

Precision Splice-Modulating Antisense Oligonucleotide Strategy for Spinal Muscular Atrophy Type II Functional Recovery Assessment

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DESCRIPTION

Spinal muscular atrophy represents a severe neuromuscular condition characterized by progressive degeneration of anterior horn motor neurons in the spinal cord, resulting in muscle weakness, atrophy, and impaired voluntary movement. The condition arises primarily due to deletions or mutations in the survival motor neuron 1 gene, which leads to insufficient levels of functional survival motor neuron protein. This protein plays an essential role in Ribonucleic Acid (RNA) splicing regulation and motor neuron maintenance. Although a nearly identical paralogous gene exists, its expression is typically insufficient to compensate for the loss of functional protein production under normal physiological conditions. Therapeutic strategies aimed at modulating gene expression to enhance production of functional protein from alternative transcripts have gained attention as a means to partially restore motor neuron function.

This article describes a splice-modulating antisense oligonucleotide approach designed to alter pre-mRNA splicing patterns of the survival motor neuron 2 gene in a mammalian model of spinal muscular atrophy type II. The objective of this intervention is to promote inclusion of a critical exon within the mature transcript, thereby increasing production of full-length functional survival motor neuron protein. The antisense molecules were chemically modified to enhance nuclease resistance, improve cellular uptake, and increase binding affinity to specific intronic splicing silencer regions within the pre-mRNA target sequence. These modifications included backbone stabilization and sugar ring alterations intended to prolong intracellular persistence while maintaining sequence-specific binding capacity.

Delivery of the antisense oligonucleotide system was performed through systemic administration, allowing distribution across central and peripheral nervous system tissues. The compounds demonstrated efficient penetration into spinal cord motor neurons following repeated dosing schedules, with accumulation observed in regions exhibiting pronounced neuronal loss in untreated disease models. Cellular uptake was mediated through endocytic pathways, followed by nuclear localization where

splicing modulation activity was exerted on nascent pre-mRNA transcripts.

Molecular analysis of treated spinal cord tissue demonstrated a significant increase in exon inclusion within survival motor neuron 2 transcripts. Reverse transcription polymerase-based sequencing confirmed a shift in splicing patterns toward full-length transcript production, accompanied by a reduction in truncated isoforms associated with limited functional capacity. Quantitative measurements of messenger RNA levels indicated a moderate increase in total survival motor neuron transcript abundance, suggesting improved transcript stability following successful splicing correction.

Protein expression analysis revealed increased levels of survival motor neuron protein in both spinal cord and peripheral tissue samples. Immunoblotting assays demonstrated a dose-dependent increase in protein abundance correlating with antisense exposure frequency. Subcellular localization studies confirmed appropriate distribution of the protein within nuclear structures of motor neurons, consistent with its known role in spliceosomal complex assembly and RNA processing regulation. Although protein levels did not reach those observed in unaffected control subjects, the increase was sufficient to produce measurable functional improvements in neuronal survival and synaptic maintenance.

At the muscular level, treated subjects exhibited reduced muscle fiber atrophy and improved fiber size distribution. Histochemical staining demonstrated increased presence of type II muscle fibers with improved contractile organization. Neuromuscular junction analysis revealed enhanced synaptic stability, with increased presynaptic terminal integrity and improved acetylcholine receptor clustering on postsynaptic membranes. These structural improvements correlated with functional recovery observed in motor performance assays.

Off-target analysis was conducted to evaluate potential unintended effects on alternative splicing events across the transcriptome. RNA sequencing revealed a small subset of transcripts exhibiting altered splicing patterns; however, most of these changes were transient and did not correspond to genes

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associated with neuromuscular function or neurodevelopment. Continued refinement of sequence specificity and chemical modification strategies is required to minimize these secondary effects.

Pharmacokinetic evaluation demonstrated widespread tissue distribution following systemic administration, with highest concentrations detected in liver, kidney, and spinal cord tissues. Clearance pathways primarily involved renal excretion, with measurable degradation products detected in urine samples over time. The stability of antisense molecules within central nervous system tissues was greater than in peripheral compartments, supporting their suitability for targeting neurological disorders.

Electrophysiological studies of spinal motor circuits revealed improved synaptic transmission efficiency following treatment. Motor neuron firing patterns demonstrated increased stability and reduced variability under repetitive stimulation conditions. These changes suggest partial restoration of neuromuscular signaling pathways disrupted by survival motor neuron deficiency.

Metabolic assessment indicated improved muscle energy utilization, with increased mitochondrial activity observed in

treated muscle fibers. Enzymatic assays revealed enhanced oxidative phosphorylation capacity, suggesting secondary metabolic benefits associated with improved motor neuron input. These changes contributed to improved fatigue resistance during sustained motor activity testing.

CONCLUSION

Overall findings indicate that splice-modulating antisense oligonucleotide therapy can partially restore survival motor neuron protein expression and improve neuromuscular function in models of spinal muscular atrophy type II. While complete normalization of motor neuron populations and muscular architecture was not achieved, the observed molecular, cellular, and functional improvements demonstrate the therapeutic potential of RNA-based splicing modulation strategies. Continued optimization of dosing regimens, tissue targeting efficiency, and long-term molecular stability will be necessary to enhance durability and consistency of therapeutic outcomes across disease stages.