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Use of antisense oligonucleotides to enhance exon 7 incorporation in the pre-mRNA splicing of SMN2

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Spinal muscular atrophy (SMA) is a neurodegenerative disorder that is relatively common in humans and is caused by loss of the survival motor neuron 1 (SMN1) gene. SMA is the leading cause of hereditary infant mortality by causing anterior horn cell degeneration in the spinal cord resulting in trunk and limb paralysis. The survival motor neuron 2 (SMN2) gene is located proximally to the SMN1 gene on chromosome 5q and the two genes are almost identical. Interestingly, only mutations in SMN1 cause SMA, whereas mutations in SMN2 have no clinical consequence. A differential pre-mRNA splicing event results in SMN2s failure to compensate fully for SMN1 mutations. Exon 7 is excised during SMN2 RNA splicing and this causes expression of a non-functional gene product. SMN2 produces the SMN protein at low levels (~10% compared to SMN1) but not enough to compensate for the loss of SMN1. Previous studies have shown that the use of antisense oligonucleotides can significantly decrease recognition of the 3' splice site of exon 8, resulting in an increase in increased levels exon 7 inclusion in SMN2-derived transcripts. Here, we have developed in vivo expression vectors that generate antisense oligonucleotides spanning two regions of the SMN2 transcript: a repressor exon 7 splicing "repressor" region and the exon 8 splice acceptor site. We are adding a pol III terminating sequence downstream of the antisense sequences to ensure that the clones are only making short oligos. If successful, these vectors could be used to increase SMN protein expression in an SMA context and may open a new area of SMA therapeutics, as well as providing a fundamental basis for treating other genetic disorders where splicing events are the main cause of disease.