Generation of Novel, Orally Active Selective Macrocyclic Peptide Inhibitors of Myostatin for Neuromuscular Diseases

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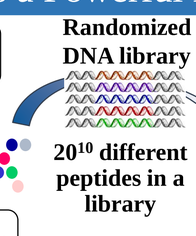
Background

- Myostatin is a one of major negative regulators of muscle growth.[1]
- Although clinical significance of myostatin signal blockade has yet to be validated for neuromuscular diseases such as DMD, sarcopenia and IBM,[2] selective second generation myostatin inhibitors are currently in P3 trials for SMA, suggesting the importance of myostatin's MOA for muscle function improvement.[3,4]
- Biologics (antibody, Fc-fusion protein etc.) requires repeated injections (IV or SC) which is a burden for patients.
- Macrocyclic peptide has a potency of oral administration, which provides a huge benefit for patients and caregivers.
- Selective myostatin inhibitor macrocyclic peptides were identified by PeptiDream's proprietary PDPS (Peptide Discovery Platform System) technology, which displays peptides with hugely diversity (>10^{13}).[5]
- In vitro and In vivo efficacy of a milestone peptide 99m was evaluated.

PDPS is a Powerful Peptide Discovery Platform

1. Amino Acid (AA) Building Blocks PeptiDream's 3000+ AAs

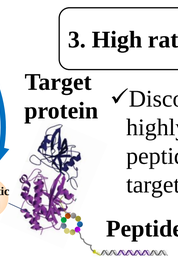


2. Trillions of peptides in each library

✓Robust, cell-free synthesis derived by nature's way of making peptides

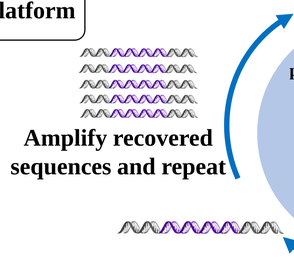
3. High rate of hit finding success

✓Discover high affinity and highly selective macrocyclic peptide binders to almost any target quickly and efficiently



4. Continuously evolving platform

✓Continuous expansion of AA building blocks that libraries can be made with and optimized from (grew from 200 to >3,000)
✓Automation of the platform (high throughput)



99m is the most updated, selective and robust (endocrine and autocrine/paracrine) myostatin blocker

- ActivinA, myostatin and GDF11 signal through ActRIIB and ALK4/5 receptors.
- 99m provides robust myostatin blockade.

1st generation
- non-selective inhibition

1. adnectin (Biohaven) (IV)
2. domagrozumab (Pfizer) (IV)

- anti-ActRIIB

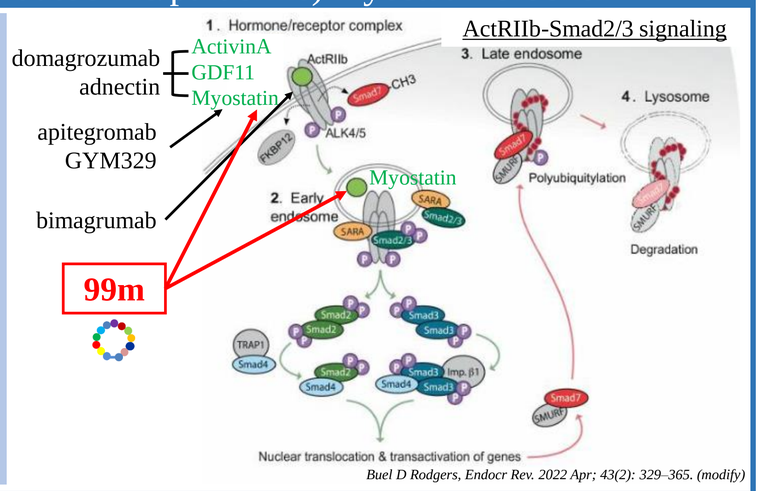
3. bimagrumab (Novartis) (IV)

2nd generation
- selective myostatin inhibition

1. apitegromab (Scholar Rock) (IV)
2. GYM329 (Chugai/Roche) (SC)

3rd generation
- selective myostatin inhibition and oral

1. 99m (PeptiDream) (SC, PO)



Buel D Rodgers, Endocr Rev. 2022 Apr; 43(2): 329–365. (modify)

99m is highly selective myostatin inhibitor against GDF11 and Activin A

Affinity of 99m to myostatin and GDF11 (SPR)

Method
Instrument: BiacoreT200
Chip: CM3
Buffer: HBS-EP + 1%DMSO
Temperature: 25℃
Curve: Fc=2-1, Fc=3-1

Result

	Ka (1/Ms)	kd (1/s)	KD (M)
Myostatin	$3.15x10^{5}$	$5.28x10^{-4}$	$1.68x10^{-9}$
GDF11	$1.75x10^{5}$	$3.34x10^{-3}$	$1.91x10^{-8}$

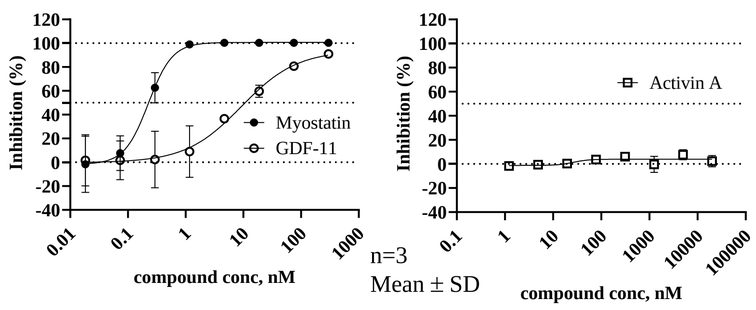
- SPR: 99m has 10-fold KD difference in myostatin vs. GDF11.

Inhibitory properties of 99m in cell reporter gene assay

Method
Seed TGFb reporter HEK blue cells in a PDL-coated plate (40,000 cells/well) and incubate for 24 hours.
Change growth medium (DMEM, 10% FBS, 50 µg/mL Normocin) to assay medium (DMEM, 0.1% BSA, 50 µg/mL Normocin) and incubate for 2 hours for equivalation.
Stimulation with ligand and 99m:
Pre-incubate 99m and of myostatin (final conc: 4.5 ng/mL), GDF-11 (final conc: 4.5 ng/mL) or Activin A (final conc: 10 ng/mL) for 30 minutes in assay medium at 37 degree. Add pre-incubated mixture to the cells and incubate for 24 hours.
Measurement:
After 24 hours incubation, 30 µL of supernatant was used to measure the absorbance signal (620 nm).

Result

	IC_{90} (nM)
Myostatin	0.64
GDF11	701.73
Activin A	>20,000
GD11/myostatin selectivity	1088



n=3
Mean ± SD

- Activity (cell reporter gene assay): 1088-fold IC90 difference of myostatin vs. GDF11.

Off-target panel profile (167 receptors)

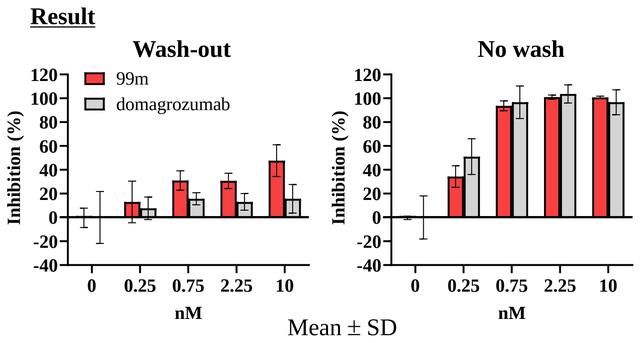
Method
- 99m 10 µM. 99m binding to off-target sites was evaluated in a screening enzyme and radioligand binding studies for 167 targets.

Result
99m has a clean off-target panel profile (167 receptors).

99m provides robust intracellular myostatin blockade

Method
Seed TGFb reporter HEK blue cells in a PDL-coated plate (18,000 cells/well) and incubate for 48 hours
Change growth medium (DMEM, 10% FBS, 50 µg/mL Normocin) to assay medium (DMEM, 0.1% BSA, 50 µg/mL Normocin).
Wash-out sample (n=3):
Add 99m and domagrozumab to the cells. After 3 hours, wash with PBS 2 times and add 3 ng/mL of myostatin to the cells. Incubate for 20 hours.
No wash sample (n=3):
Pre-incubate 99m or domagrozumab and 3 ng/mL myostatin for 1 hour in assay medium.
Add pre-incubated solution to the cells and incubate for 20 hours.
Measurement:
After incubation, 30 µL of supernatant was used to measure the absorbance signal (620 nm).

Result



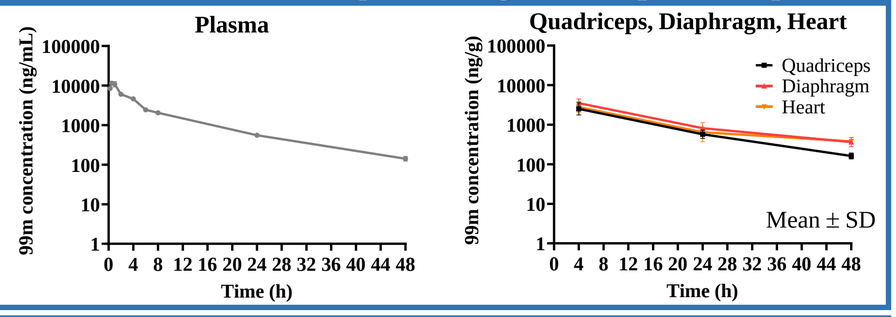
Mean ± SD

- Pre-treatment of 99m inhibited myostatin signal in vitro cell reporter gene activity in a wash-out assay, while domagrozumab showed weaker inhibition.
- Cell penetration and intracellular blockade by 99m suggest multiple modes of myostatin inhibition and potentially greater impact on muscle function.

In vivo PK: 99m distributes into muscle at equal or higher compared to plasma

Method
Instrument: HPLC
Animal: CD1 Mouse (n=3)
ROA: SC, single shot
Dose: 10 mg/kg
Vehicle: PBS

- 99m was long PK and distributes into muscles.

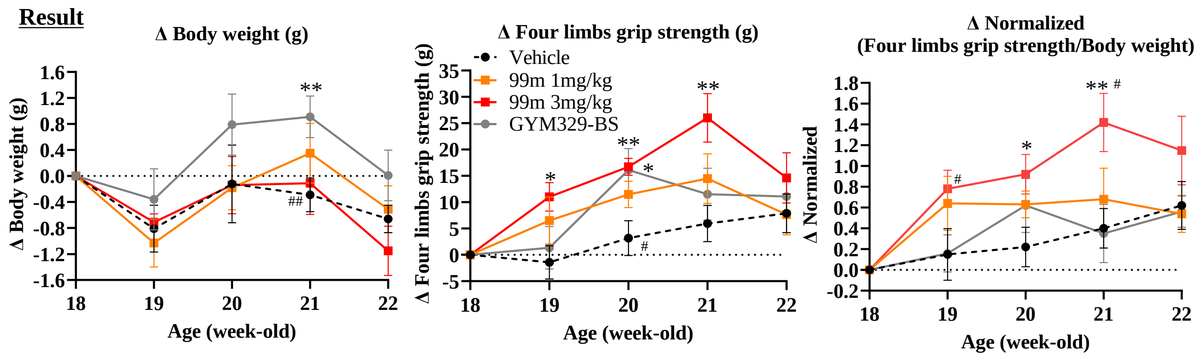


Grip strength improvement by 99m superior to GYM329-BS

Method
- Mouse: DBA/2-*mdx*, female, 18 weeks-old
- Duration of Treatment: 4 weeks
- Group:
  1. Vehicle (n=9)
  2. 99m 1 mg/kg, SC, QW (n=8)
  3. 99m 3 mg/kg, SC, QW (n=8)
  4. GYM329-BS 10 mg/kg, IV, single (n=8)
- Key assessments:
  1. Body weight (BWT) once per week
  2. Four limbs grip strength once per week
  3. Normalized grip strength (grip strength/BWT) once per week

Mean ± SEM
Student t test (vs. Vehicle)
*: P<0.05, **: P<0.01
(vs. GYM329-BS)
#: P<0.05, ##: P<0.01

Result

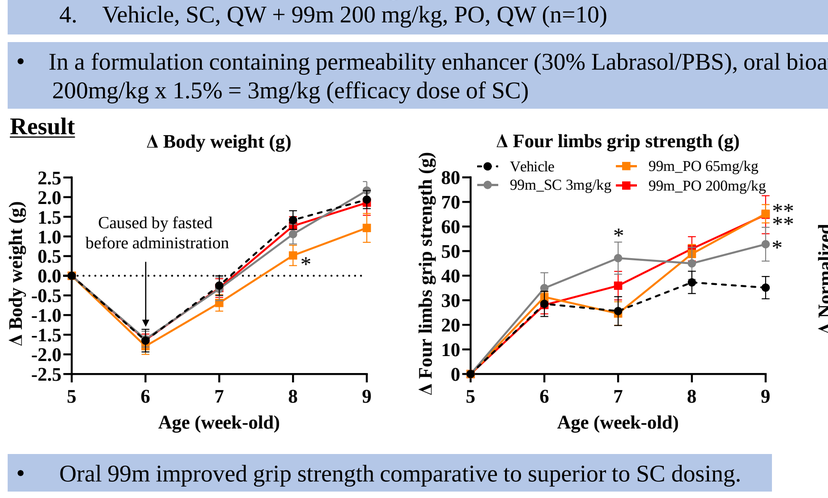


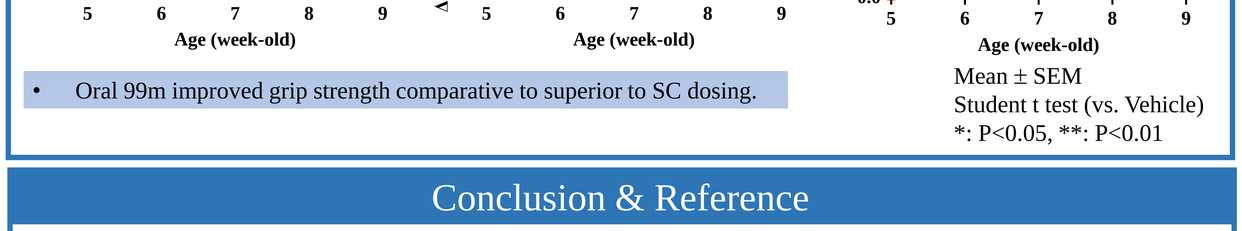
- 99m shows superior improvement in grip strengths (appendicular and normalized), suggesting improvement of muscle quality in DMD model mouse vs. GYM329-BS.

99m prevents necrosis, fibrosis and fat deposition of diaphragm

Method
- Mouse: DBA/2-*mdx*, male, 24 weeks-old
- Duration of Treatment: 4 weeks
- Group:
  1. Vehicle (n=5)
  2. 99m 3 mg/kg, SC, QW (n=5)
  3. GYM329-BS 10 mg/kg, IV, single (n=5)
- Diaphragm histopathology (frozen section):
  1. Necrosis: mIgG immunofluorescence
  2. Fat deposition: oil red O staining
  3. Fibrosis: Van Gieson staining

Result





- 99m 3mg/kg, SC, QW administration to DBA/2-*mdx* mice significantly inhibited myonecrosis of the diaphragm and its subsequent fibrosis and fat deposition.
- Anti-latent myostatin antibody (GYM329-BS) did not show significant suppression in evaluated parameters.

Mean ± SEM
Student t test (vs. Vehicle)
*:P<0.05, **:P<0.01

Oral 99m improves grip strength compared to SC administration

Method
- Mouse: B10-*mdx*, female, 5 weeks-old
- Duration of Treatment: 4 weeks
- Group:
  1. Vehicle, SC, QW + Vehicle, PO, QW (n=13)
  2. 99m 3 mg/kg, SC, QW + Vehicle, PO, QW (n=11)
  3. Vehicle, SC, QW + 99m 65 mg/kg, PO, QW (n=10)
  4. Vehicle, SC, QW + 99m 200 mg/kg, PO, QW (n=10)
- Key assessments:
  1. Body weight (BWT) once per week
  2. Four limbs grip strength once per week
  3. Normalized grip strength (grip strength/BWT) once per week

- In a formulation containing permeability enhancer (30% Labrasol/PBS), oral bioavailability of 99m was 1.5% in mice. 200mg/kg x 1.5% = 3mg/kg (efficacy dose of SC)

Result

- Oral 99m improved grip strength comparative to superior to SC dosing.

Mean ± SEM
Student t test (vs. Vehicle)
*: P<0.05, **: P<0.01

Conclusion & Reference

Conclusion
- 99m's potent, selective inhibition of myostatin signaling with IC90 of 0.64 nM and 1088-fold selectivity over GDF11 in vitro.
- Weekly SC administration of 99m shows improvement of grip strength superior to anti-latent myostatin antibody (GYM329-BS) in DBA/2-*mdx* mice.
- Weekly oral dosing of 99m shows grip strength improvement comparable to weekly SC dosing in B10-*mdx* mice.

Reference
1. *McPherron, Nature 387, 1997*
2. *Se-Jin Lee, J Clin Invest. 2021;131(9)*
3. *https://www.roche.com/solutions/pipeline*
4. *https://scholarrock.com/our-pipeline/*
5. *https://www.peptidream.com/en/science/pdps/*