ADVANCES IN SKELETAL MUSCLE BIOLOGY IN HEALTH AND DISEASE

UNIVERSITY *of* FLORIDA

KER-065, a novel ActRII-Fc ligand trap, increased functional muscle and bone mass with reduced BMP9 binding

Remya Nathan[1] , Morgan Cahill[1], Chris Materna[1], Tandicka Nurse[1], Evan Lema[1] , Alana Gudelsky[1], Emily Ledoux[1], Radina Todorova[1], Savanna Macaluso[1], Claire Tseng[1] , Ffolliott Martin Fisher[1] , Lorena Lerner[1] , Jasbir Seehra[1], Jenn Lachey[1]. 1. Keros Therapeutics Inc., Research, Lexington, USA

KEROS THERAPEUTICS

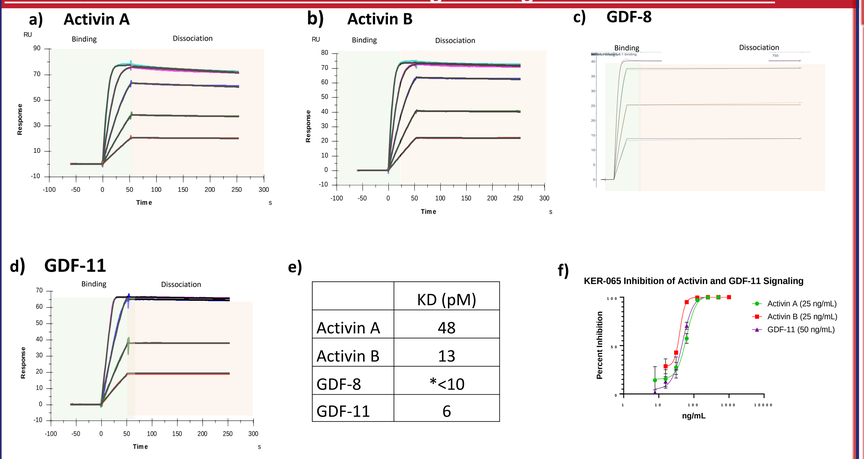
Introduction

Neuromuscular diseases (NMDs) are characterized by muscle atrophy and loss of functional muscle strength; however, bone loss is also prevalent, which can lead to debilitating fractures. Ligands in the TGF-β superfamily, including myostatin and activins A and B, signal through activin type II receptors to negatively regulate muscle and bone. [1][2][3]

ActRIIB is a transmembrane receptor for TGF-β superfamily members including myostatin and activins. Inhibiting the signaling of these ligands with a soluble form of ActRIIB (ActRIIB-Fc) increased lean mass in healthy volunteers and increased muscle function in patients with Duchenne muscular dystrophy (DMD) [4][5][6]. However, Phase 2 clinical trials were halted due to adverse events of nose and gum bleeding. These effects were attributed to BMP9 inhibition which is critical for vascular remodeling. Keros Therapeutics has generated novel investigational therapeutics based on the pharmacology of ActRIIB-Fc with reduced BMP9 binding while maintaining the muscle and bone anabolic properties of wild-type ActRIIB. KER-065 is a chimera comprised of modified extracellular domains of ActRIIA and ActRIIB fused to the Fc of human IgG to maximally bind activins and GDFs with minimal BMP9 binding. RKER-065 is the research form of KER-065, having a murine (IgG2a) Fc in place of the human (IgG1) Fc domain. Treatment of wild-type mice with RKER-065 demonstrated increased muscle and bone mass.

1. McPherron, A.C, et al., Nature, 1997. **2**. Lin, J., et al., Biochem Biophys Res Commun, 2002. **3.** Bhattacharya, I., et al., Clin Pharmacol Drug Dev, 2018. **4.** Amthor, H., et al., Dev Biol, 2004. **5.** Attie, K.M., et al., Muscle Nerve, 2013. **6**. Campbell, C., et al., Muscle Nerve, 2017. **7**. Abdulkadyrov, K.M., et al., Br J Haematol, 2014. **8**. Raftopoulos, H., et al., Support Care Cancer, 2016.

KER-065 observed to bind and inhibit negative regulators of muscle mass



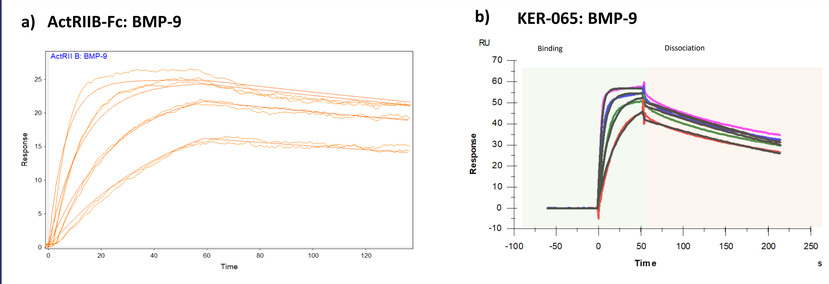
e)

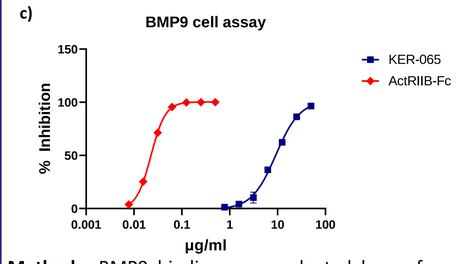
	KD (pM)
Activin A	48
Activin B	13
GDF-8	*<10
GDF-11	6

Methods: Ligand binding was evaluated by surface plasmon resonance (SPR). Ligand inhibition was also evaluated in a cell-based luciferase reporter assay.

Results: KER-065 was engineered to bind activins A and B, GDF-8, and GDF-11 with high affinity and reduced BMP9. SPR sensorgrams demonstrated that KER-065 did bind a) activin A, b) activin B, c) GDF-8, d) GDF-11 with high affinity. e) KD values for SPR results using a Biacore T200 and 8k.* no dissociation was observed. f) HEK-293-SBE-luc cell reporter assays demonstrated KER-065-mediated inhibition of activin A, activin B, and GDF-11 ligands with IC50s of 54.3, 34.4, and 45.4 ng/mL, respectively. Due to the sequence homology between GDF-8 and GDF-11, we expect GDF-8 inhibitory results to be similar to GDF-11.

KER-065 had reduced affinity to BMP9 compared to ActRIIB-Fc





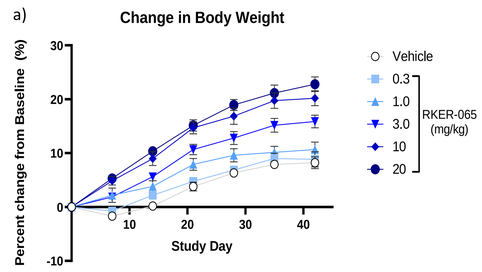
d)

Test Article	IC50 (4 param)
KER-065	9.1 µg/ml
ActRIIB-Fc	0.023 µg/ml

Methods: BMP9 binding was evaluated by surface plasmon resonance (SPR). KER-065 was also evaluated alongside ActRIIB-Fc in a luciferase reporter assay with C2C12-BRE-luc reporter cells for its ability to inhibit BMP-9 signaling.

Results: a) ActRIIB-Fc sensorgram of BMP9 binding profiles by Biacore. b) KER-065 sensorgram of BMP9 binding showed an increase in the rate of dissociation as compared to ActRIIB-Fc. c) C2C12-BRE-luc cell reporter assays showed that ActRIIB-Fc potently inhibited BMP9 signaling, whereas KER-065-mediated inhibition was reduced. d) Calculated IC50s demonstrate that KER-065 exhibited a 400-fold lower inhibition of BMP9 compared to ActRIIB-Fc.

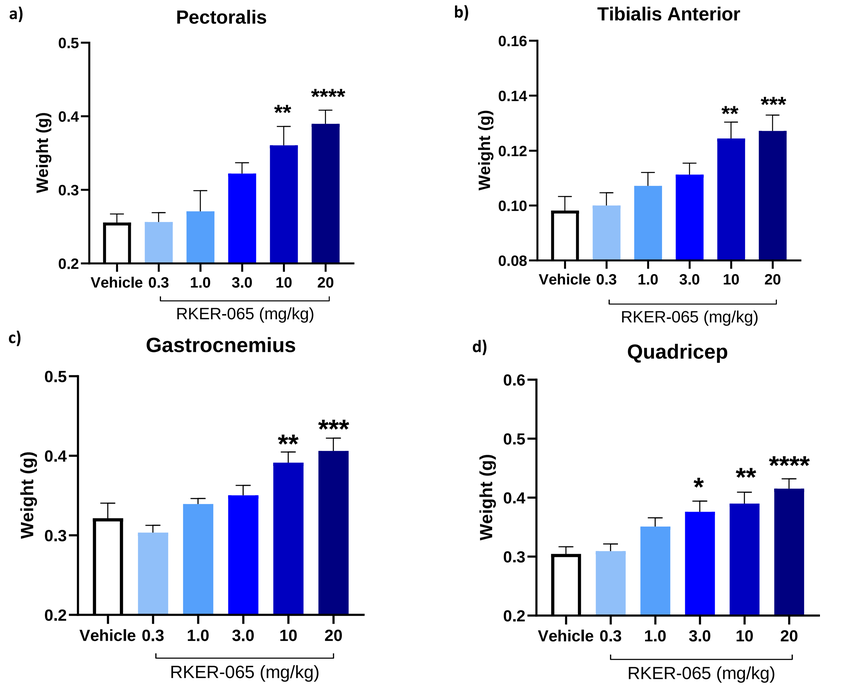
RKER-065 demonstrated dose-dependent increases in percent body weight and lean mass in mice



Methods: To compare the effects on muscle, 12-week-old male C57BL/6 mice were treated with vehicle (Veh; volume by weight), 0.3, 1, 3, 10, or 20 mg/kg RKER-065 dosed weekly (QW) for 6 weeks. Body mass was measured once a week while lean mass was assessed via nuclear magnetic resonance (NMR) at baseline and study termination. Mice were sacrificed 48 hours post final dose. For lean mass, an ordinary one-way ANOVA and Dunnett's multiple comparisons tests. Data is shown as the mean ±SEM, **p<0.01, ***p<0.001, **** p<0.0001, ns=not significant.

Results: a) RKER-065 increased body weight in a dose-dependent manner. b) Observed increase in body weight was associated with a significant increase in lean mass at 3, 10, and 20 mg/kg.

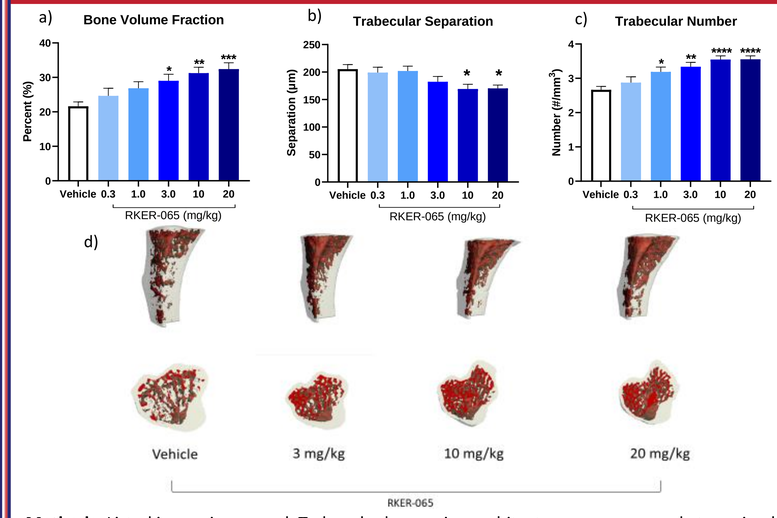
RKER-065 promoted dose-dependent skeletal muscle growth and a significant increase in muscle strength in mice



Methods: a-d) At study termination outlined in the previous panel and muscles were collected and weighed. Weights listed are the average of bilateral muscles. **e)** Forelimb grip strength was measured at baseline and study termination. Statistical analysis is shown versus vehicle and was done using an ordinary one-way ANOVA and Dunnett's multiple comparisons tests. Data are shown as the mean ±SEM, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. **f)** Circulating mFc exposure measured via in-house ELISA that detects circulating levels of protein in serum.

Results: Dose-dependent increase observed in muscle mass of a) pectoralis, b) tibialis anterior, c) quadriceps d) gastrocnemius. e) Observed muscle mass increases were associated with a significant increase in functional strength. f) ELISA results demonstrated a dose-dependent exposure.

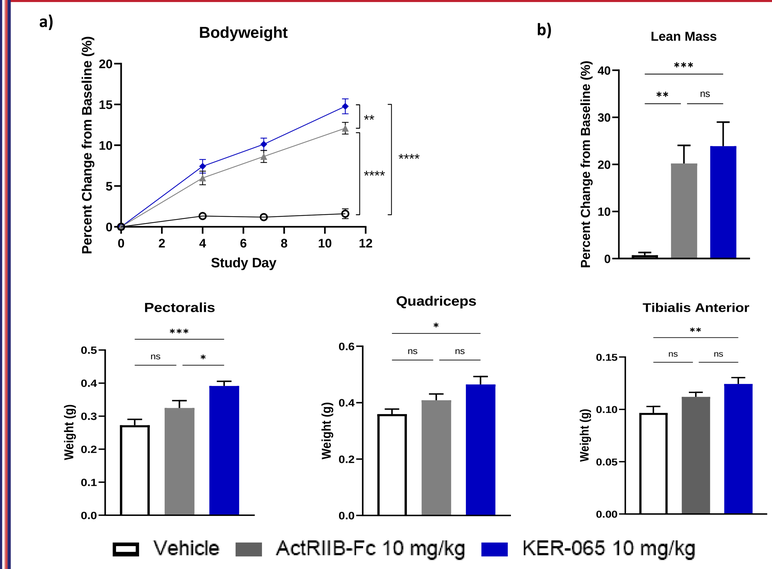
RKER-065 demonstrated dose-dependent increases in trabecular bone in mice



Methods: Listed in previous panel. Trabecular bone microarchitecture was measured at proximal tibia via µCt (GX2). Statistical analysis is shown versus vehicle and was done using an ordinary one-way ANOVA and Dunnett's multiple comparisons test. Data are shown as the mean ±SEM, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Results: a-c) RKER-065 treatment increased bone volume, reduced trabecular separation and increased trabecular number. d) Representative images of trabecular bone within proximal tibia

KER-065 increased bodyweight and lean mass in WT mice compared to vehicle and ActRIIB-Fc



Methods: To compare the effects on muscle, 12-week-old male C57BL/6 mice were treated with ActRIIB-Fc, KER-065 (10 mg/kg) or Vehicle (Veh; volume by weight) QW for 12 days. Body mass was measured twice a week while lean mass was assessed via nuclear magnetic resonance (NMR) at study termination after a total of 4 doses. Mice were sacrificed 48-72 hours post final dose. Statistical analysis was done using a 2-way ANOVA and Tukey's multiple comparisons for body mass. For lean mass, an ordinary one-way ANOVA and Dunnett's multiple comparisons tests. Data is shown ±SEM, *p<0.05, **p<0.01, ***p<0.001,****p<0.0001, ns=not significant.

Results: a) Administration of RKER-065 significantly increased the percent change in body weight from baseline when compared to vehicle and ActRIIB-Fc. b) This increase in weight can be attributed to a significant increase in lean mass. c-e) The observed increase in total lean mass is underscored by observed increases in the mass of individual muscle types.

Conclusions

- **KER-065 was engineered to have reduced BMP9 binding while retaining strong affinity to activins A and B, GDF-8 and GDF-11.**
- **Preclinical studies demonstrate RKER-065 treatment led to robust increase in body mass, muscle mass, functional strength, and bone formation.**
- **These results demonstrate Keros Therapeutics can leverage its proprietary discovery approach to generate investigational ligand traps that have the potential to treat musculoskeletal disorders while potentially limiting the adverse events associated with BMP9 inhibition.**

Correspondence

Justin Frantz
jfrantz@soleburystrat.com
617-221-9100