Poster 323

Taldefgrobep Alfa Reduces Lipids and Increases Mitochondrial Content in Adipocytes

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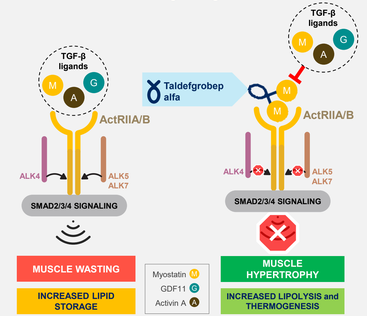
INTRODUCTION

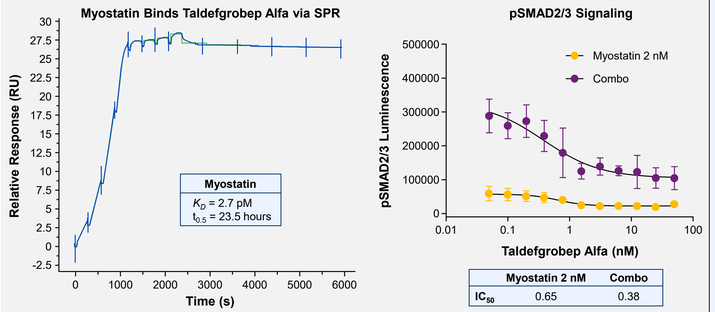
- Antiobesity medications, including glucagon-like peptide-1 (GLP-1) receptor agonists, reduce total body weight through loss of fat and lean muscle mass; however, lean muscle loss may have long-term adverse consequences[1-4]
- Inhibition of myostatin and activin A signaling induces significant fat loss and increase in lean mass,[5,6] an ideal combination with GLP-1 receptor agonist therapy
- Taldefgrobep alfa, a novel myostatin inhibitor, binds and sequesters myostatin, forming a stable taldefgrobep alfa/myostatin complex, which potently binds activin II receptors and competes with receptor ligands, limiting downstream muscle wasting[7,8] (**Figure 1**)
- Myostatin binds with low picomolar affinity to a taldefgrobep alfa-laden surface plasmon resonance surface, and taldefgrobep inhibits pSMAD2/3 signaling in a cell-based luciferase reporter assay (**Figure 2**)
- In mouse models of obesity, treatment with taldefgrobep alfa led to improvements in lean mass and loss of fat[7,8]

Figure 1. Taldefgrobep Alfa: Reduces Free Myostatin and Blocks ActRIIA/B Signaling



ActRIIA/B, myostatin/activin receptor type II A/B receptor; ALK, activin-like kinase; GDF11, growth differentiation factor 11; TGF-β, transforming growth factor-beta.

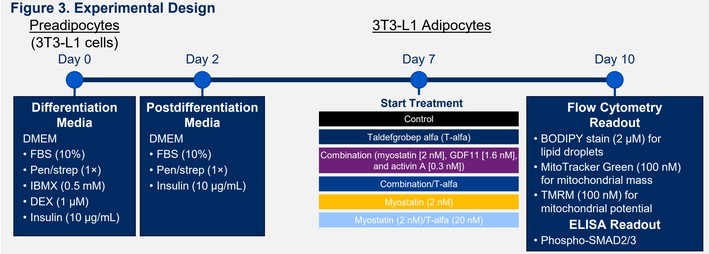
Figure 2. Taldefgrobep Alfa Binds to Myostatin and Inhibits pSMAD2/3 Signaling



	Myostatin 2 nM	Combo
IC_{50}	0.65	0.38

Combo (myostatin [2 nM], GDF11 [1.6 nM], and activin A [0.3 nM]); GDF11, growth differentiation factor 11; IC_{50}, half-maximal inhibitory concentration; RU, resonance unit; SPR, surface plasmon resonance.

Figure 3. Experimental Design



BODIPY, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene; DEX, dexamethasone; DMEM, Dulbecco's Modified Eagle Medium; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; GDF11, growth differentiation factor 11; IBMX, 3-Isobutyl-1-methylxanthine; Pen/strep, penicillin/streptomycin; T-alfa, taldefgrobep alfa; TMRM, tetramethylrhodamine methyl ester.

OBJECTIVE

- Elucidate the role of taldefgrobep alfa/myostatin complex in regulating SMAD2/3-mediated adipocyte metabolism

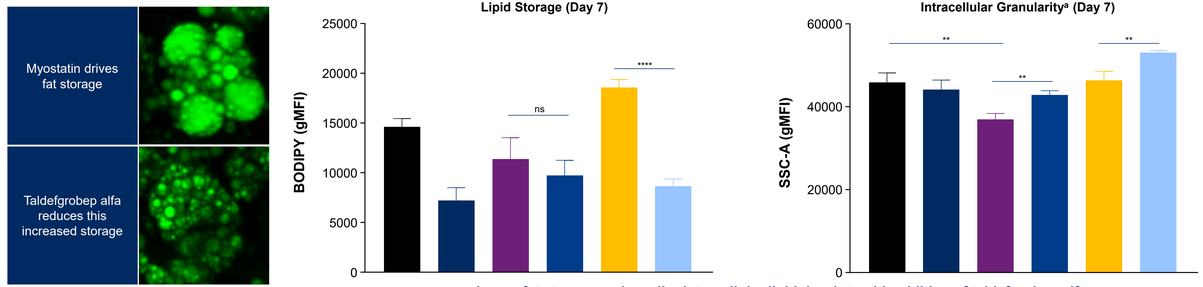
METHODS

- 3T3-L1 fibroblasts were differentiated into adipocytes, followed by the addition of taldefgrobep alfa and activin II receptor ligands, including myostatin, growth differentiation factor 11, and activin A (**Figure 3**)
- Post differentiation, adipocytes were assessed for:
  - Lipid content and droplet size with BODIPY staining and flow cytometry
  - Mitochondrial activity with co-staining with MitoTracker Green and tetramethylrhodamine methyl ester
  - SMAD2/3 signaling with enzyme-linked immunosorbent assay

RESULTS

- Addition of activin II receptor ligands led to an increase in lipid accumulation in adipocytes. Addition of taldefgrobep alfa reduced this fat storage, and smaller intracellular lipid droplets were observed (**Figure 4**)
- Mitochondrial mass was reduced with activin II receptor ligands but increased in the presence of taldefgrobep alfa (**Figure 5**)
- The addition of taldefgrobep alfa to adipocytes led to a decrease in SMAD2/3 signaling (**Figure 6**)

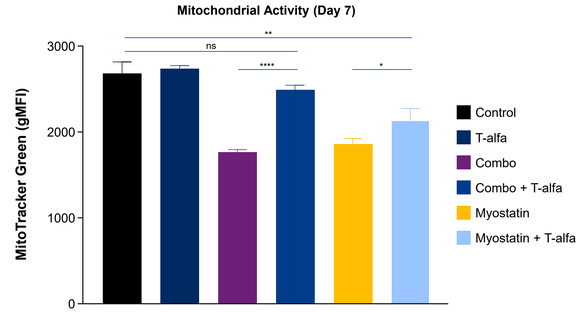
Figure 4. Taldefgrobep Alfa Treatment Reduces Fat Storage in Adipocytes



Lower fat storage and smaller intracellular lipid droplets with addition of taldefgrobep alfa

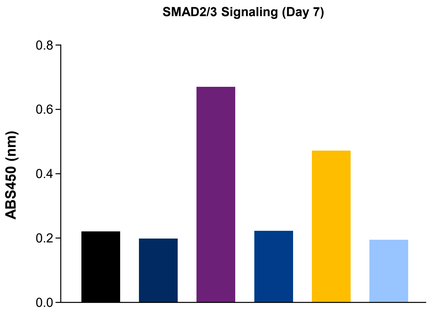
[a]SSC measures light scattered by intracellular components, providing information about internal complexity and granularity of cellular structures.[9] Significance tested with two-tailed *t* test.**P < 0.01; ****P < 0.0001.
BODIPY, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene; Combo (myostatin, GDF11, and activin A); GDF11, growth differentiation factor 11; gMFI, geographic mean fluorescence intensity; ns, not significant; SSC-A, side scatter area; T-alfa, taldefgrobep alfa.

Figure 5. Taldefgrobep Alfa Increases Mitochondrial Activity in Adipocytes



Significance tested with two-tailed *t* test. *P < 0.05; **P < 0.01; ****P < 0.0001.
Combo (myostatin, GDF11, and activin A); GDF11, growth differentiation factor 11; gMFI, geographic mean fluorescence intensity; ns, not significant; T-alfa, taldefgrobep alfa.

Figure 6. Taldefgrobep Alfa Decreases SMAD2/3 Signaling in Adipocytes



ABS, absorbance; Combo (myostatin, GDF11, and activin A); GDF11, growth differentiation factor 11; T-alfa, taldefgrobep alfa.

CONCLUSIONS

- Taldefgrobep alfa/myostatin complexes directly affect activin II receptor signaling cascades in adipose tissue to reduce fat storage
- Mitochondrial mass was reduced with activin II receptor ligands but increased in the presence of taldefgrobep alfa, suggesting a breakdown of stored fat through an increased metabolic rate with taldefgrobep alfa treatment
- SMAD signaling negatively regulates lipolysis in adipose tissue. Adding taldefgrobep alfa with activin II receptor ligands decreased SMAD2/3 signaling compared with ligands alone, showing that taldefgrobep alfa directly modulates this cascade in adipocytes, leading to reduced adipocyte size
- Our data support the role of activin receptor-mediated signaling in regulating adipose homeostasis, and inhibiting SMAD signaling with taldefgrobep alfa leads to decreased adipose mass

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DISCLOSURES: EHM, **NN**, **DP**, **VG**, **CS**, **BML**, **CJ**, **CB**, and **BC** are employed by and/or hold stock/stock options in Biohaven Pharmaceuticals. **DM** is a former employee of Biohaven Pharmaceuticals.

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