A comparison of anabolic effects on muscle mass of wild-type ActRIIB-Fc and the novel ActRII-Fc ligand trap KER-065

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Introduction

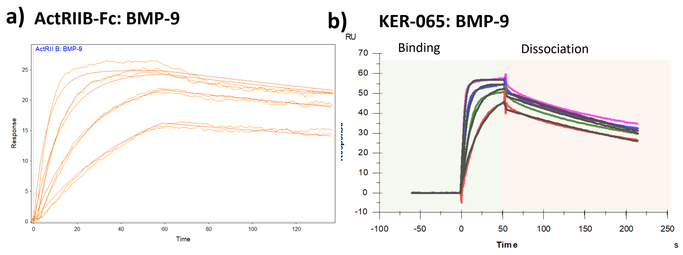
TGF-β superfamily ligands, including myostatin and activins, that signal through ActRII receptors have been described to negatively regulate muscle mass[1][2][3]. Inhibition of these ligands has been demonstrated, through various therapeutic modalities, to increase muscle mass and strength [4].

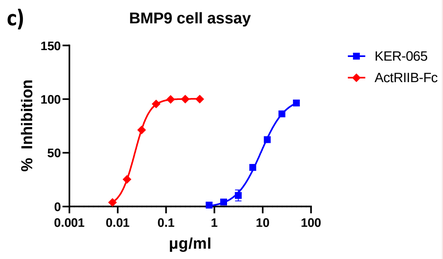
ActRIIB-Fc, a soluble form of ActRIIB, functions as a ligand trap for these catabolic ligands to promote muscle growth. Inhibition of these negative regulators of myogenesis with a soluble form of ActRIIB (ActRIIB-Fc) increased lean mass in healthy volunteers [5] and increased muscle mass and muscle function in patients with Duchenne muscular dystrophy (DMD) [6]. However, the Phase 2 trial was halted due to adverse events of nose and gum bleeding. This effect was attributed to ActRIIB-Fc inhibition of BMP9, a ligand involved in vascular remodeling.

Keros Therapeutics has generated novel investigational therapeutics based on the pharmacology of ActRIIB-Fc with reduced BMP9 binding while preserving the muscle and bone anabolic properties of WT ActRIIB-Fc. Ker-065 is a chimera of the extracellular domains of ActRIIA and ActRIIB fused to a human fc designed to maximally bind activins and GDFs with minimal BMP9 binding. RKER-065 is a research form of KER-065 that has increased muscle and bone in wild type mice.

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.

1. KER-065 has reduced affinity to BMP9 compared to ActRIIB-Fc





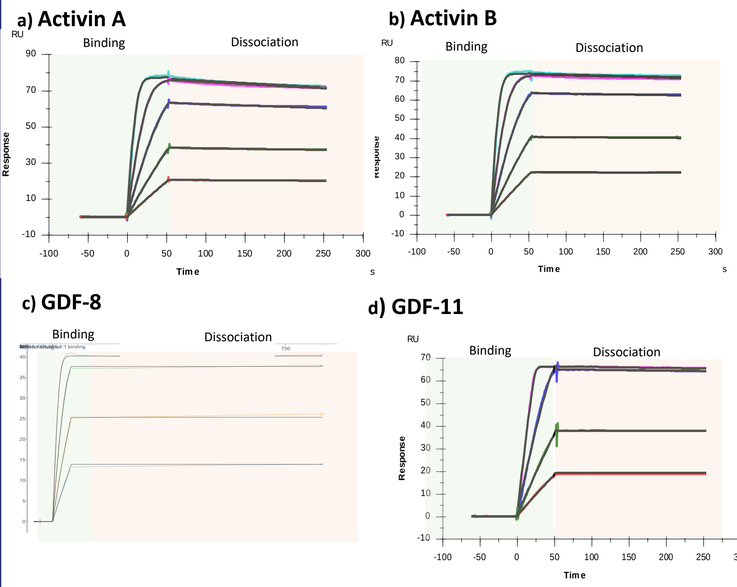
Methods: BMP9 binding was evaluated by surface plasmon resonance (SPR). KER-065 was also evaluated alongside ActRIIB-Fc in a luciferase reporter assay with C2C12-BRE-luc reporter cells for its ability to inhibit BMP-9 signaling.

d)

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

Results: a) ActRIIB-Fc sensorgram of BMP9 binding profiles by Biacore at a KD value of 111 pM. b) RKER-065 sensorgram of BMP9 binding showing an increase in the rate of dissociation at a KD value of 612 pM 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.

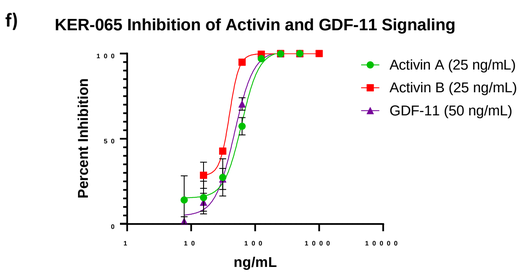
2. KER-065 observed to bind and inhibit negative regulators of muscle mass



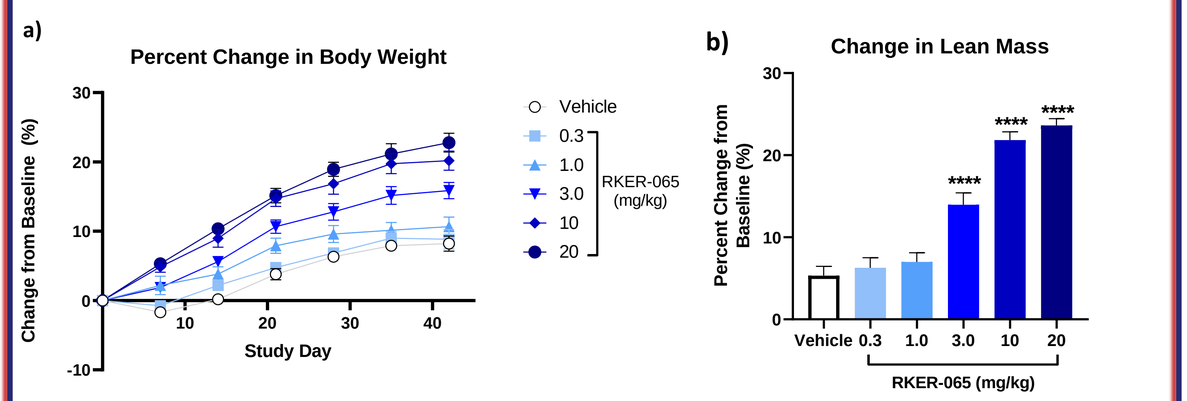
KER-065 was engineered to bind activins A and B, GDF-8, and GDF-11 with high affinity and reduced BMP9. SPR sensorgrams demonstrated that KER-065 did bind a) activin A, b) activin B, c) GDF-8, d) GDF-11 with high affinity. e) KD values for SPR results using a Biacore T200 and 8k.* no dissociation was 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.

e)

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



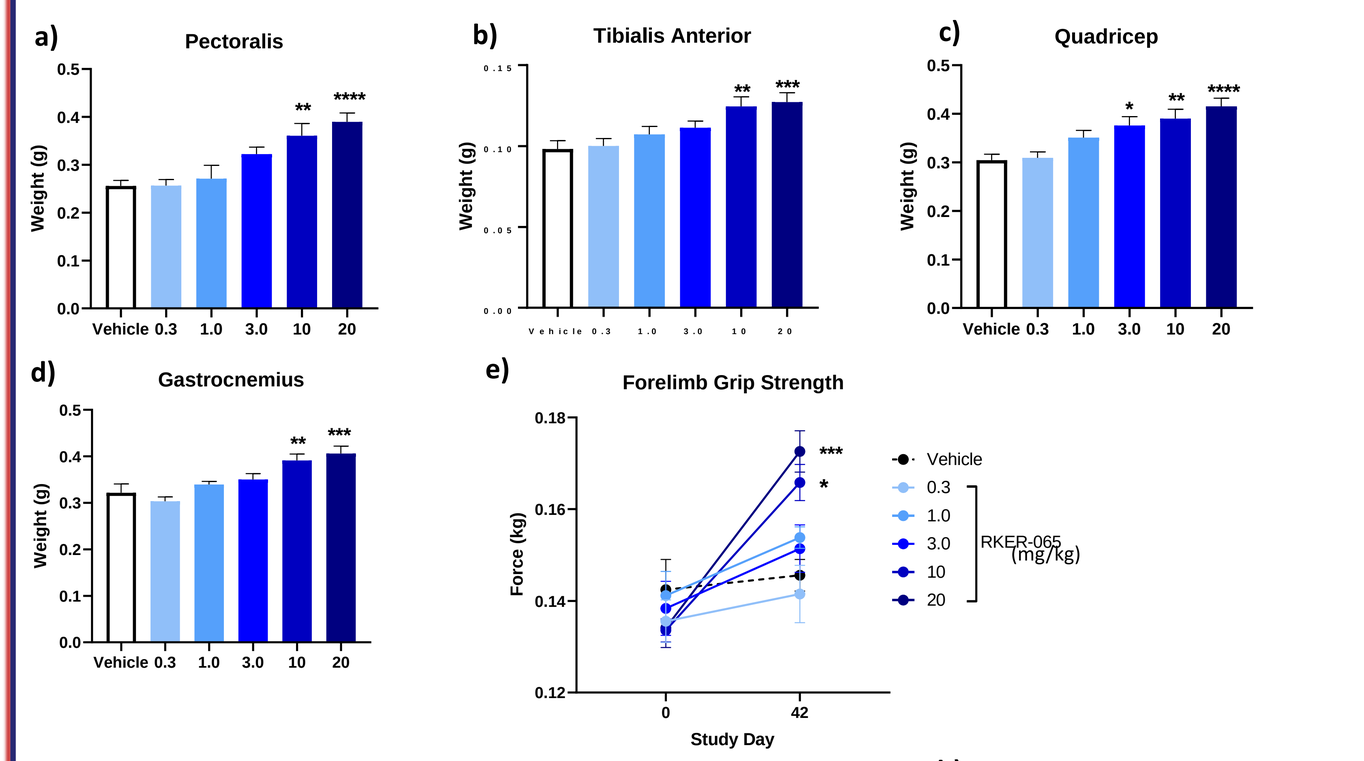
3. RKER-065 demonstrated dose-dependent increases in total body weight and lean mass



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 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 test. Data is shown as the mean ±SEM, **p<0.01, ***p<0.001, **** p<0.0001, ns=not significant.

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

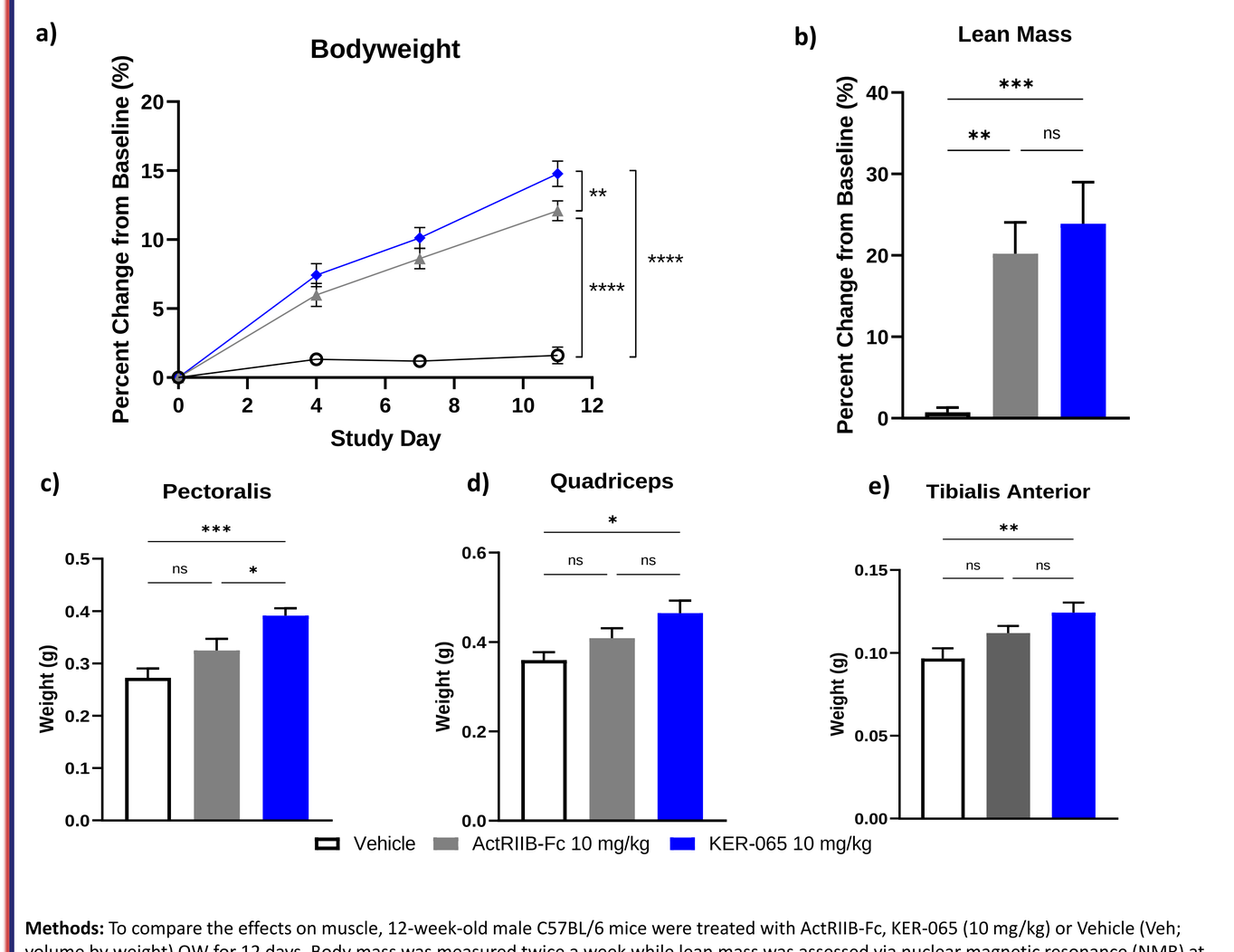
4. RKER-065 promoted dose-dependent skeletal muscle growth, a significant increase in muscle strength and trabecular bone



Methods: a-d) At study termination outlined in previous panel, mice were euthanized using CO_2 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. **f-g)** 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: Dose-dependent increase observed in muscle mass of a) pectoralis, b) tibialis anterior, c) quadriceps d) gastrocnemius. (e) Muscle mass increases are associated with a significant increase in functional strength. RKER-065 treatment increased bone volume, reduced trabecular separation and increased trabecular number (f-h).

5. KER-065 increased body weight 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 test. Data is shown ±SEM, *p<0.05, **p<0.01, ***p<0.001, ns=not significant.

Results: a) KER-065 percent change in bodyweight from baseline was significantly increased when compared to vehicle and ActRIIB-Fc. b) This increase in weight can be attributed to a significant increase in lean mass. c-e) As seen in previous studies, increases in skeletal muscle mass correlated with increases in lean mass.

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 suggest Keros Therapeutics can leverage its proprietary discovery approach to generate ligand traps that could potentially treat musculoskeletal disorders while limiting the adverse events associated with BMP9 inhibition.**

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