C_{max} and $AUC_{0\text{-}t}$ after intravenous dosing were 2490 ng/mL and 8655 hours ng/mL, respectively. Compound plasma total body clearance and volume of distribution after subcutaneous or intravenous administration were ≥4-fold greater than mouse glomerular filtration rate (GFR) and ~110-fold greater than mouse blood volume, respectively, suggesting primarily GFR clearance and wide tissue distribution. Data will be presented supporting distribution of our investigational PNA therapy targeting *DMPK* pre-mRNA conjugated to a novel delivery technology to the brain and throughout the body following systemic administration in BALB/c mice, consistent with previously observed CNS pharmacologic activity.

Introduction

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

PATrOL™ is a 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 effects. With our delivery technology, our PNAs display rapid tissue uptake (including into the brain) after IV administration with slow renal elimination.



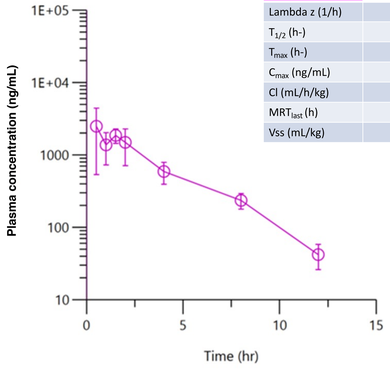
Methods and Materials

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

- NT-0231.F was administered intravenously (30 mg/kg total mass, 20 mg/kg oligo mass) 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 and 7, 14, 21, and 28 days post-dose
- NT-0231.F was measured by liquid chromatography–mass spectrometry with calibration curves ranging from 20 to 2000 ng/mL with a lower limit of quantification of 20 ng/mL (plasma) and 300 ng/mL (tissues), respectively
- Noncompartmental model-independent PK metrics were calculated from the mean plasma concentration-time data

Results, Serum Levels

Figure 1. PK serum profile after a single 30-mg/kg IV dose (mean ± SD) illustrates rapid uptake from the circulation into tissues.



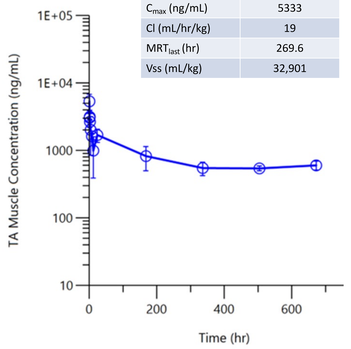
Parameter	
Lambda z (1/h)	0.35
$T_{1/2}$ (h-)	2.0
T_{max} (h-)	0.5
C_{max} (ng/mL)	2490
Cl (mL/h/kg)	0.0036
MRT_{last} (h)	2.7
Vss (mL/kg)	0.010

Lambda z, terminal elimination; $T_{1/2}$, half-life; T_{max}, time to maximum concentration; C_{max}, peak concentration; CL, clearance; MRT_{last}, mean residence time, last; Vss, volume of distribution at steady state.

Results, Tissue Levels

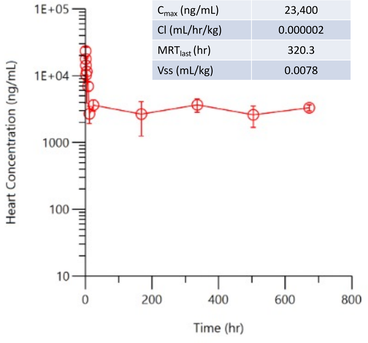
Figure 2. Tissue exposure after a single IV infusion of NT-0231.F in BALB/c mice (mean ± SD) illustrate the broad biodistribution of the lead compound across tissues affected in patients with DM1, and support a whole-body solution to this disease, including potentially for the CNS manifestations.

Tibialis Anterior Muscle



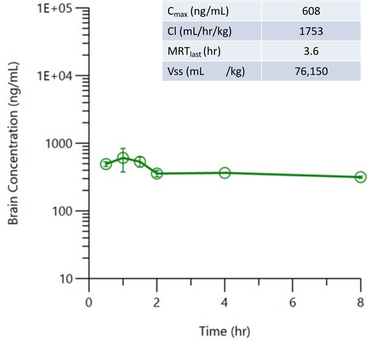
Parameter	
Lambda z (1/hr)	0.00057
$T_{1/2}$ (hr)	1212
T_{max} (hr)	0.5
C_{max} (ng/mL)	5333
Cl (mL/hr/kg)	19
MRT_{last} (hr)	269.6
Vss (mL/kg)	32,901

Heart



Parameter	
Lambda z (1/hr)	0.00030
$T_{1/2}$ (hr)	2294
T_{max} (hr)	0.5
C_{max} (ng/mL)	23,400
Cl (mL/hr/kg)	0.000002
MRT_{last} (hr)	320.3
Vss (mL/kg)	0.0078

Brain



Parameter	
Lambda z (1/hr)	0.023
$T_{1/2}$ (hr)	31
T_{max} (hr)	1.0
C_{max} (ng/mL)	608
Cl (mL/hr/kg)	1753
MRT_{last} (hr)	3.6
Vss (mL /kg)	76,150

Conclusions

- NT-0231.F is rapidly cleared from the vascular space after IV administration (Figure 1), with total plasma clearance ~ 4 than the mouse GFR, with wide distribution to body organs (V_{ss} ~110-fold greater than the mouse blood volume)
- NT-0231.F is rapidly accumulated by body organs, with T_{max} between 0.5 and 1 hour, consistent with the expected rapid distribution from the systemic compartment to tissues (Figure 2). C_{max} in organs ranged from 608 to 23,400 ng/mL
- Although NT-0231.F is rapidly cleared from plasma ($T_{1/2}$ 2.0 hours), all organs evaluated in this study displayed an extended elimination phase of between 31 and 2294 hr. The extended elimination phase aligns with the sustained function improvement observed in animal models of DM1 (see abstract 1049)
- **The preliminary plasma and tissue PK profiles in mice are consistent with the expected PK characteristics of NT-0231.F, demonstrating a rapid distribution phase followed by a slow elimination phase, with tissue concentrations measurable for at least 4 weeks after a single IV dose**

Contact

- Noel R. Monks: NeuBase Therapeutics, Inc., 350 Technology Drive, Pittsburgh, PA 15219
- Email: nmonks@neubasetherapeutics.com
- Website: https://www.neubasetherapeutics.com
- Phone: 1.412.763.3350