Spherical Nucleic Acids Show Increased Distribution and Longer Persistence than Linear Oligonucleotides in Rat Brain Following IT Administration

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BACKGROUND

- SNAs are dense, radial arrangements of oligonucleotides (Figure 1) that have useful properties compared to linear oligonucleotides, notably increased cellular uptake via scavenger receptors and lipid raft engagement.
- Spinal muscular atrophy (SMA) is an autosomal recessive motor neuron disorder caused by reduced levels of the survival of motor neuron (SMN) protein. Therapeutics that restore SMN protein levels have had a major impact in SMA.
- Nusinersen, a linear antisense oligonucleotide that increases SMN protein levels, is an approved treatment for SMA.
- Intracerebroventricular injection of SNA-formulated nusinersen (nusinersen-SNA) in the Δ7SMA mouse model substantially increased median survival versus nusinersen (Figure 2).
- We hypothesized that the increased survival was due, at least in part, to the improved retention of SNAs in CNS tissues.

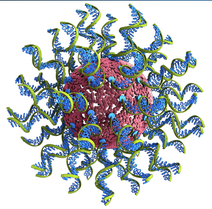


Figure 1: Spherical nucleic acid structure

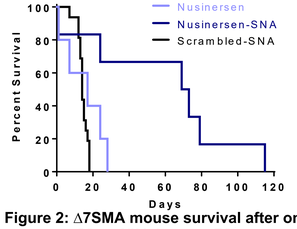


Figure 2: Δ7SMA mouse survival after one 20 µg ICV dose on P0[1]

OBJECTIVE

Compare and characterize the pharmacokinetics and central nervous system distribution of intrathecally (IT) administered nusinersen and nusinersen-SNA using single-photon emission computed tomography combined with computed tomography (SPECT/CT) in rats.

METHODS

- ^{125}I-labeled nusinersen-SNA and nusinersen were injected IT into Sprague Dawley rats as a single 70 µL bolus emitting 190 to 375 µCi of radioactivity.
- Animals were imaged by SPECT/CT at 0-1, 6, 24, 72, and 168 hours (hr) after dosing.
- Animals were sacrificed after the final (168 hr) time point and whole blood was drawn via cardiac puncture (up to 5 mL) for gamma counting in blood and plasma.
- Whole-body field of view imaging was used with feet first/prone positioning for both SPECT and CT. SPECT scans were acquired in 4 × 9 minute frames for the 0-1 hr time points, and in 1 × 30 minute frames for the 6, 24, 72, and 168 hr time points.
- Regions of interest were defined using VivoQuant software (inviCRO, LLC). A 13-region rat brain atlas was placed onto each image, and radioactivity signals were decay-corrected from the time of measurement to the time of injection.

Distribution and Persistence

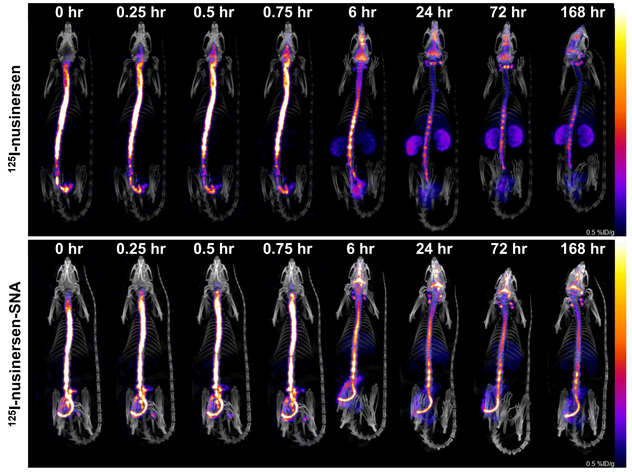
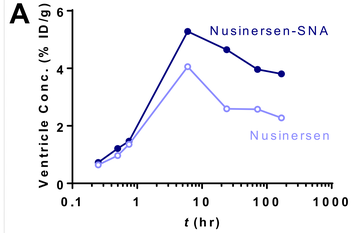


Figure 3. Representative SPECT/CT time-course imaging of rats treated with nusinersen and nusinersen-SNA. Nusinersen (top) and nusinersen-SNA (bottom) were detected throughout the spinal cord immediately after dosing, with penetration into the cranial cavity beginning at 0.5 hr. By 24 hr, nusinersen signals became prominent in the kidneys and began to decrease in the CNS. In contrast, nusinersen-SNA signals persisted throughout the CNS, through 168 hr post-injection. Yellow and blue colors indicate regions of greater and lesser oligonucleotide concentration, respectively.



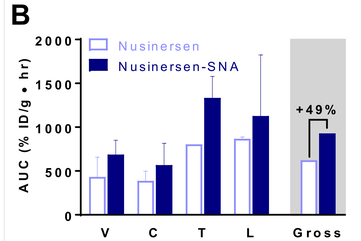
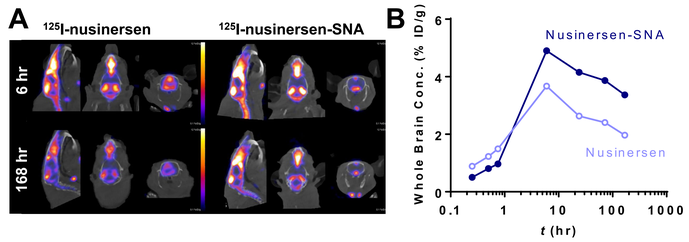


Figure 4. Distribution of nusinersen and nusinersen-SNA in CNS biofluids. (A) Time-course SPECT intensities of nusinersen and nusinersen-SNA in ventricular regions in units of percent injected dose / g [% ID/g]) **(B)** Area under the curve (AUC) binning of all time points revealed increased bioavailability of nusinersen-SNA in ventricular (V), cervical (C), thoracic (T) and lumbar (L) CSF. Data are means ± SD.



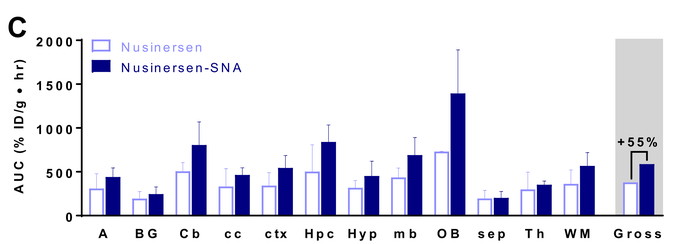


Figure 5. Distribution of nusinersen and nusinersen-SNA in CNS structures. (A) Magnified SPECT/CT imaging of rat brains 6 hr (top row) and 168 hr (bottom row) after administration of nusinersen and nusinersen-SNA. Yellow and blue colors indicate regions of greater and lesser oligonucleotide concentration, respectively. **(B)** Time-course SPECT intensities of nusinersen and nusinersen-SNA in whole rat brain. **(C)** AUC binning of all time points reveals increased bioavailability of nusinersen-SNA in the amygdala (A), basal ganglia (BG), cerebellum (Cb), corpus callosum (cc), cerebral cortex (ctx), hypothalamus (Hyp), hippocampus (Hpc), midbrain (mb), olfactory bulb (OB), septum (sep), thalamus (Th) and white matter (WM). Data are means ± SD.

Clearance / Excretion

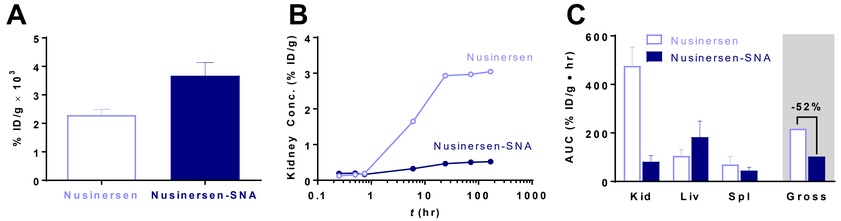


Figure 6. Clearance of nusinersen and nusinersen-SNA. (A) *Ex vivo* measurement of nusinersen and nusinersen-SNA in blood revealed elevated association of nusinersen-SNA with blood plasma 168 hr after dosing. **(B)** Time-course SPECT intensities of nusinersen and nusinersen-SNA in rat kidneys. **(C)** AUC binning of all time points reveals reduced evacuation of nusinersen-SNA in kidneys (Kid) and spleen (Spl) relative to nusinersen. Data are means ± SD.

CONCLUSIONS

- Immediate and pronounced CNS distribution, particularly in the spinal cord, was observed in both nusinersen and nusinersen-SNA IT injected rats.
- Nusinersen-SNA persists throughout the CNS, including in the brain and spinal cord, longer than nusinersen. These results explain, at least in part, our previous observations that nusinersen-SNA improves survival duration of Δ7SMA mice after a single injection.
- In line with these findings, the presence of nusinersen in the kidneys was significantly increased at 6, 24, 72, and 168 hours relative to nusinersen-SNA.
- Subsequent studies are currently investigating the CNS biodistribution of nusinersen and nusinersen-SNA in non-human primates at comparable and extended time points.
- **Collectively, these data strongly support the therapeutic potential of SNAs for treating CNS disorders.**

1: A. Burghes, V. McGovern, K. Corlett, S. R. Nallagatla, B. R. Anderson, R. S. Kang and E. R. Kandimalla. "Nusinersen in spherical nucleic acid (SNA) format improves efficacy both in vitro in SMA patient fibroblasts and in Δ7 SMA mice and reduces toxicity in mice." Cure SMA Conference 2018. Disclosure: The research being reported in this poster was supported by Exicure, Inc. Exicure employees received compensation and stock options. Exicure, Inc. is developing products related to the research being reported.