The Fast Skeletal Muscle Troponin Activator, *Reldesemtiv*, in Combination With SMN-C1 Improves Muscle Function in a Mouse Model of Spinal Muscular Atrophy

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ABSTRACT

Reldesemtiv is a small molecule, selective fast skeletal muscle troponin activator that increases muscle force in response to neural stimulation by increasing sarcomere calcium sensitivity. SMN-C1 is a small molecule regulator of *SMN2* splicing that results in increased survival of motor neuron (SMN) protein expression. The objective of this study was to investigate the effects of *reldesemtiv* combined with SMN-C1 treatment on skeletal muscle function in the Hung Li mouse model of spinal muscular atrophy (SMA). Hung Li SMA mice were treated with SMN-C1 (10 mg/kg, IP) daily from post-natal day 1 through study completion. At 12–13 weeks of age, wild-type FVB, Hung Li SMA untreated, and Hung Li SMN-C1–treated mice were administered either vehicle or *reldesemtiv* (30 mg/kg, IP) to assess the effects of SMN-C1 alone and in combination with *reldesemtiv*. All groups were evaluated in an *in vivo* plantar flexor assay to assess 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 neural stimulation frequencies (30 to 200 Hz) compared with wild-type FVB mice. In response to sub-tetanic 30 Hz nerve stimulation, force in untreated SMA mice was 60% of wild-type FVB levels (wild-type FVB: 67.8 ± 9.2 mN vs. Hung Li SMA untreated: 40.7 ± 3.2 mN; mean ± SEM, n = 6/group; $p < 0.01$). In SMN-C1–treated mice, 30 Hz force was 76% of wild-type FVB levels (Hung Li SMA SMN-C1–treated: 51.4 ± 3.6 mN). In SMN-C1–treated mice, *reldesemtiv* significantly increased isometric force in response to 30 Hz nerve stimulation to 176% of wild-type FVB levels (SMN-C1 + *reldesemtiv*: 119 ± 17.1 mN; mean ± SEM, n = 6; $p < 0.01$). Treatment with SMN-C1 plus *reldesemtiv* also resulted in a leftward shift of the force-frequency curve, demonstrating an increase in Ca^{2+} sensitivity in muscles from SMN-C1–treated mice. In summary, treatment with *reldesemtiv* significantly increased force at sub-tetanic stimulation frequencies in SMN-C1–treated SMA mice. These results support the hypothesis that *reldesemtiv* in combination with *SMN2* modulating therapies, such as SMN-C1, can further improve muscle function in SMA.

INTRODUCTION

- Spinal muscular atrophy (SMA) is a neuromuscular disorder characterized by the loss of motor neurons with a consequential decline in motor nerve function, muscle atrophy, and weakness
- SMN-C1 is a small molecule that modifies splicing of *SMN2* to increase survival of motor neuron (SMN) protein expression[1]
- *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 SMN-C1 treatment on skeletal muscle function in the Hung Li mouse model of SMA

METHODS

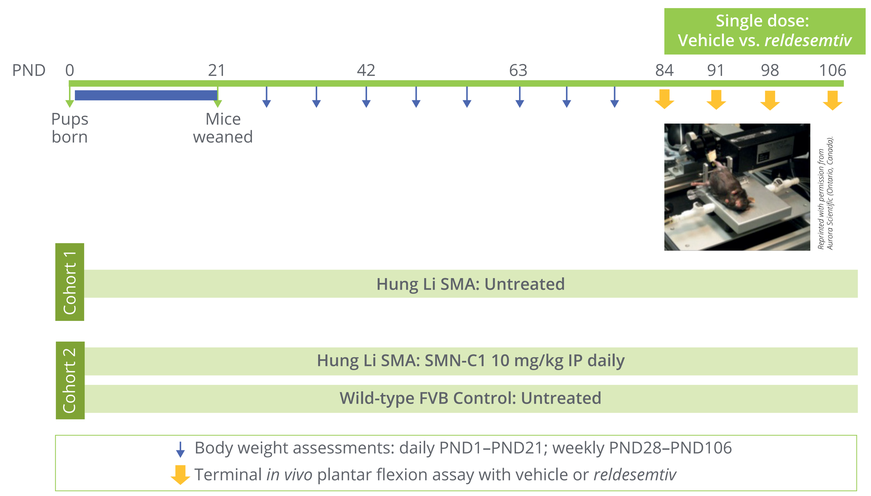
Mouse models

- Hung Li SMA mice have been previously characterized[2] and were obtained from the Jackson Laboratory (stock no. 005058; Bay Harbor, ME, USA)
- Breeder pairs of Hung Li SMA mice were mated and newborn pups were either untreated or treated daily with SMN-C1 (10 mg/kg, IP) starting on post-natal day 1 (PND1) until study termination (PND106) (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 SMN-C1–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% DMA: 50% propylene glycol: 16% 2-hydroxypropyl-beta-cyclodextrin) 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 dorsi flexors. The foot was taped to a footplate attached to a force transducer to measure force production (Aurora 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 isokinetic force response to 300 repetitive stimulations (30 Hz, 1ms pulse width, 200 ms train duration, 1 stim/1.5 sec, 180°/sec over 40° range)

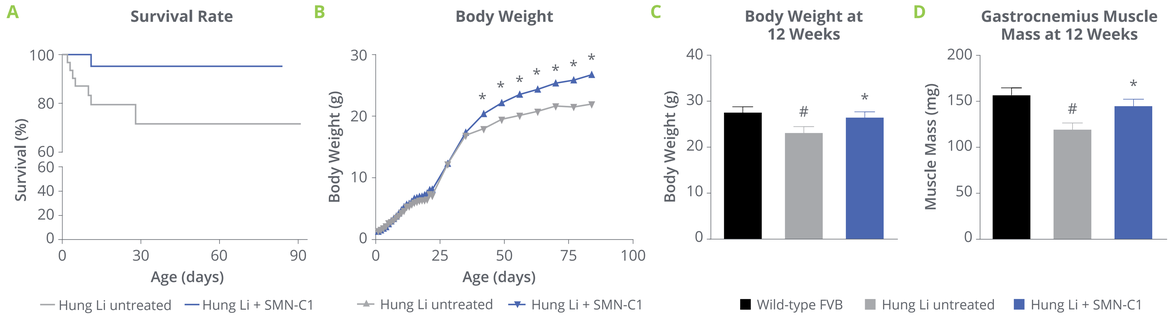
Study design for assessing effects of SMN-C1 and *reldesemtiv* in Hung Li SMA mice



- Hung Li SMA and wild-type FVB (control) mice were generated from breeder pairs
- In Cohort 1, Hung Li SMA mice were untreated
- In Cohort 2, Hung Li SMA mice were treated with SMN-C1 (10 mg/kg, IP) daily from PND1 through end of study (PND106)
- Control wild-type FVB mice were not treated
- Between PND85 and PND106, wild-type FVB control and SMA mice were evaluated for muscle function using an *in vivo* muscle plantar flexor assay following a single dose of vehicle or *reldesemtiv* (30 mg/kg, IP)

RESULTS

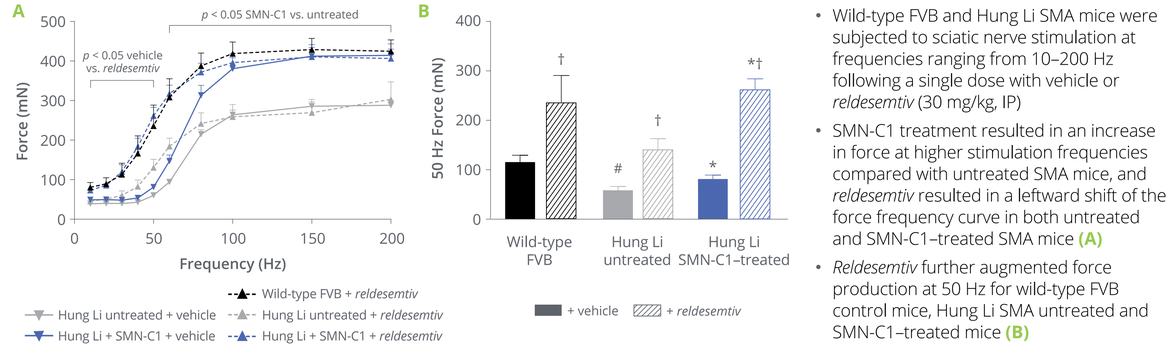
Increased survival, body mass, and muscle mass in Hung Li SMA mice treated with SMN-C1



- Hung Li SMA mice treated with SMN-C1 (10 mg/kg daily, IP) from PND1 to PND91 had an increased survival rate **(A)** and increased body weight from PND42 to PND84 **(B)**
- At the time of the final muscle function studies, body weight **(C)** and muscle mass **(D)** were significantly reduced in Hung Li SMA mice compared with wild-type FVB mice, but were not different in SMN-C1–treated Hung Li SMA mice

All data are expressed as mean ± standard error of the mean; survival rate: n = 45 for Hung Li untreated, n = 20 for Hung Li + SMN-C1; body weight: n = 32 for Hung Li untreated, n = 19 for Hung Li + SMN-C1; body weight at 12 weeks and gastrocnemius muscle mass at 12 weeks: n = 12 for all groups.
* $p < 0.05$ vs. Hung Li SMA untreated mice; # $p < 0.05$ vs. wild-type FVB mice.

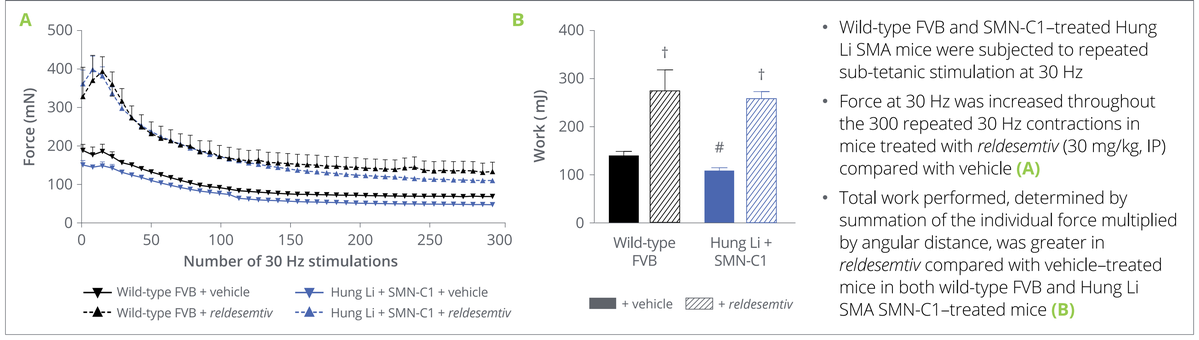
SMN-C1 increases high frequency force and *reldesemtiv* causes a leftward shift of the force-frequency curve in Hung Li SMA mice



- Wild-type FVB and Hung Li SMA mice 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)
- SMN-C1 treatment resulted in an increase in force at higher stimulation frequencies compared with untreated SMA mice, and *reldesemtiv* resulted in a leftward shift of the force frequency curve in both untreated and SMN-C1–treated SMA mice **(A)**
- *Reldesemtiv* further augmented force production at 50 Hz for wild-type FVB control mice, Hung Li SMA untreated and SMN-C1–treated mice **(B)**

All data are expressed as mean ± standard error of the mean; n = 8 for Hung Li untreated + vehicle or *reldesemtiv*; n = 6 for all other groups.
* $p < 0.05$ vs. Hung Li SMA untreated mice; # $p < 0.05$ vs. wild-type FVB mice; † $p < 0.05$ vehicle vs. *reldesemtiv*.

Reldesemtiv increases force throughout a bout of repeated muscle contractions and increases total work performed



- Wild-type FVB and SMN-C1–treated Hung Li SMA mice were subjected to repeated sub-tetanic stimulation at 30 Hz
- Force at 30 Hz was increased throughout the 300 repeated 30 Hz contractions in mice treated with *reldesemtiv* (30 mg/kg, IP) compared with vehicle **(A)**
- Total work performed, determined by summation of the individual force multiplied by angular distance, was greater in *reldesemtiv* compared with vehicle–treated mice in both wild-type FVB and Hung Li SMA SMN-C1–treated mice **(B)**

All data are expressed as mean ± standard error of the mean; n = 6 for all groups.
# $p < 0.05$ vs. wild-type FVB; † $p < 0.05$ vehicle vs. *reldesemtiv*.

SUMMARY OF FINDINGS

- **Hung Li SMA mice treated with SMN-C1 (10 mg/kg daily, IP) had increased survival, body weight, and muscle mass after PND42**
- **Treatment with SMN-C1 increased isometric muscle force *in vivo* in response to nerve stimulation, with increased absolute force significant in the mid- to high-stimulation frequencies (50–200 Hz)**
- **Single doses of *reldesemtiv* (30 mg/kg, IP) increased isometric force *in vivo* in response to sub-tetanic nerve stimulation in both untreated and SMN-C1–treated Hung Li SMA mice in the low- to mid-stimulation frequencies (10–60 Hz)**
- **There was an additive benefit of SMN-C1 and *reldesemtiv* treatment in Hung Li SMA mice at the mid-frequency range (50–60 Hz)**
- **These results suggest that *reldesemtiv* treatment in combination with *SMN2* modulating therapies such as SMN-C1 can further improve muscle function in SMA**

References

1. Zhao, et al. *Hum Mol Genet*. 2016;5:1885–1899.

2. Hsieh-Li, et al. *Nat Genet*. 2000;24:66–70.

Disclosures

Edell, **Ferrufino**, **Thomsen**, **Hwee**, **Morgan**, **Malik**, and **Chin** were all employees of Cytokinetics, Inc. at the time of the study and were compensated financially for their work.

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In collaboration with Astellas Pharma, Inc., Cytokinetics is developing *reldesemtiv* as a potential treatment for people living with SMA and certain other debilitating diseases and conditions associated with skeletal muscle weakness and/or fatigue.

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