# MF-300, an orally bioavailable small molecule inhibitor of 15-PGDH, improves muscle force in preclinical models of neuromuscular dysfunction and disease

Epirium Bio™

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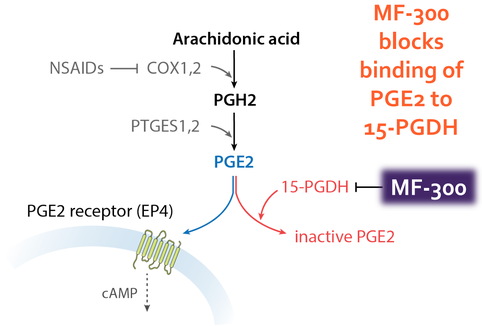
## Introduction

- Prostaglandin E2 (**PGE2**) is a lipid signaling molecule critical for muscle regeneration and function (1, 2).
- Therapeutic inhibition of the PGE2-degrading enzyme, 15-Prostaglandin Dehydrogenase (**15-PGDH**), improved muscle strength in aged mice (2).
- **MF-300** is an orally bioavailable small molecule inhibitor of 15-PGDH.
- Administration of MF-300 in mice increased strength in two models of Spinal Muscular Atrophy (**SMA**) as well as an inducible model of muscle atrophy (sciatic nerve crush).

## Figure 1) PGE2 Pathway




MF-300 blocks binding of PGE2 to 15-PGDH

**Effects on skeletal muscle**

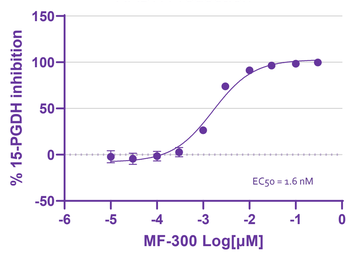
- *Increased stem cell proliferation (1)*
- *Increased muscle force (1, 2)*
- *Improved mitochondrial function (2)*

## Figure 2) MF-300 MoA and PK

MF-300 inhibits 15-PGDH, in vitro

**15-PGDH Inhibition (Biochemical Assay)**

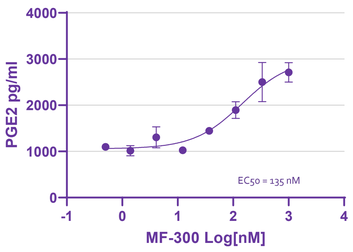
NADH Production




MF-300 inhibits 15-PGDH in cell-based assay

**PGE2 Stability (Cell-based Assay)**

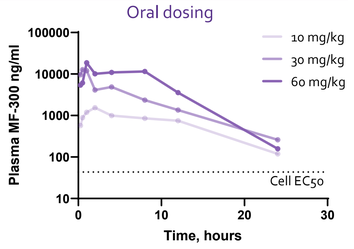
A549 Cells




MF-300 is orally bioavailable

**Single dose, 24-hour plasma PK (mouse)**

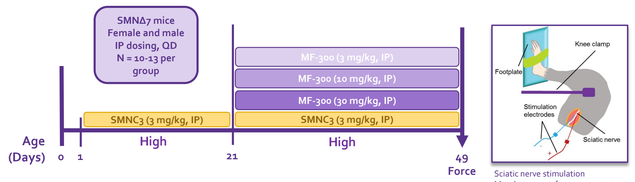
Oral dosing



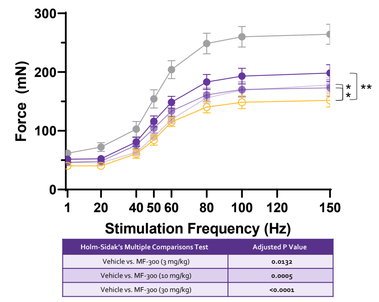

## Figure 3) MF-300 administration (IP) in SMN Δ7 mice

### MF-300 significantly increased force in Δ7 (high/high) mice

- SMN Δ7 Mouse Model – Severe phenotype modulated by therapeutic administration of the SMN splice enhancer, SMN-C3 (5).
- High/High = moderate phenotype (SMN-C3 3 mpk)
- Endpoint - Isometric plantar flexor force.
- Conclusion - MF-300 increased force at all dose levels.

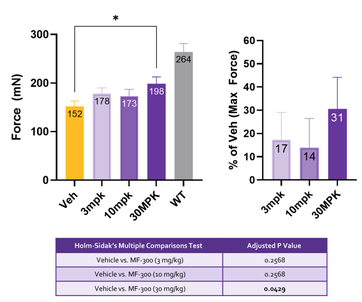



**MF-300 increased force**




| Holm-Sidak's Multiple Comparisons Test | Adjusted P Value |
|---|---|
| Vehicle vs. MF-300 (3 mg/kg) | 0.0132 |
| Vehicle vs. MF-300 (10 mg/kg) | 0.0005 |
| Vehicle vs. MF-300 (30 mg/kg) | <0.0001 |

**MF-300 increased maximum force (150 Hz)**

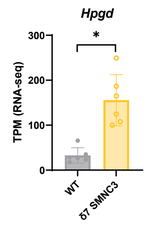



| Holm-Sidak's Multiple Comparisons Test | Adjusted P Value |
|---|---|
| Vehicle vs. MF-300 (3 mg/kg) | 0.2568 |
| Vehicle vs. MF-300 (10 mg/kg) | 0.2568 |
| Vehicle vs. MF-300 (30 mg/kg) | 0.0429 |

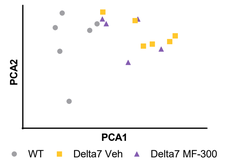
### Preliminary RNA-seq analysis of MF-300 treated muscle from Δ7 (high/high) mice

- RNA-seq analysis – gastrocnemius muscles (N=6/group) from above study.
- Poly A selection, paired end reads, read depth ~30x10$^6$ per sample.
- Preliminary results: CREB promoter sequences are significantly enriched in DEGs from MF-300 treated muscle.
- GO and Ingenuity Pathway Analysis predicts corrective effects of MF-300 on Mitochondrial Activity, among other pathways.

**HPGD, the gene for 15-PGDH, is upregulated in SMA muscle**



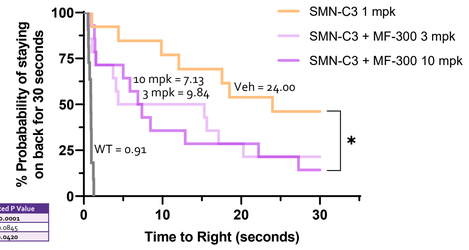

**Principal Components Analysis suggests MF-300 treatment may shift Δ7 gene expression towards WT**




### MF-300 improved time-to-right in Δ7 (med/med) mice

- SMN Δ7 med/med model (SMN-C3 1 mpk)
- SMN-C3 ± MF-300 IP dosing beginning PND3
- Time-to-Right assessed on PND12. Animals given 30 seconds to "right."
- Data presented as Kaplain-Meyer curves, MF-300 improved probability that animal righted in 30 sec..

SMNΔ7 mice
IP dosing, QD
SMNΔ7 N=14/group
WT N=10



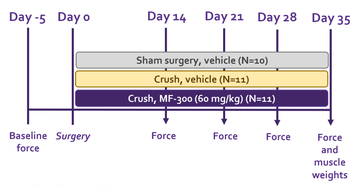

| Log-rank (Mantel-Cox) test | Adjusted P Value |
|---|---|
| WT veh vs D7 SMC3 | <0.0001 |
| D7 SMNC3 vs D7 SMNC3 MF-300 3 mpk | 0.0845 |
| D7 SMNC3 vs D7 SMNC3 MF-300 10 mpk | 0.0420 |

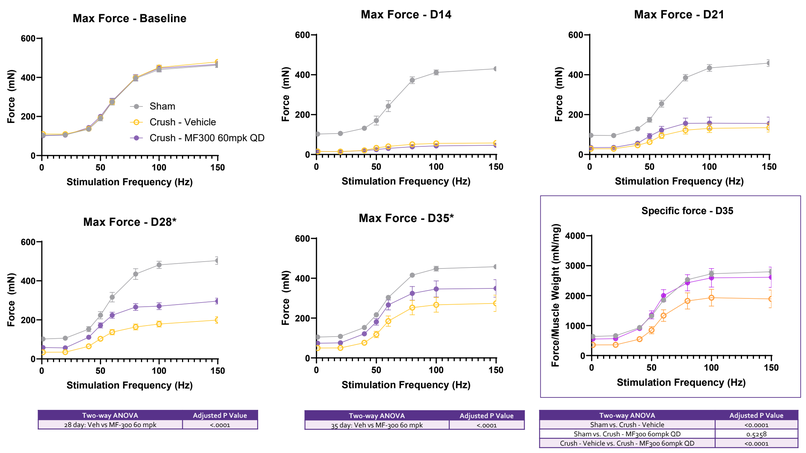
## Figure 5) MF-300 administration (PO) in a sciatic nerve crush model

### MF-300 accelerated rate of force recovery following nerve crush injury

- Healthy animals - surgery to expose the sciatic nerve.
- Nerve crush for 30 sec (sham = no crush injury).
- In vivo muscle force (isometric plantar flexor) - baseline and then once weekly starting at 14 days post injury.
- MF-300 accelerated force recovery at 28- and 35-days post injury.

C57Bl/6
Male, 10-12 weeks old
QD dosing, PO



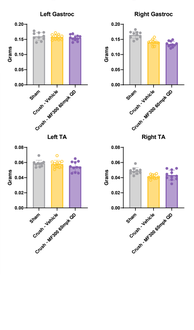




| Two-way ANOVA | Adjusted P Value |
|---|---|
| 28 day: Veh vs MF-300 60 mpk | <.0001 |

| Two-way ANOVA | Adjusted P Value |
|---|---|
| 35 day: Veh vs MF-300 60 mpk | <.0001 |

| Two-way ANOVA | Adjusted P Value |
|---|---|
| Sham vs. Crush - Vehicle | <0.0001 |
| Sham vs. Crush - MF300 60mpk QD | 0.5258 |
| Crush - Vehicle vs. Crush - MF300 60mpk QD | <0.0001 |

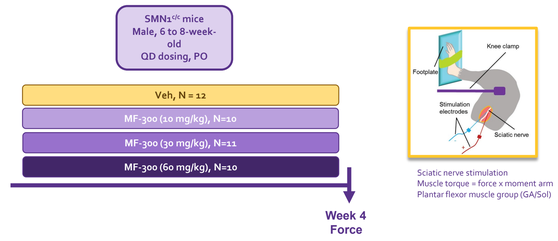
**MF-300 did not affect muscle mass**



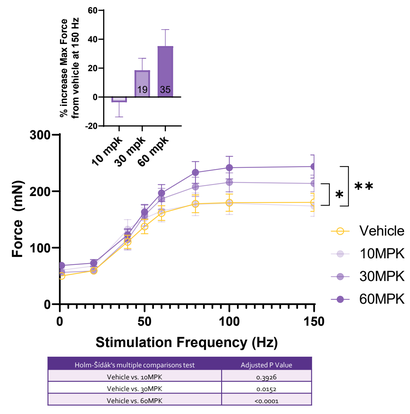

## Figure 4) MF-300 administration (PO) in SMN1$^{C/C}$ mice

### MF-300 dose responsive increase in force in SMN1$^{C/C}$ mice

- SMN1$^{C/C}$ is a genetic mouse model for mild phenotypic SMA.
- Endpoint - Isometric plantar flexor force.
- Conclusions:
  - MF-300 increased force in a dose responsive manner.
  - Increased force was independent of any effect on muscle mass.

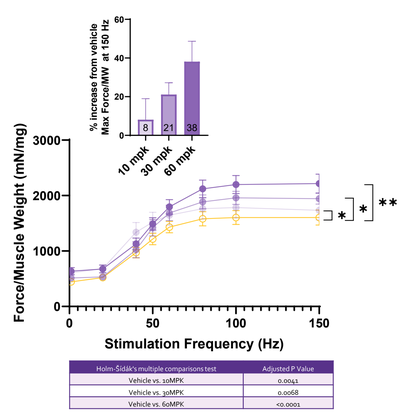



**MF-300 increased force**




| Holm-Šídák's multiple comparisons test | Adjusted P Value |
|---|---|
| Vehicle vs. 10MPK | 0.3926 |
| Vehicle vs. 30MPK | 0.0152 |
| Vehicle vs. 60MPK | <0.0001 |

**MF-300 increased force normalized to muscle mass**



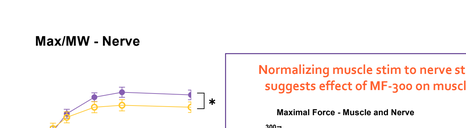

| Holm-Šídák's multiple comparisons test | Adjusted P Value |
|---|---|
| Vehicle vs. 10MPK | 0.0041 |
| Vehicle vs. 30MPK | 0.0068 |
| Vehicle vs. 60MPK | <0.0001 |

### MF-300 increased force of both nerve and direct muscle stimulated contraction

- In –life study design as above with 1) Veh and 2) MF-300 60 mpk groups.
- Force generated by nerve vs muscle stimulation was compared to localize effect of MF-300.
- Observed greater increase in muscle stimulated force over nerve with MF-300 compared to Veh.
- Conclusion - MF-300's effect on force after 4-week treatment is, at least in part, intrinsic to muscle. These data do not exclude potential effect on NMJ.

**Experimental Rationale**

**Based upon published data showing that α-bungaratoxin does not reduce direct muscle stimulated force in mice (3)*





Normalizing muscle stim to nerve stim suggests effect of MF-300 on muscle




| Two-way ANOVA | P Value |
|---|---|
| (A) Max force – Nerve | <0.0001 |
| (B) Max force/MW – Nerve | .0191 |
| (C) Max force – Muscle | <0.0001 |
| (D) Max force/MW – Muscle | <0.0001 |

## Conclusions

- Therapeutic administration of MF-300 increased muscle force and function in mouse models of SMA. Preliminary RNA-seq analysis suggests that MF-300 corrected dysregulated expression of multiple genes in Δ7 muscle.
- Increased muscle force was independent of changes in muscle mass. Comparing nerve- to muscle-stimulated contraction suggests that MF-300 has a muscle intrinsic effect on force after 4 weeks of treatment. An effect on NMJ is also possible, given these data.
- MF-300 accelerated force recovery following sciatic nerve injury in healthy mice. Timing of effect on force recovery suggests improved regeneration of functional neuromuscular junctions (4) and/or enhanced sensitivity of the muscle to contractile stimuli.

## References

1. Ho et al., Prostaglandin E2 is essential for efficacious skeletal muscle stem-cell function, augmenting regeneration and strength. *PNAS* 2017
2. Palla et al., Inhibition of prostaglandin-degrading enzyme 15-PGDH rejuvenates aged muscle mass and strength. *Science* 2021
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4. Magill et al., Reinnervation of the tibialis anterior following sciatic nerve crush injury: A confocal microscopic study in transgenic mice. *Exper. Neuro.* 2007
5. Naryshkin et al., SMN2 splicing modifiers improve motor function and longevity in mice with spinal muscular atrophy. *Science* 2014

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