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

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Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive motor neuron disorder caused by reduced levels of SMN protein[1,2]. Therapeutics that restore SMN protein levels have had major impact in SMA[3,4]. Currently Spinraza (Nusinersen) a MOE based antisense oligonucleotide directed against the ISSN1 sequence which blocks the binding of a negative regulator of SMN2 splicing is an approved treatment for SMA. In SMA mice (Taiwanese) when given Nusinersen via ICV at a dose of 20μg/g the mice had improved mean survival from 10 days to 17 days with administration in the periphery further improving survival[5]. The current clinical paradigm is to give a dose of 12 mg but with repeated dosing via intrathecal injection. A single 12 mg dose, assuming the average weight of Newborn is 3.5 kg, translates to 3.57μg/g in a newborn mouse. Spherical Nucleic Acids (SNA) are nanoscale constructs consisting of densely packed synthetic nucleic acid molecules that are radially arranged in three dimensions around a liposomal core[6]. These constructs can enter cells by engaging scavenger receptors and lipid rafts[7]. This results in improved uptake by a defined pathway. As it is important to maximize the amount of SMN obtained, we have investigated the use of Nusinersen in SNA format (Nusinersen-SNA) to improve exon 7 inclusion in Δ7SMA mouse model as well as reduce toxicity when delivered via the CSF.

Nusinersen-SNA increases full-length SMN mRNA levels in SMA patient fibroblasts

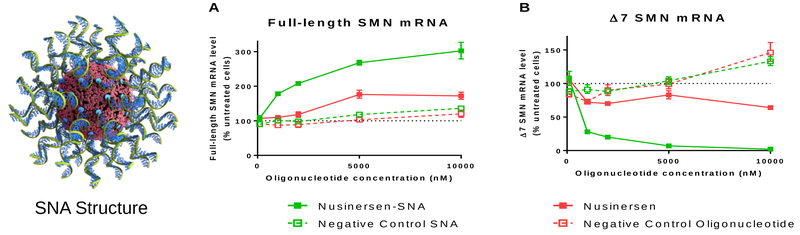


Figure 1. Effect of Nusinersen-SNA and Nusinersen on SMN mRNA levels *in vitro*. SMA patient fibroblasts (GM09677C) were treated for 48 hours and then qRT-PCR was used to measure the levels of SMN mRNA. Mean ± SEM of n=3 replicate wells each measured in duplicate. **(A)** Full-length SMN mRNA. **(B)** Δ7 SMN mRNA.

Nusinersen-SNA increases full-length SMN mRNA and SMN protein levels in SMA patient fibroblasts

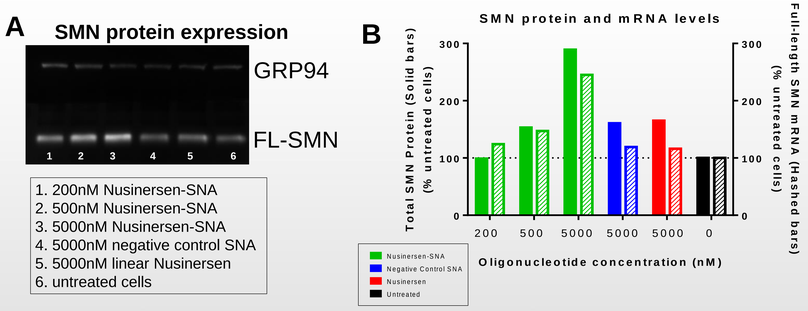


Figure 2. Effect of Nusinersen-SNA and Nusinersen on SMN protein levels *in vitro*. SMA patient fibroblasts (GM09677C) were treated with SNAs for 72 hours and, then assessed by western blot and qRT-PCR. (**A**) Western blot showing total SMN protein and loading control GRP94. GRP94 protein loading control was detected with ADI-SPA-850-F (Enzo Life Sciences). SMN was detected with VMA00249 (Bio-Rad). (**B**) Densitometric quantification of SMN western blot (solid bars) and qRT-PCR of full-length SMN mRNA (hashed bars) from identically treated wells. SMN qRT-PCR was performed on SMA patient fibroblasts (GM09677C) that were plated in 96-well plates and treated in triplicate with SNAs in complete media. After cell lysis, cDNA was derived from extracted RNA and assessed by qRT-PCR with technical duplicates for each sample. Full-length SMN2 was measured relative to GAPDH.

Survival of Nusinersen-SNA treated Δ7SMA mice

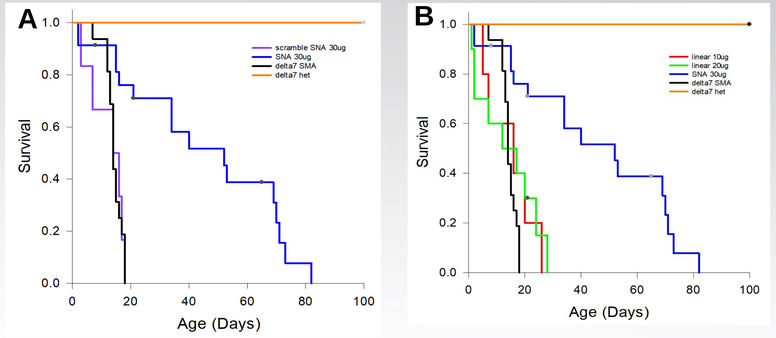


Figure 3. Survival of Nusinersen-SNA treated Δ7SMA mice. Mice were genotyped at P0 (day of birth) and injected via Intracerebroventricular injection (ICV) on P0 as described previously[8]. The recorder of events was blinded to genotype and treatment. (**A**) Survival of Δ7SMA mice treated with the 30μg dose Nusinersen-SNA increase survival to a maximum of 82 days while scramble SNA has no effect on survival. (**B**) Linear Nusinersen improved survival of Δ7 SMA mice to a maximum of 28 days. The data is summarized in the table below.

Treatment with Nusinersen	# of mice	Mean survival (days)	Maximum survival (days)	Log rank p value
Linear 10μg	5	14.8±4.0	26	NS
Linear 20μg	10	14.0±3.0	28	NS (censored)
Linear 30μg	5	2.2±0.1	2	Toxicity
SNA 10μg	8	25.7±3.3	40	0.00064
SNA 20μg	9	57.0±14.0	115	.002 censored
SNA 30μg	23	45.6±6.1	82	0.000017 censored
Scrambled SNA 30μg	6	12.5±2.5	18	NS
untreated Δ7SMA	16	14.3±0.7	18	Tested against

Weight curves of Nusinersen-SNA treated Δ7 mice

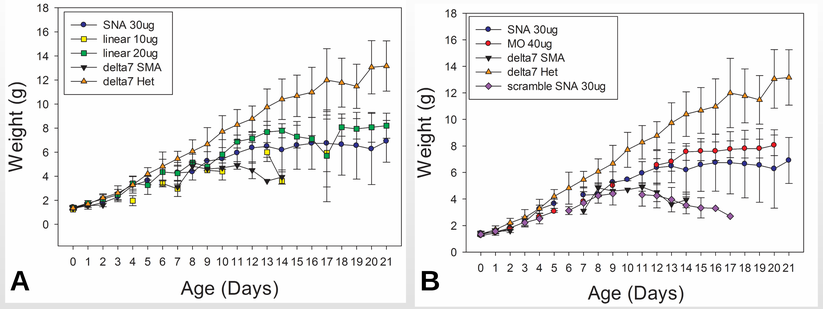


Figure 4. Weight curves to 21 days of age in treated and untreated control mice. Mice were weighed each day. (A) Weights are similar in Δ7SMA mice treated with linear or Nusinersen-SNA treated mice. (**B**) Weights are similar in Δ7SMA mice treated with morpholino to ISS-N1 or Nusinersen-SNA . The scramble-SNA did not alter the weight of the Δ7SMA mice.

Nusinersen-SNA increases full-length SMN mRNA

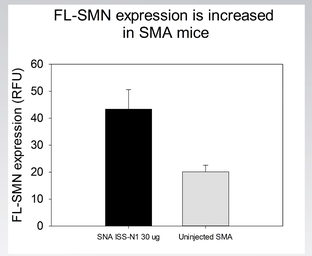
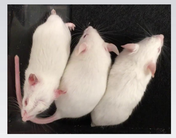


Figure 5. Digital droplet RT-PCR of 3 biological replicates of spinal cord from treated and untreated P9 mice to measure the level of full-length SMN. Notice the two-fold increase in full-length SMN upon treatment. SMN mRNA quantification was obtained with ddPCR (BioRad) as previously described[8]. FL-SMN was detected with SMN-FL specific probe and normalized to expression of YWHAZ12 in a multiplex assay.

Figure 6. Phenotype of Nunsinersen-SNA treated mice. The mice with no tail are SNA-30μg treated Δ7SMA mice at 62 days of age compared to a Δ7 het sib (middle mouse). Necrosis of the ears is also present at this age.



Conclusions

- **SNAs increase uptake of MOE Nusinersen in cell models lacking *SMN1* but containing *SMN2* resulting in increased amounts of full-length mRNA and SMN protein from *SMN2*.**
- **SNAs when delivered to CSF in the Δ7SMA mouse model allow increased dosing of Nusinersen and increased efficacy with prolonged survival of SMA mice.**
- **SNAs when delivered to CSF in the Δ7SMA mouse model have increased full-length SMN mRNA levels in spinal cord tissue.**

Future Directions

- **Complete enrollment in all treatment groups**
- **Perform EMG, compound muscle action potential (CMAP) and motor unit number estimation (MUNE) to assess the extent of motor neuron correction.**
- **Determine Nusinersen-SNA bio-distribution and SMN levels in all treatment groups using ELISA and Western blot.**

References

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