The Fast Skeletal Muscle Troponin Activator Reldesemtiv in Combination With Nusinersen Improves Muscle Function in a Mouse Model of Spinal Muscular Atrophy

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ABSTRACT

Reldesemtiv, formerly known as CK-2127107, is a small molecule, selective fast skeletal muscle troponin activator that sensitizes the sarcomere to calcium, leading to increased muscle force in response to sub-tetanic nerve stimulation. Nusinersen, an anti-sense oligonucleotide (ASO) that modifies pre-mRNA splicing of the SMN2 gene to increase production of full-length survival of motor neuron (SMN) protein, is an approved therapy for spinal muscular atrophy (SMA). The objective of this study was to investigate the effects of combining treatment with reldesemtiv and nusinersen on skeletal muscle function in the Hung Li mouse model of SMA. Hung Li SMA mice were treated with either a mismatch control ASO (ASO-CON) or nusinersen (ASO-10-27) on post-natal days 1 and 3 (160 µg/g, SC). At 8–10 weeks of age, wild-type FVB, ASO-CON, and nusinersen groups were dosed with either vehicle or reldesemtiv (30 mg/kg, IP) to assess the effects of nusinersen alone and in combination with reldesemtiv. All groups were evaluated in an in vivo planar flexor assay for isometric muscle force production in response to sciatic nerve stimulation. Isometric muscle force was significantly lower in Hung Li SMA mice at submaximal and tetanic rates of nerve stimulation (30 to 200 Hz) compared with wild-type FVB mice (p < 0.05). At the representative sub-tetanic frequency of 30 Hz, ASO-CON muscle force was 71% of wild-type FVB levels (wild-type: 57.0 ± 2.9 mN vs. ASO-CON: 39.4 ± 3.0 mN; mean ± standard error of the mean; SEM, n = 6/group; p < 0.05). Nusinersen treatment improved force to levels not significantly different from wild-type FVB mice (nusinersen: 49.1 ± 3.2 mN). In nusinersen-treated mice, reldesemtiv significantly increased isometric force in response to 30 Hz nerve stimulation to 267% of wild-type levels (nusinersen + reldesemtiv: 147.2 ± 17.1 mN, mean ± SEM, n = 6, p < 0.001). Treatment with nusinersen plus reldesemtiv also resulted in a leftward shift of the force-frequency curve, indicating an increase in Ca²⁺ sensitivity of the muscle. In summary, a single dose of reldesemtiv significantly increased isometric muscle force at 30 Hz to 267% of wild-type levels in Hung Li SMA mice. These results support the hypothesis that reldesemtiv in combination with nusinersen modulating therapies, such as nusinersen, can provide additional benefit to improving muscle function in SMA.

INTRODUCTION

• Spinal muscular atrophy (SMA) is a neuromuscular disorder characterized by the loss of motor neurons with a consequent decline in motor neuron function, muscle atrophy, and weakness.
• Nusinersen (ASO-10-27) is an anti-sense oligonucleotide (ASO) that modifies splicing of SMN2 to increase survival of motor neuron (SMN) protein expression.1 Nusinersen was recently approved for the treatment of all types of SMA.2
• Reldesemtiv is a small molecule, fast skeletal muscle troponin activator that sensitizes the sarcomere to calcium, leading to increased muscle force in response to sub-tetanic rates of nerve stimulation.
• The objective of this study was to investigate the effects of reldesemtiv combined with nusinersen treatment on skeletal muscle function in the Hung Li mouse model of SMA.

METHODS

Mouse models
• Hung Li SMA mice have been previously characterized and were obtained from the Jackson Laboratory (stock no. 005558; Bay Harbor, ME, USA).
• Breeder pairs of Hung Li SMA mice were mated and newborn pups were randomized by body weight to receive either nusinersen (160 µg/g, SC) or a mismatch oligo control (ASO-CON) on post-natal day 1 (PND1) and PND3 (see Study design figure below). Breeder pairs for wild-type FVB control mice were also mated to produce offspring but were not treated.
• The effect of reldesemtiv on in vivo muscle force production was assessed in wild-type FVB and SMA (untreated and nusinersen-treated) mice.

Terminal assessment of muscle force in vivo
• Isometric ankle plantar flexor muscle force was measured in vivo in Hung Li SMA and wild-type FVB control mice in the presence of vehicle (10% DMSO, 50% propylene glycol) or reldesemtiv (30 mg/kg, IP).
• Mice were placed under anesthesia with inhaled isoflurane (1%–3%). A single incision was made slightly distal to the mid-thigh to expose the sciatic nerve. Through this incision, an electrode was attached to the sciatic nerve and the peroneal nerve was severed to prevent co-contraction of the ankle dorsiflexors. The foot was taped to a footplate attached to a force transducer to measure force production (Kurz Scientific, Ontario, Canada).
• Muscle contractile properties were assessed by applying an electrical current to the nerve and recording the resulting muscle force. An isometric force-frequency relationship (10–200 Hz, 1 ms pulse width, 350 ms train duration) was assessed with the ankle joint at 90° of flexion.
• Muscle fatigue was assessed by measuring the isometric force response to 300 repetitive stimulations (30 Hz, 1 ms pulse width, 200 ms train duration, 1 stim/1.5 sec, 180/sec over 40° range)

Study design for assessing the effects of nusinersen and reldesemtiv in Hung Li SMA mice

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>Vehicle + rldesemtiv</th>
<th>ASO-CON</th>
<th>ASO-CON + rldesemtiv</th>
<th>ASO-10-27</th>
<th>ASO-10-27 + rldesemtiv</th>
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<tbody>
<tr>
<td>PND0</td>
<td>21</td>
<td>42</td>
<td>63</td>
<td>8</td>
<td>10</td>
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<tr>
<td>PND1</td>
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<td>PND2</td>
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Nusinersen and reldesemtiv treatment groups are indicated as vehicle (10% DMSO, 50% propylene glycol) or vehicle + rldesemtiv (30 mg/kg, IP) or as either ASO-CON or nusinersen. *p < 0.05 vs. wild-type FVB mouse; †p < 0.05 vs. ASO-CON Hung Li SMA mouse; +p < 0.05 vs. wild-type FVB mouse. 

RESULTS

Increased tail length, body weight, and muscle mass in Hung Li SMA mice treated with nusinersen (ASO-10-27)

<table>
<thead>
<tr>
<th>Tail Length (mm)</th>
<th>Body Weight (g)</th>
<th>Gastrocnemius Muscle Mass (mg)</th>
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</thead>
<tbody>
<tr>
<td>Hung Li SMA</td>
<td>Wild-type FVB</td>
<td>Hung Li + 10 µg/g nusinersen</td>
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<tr>
<td>Wild-type FVB</td>
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<td>100</td>
</tr>
<tr>
<td>Hung Li + 10 µg/g nusinersen</td>
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<td>100</td>
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<td>Hung Li + 40 µg/g nusinersen</td>
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• Hung Li SMA mice were treated with ASO-CON or nusinersen (40, 80, or 160 µg/g, SC) on PND1 and PND3. Hung Li mice treated with nusinersen had a dose-dependent increase in tail length (A and B). There was a significant difference in tail length by PND14.
• Nusinersen-treated Hung Li SMA mice also had an increase in body weight (C) and muscle mass (D) compared with ASO-CON-treated mice; data shown only for 160 µg/g dose for simplicity. Body weight and muscle mass data shown are values at terminal in vivo planar flexor assessment.

Study design for assessing the effects of nusinersen and reldesemtiv in Hung Li SMA mice

<table>
<thead>
<tr>
<th>Study</th>
<th>Nusinersen (160 µg/g, SC)</th>
<th>PND0 &amp; PND2</th>
<th>Single dose vehicle vs. rldesemtiv</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
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<tr>
<td></td>
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<td></td>
<td>Vehicle + rldesemtiv</td>
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<td></td>
<td>ASO-CON</td>
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<td>ASO-CON + rldesemtiv</td>
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</table>

Complete group of wild-type FVB control and rldesemtiv that were not treated were generated.

REFERENCES


SUMMARY OF FINDINGS

• Hung Li SMA mice treated with nusinersen had increased body weight, tail length, and muscle mass, conferring efficacy of treatment.
• Wild-type FVB and Hung Li SMA mice treated with either ASO-CON or nusinersen were subjected to sciatic nerve stimulation at frequencies ranging from 10–200 Hz following a single dose with vehicle or reldesemtiv (30 mg/kg, IP).
• Nusinersen treatment resulted in an increase in force at higher stimulation frequencies (50–200 Hz) compared with ASO-CON-treated SMA mice, and reldesemtiv resulted in a leftward shift of the force-frequency curve in both ASO-CON and nusinersen-treated SMA mice. Wild-type FVB mice without reldesemtiv are not shown to simplify figure (A).
• Reldesemtiv further augmented force production at 50 Hz for control wild-type FVB mice, and Hung Li SMA mice treated with ASO-CON and nusinersen (B).

CONCLUSIONS

• Nusinersen treatment resulted in a dose-dependent increase in sciatic nerve stimulation at frequencies ranging from 10–200 Hz following a single dose with vehicle or reldesemtiv (30 mg/kg, IP).
• Nusinersen treatment increased isometric force in response to 10–200 Hz nerve stimulation.
• Nusinersen and rledesemtiv together increased isometric force in vivo to 71% of wild-type FVB levels. Nusinersen treatment increased isometric force in response to 30 Hz nerve stimulation to 267% of wild-type levels. These results support the hypothesis that rledesemtiv in combination with nusinersen modulating therapies, such as nusinersen, can provide additional benefit to improving muscle function in SMA.

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