

# Utilizing Antisense Oligonucleotides in Spinal Muscular Atrophy Gene Therapy

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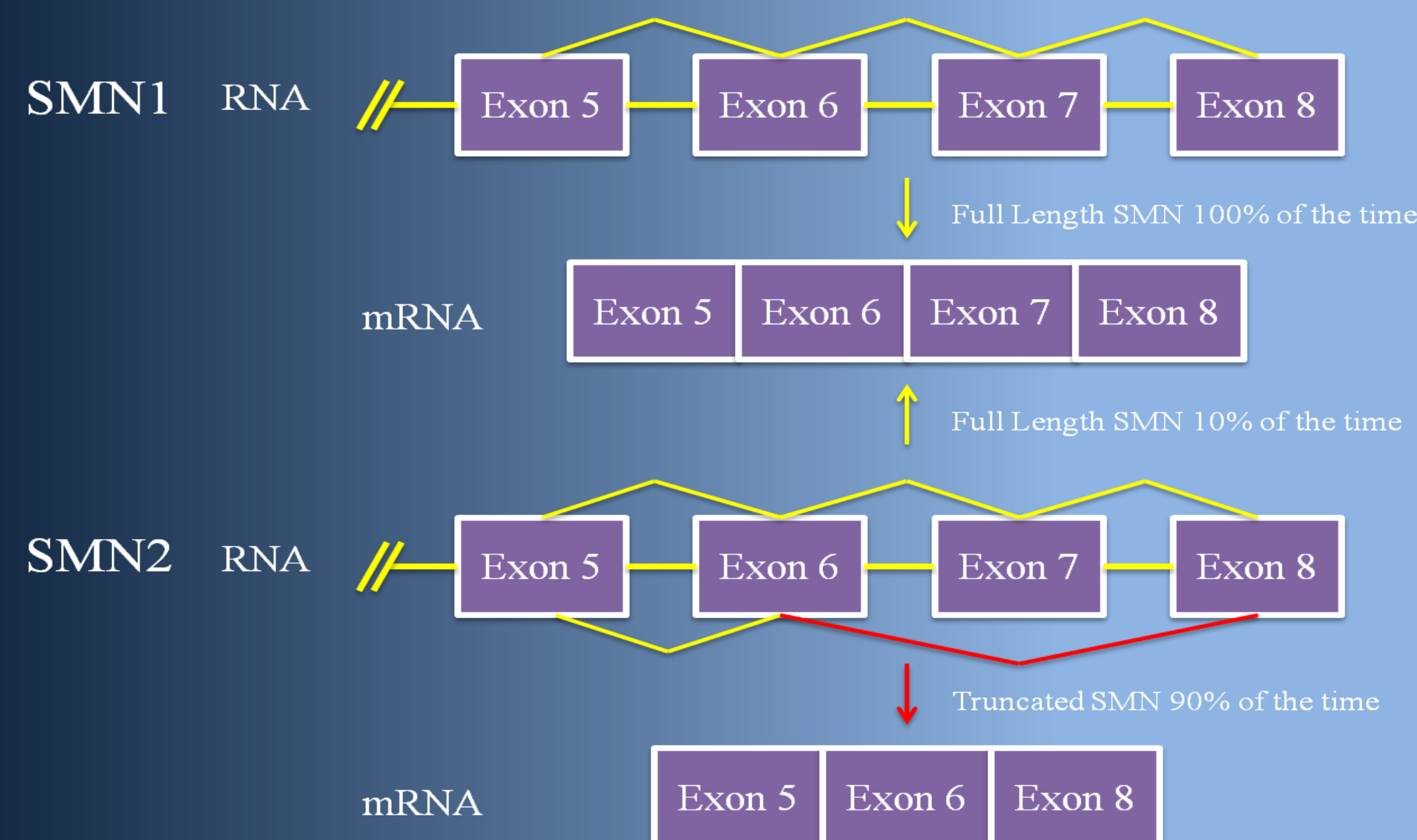
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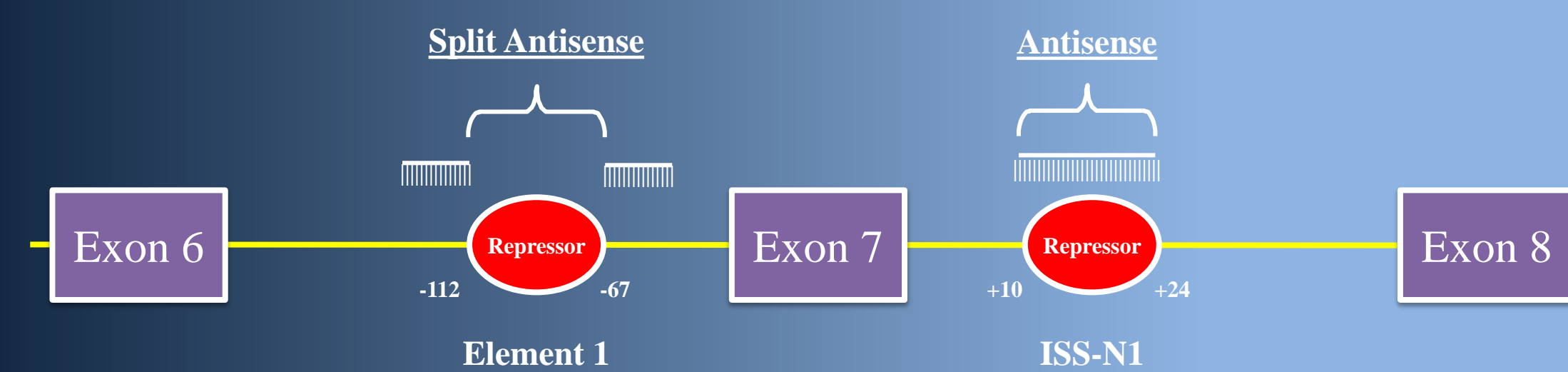
## Abstract

Spinal Muscular Atrophy (SMA) is an autosomal recessive neurodegenerative disease that is the leading cause of infantile mortality worldwide. The disease causes degeneration of  $\alpha$ -motor neurons in the anterior horn of the spinal cord leading to weakness of the lower limbs and eventual death. Patients affected with SMA suffer from a homozygous deletion of Survival Motor Neuron 1 (SMN1). A similar gene, Survival Motor Neuron 2 (SMN2), produces an identical protein to SMN1, but a single missense mutation of the SMN2 nucleotide sequence leads to formation of a functional SMN protein only 10% of the time. The truncated protein most commonly produced by the SMN2 gene, SMN $\Delta$ 7, undergoes aberrant splicing leading to exclusion of the critical exon 7 from the protein. A regulatory region upstream of exon 7 named Element 1 (E1) was identified that acts as an intronic splice suppressor and represses exon 7 inclusion in SMN2 transcripts. Using Morpholino-based antisense oligonucleotides (ASOs), which bind to a specific nucleotide sequence in RNA, E1 was targeted for inhibition to promote full-length SMN expression from SMN2. ASOs were delivered to the central nervous system via intracerebroventricular injection in SMA mice. ASO administration resulted in a significant increase of SMN in the spinal cord, as well as increased life span and development of healthy neuromuscular junction pathology. Initial success in the murine model suggests that ASOs may be a potent therapy in the treatment of SMA in human patients.

## Spinal Muscular Atrophy: Genetics

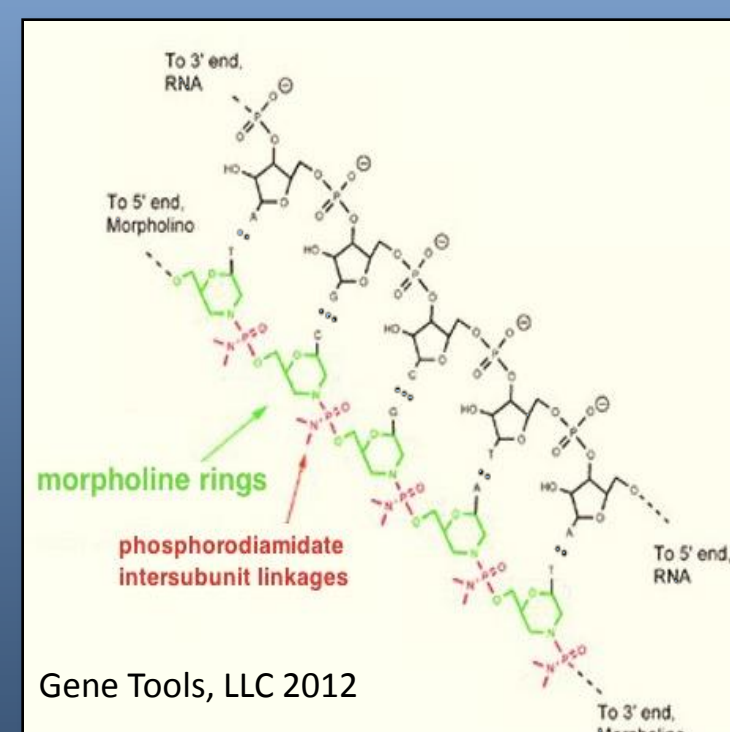


## Splicing Repressors in SMA: Targets for ASO Inhibition

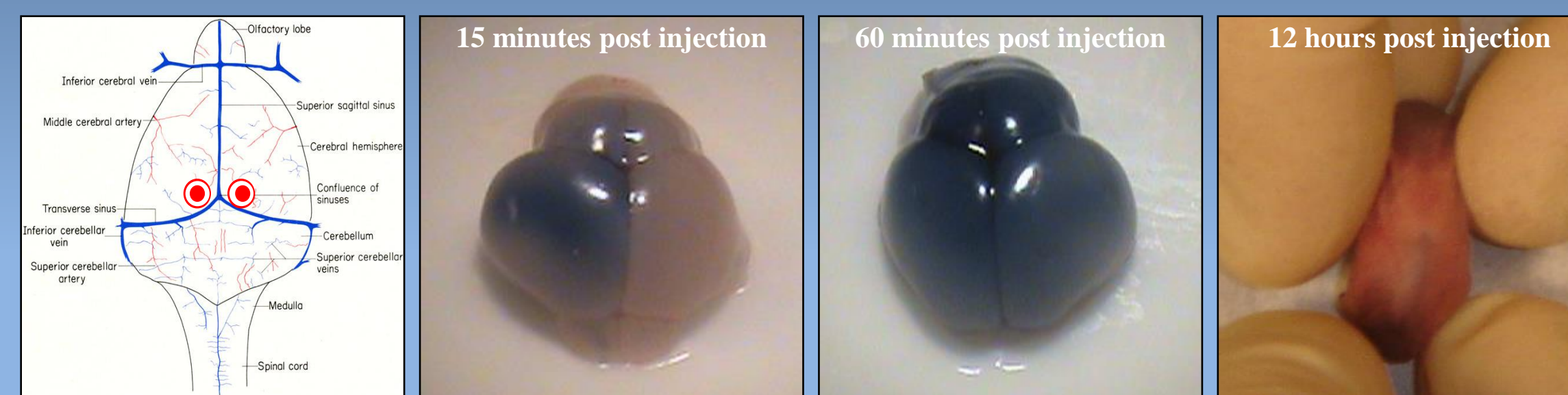


## Morpholino Modified Antisense Oligonucleotides

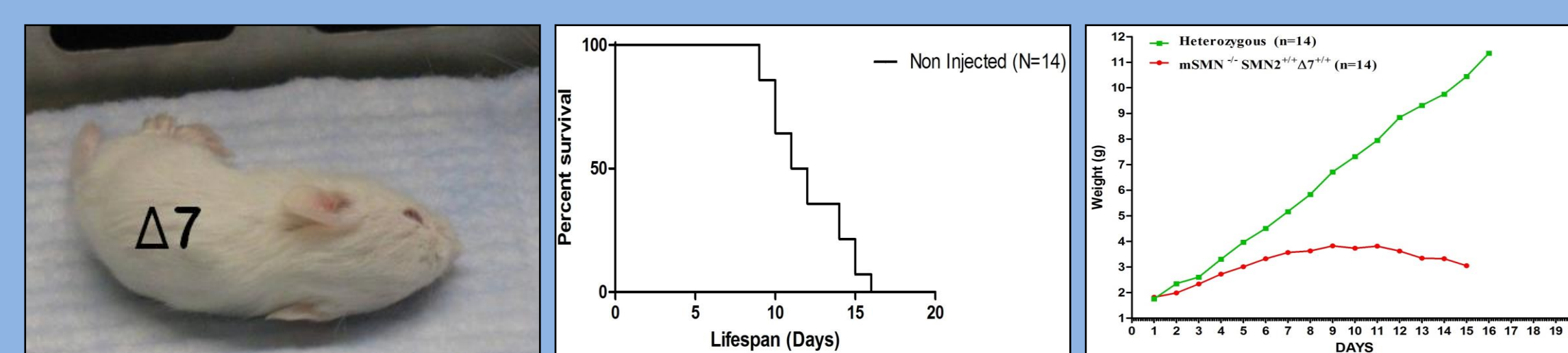
Morpholino-based ASOs are commonly used to modify RNA splicing by binding to a nucleic acid sequence and inactivating that specific section of RNA. Morpholinos have a synthetic backbone of standard nucleic acid bases that are bound to morpholine rings, making them unrecognizable to the cell. They are not degraded by nucleases and do not trigger an immune response, which allows their effects to last longer than unmodified ASOs. This makes Morpholino-based ASOs the preferred ASO for SMA treatment.



## Intracerebroventricular (ICV) Injections

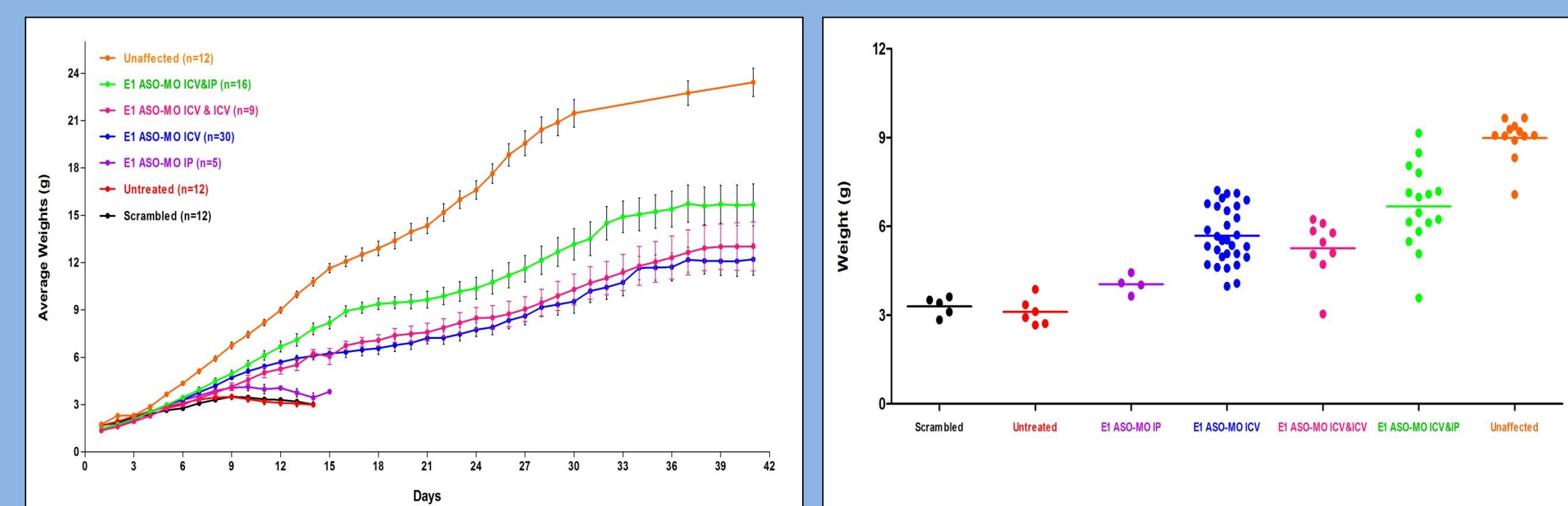


## SMN $\Delta$ 7 Mouse Models: Severe Form

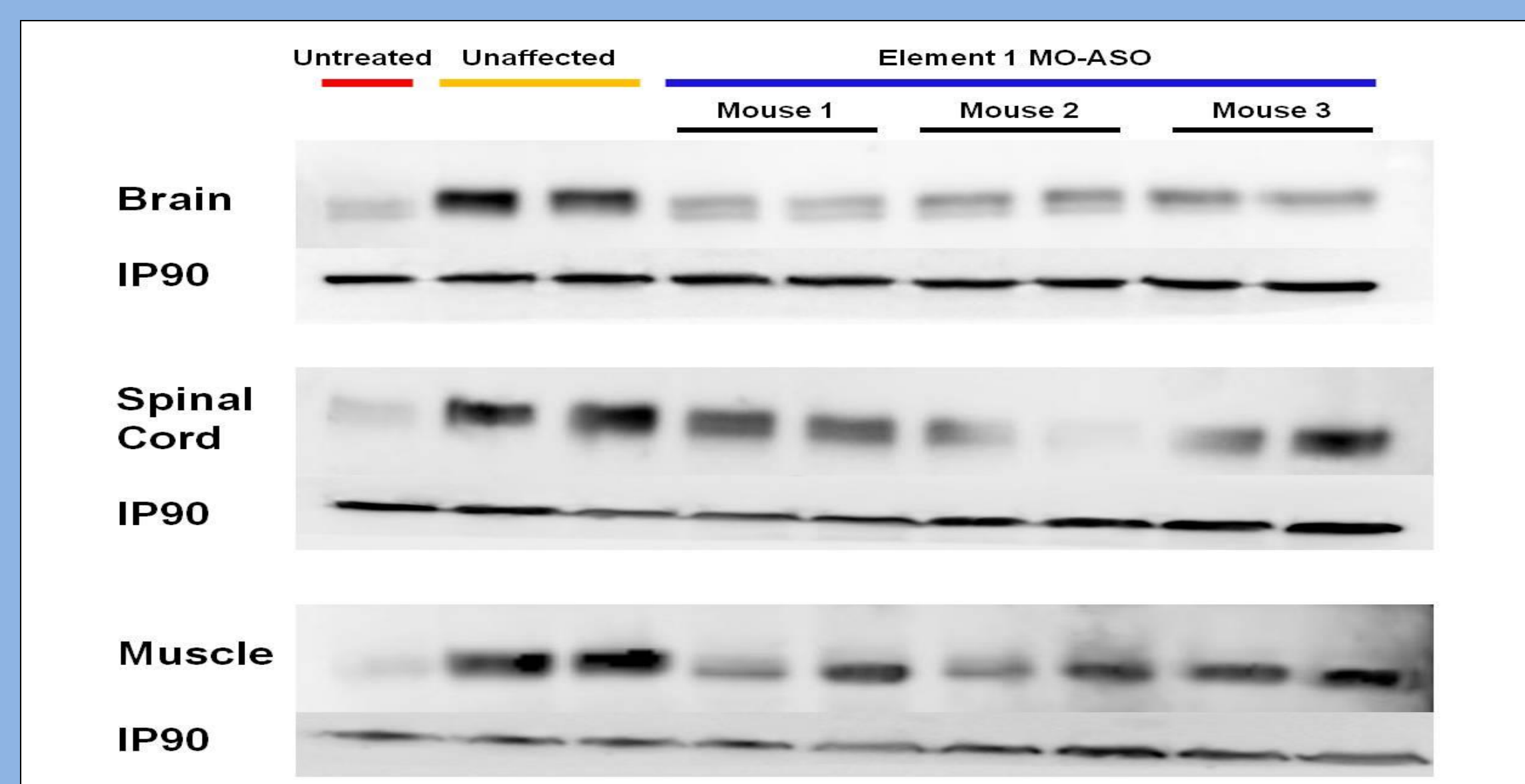


SMN  $\Delta$ 7 Model = *Smn*<sup>-/-</sup>; *SMN2*<sup>+/-</sup>;  $\Delta$ 7<sup>+/-</sup>

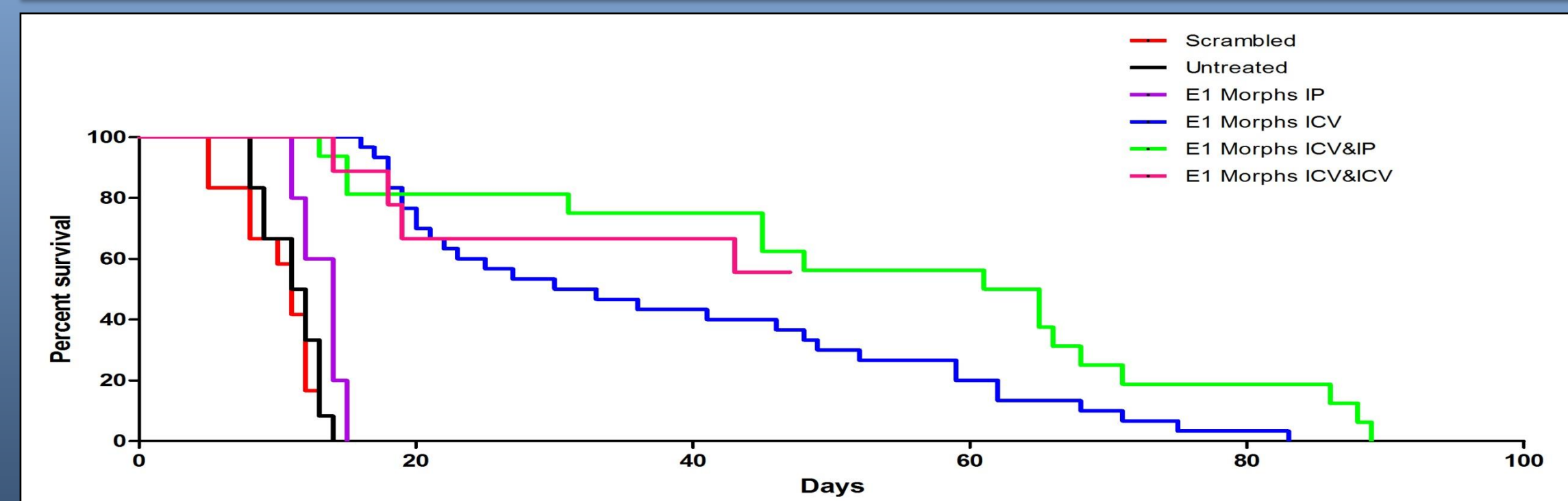
## ASO Treated SMN $\Delta$ 7 Mice: Increased Weight Gain



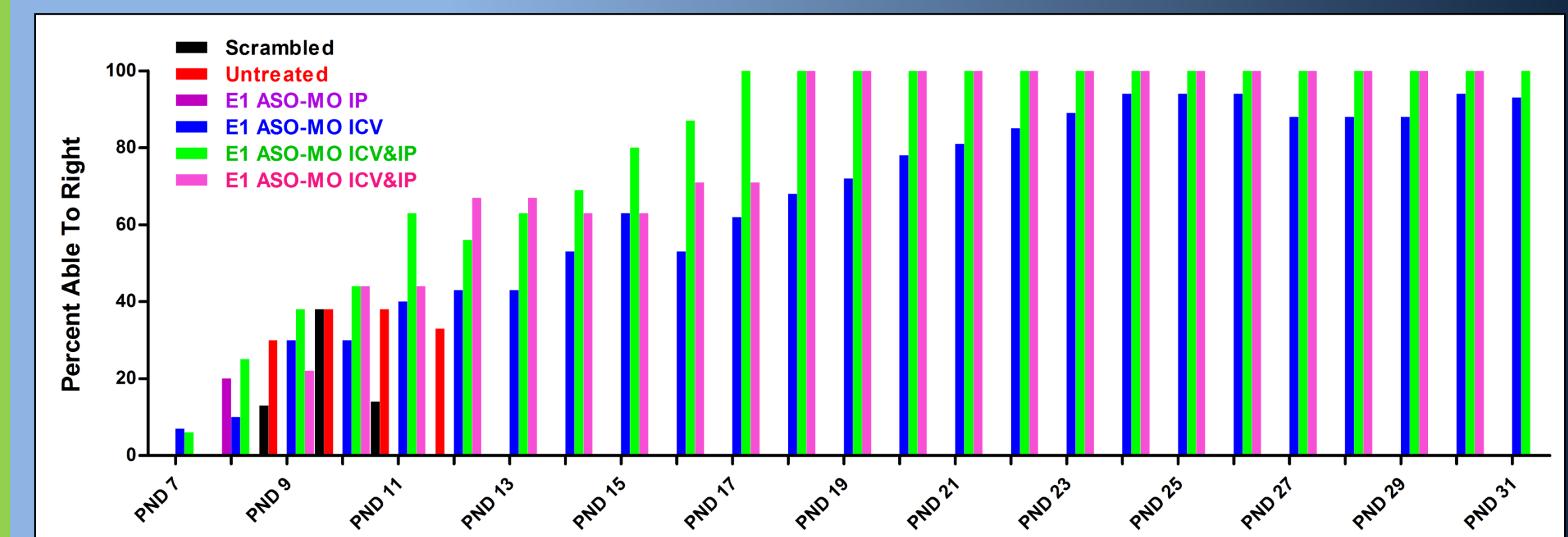
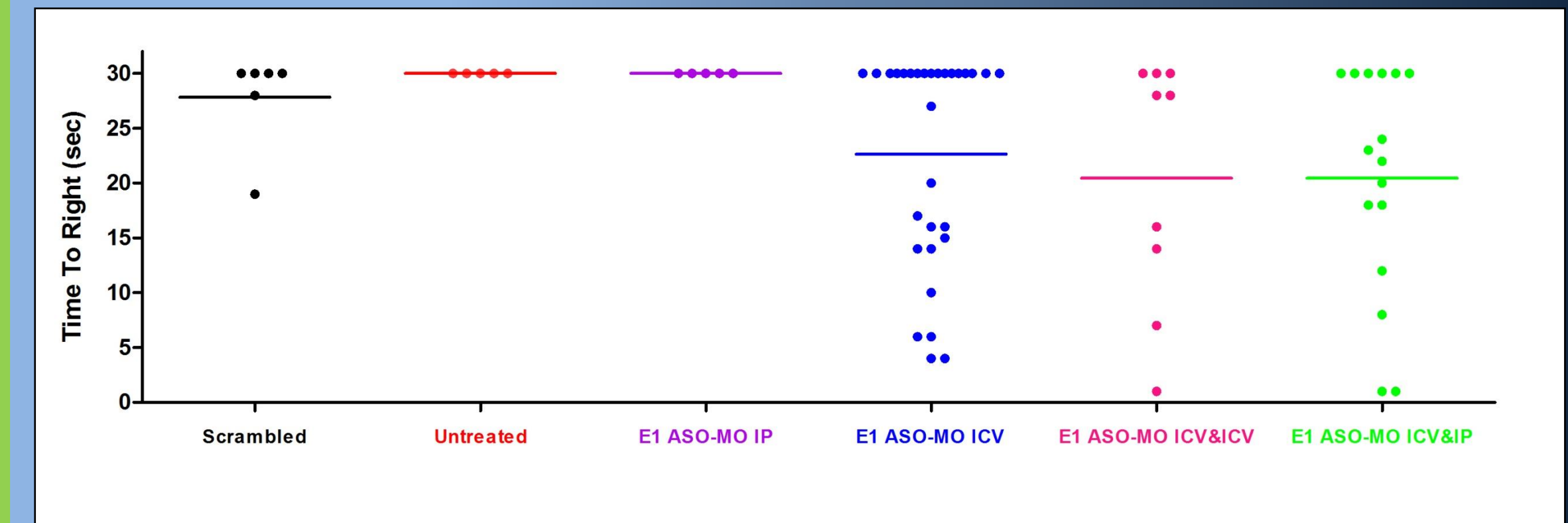
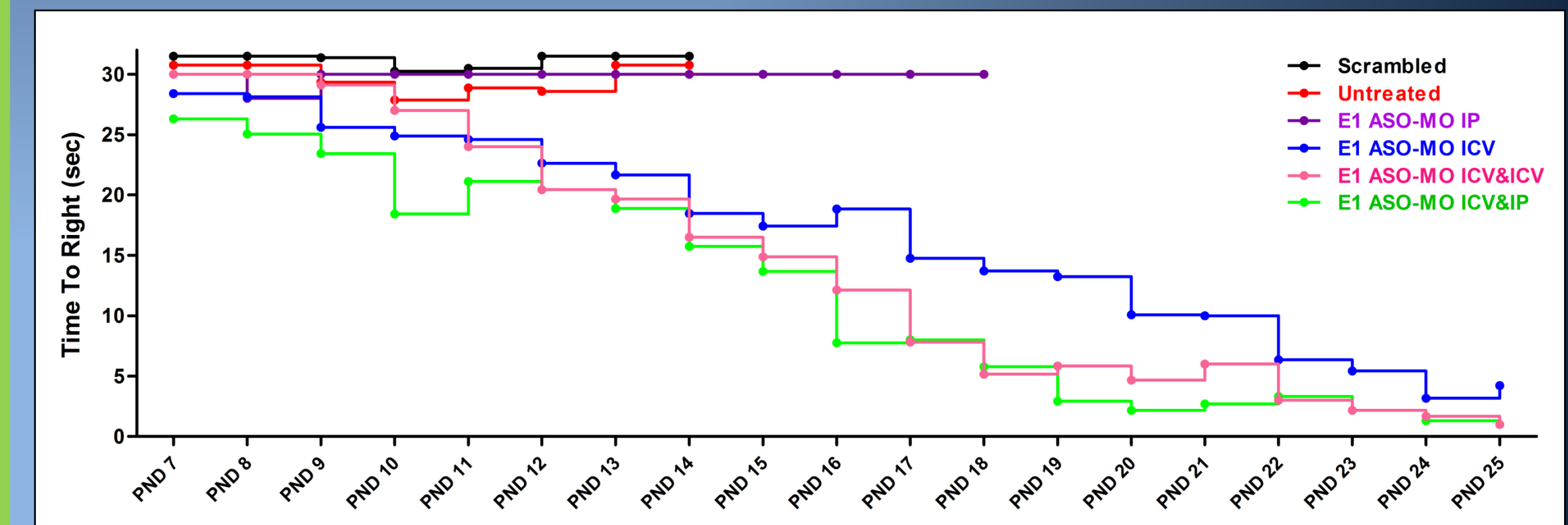
## ASO Treated SMN $\Delta$ 7 Mice: SMN Protein Induction



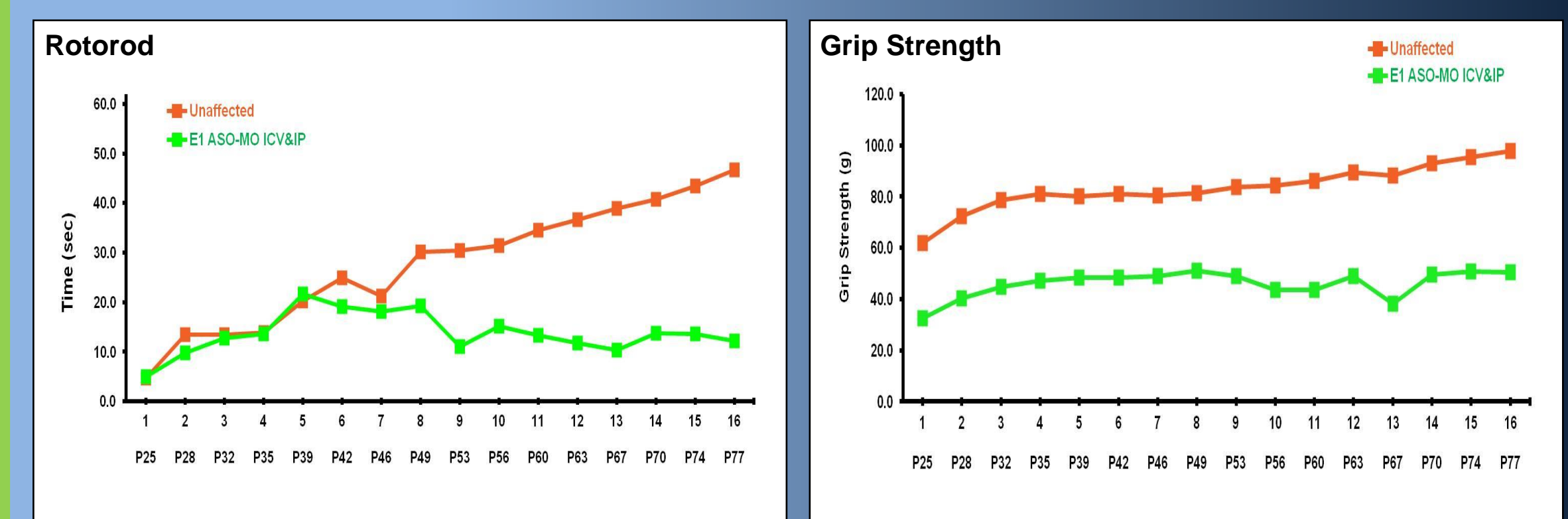
## ASO Treated SMN $\Delta$ 7 Mice: Increased Survival



## ASO Treated SMN $\Delta$ 7 Mice: Improved Time to Right



## ASO Treated SMN $\Delta$ 7 Mice: Motor Function and Strength



## Conclusions and Future Directions

SMA is especially suitable for therapeutic intervention because of the presence of SMN2, which has the ability to produce functional full-length SMN. This makes SMN2 a potent target for induction of alternative splicing events as a form of treatment therapy. ASOs are instrumental in creating these alternative splicing events that are crucial to the formation of functional SMN. Inhibition of Element 1 using Morpholino-based ASOs lead to improvement in all areas of testing in treated mice when compared to untreated SMN $\Delta$ 7 mice. This is most likely because Element 1 functions as a potent repressor of exon 7 inclusion, and inhibition of this segment of RNA by ASOs allows the inclusion of exon 7 resulting in the formation of full-length SMN. Due to the success with the severe SMN $\Delta$ 7 mouse model further research regarding Morpholino-based ASOs is being conducted using the SMN<sup>RT</sup> mouse model which represents an intermediate SMA phenotype. Success in the SMA treatment suggests ASOs may be successful therapies for additional genetic diseases.

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