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

Samantha Edell, Carolina Ferrufino, Karen Thomsen, Darren T. Hwee, Bradley P. Morgan, Fady I. Malik, Eva R. Chin

Cytokinetics, Inc., South San Francisco, CA

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* plantar 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 ± 1.3 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 Ca^{2+} sensitivity of the muscle. In summary, a single dose of *reldesemtiv* significantly increased submaximal force in nusinersen-treated SMA mice. These results support the hypothesis that *reldesemtiv* in combination with *SMN2* 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 consequential decline in motor nerve 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

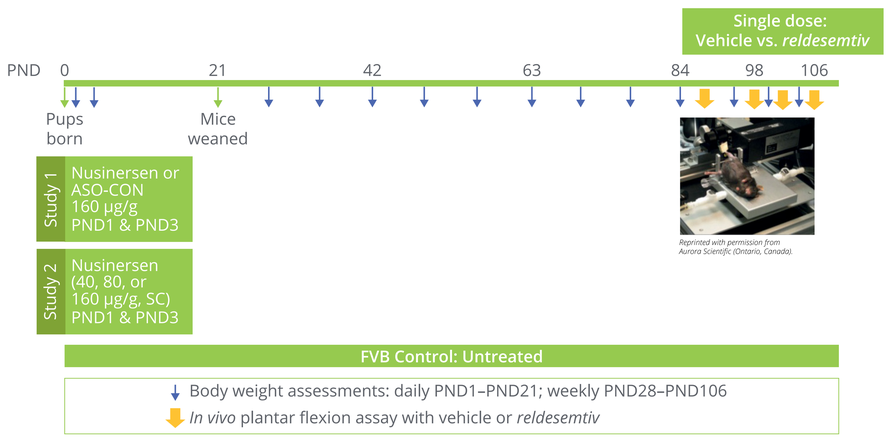
Mouse models

- Hung Li SMA mice have been previously characterized[3] 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 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% 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, 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



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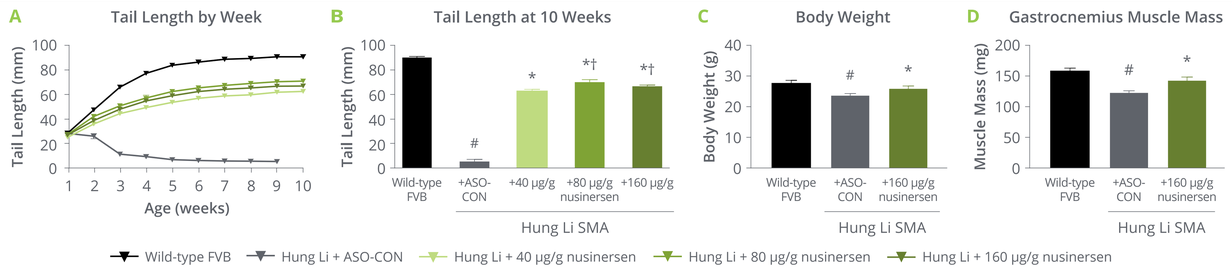
- Hung Li SMA mice were generated from 5 breeding pairs
- In Study 1, Hung Li SMA newborn pups were weighed on PND1, and then randomized within each litter to either nusinersen (160 µg/g, SC) or ASO control (ASO-CON; 160 µg/g, SC) treatment on PND1 and PND3
- In Study 2, Hung Li SMA newborn pups were randomized to receive either 40, 80, or 160 µg/g nusinersen (SC) on PND1 and PND3
- A control group of wild-type FVB offspring that were not treated was generated
- Between PND85 and PND106, wild-type FVB 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

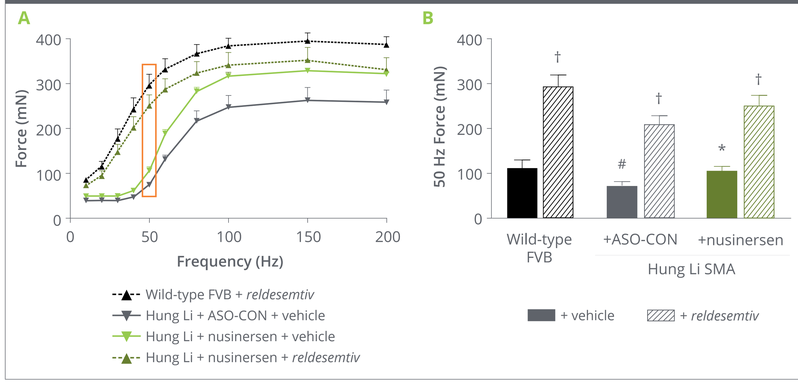
Increased tail length, body weight, and muscle mass in Hung Li SMA mice treated with nusinersen (ASO-10-27)



- 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* plantar flexor assessment

All data are expressed as mean ± standard error of the mean; Tail length measurements: n = 25 for wild-type FVB, n = 18 for all other groups; body weight and gastrocnemius muscle mass: n = 12 for all groups.
# $p < 0.05$ vs. wild-type FVB; * $p < 0.05$ for nusinersen-treated groups vs. wild type FVB; † $p < 0.05$ vs. nusinersen 40 µg/g.

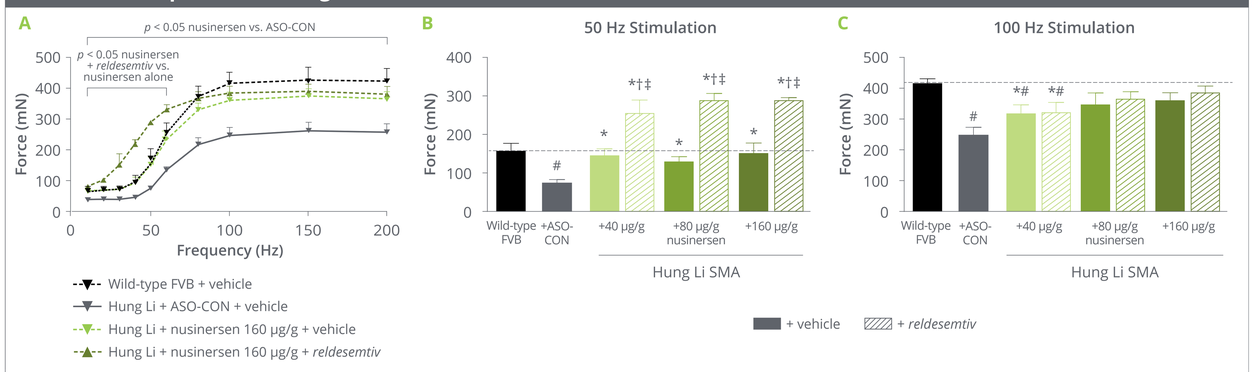
Reldesemtiv increases force in nusinersen-treated Hung Li SMA mice at sub-tetanic stimulation frequency



- 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)**

Solid bars are vehicle-treated mice; hatched bars are *reldesemtiv*-treated mice. All data are expressed as mean ± standard error of the mean; n = 6 for all groups.
# $p < 0.05$ vs. wild-type FVB mice; * $p < 0.05$ vs. ASO-CON Hung Li SMA mice; † $p < 0.05$ vehicle vs. *reldesemtiv*.

Nusinersen increases force at tetanic nerve stimulation frequencies while *reldesemtiv* increases force at sub-tetanic nerve stimulation frequencies in Hung Li SMA mice



- 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 across frequencies (10–200Hz) compared with ASO-CON–treated SMA mice **(A)**
- Force at a sub-tetanic (50 Hz) **(B)** and tetanic (100 Hz) **(C)** nerve stimulation frequency for wild-type FVB control mice, Hung Li SMA ASO-CON– and nusinersen-treated mice. In all groups, *reldesemtiv* further augmented force production at 50 Hz

Solid bars are vehicle-treated mice; hatched bars are *reldesemtiv*-treated mice. All data are expressed as mean ± standard error of the mean; n = 6 for all groups.
# $p < 0.05$ for ASO-CON–treated Hung Li SMA mice vs. wild-type FVB mice; * $p < 0.05$ for ASO-CON– vs. nusinersen-treated Hung Li SMA mice; † $p < 0.05$ vehicle vs. *reldesemtiv*; ‡ $p < 0.05$ vs. wild-type FVB mice.

SUMMARY OF FINDINGS

- **Hung Li SMA mice treated with nusinersen had increased body weight, tail length, and muscle mass, confirming efficacy of treatment**
- **Hung Li SMA mice treated with nusinersen had dose-dependent increases in isometric force *in vivo* in response to tetanic (100 Hz) nerve stimulation**
- **Single doses of *reldesemtiv* increased isometric force *in vivo* in response to sub-tetanic (50 Hz) nerve stimulation in all groups of Hung Li SMA mice (ie, ASO-CON– and nusinersen-treated at doses of 40, 80, and 160 µg/g)**
- **These results suggest that *reldesemtiv* treatment in combination with *SMN2* modulating therapies such as nusinersen can further improve muscle function in SMA by increasing muscle force production in a complementary fashion across a range of nerve stimulation frequencies**

References

1. Hua, et al. *Nature.* 2011;478:123–126.
2. SPINRAZA (nusinersen) prescribing information. Biogen, Cambridge, MA; 2018.
3. 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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