**RKER-065 ameliorated muscle and bone loss in a progressive murine model of Duchenne muscular dystrophy**

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**Introduction**

Patients with Duchenne muscular dystrophy (DMD) have reduced muscle mass and function. They also exhibit low bone strength leading to a higher risk of skeletal fractures. Treatment options include long-term corticosteroid treatment, which delays the loss of ambulation but induces muscle loss, increases adiposity and accelerates bone loss, resulting in increased risk of vertebral fractures. The TGF-β 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). Inhibition of these ligands has been demonstrated, through various therapeutic modalities, to increase muscle mass and strength (3). Furthermore, inhibition of these negative regulators of myogenesis with a soluble form of ActRIIB (ActRIIB-Fc) increased lean mass in healthy volunteers (4) and increased muscle mass and muscle function in patients with DMD (6). However, the Phase 2 trial was halted due to adverse nose and gum bleeding events. ActRIIA-Fc, a highly homologous receptor, has reduced BMP binding but does not increase muscle mass (6). KER-065 is an investigational modified ActRII ligand trap that contains sequences from both wild-type ActRIIA and ActRIIB fused to a human Fc designed to retain the muscle and bone anabolic properties of ActRIIB but with reduced BMP binding to potentially reduce the risk of bleeding events. Below, we studied the effect of KER-065, a research form of KER-065, in the progressive and phenotypically more severe D2 mdx mouse model.

**KER-065 observed to bind and inhibit negative regulators of muscle mass with reduced affinity to BMP-9**

<table>
<thead>
<tr>
<th>KD (μM)</th>
<th>IC50 (ng/mL)</th>
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<tbody>
<tr>
<td>Activin A</td>
<td>4.8</td>
</tr>
<tr>
<td>Activin B</td>
<td>13</td>
</tr>
<tr>
<td>GDF-8</td>
<td>&lt;2</td>
</tr>
<tr>
<td>GDF-11</td>
<td>6</td>
</tr>
<tr>
<td>BMP-9</td>
<td>602</td>
</tr>
</tbody>
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**Methods:** KER-065 inhibition of muscle catabolic ligands was evaluated in HEK293-SBE-Luc reporter cells. Inhibition of BMP9 induced SMAD1 signaling was evaluated in HUVEC cells by AlphaLISA. KER-065 binding kinetics were evaluated on a Biacore Rk. **Approaching instrument limits for detecting off-rate.**

**Results:** KER-065 was engineered to maintain strong (A) binding affinity to activins A and B, GDF-8, and GDF-11 whilst having a decreased binding affinity for BMP9, as shown in Table A (equilibrium dissociation constant, KD). Cell reporter assays confirmed inhibition of these ligands (IC50, Table A); (B) corresponding percent inhibition values were graphed on the right. Inhibition of BMP-induced SMAD signaling was dramatically reduced as compared to the other ligands.

**RKER-065 increased lean mass and grip strength in a severe MDX mouse model**

**Methods:** Briefly, 11-week-old DBA2J (WT) mice were dosed with vehicle (veh) while D2 mdx mice were dosed with vehicle (mdx-veh) or RKER-065 (mdx-065) IP GW at 10 mg/kg for 28 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 48 hours post final dose. Statistical analysis for change in bodyweight was done by 2-way ANOVA with Sidak’s multiple comparisons test. Graphs plotted are mean ± SEM. Statistical analysis for lean mass and grip strength was done by Ordinary one-way ANOVA. Data shown is *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns—not significant.

**Results:** Rker-065 treatment led to (A) significant increase in body weight as D2 compared to D2.mdx-Veh; (B) significant increase in lean mass, as compared to mdx-veh; (C) an increase in forelimb grip strength supportive of improved muscle function.

**RKER-065 increased individual muscle weights and utrophin in D2.mdx-065 mice**

**Methods:** (A-B) Mice were sacrificed post final dose, muscles dissected and weighed. Graphs are mean ± SEM. Statistical analysis was done by Ordinary one-way ANOVA with Tukey’s multiple comparisons test. (C) IFC for utrophin in tibialis anterior (TA) done at Myologica, LLC. Data shown is *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 (A) pectoralis and (B) TA as compared to mdx-veh. (C) Compensatory expression of utrophin was higher in the TA of D2.mdx-065 mice than D2.mdx-veh.

**RKER-065 demonstrated increases in bone mineral density and trabecular bone**

**Methods:** Briefly, 11-week-old DBA2J (WT) were dosed with vehicle (veh) while D2 mdx mice were dosed with vehicle (mdx-veh) or RKER-065 (mdx-065) IP GW at a dose of 10 mg/kg for 42 days to observe effects on bone. At study termination, hind limbs were collected and imaged via μCT (GX). Trabecular bone microarchitecture was measured at distal femur using Analyze 14.0 software. Bone mineral density (BMD) was measured using rodent dual-energy X-ray absorptiometry (DXA). Graphs plotted are mean ± SEM. Statistical analysis done by Ordinary One-Way ANOVA. Data shown is *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns—not significant.

**Results:** Figure (A-C) images of distal femur displaying bone morphometric changes. D2.mdx-veh mice displayed (D) significant decreases in bone mineral density, while mdx-065 had no significant difference compared to WT-veh. RKER-065 treatment led to (E) a significant increase in bone volume fraction by D42 as compared to D2.mdx-Veh, (F) a significant increase in trabecular thickness as compared to mdx-veh, (G) a significant decrease in trabecular spacing, and (H) a significant increase in trabecular number.

**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 a robust increase in muscle mass, functional strength, and bone formation in the D2 mdx mouse model.
- This is clinically relevant as DMD patients have severe muscle loss, muscle function and are at a higher risk of fractures. These studies provide proof-of-concept for KER-065, which is being developed for the treatment of neuromuscular disorders, with an initial focus on Duchenne muscular dystrophy.

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