

**GENE THERAPY IN SPINAL MUSCULAR ATROPHY: RNA-BASED
STRATEGIES TO MODULATE THE PRE-mRNA SPLICING of SURVIVAL
MOTOR NEURON**

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ABSTRACT

Gene expression is the required process in eukaryotes in which DNA is transcribed into pre-mRNA, spliced to produce "mature" mRNA and translated into proteins. Inaccuracies in splicing events are receiving more attention as there is the finding that nearly 59% of all genes express alternative forms of mRNA. Spinal Muscular Atrophy (SMA) the disease that this project studies is not caused by alternative RNA splicing, but rather a duplicate gene product is skewed due to an inherent alternative splicing event. To expand on the knowledge of how a mirror copy of the causative gene (SMN2) can be regulated, we developed multiple types of small RNA molecules to manipulate the alternative splicing process. This manipulation had an ultimate goal of increasing the inclusion of a required exon, exon 7. It is known in SMA if there is an increase in full length expression there is a correlative decrease in disease severity. To better understand how aberrant RNA processing events occur and how we could manipulate these signals, we identified two *trans* elements associated with survival motor neuron (SMN) pre-mRNA splicing, and targeted therapeutic RNA to this region. In addition, we used a translational approach to restore proper SMN pre-mRNA splicing by the development of bifunctional RNAs, antisense RNAs, and a multiple-antisense therapy targeting various regulator regions in and around the required exon. This work has shown the feasibility of multiple types of therapeutic RNA modalities in several assays both *in vitro* and in a disease-relevant context, the SMA mouse models.