The Fast Skeletal Muscle Troponin Activator, CK-2127107, Improves Muscle Function in Mouse Models of Spinal Muscular Atrophy

Darren T. Hwee[1], Ph.D., Christine J. DiDonato[2], Ph.D., Fady I. Malik[1], M.D., Ph.D. and Eva R. Chin[1], Ph.D.

[1]Cytokinetics, Inc., South San Francisco, CA., [2]Lurie Children's Hospital of Chicago/Northwestern University, Chicago, IL.

ABSTRACT

CK-2127107 is small molecule fast skeletal muscle-specific troponin activator that sensitizes the sarcomere to calcium, leading to increased muscle force in response to sub-tetanic nerve stimulation. The objective of this study was to investigate the effect of CK-2127107 on skeletal muscle function in two mouse models of spinal muscular atrophy (SMA) with varying disease severity. The 2B/2B-Neo and Hung Li models of SMA, corresponding to an intermediate and adult-onset SMA phenotype, respectively, were evaluated *in situ* for plantarflexor isometric muscle force production in response to sciatic nerve stimulation. 2B/2B-Neo SMA mice, characterized by reduced compound muscle action potentials and motor unit number estimation, had hindlimb muscle atrophy compared to sibling controls (2B/CON).

Isometric muscle force *in situ* was significantly lower in 2B/2B-Neo SMA mice at all submaximal and tetanic rates of nerve stimulation (10 to 200Hz) compared to 2B/CON (n=11-12/group; $p < 0.0001$). In 2B/2B-Neo SMA mice, CK-2127107 (30 mg/kg, IP) significantly increased isometric force in response to 30Hz nerve stimulation (Vehicle: 27 ± 2.8mN vs. CK-2127107: 69 ± 2.7mN, mean ± S.E.M, n=4-5/group $p<0.001$) and resulted in a leftward shift of the force-frequency curve. The adult-onset Hung Li mice also had significant muscle atrophy and a decrease in muscle force production compared to controls (HL/CON), including reduced force at 30Hz (n=8-10; $p<0.05$). In the Hung Li SMA mice CK-2127107 (30 mg/kg, IP) significantly increased isometric force in response to 30Hz nerve stimulation (Vehicle: 89 ± 6.8 mN vs. CK-2127107: 151 ± 10.2 mN, mean ± S.E.M, $p<0.05$, n=4-8/group, $p<0.001$) and resulted in a leftward shift of the force-frequency curve. In summary, single doses of CK-2127107 significantly increased submaximal force *in situ* in two models of SMA mice. These results suggest that CK-2127107 and other fast skeletal muscle troponin activators may be viable therapeutics for improving muscle function in spinal muscular atrophy.

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
- The small molecule CK-2127107 is a specific 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 effect of CK-2127107 on skeletal muscle function in two mouse models of spinal muscular atrophy (SMA) with varying disease severity

METHODS

- SMA mouse models:
  - 2B/2B-Neo intermediate SMA mice were developed and maintained at Lurie Children's Hospital of Chicago (Chicago, IL).
    - § As a confirmation of neuromuscular dysfunction, compound muscle action potentials (CMAPs) and motor unit number estimation (MUNE) were measured in control and 2B/2B-Neo mice according to previously described methods[1,2].
  - Hung Li SMA mice have been previously characterized[3] and were obtained from the Jackson Laboratory (stock no. 005058).
- Assessment of muscle force *in situ*
  - Isometric ankle plantarflexor muscle force was measured *in situ* in 2B/2B-Neo SMA, Hung Li SMA, and their respective control mice in the presence of vehicle (10% DMA: 50% propylene glycol (PG): 16% Cavitron, IP) or CK-2127107 treatment (10 and 30 mg/kg, IP).
  - Mice were placed under anesthesia with inhaled isoflurane (1-3%). An incision was made on the mid-thigh region of the right leg to expose the sciatic nerve. To prevent co-contraction of the ankle dorsiflexors, a second incision was made lateral to the patella to isolate and sever the peroneal nerve. An electrode was attached to the sciatic nerve and the foot was taped to a footplate attached to a force transducer (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, 1ms pulse width, 350 ms train duration) was assessed with the ankle joint at 90° of flexion.

RESULTS

SMA MOUSE MODEL CHARACTERISTICS

	CMAP (mV)	MUNE (#)	Body Mass (g)	GA Muscle Mass (mg)
Control (2B/2B-Neo) (n=12)	33.1 ± 1.3	349.2 ± 9.6	26.7 ± 0.7	168.1 ± 4.9
2B/2B-Neo (n=12)	25.9 ± 0.9***	193.3 ± 13.8****	19.4 ± 0.6****	72.5 ± 2.4****
Control (Hung Li) (n=19)	–	–	30.9 ± 0.5	174.1 ± 1.7
Hung Li (n=20)	–	–	27.4 ± 0.5****	165.7 ± 2.6*

Table 1. SMA mouse models demonstrate indices of neuromuscular dysfunction. Relative to age-matched control mice, 2B/2B-Neo mice had lower compound muscle action potential (CMAP) and motor unit number estimation (MUNE) values. Both 2B/2B-Neo and Hung Li SMA mice had significantly lower body and gastrocnemius (GA) muscle mass. Data is expressed as mean ± SEM.

*p<0.05, ***p<0.001, ****p<0.0001 vs. respective control

RESULTS (CONTD.)

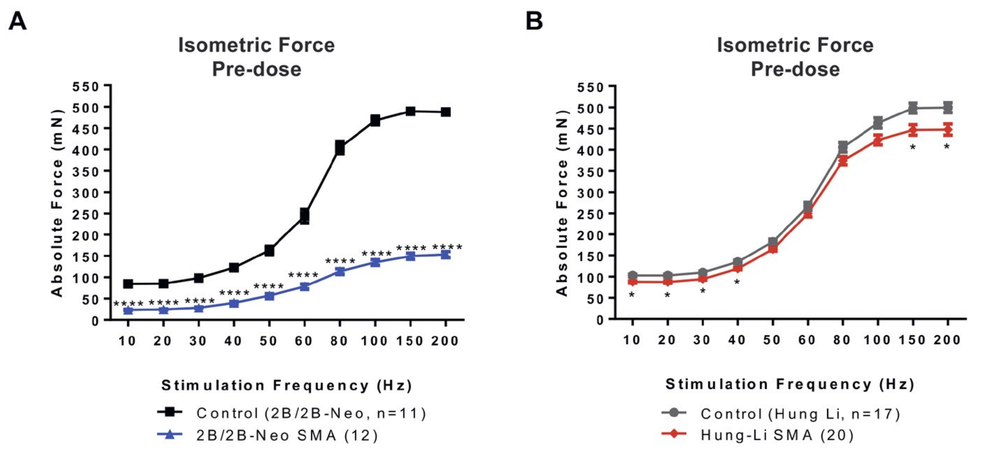


Figure 1. SMA mice produced less force *in situ* than control mice. Control and SMA mice were subjected to sciatic nerve stimulation at frequencies ranging from 10-200 Hz. **A.** 2B/2B-Neo SMA mice produced significantly lower isometric absolute force at all stimulation frequencies. **B.** Hung Li SMA produced significantly lower isometric absolute force at select sub-tetanic and tetanic stimulation frequencies. All data are expressed as mean ± SEM.

*p<0.05, ****p<0.0001 Control vs. SMA at respective stimulation frequency.

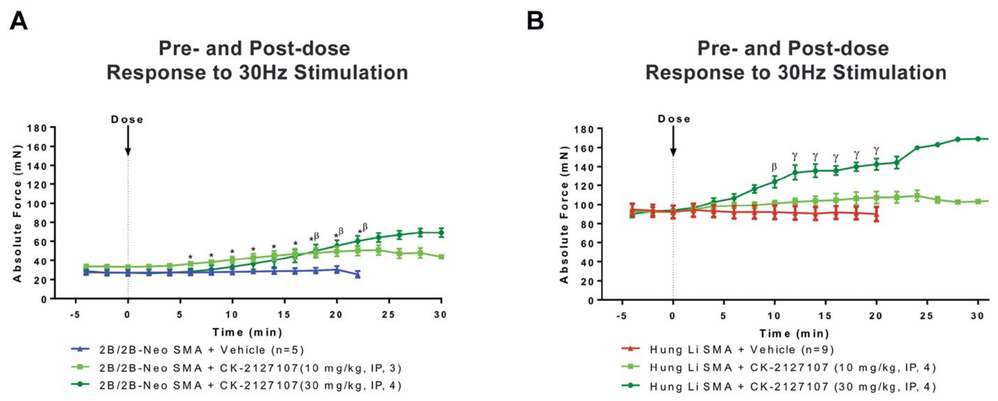


Figure 2. CK2127107 increased the muscle force response to 30Hz nerve stimulation. A. 2B/2B-Neo SMA and **B.** Hung Li SMA mice were dosed with vehicle or CK-2127107 (10 or 30 mg/kg, IP) and subjected to 30 Hz stimulations every minute. The force response to vehicle treatment was unchanged during the stimulation period. CK-2127107 increased muscle force in a dose-dependent manner in both SMA groups. All data are expressed as mean ± SEM.

*p<0.05 vehicle vs. 10 mg/kg CK-2127107. $^{\beta}p<0.05$, $^{\gamma}p<0.01$ vehicle vs. 30 mg/kg CK-2127107

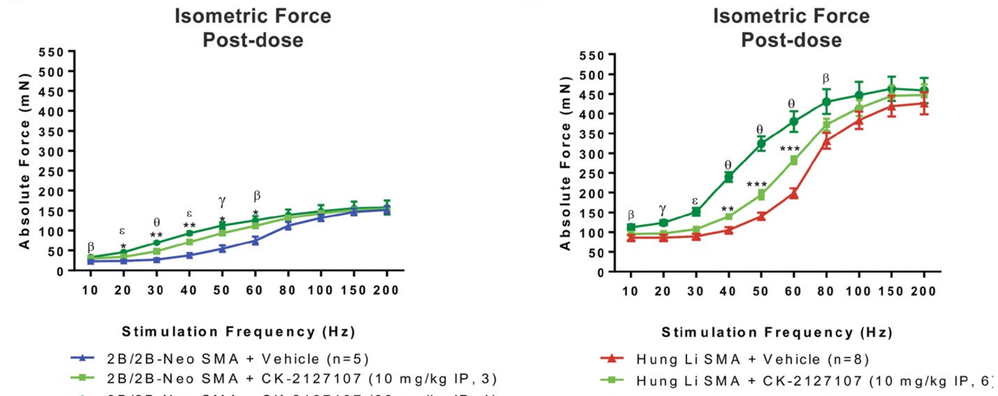


Figure 3. CK-2127107 caused a leftward shift in the force-frequency response in both SMA mouse models. SMA mice were subjected to sciatic nerve stimulation at frequencies ranging from 10-200 Hz following a single dose with vehicle or CK-2127107 (10 or 30 mg/kg, IP). At sub-tetanic stimulation frequencies, CK-2127107 increased force in a dose-dependent manner in both the **A.** 2B/2B-Neo SMA and **B.** Hung Li SMA mice. All data are expressed as mean ± SEM.

*p<0.05, **p<0.01, ***p<0.001 vehicle vs. 10 mg/kg CK-2127107. $^{\beta}p<0.05$, $^{\gamma}p<0.01$, $^{\varepsilon}p<0.001$, $^{\theta}p<0.0001$ vehicle vs. 30 mg/kg CK-2127107.

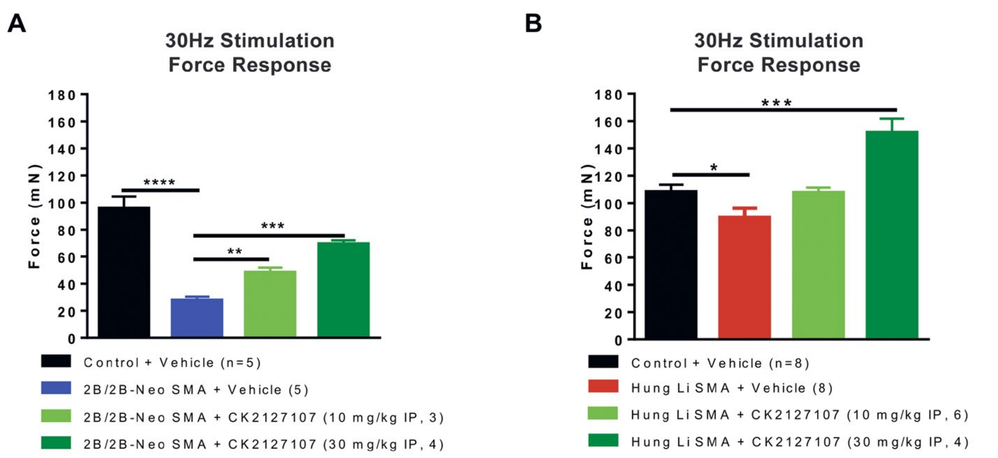
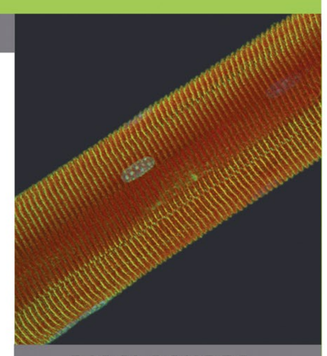


Figure 4. CK-2127107 improved the force response to sub-tetanic nerve stimulation. Relative to its respective control mice, **A.** 2B/2B-Neo and **B.** Hung Li SMA mice produced significantly lower force in response to a 30 Hz nerve stimulation. CK-2127107 significantly increased force at a 30 Hz stimulation in a dose-dependent manner. Data is expressed as mean ± SEM.

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. respective control.

SUMMARY OF FINDINGS

- 2B/2B-Neo and Hung Li SMA mice exhibited significant nerve dysfunction and/or muscle atrophy and a decrease in maximum muscle force production
- Single doses of CK-2127107 increased isometric force *in situ* in response to sub-tetanic nerve stimulation in both SMA mouse models
- These results suggest that CK-2127107 and other fast skeletal muscle troponin activators may be viable therapeutics for improving muscle function in spinal muscular atrophy



DISCLOSURES

D. Hwee, F. Malik, and E. Chin are currently employees of Cytokinetics, Inc. and were compensated financially for their work.

C. DiDonato is supported by funding from NIH, Cure SMA and MDA.

REFERENCES

1. Gugliotti *et al*. Hum Mol Genet. 2013 Oct 15;22(20):4084-101.
2. Arnold *et al*. Ann Clin Transl Neurol. 2014 Jan 1;1(1):34-44.
3. Hsieh-Li *et al*. Nat Genet. 2000 Jan;24(1): 66-70.

Cytokinetics

astellas