Inhibition of myostatin activation by SRK-015 promotes muscle strength in a multiple mouse models of SMA

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Abstract

Pharmacological inhibition of myostatin is a promising therapy for many muscle diseases. Multiple anti-myostatin therapies are currently in the clinic, many of which also inhibit related family members, such as GDF11 and Activin A. This lack of selectivity has the potential to result in unwanted side effects, some of which may be particularly important to avoid in pediatric populations.

Myostatin is expressed as an inactive proprotein and undergoes two cleavage steps to release and activate the mature growth factor. While the mature form of myostatin is highly homologous to other TGFβ family members, most notably GDF11, their pro-domains are very divergent. We therefore targeted the pro-form of myostatin to generate highly specific antibodies that prevent release from the prodomains. One such antibody, SRK-015, inhibits the second cleavage step, preventing activation of mature myostatin.

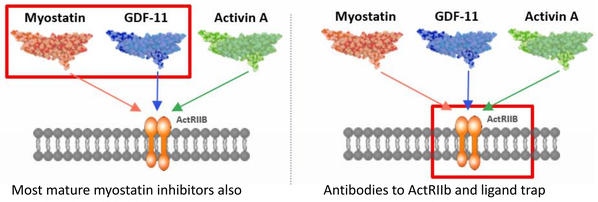
We have confirmed that SRK-015 specifically binds pro- and latent myostatin and does not recognize mature myostatin or any forms of GDF11 or Activin A. We have also shown that SRK-015 increases muscle mass and force in healthy mice and prevents muscle loss in a dexamethasone-induced model of atrophy.

Here we demonstrate that the parental clone of SRK-015, SRK-015P, improves muscle function in multiple models of SMA. We first assessed the ability of SRK-015P to increase muscle function in two variants of the Δ7 model. The first variant aimed to approximate type II SMA: Δ7mice were administered a subtherapeutic dose of the SMN splice modulator SMN-C1 from birth until day 24, after which the dose was increased to a high, therapeutic dose, and SRK-015P treatment initiated. The second variant aimed to model type III/IV SMA: Δ7 mice are given high dose SMN-C1 from birth, with SRK-015P treatment again beginning at day 24. In both models 4 weeks of SRK-015P treatment resulted in significant improvements in muscle force. Additionally, we observed significant increases in cortical and trabecular bone volume in Δ7 mice administered high dose SMN-C1 from birth and 4 weeks of SRK-015P treatment.

The data presented here indicate that blocking myostatin activation with SRK-015 has therapeutic potential for SMA, both as a monotherapy and as an adjunct to splice modulator therapies. In addition, the specificity of this antibody for myostatin may be of particular relevance for safety in a chronic treatment setting for pediatric populations.

Myostatin specificity is difficult to achieve

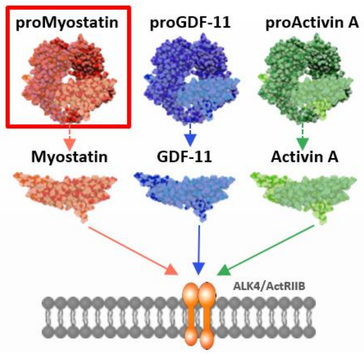
TRADITIONAL APPROACHES



Most mature myostatin inhibitors also inhibit mature GDF11 (and in many cases other growth factors as well)

Antibodies to ActRIIb and ligand trap approaches inhibit signaling of multiple ligands

SCHOLAR ROCK APPROACH: INHIBIT MYOSTATIN PROFORMS



Benefits of Scholar Rock Approach

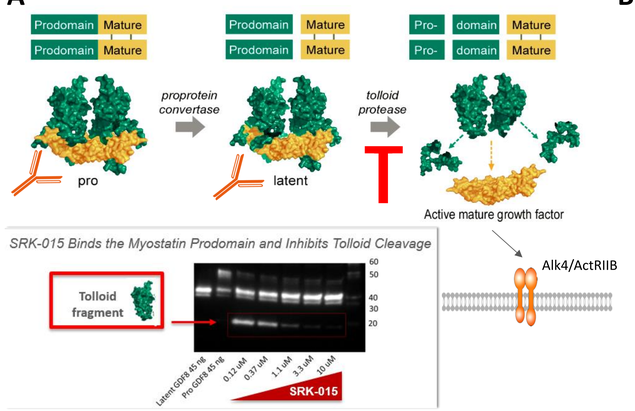
Identity between Myostatin and GDF11

Mature growth factor	Pro-domain
90%	47%

Superior selectivity due to sequence divergence of prodomains.

SRK-015 is an investigational product under development for SMA and other disorders. The safety and efficacy of this molecule have not been established.

SRK-015: A fully human antibody that blocks cleavage of the Myostatin prodomain



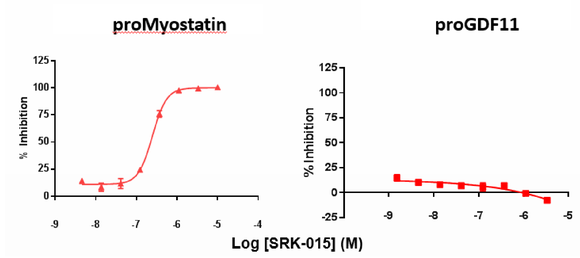
B

SRK-015 binding to myostatin and related proteins

	SRK-015 KD (nM)
ProMyostatin	**2.9**
Latent Myostatin	**2.4**
Myostatin	-
ProGDF11	-
GDF11	-
ProActivin A	-
Activin A	-
BMP9	-
BMP10	-
TGFβ1	-

C

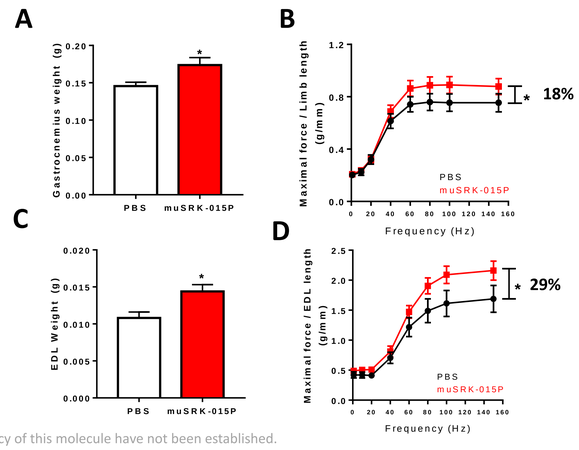
Inhibition of proteolytic activation Cell based assay



(A) Schematic of myostatin processing. SRK-015 binds to both pro- and latent myostatin and inhibits the tolloid-mediated cleavage step. Incubation of recombinant purified latent myostatin (inset) with increased concentrations of SRK-015 and tolloid protease results in inhibition of cleavage. (B) SRK-015 binds to pro- and latent myostatin with high affinity, with no binding detected to related family members. Binding was determined by biolayer interferometry (ForteBio Octet).

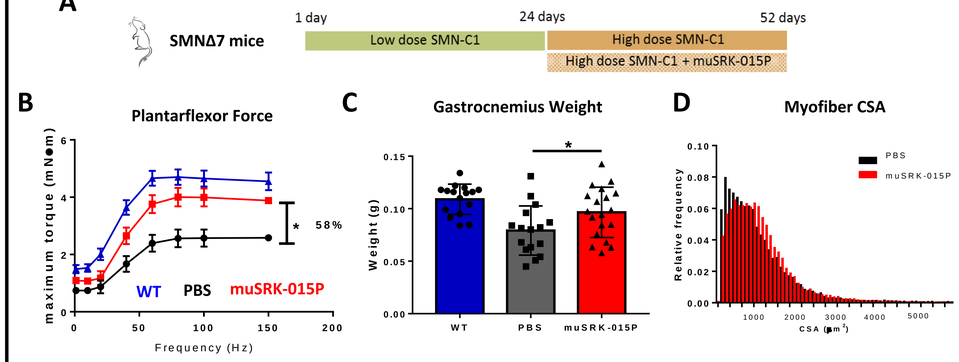
(C) SRK-015 inhibits proMyostatin activation, but not proGDF11 activation, in a cell based assay. Recombinant proMyostatin or proGDF11 was incubated SRK-015 and the required proteases (Furin and mTLL2 for proMyostatin, PCSK-5 and BMP-1 for proGDF11) for 24 hours. Following proteolysis, the reaction mixture was incubated with a cell line expressing a Smad2/3 responsive luciferase reporter element, which allows myostatin or GDF11 induced signaling to be measured via luciferase activity. SRK-015 fully inhibits the proteolytic activation of proMyostatin, while having no effect on proGDF11 activation.

SRK-015P improves muscle mass and strength in healthy mice



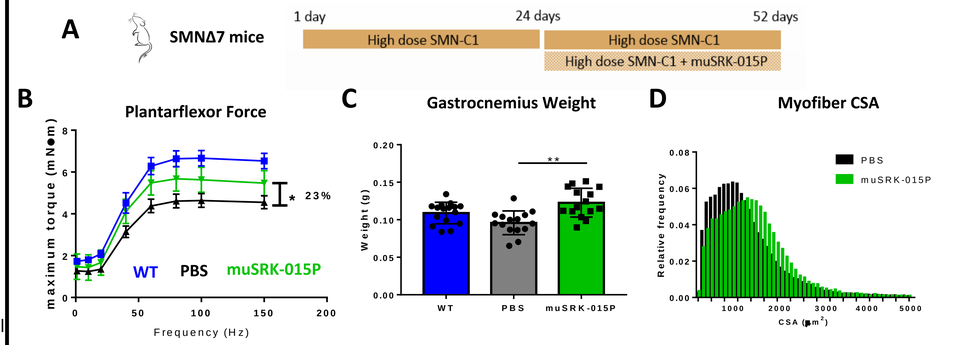
C57BL/6 mice (9 weeks old) were treated for 4 weeks with muSRK-015P (the parental clone of SRK-015) on a mouse IgG1 framework. (A) Gastrocnemius weight. (B) In vivo plantarflexor functional performance normalized to limb length. (C) EDL weight. (D) In vitro EDL performance normalized to EDL length. N=9 (EDL muSRK-015P) or N=10 all other groups. Data are mean ± SEM and were analyzed by two way ANOVA. * Main effect P=0.003 (Plantarflexor) or P=0.0003 (EDL).

Muscle mass function in an intermediate model of SMA is improved upon treatment with SRK-015P



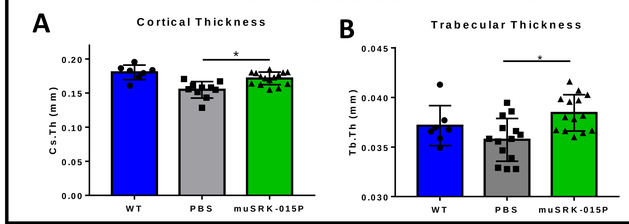
(A) Schematic of treatment paradigm. Δ7 mice were treated with a low dose (0.1 mg/kg/day) of the SMN splice modulator SMN-C1 from post-natal days 2 to 24. At day 24, mice were switched to a high dose (3 mg/kg/day) of SMN-C1, and treatment with PBS or muSRK-015P (20 mg/kg/week) initiated. After 4 weeks, in vivo plantarflexor muscle function was assessed, and the gastrocnemius muscle was isolated and weighed. (B) Δ7 mice treated with muSRK-015 exhibit a 58% increase in the force generation of the plantarflexor muscle group. Data are mean ± SEM. *P<0.0001 by 2-way ANOVA. (C) Gastrocnemius muscles from animals treated with muSRK-015P display a 22% increase in weight compared to PBS control. Data are mean ± SD. *P=0.039 by T-Test. (D) Frequency distribution of myofiber cross sectional area. N=16 (WT, PBS) or N=19 (muSRK-015P).

SRK-015P increases muscle mass and function in a mild model of SMA



(A) Schematic of treatment paradigm. Δ7 mice were treated with a high dose (3 mg/kg/day) of SMN-C1 from post-natal day 2. At day 24 treatment with PBS or muSRK-015P (20 mg/kg/week) began. 4 weeks later, in vivo plantarflexor muscle function was assessed, and the gastrocnemius muscle was isolated and weighed. (B) High dose Δ7 mice treated with muSRK-015 exhibit a 23% increase in the force generation of the plantarflexor muscle group. Data are mean ± SEM. *P<0.0001 by 2-way ANOVA. (C) Gastrocnemius muscles from animals treated with muSRK-015P display a 28% increase in weight compared to PBS control. Data are mean ± SD. *P=0.0002 by T-Test. (D) Frequency distribution of myofiber cross sectional area. N=11 (WT) or N=16 (PBS, muSRK-015P).

SRK-015P increases bone mass in a mild model of SMA



Tibias from high dose SMNC-1 treated mice were collected and analyzed by μCT. (A) muSRK-015 increases cortical thickness. *P=0.0018 by 1-Way ANOVA. (B) muSRK-015P increases trabecular thickness. *P=0.0032 by 1-Way ANOVA. Data are mean ± SD. N=11 (WT) or N=16 (PBS, muSRK-015P).

Summary

- SRK-015 is a specific inhibitor of myostatin activation. The lack of binding to related family members may reduce the potential for unwanted side effects that may occur with less specific inhibitors.
- Inhibition of myostatin activation is an effective way to increase muscle mass and strength in multiple pre-clinical models, including mouse models of SMA with varying degrees of severity.
- Preparations are under way to move SRK-015 into clinical trials for SMA in patients being treated with SMN -elevating therapies and as a monotherapy for selected groups of patients.