IOWA STATE UNIVERSITY
Department of Biology

Honors Poster Presentation
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Off-target Effects of Spinal Muscular Atrophy (SMA) Therapeutics

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by progressive loss of spinal motor neurons resulting in muscular atrophy and issues in various other tissues. SMA is caused by mutation or deletion of the *Survival Motor Neuron 1* (*SMN1*) gene. *SMN1* is responsible for producing survival motor neuron (SMN) protein. SMA is the most common genetic cause of death in infants and young children. While most people have a second gene, *SMN2*, that produces SMN protein, differences between *SMN1* and *SMN2* cause exon 7 to be spliced out in the pre-mRNA stage. This truncated version of the SMN protein, called SMN2Δ7, rapidly degenerates and the full-length SMN protein generated by *SMN2* is insufficient to meet the body's needs. Risdiplam (Ris), an FDA-approved treatment for SMA, and Branaplam (Bra), an SMA treatment in clinical trials, can both increase inclusion of exon 7. Critically, they can cross the blood-brain barrier to promote *SMN2* exon 7 inclusion in motor neurons. However, they also affect splicing of other pre-mRNAs. Earlier work by our lab identified several genes that display aberrant splicing in cells treated with Ris or Bra.

Objectives

- To identify the off-target splicing effects of small molecule therapeutics of SMA.
- To better understand the mechanisms of action for Ris and Bra to improve therapeutic function.

Materials and Methods

The techniques used to complete my project included gel electrophoresis to separate DNA fragments according to length for analysis or cloning, ImageJ to quantify gel band intensity, polymerase chain reactions (PCR) to amplify DNA fragments, ligation to insert DNA fragments into plasmid backbones, transformation to introduce plasmids into bacterial cells, plasmid purification to isolate plasmids from bacterial cells, transfection to introduce a plasmid into mammalian cells, TRIzol RNA extraction to collect and isolate total RNA from mammalian cells, DNase treatment to degrade genomic DNA and plasmid in the sample, reverse transcription PCR (RT-PCR) to generate a complementary DNA strand for an RNA fragment followed by PCR, Sanger sequencing (DNA) to verify isolated fragment identity.

Results

Low Ris: 50nM, High Ris: 1000 nM, Low Bra: 2nM, High Bra: 40 nM
Below is some of the data collected by generating minigenes containing an exon that displayed aberrant splicing when exposed to Ris or Bra.

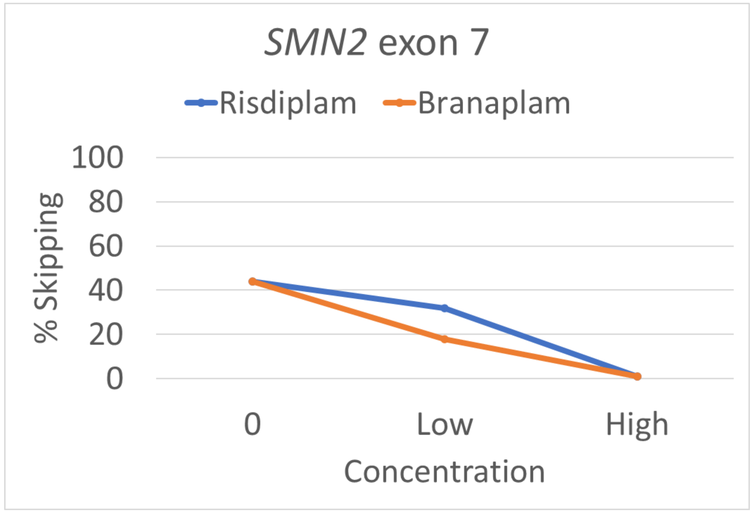

Figure 1. *SMN2* exon 7 splicing in presence of SMA therapeutics.

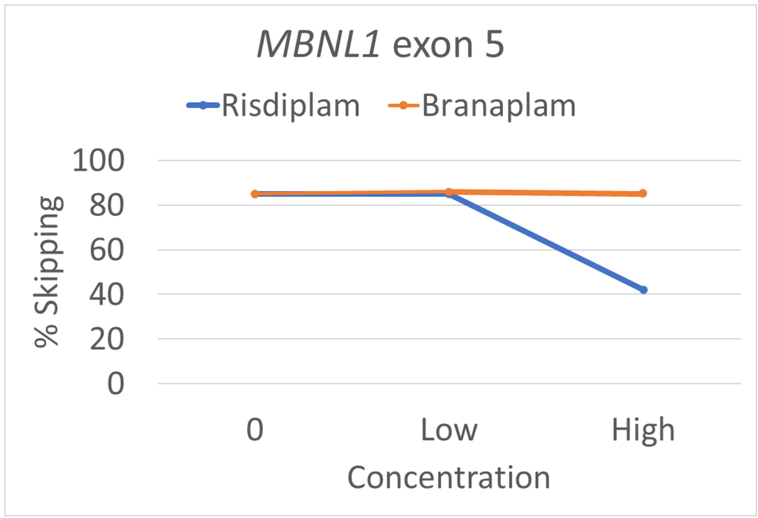

Figure 2. *MBNL1* exon 5 splicing in presence of SMA therapeutics.

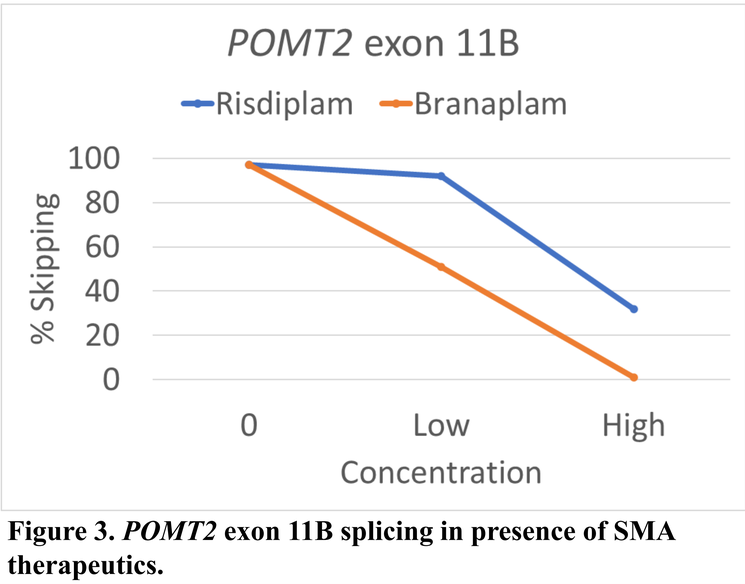

Figure 3. *POMT2* exon 11B splicing in presence of SMA therapeutics.

Conclusions

- Both Ris and Bra promote inclusion of *SMN2* exon 7. Bra is significantly more effective for promoting inclusion at lower concentrations than Ris. Low concentrations of both moderately reduce skipping suggesting a low Ris and Bra combination may be effective for SMA treatment
- High Ris is the only concentration that affected *MBNL1* exon 5 splicing. *MBNL1* regulates pre-mRNA alternative splicing. By altering *MBNL1* splicing, high Ris could have downstream splicing consequences on other genes.
- Splicing of *POMT2* exon 11B shows Ris and Bra can influence splicing of the same genes. In this case, Bra showed a much more dramatic increase in inclusion. *POMT2* is responsible for piece of the protein O-mannosyltransferase (POMT) enzyme complex. POMT is important in movement of skeletal muscles and the testes.
- Low Ris shows no effect on *MBNL1* splicing and a very weak effect on *POMT2* splicing. However, high concentrations of Ris showed significant changes to splicing. This indicates the off-target effects of Ris may mechanistically change based on concentration
- Of all the aberrant splicing events, low concentrations of Ris and Bra showed far fewer and weaker off-target effects than higher concentrations. Low Bra only triggered aberrant splicing in 9% of the genes affected by high Bra.