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RKER-065, a novel muscle and bone anabolic, increased muscle, grip strength and trabecular bone in a mouse model of Duchenne muscular dystrophy

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Introduction

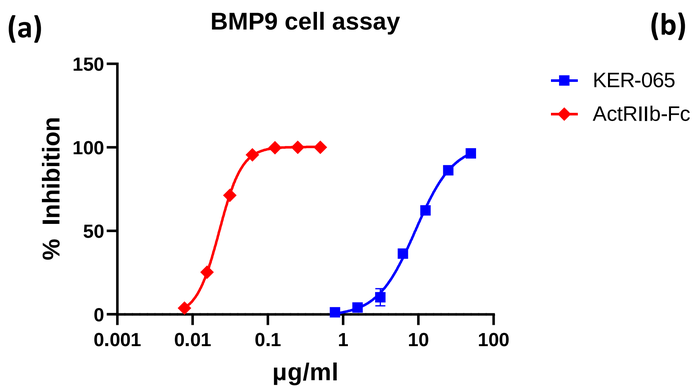
Patients with Duchenne muscular dystrophy (DMD) have reduced muscle mass and muscle function. They also exhibit low bone strength which leads to higher risk of skeletal fractures. Current standard of care includes long-term corticosteroid treatment which further reduces muscle and bone mass. One therapeutic strategy being explored in DMD is the use of muscle and bone anabolic agents to increase or maintain lean and bone mass. Multiple TGFβ superfamily ligands, including myostatin along with activins A and B, signal through TGF-β superfamily receptors ActRIIA and ActRIIB 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].

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 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 [7], a ligand involved in vascular remodeling. ActRIIA-Fc, a highly homologous receptor also in clinical development, increases red blood cells without affecting muscle mass and lacks the effect on vascular remodeling seen with ActRIIB-Fc [8][9]. Keros Therapeutics has generated novel therapeutic pre-clinical candidates based on the pharmacology of ActRIIA-Fc and ActRIIB-Fc, which act as muscle and bone anabolic agents, but with reduced BMP9 binding that may have a sparing effect on the BMP signaling axis.

KER-065 is a chimera of the extracellular domains of ActRIIA and ActRIIB fused to a human Fc. KER-065 was designed to have minimal BMP9 binding whilst maximizing muscle and bone anabolism. RKER-065 is a research form of KER-065 that, in the study presented, increased muscle and bone in a murine model of DMD.

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**. Li, J., et al., J Clin Invest, 2021. **8.** Abdulkadyrov, K.M., et al., Br J Haematol, 2014. **9**. Raftopoulos, H., et al., Support Care Cancer, 2016.

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

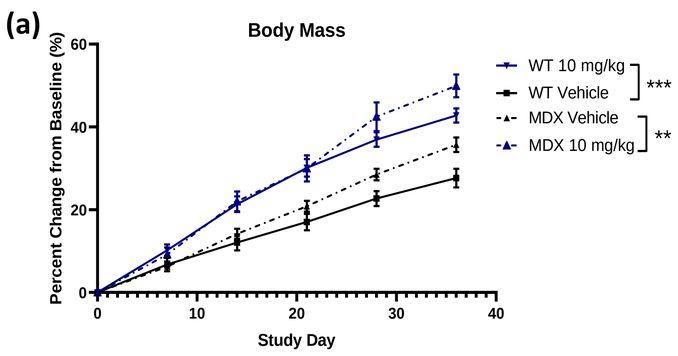


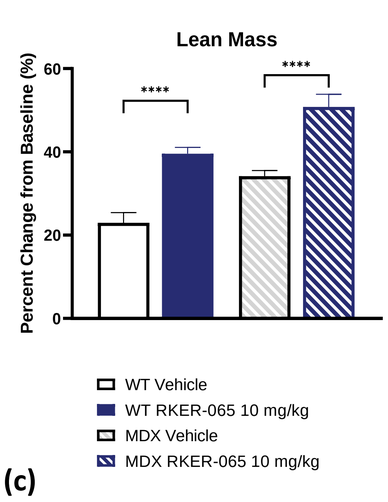
Test Article	IC50 (4 param)
KER-065	9.1 µg/ml
ActRIIB-hFc	0.0227 µg/ml

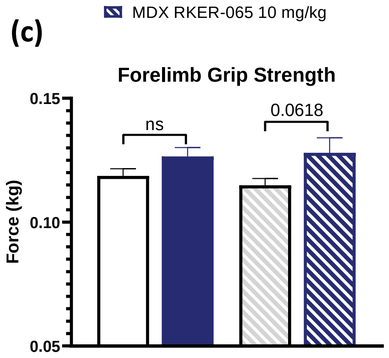
Methods: KER-065 was evaluated alongside ActRIIB-Fc in a luciferase reporter assay with C2C12-BRE-luc reporter cells for its ability to inhibit BMP9 signaling.

Results: (a) Cell reporter assays showed that ActRIIB-Fc potently inhibited BMP9 signaling whereas the inhibitory profile of KER-065 was greatly reduced; **(b)** Calculated IC50 highlighting that KER-065 showed a 400-fold lower inhibition of BMP9, compared to the IC50 of ActRIIB-Fc.

2. RKER-065 increased lean mass and strength in dystrophic MDX mice



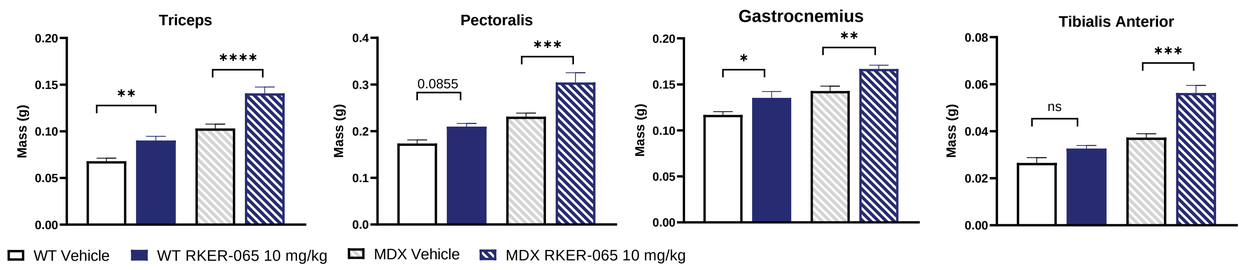




Methods: Briefly, 5-week-old, female C57BL/10 and MDX mice were injected IP once a week (total of 6 doses), with vehicle (TBS) or RKER-065-mFc, at a dose of 10mg/kg for 36 days. Body mass was measured once a week and lean mass was assessed at baseline and study termination using nuclear magnetic resonance (NMR). Forelimb grip strength was also assessed at study termination. Mice were sacrificed 48hrs post final dose. For MDX studies, statistical analysis was done by 2-way ANOVA and individual comparisons shown from a Sidaks (repeat measures and Tukey for body mass) multiple comparison test. Data is shown ±SEM, ** p<0.01, *** p<0.001, **** p<0.0001, ns= not significant;

Results: RKER-065 treatment led to **(a)** robust increases in body mass of mice shown as percent increase from baseline; **(b)** increase in lean mass in both WT C57BL/10 and MDX mice; **(c)** Increase in lean mass is associated with a trend toward an increase in forelimb grip strength supportive of improved muscle function.

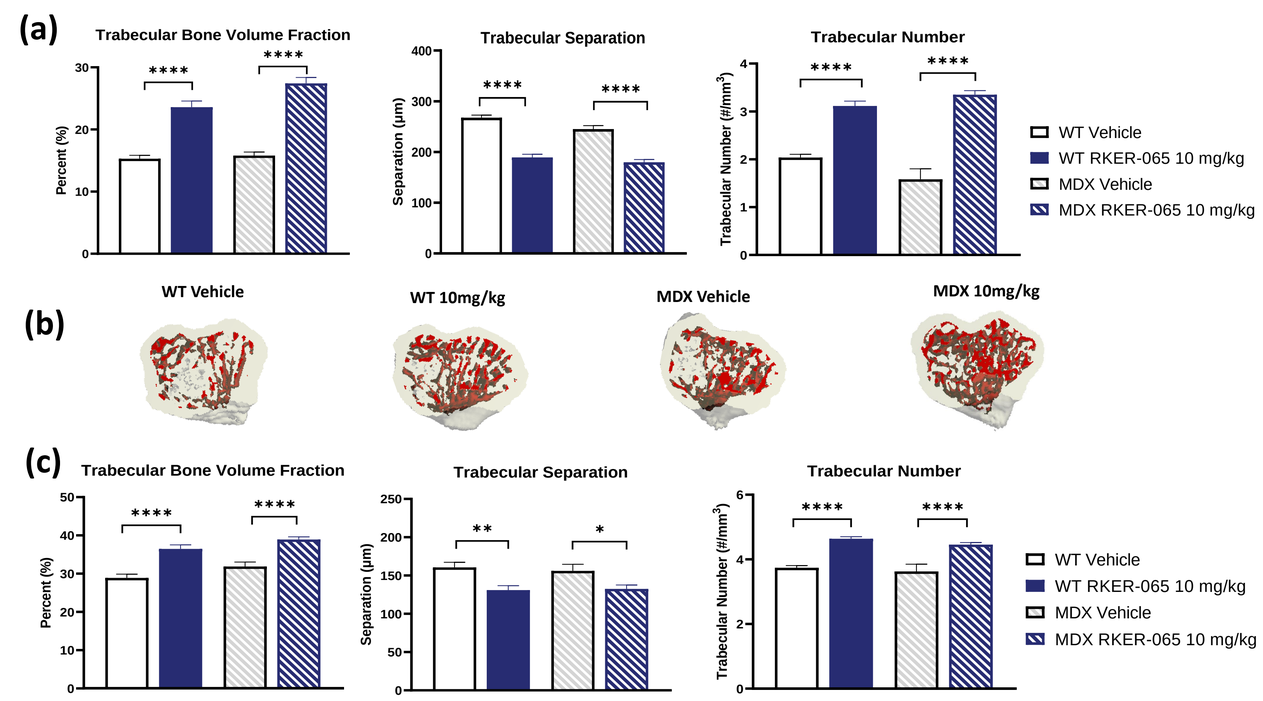
3. RKER-065 increased individual muscle mass in WT and MDX mice



Methods: At study termination, mice were euthanized, and muscles dissected and weighed. Data is shown as mean ±SEM, * p≤0.05, ** p<0.01, *** p<0.001, **** p<0.0001, ns= not significant.

Results: Significant increases in muscle mass were observed in the dystrophic mice treated with RKER-065.

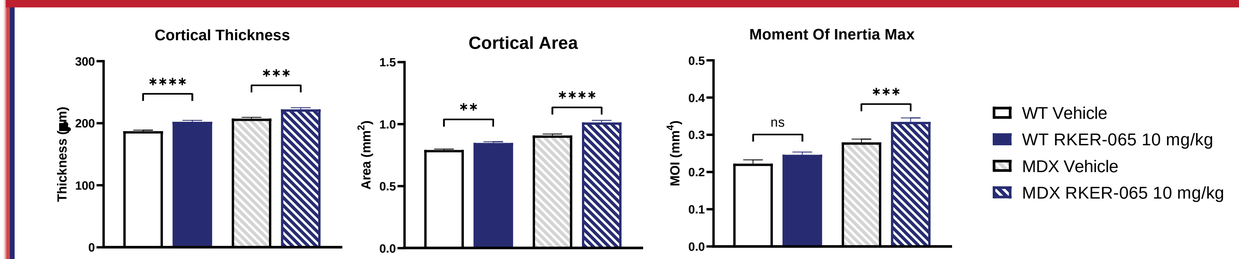
4. RKER-065 increased trabecular bone in the tibia and L5 vertebra in WT and MDX mice



Methods: Tibias and L5 vertebrae were scanned using GX2 µCT (10 mm FOV, 90 kV, 88 µA, 4 minutes, Perkin Elmer). Trabecular bone was evaluated using Analyze 14.0 Bone Micro-architecture Analysis software (AnalyzeDirect). Representative images produced using Scanco Software (Scanco Medical AG, Brüttisellen, Switzerland). Data is shown as mean ±SEM, * p≤0.05, ** p<0.01, *** p<0.001, **** p<0.0001, ns= not significant.

Results: (a) Trabecular morphometry data from the proximal tibia showed significant improvements in bone parameters supportive of increased bone strength, and **(b)** 3D representations of the regions assessed; **(c)** Similar improvements observed in the trabecular bone of the lumber spine.

5. RKER-065 increased tibial cortical bone in WT and MDX mice



Methods: The mid diaphysis of each scanned tibia was evaluated using Analyze 14.0 to delineate cortical bone morphometry. Data is shown as mean ±SEM, * p≤0.05, ** p<0.01, *** p<0.001, **** p<0.0001, ns= not significant.

Results: RKER-065 significantly increased cortical thickness and area in WT mice with an additional increase in the moment of inertia in the MDX mice.

Conclusions

- **Taken together, these data show that KER-065 can increase muscle mass, improve muscle function and prevent bone loss in MDX mice.**
- **The reduced inhibition of BMP9 by KER-065 has the potential to reduce the safety signal associated with abnormal vascular remodeling linked with attenuated BMP9 signaling in the vasculature.**
- **These studies demonstrate proof-of-concept of the platform, and pharmacological approach at Keros Therapeutics to generate product candidate muscle and bone anabolic agents with the potential for development as therapeutics for the treatment of musculoskeletal disorders.**

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