POSTER 3005

Taldefgrobep Alfa Inhibition of Activin II Receptor Signaling Drives Lipid Oxidation and Muscle Growth, Providing a Novel Therapeutic Approach to Obesity

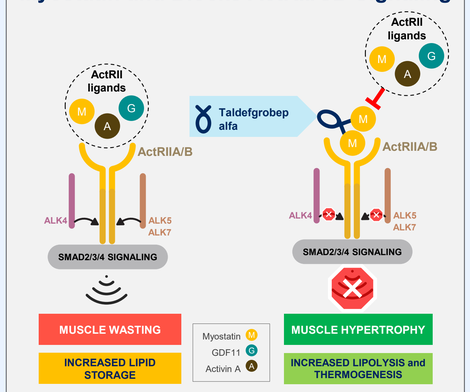
David Pirman, PhD[1]; Clifford Bechtold, MS[1]; Eric H. Ma, PhD[1]; Nadee Nissanka, PhD[1]; Brian Linhares, PhD[1]; Charlotte Spliid, PhD[1]; Ansarullah, PhD[2]; Volkan Granit, MD, MSc[1]; Christopher Jensen, PharmD[1]; Camille Schrier, PharmD[1]; Peter Ackerman, MD[1]; Bruce Car, DVM, PhD[1]; Vladimir Coric, MD[1]; Se-Jin Lee, MD, PhD[3,4]

[1]Biohaven Pharmaceuticals, New Haven, CT, USA; [2]The Jackson Laboratory, Bar Harbor, ME, USA; [3]The Jackson Laboratory, Farmington, CT, USA; [4]University of Connecticut School of Medicine, Farmington, CT, USA

INTRODUCTION

- Obesity is a disease of excess or abnormal adipose tissue, the key driver of its pathogenic process[1-3]
- Currently approved antiobesity medications, including glucagon-like peptide-1 (GLP-1) receptor agonists, achieve reductions in total body weight based on a composite loss of fat mass and loss of lean muscle mass; however, the loss of lean muscle mass with these therapeutic agents may have long-term adverse health consequences[4-7]
- Alterations in phosphocreatine and proline catabolism pathways have been shown in previous work to have an impact on obesity, making these important biomarkers in the assessment of adipocyte metabolism[8,9]
- Inhibition of myostatin and activin A signaling induces significant fat loss while increasing lean mass; these body composition changes are optimal in the management of people living with overweight and obesity[9,10]
- Taldefgrobep, a novel myostatin inhibitor, targets and binds mature myostatin to form a stable complex, which potently binds activin II receptors (ActRII) and competes with receptor ligands; this limits downstream signaling, including SMAD2/3 phosphorylation (**Figure 1**)[11-13]

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



ActRIIA/B, myostatin/activin type IIA/B receptor; ALK, activin-like kinase; GDF11, growth differentiation factor 11.

OBJECTIVE

- Explore and evaluate the role of SMAD2/3-mediated adipocyte regulation with taldefgrobep in the treatment of overweight and obesity through in vitro and in vivo experimentation

METHODS

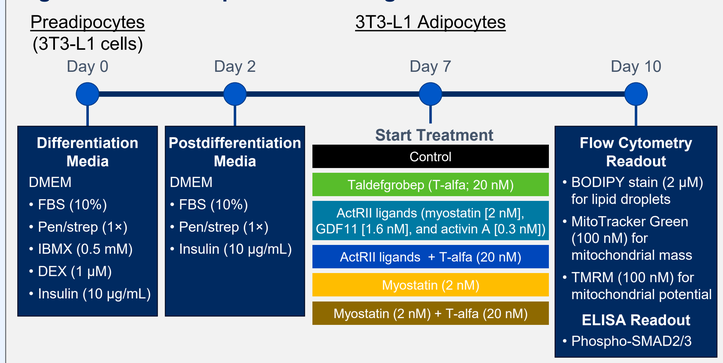
In vitro

- 3T3-L1 fibroblasts were differentiated into adipocytes, followed by the addition of taldefgrobep alfa and ActRII ligands, including myostatin, growth differentiation factor 11, and activin A (**Figure 2**)
- Post differentiation, adipocytes were assessed for:
  - Lipid content and droplet size by BODIPY staining and flow cytometry
  - Mitochondrial activity by co-staining with MitoTracker™ Green and tetramethylrhodamine methyl ester
  - SMAD2/3 signaling by enzyme-linked immunosorbent assay
  - Intracellular metabolite abundance measurement using ThermoFisher Q Exactive Orbitrap Mass Spectrometer from 3T3-L1 adipocytes extracted with -20°C methanol/acetonitrile/water (40/40/20)

In vivo

- Six-week-old C57BL/6J male mice received a high-fat diet (60% fat; Research Diets D12492) for 13 weeks prior to their subcutaneous treatment assignment: vehicle twice weekly (BIW), taldefgrobep 100 mg/kg BIW, semaglutide 20 µg/kg once daily (QD), semaglutide 40 µg/kg QD, taldefgrobep 100 mg/kg BIW with semaglutide 20 µg/kg QD or 40 µg/kg QD
- Body composition (EchoMRI™) was assessed at baseline, 4 weeks of treatment, and study end
- Results from 8 weeks of dosing are presented

Figure 2. In Vitro Experimental Design



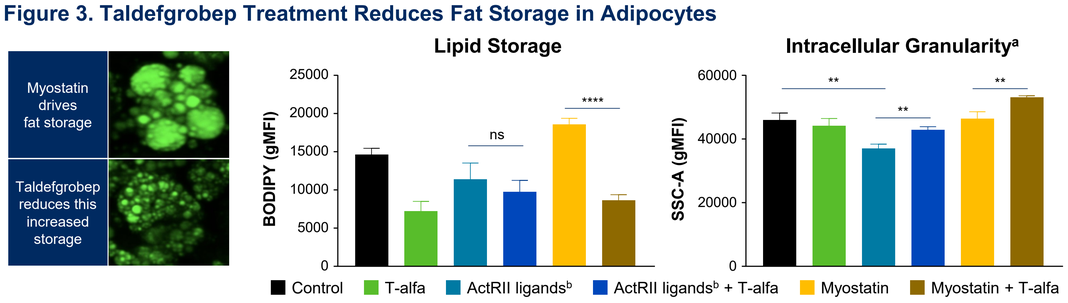
ActRII, activin II receptor; BODIPY, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene; DEX, dexamethasone; DMEM, Dulbecco's Modified Eagle Medium; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; GDF11, growth differentiation factor 11; IBMX, 3-Isobutyl-1-methylxanthine; Pen/strep, penicillin/streptomycin;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

RESULTS

In vitro

- Taldefgrobep treatment attenuated ActRII ligand–induced lipid accumulation in adipocytes, resulting in smaller intracellular lipid droplets (**Figure 3**)
- Taldefgrobep also decreased SMAD2/3 signaling induced by ActRII ligands, a known regulator of lipid homeostasis in adipose tissues (**Figure 4**)
- Mitochondrial content was reduced with ActRII stimulation but increased with taldefgrobep (**Figure 5**)

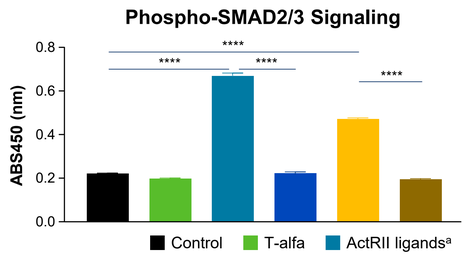
Figure 3. Taldefgrobep Treatment Reduces Fat Storage in Adipocytes



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

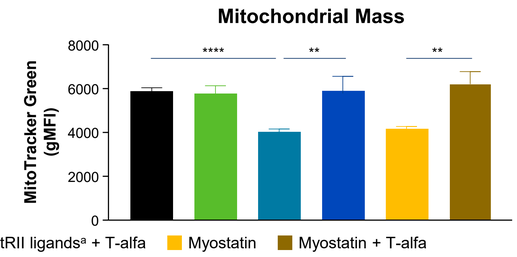
[a]SSC measures light scattered by intracellular components, providing information about internal complexity and granularity of cellular structures.[14] [b]Myostatin, GDF11, and activin A.
Significance tested with two-tailed *t* test. ***P* < 0.01; *****P* < 0.0001.
ActRII, activin II receptor; BODIPY, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene; GDF11, growth differentiation factor 11; gMFI, geographic mean fluorescence intensity; ns, not significant; SSC-A, side scatter area; T-alfa, taldefgrobep.

Figure 4. Taldefgrobep Decreases Phospho-SMAD2/3 Signaling in Adipocytes



[a]Myostatin, GDF11, and activin A. *****P* < 0.0001.
ABS450, absorbance at 450 nm; ActRII, activin II receptor; GDF11, growth differentiation factor 11; T-alfa, taldefgrobep.

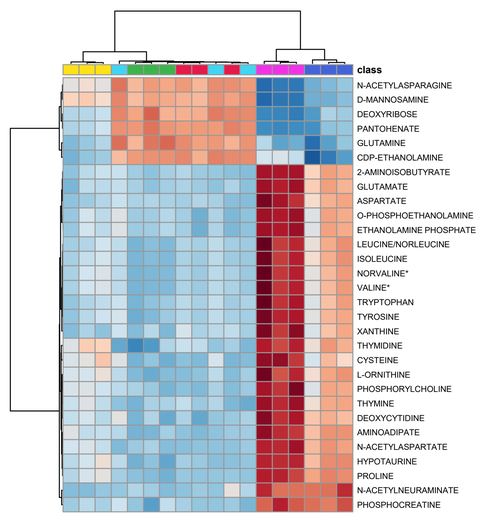
Figure 5. Taldefgrobep Increases Mitochondrial Mass in Adipocytes



[a]Myostatin, GDF11, and activin A. Significance tested with two-tailed *t* test. ***P* < 0.01; *****P* < 0.0001.
ActRII, activin II receptor; GDF11, growth differentiation factor 11; gMFI, geographic mean fluorescence intensity; T-alfa, taldefgrobep.

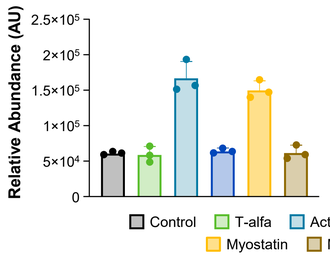
- ActRII ligands alone alter the intracellular metabolite levels relative to adipocytes without ActRII ligands. This change is reversed to control levels with the addition of taldefgrobep (**Figure 6**)
- Data suggest increased phosphocreatine levels in patients with obesity could be due to ActRII ligands; adipocyte phosphocreatine levels increased in the presence of ActRII ligands and reversed in the presence of taldefgrobep (**Figure 7**)
- Proline buildup in ActRII-stimulated adipocytes suggested decreased proline catabolism in the presence of ActRII ligands, preventing a switch to fat-burning metabolism; proline levels are reduced in the presence of taldefgrobep (**Figure 8**)

Figure 6. Taldefgrobep Reversed ActRII Ligand–Induced Alterations in Intracellular Metabolite Levels in Adipocytes



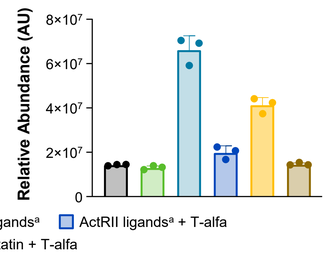
[a]Myostatin, GDF11, and activin A.
ActRII, activin II receptor; GDF11, growth differentiation factor 11; T-alfa, taldefgrobep.

Figure 7. Taldefgrobep Normalizes Intracellular Phosphocreatine Levels in the Presence of ActRII Ligands



[a]Myostatin, GDF11, and activin A.
ActRII, activin II receptor; AU, arbitrary units; GDF11, growth differentiation factor 11; T-alfa, taldefgrobep.

Figure 8. Taldefgrobep Normalizes Intracellular Proline Levels in the Presence of ActRII Ligands

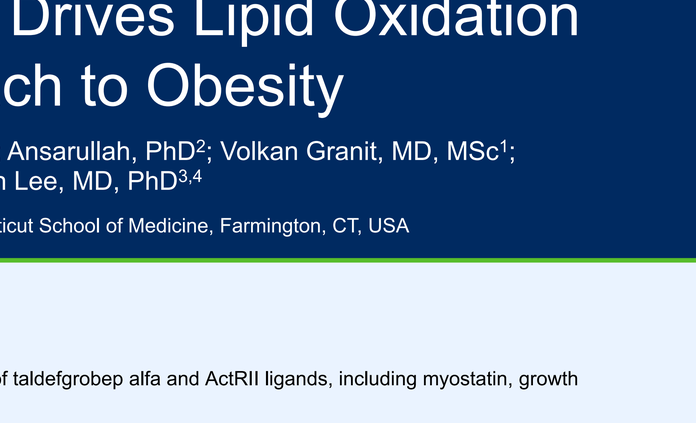


[a]Myostatin, GDF11, and activin A.
ActRII, activin II receptor; AU, arbitrary units; GDF11, growth differentiation factor 11; T-alfa, taldefgrobep.

In vivo

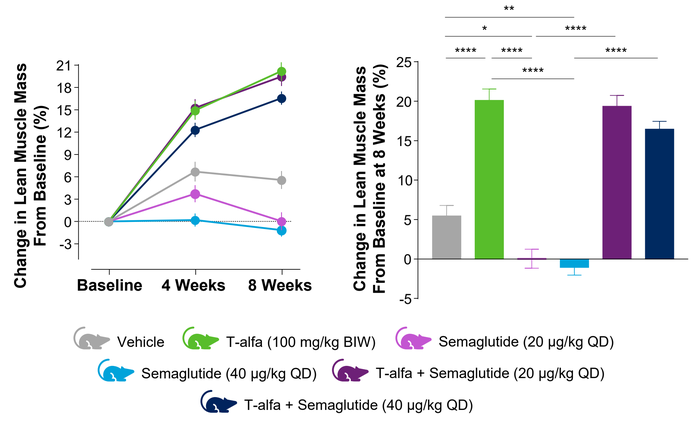
- Through 8 weeks of treatment, all taldefgrobep groups demonstrated significant and durable reductions in fat mass and increased lean mass (**Figures 9** and **10**)
- The addition of taldefgrobep to semaglutide resulted in greater reductions in fat mass and increases in lean mass relative to semaglutide alone

Figure 9. Taldefgrobep Monotherapy and Combination Therapy Resulted in Greater Reductions in Fat Mass Than Semaglutide Alone



n = 15 for vehicle; n = 16 for all other groups. Error bars represent standard error of the mean. Significance evaluated using Tukey's multiple comparisons test. ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001.
BIW, twice weekly; QD, once daily; T-alfa, taldefgrobep.

Figure 10. Taldefgrobep Monotherapy Increased Lean Muscle Mass and Combination Therapy Prevented Muscle Loss Observed With Semaglutide Alone



n = 15 for vehicle; n = 16 for all other groups. Error bars represent standard error of the mean. Significance evaluated using Tukey's multiple comparisons test. **P* < 0.05; ***P* < 0.01; *****P* < 0.0001.
BIW, twice weekly; QD, once daily; T-alfa, taldefgrobep.

CONCLUSIONS

- Our data support the role of activin receptor–mediated signaling in regulating adipose homeostasis and that inhibiting SMAD signaling with taldefgrobep leads to decreased adipose mass
- Taldefgrobep alfa/myostatin complexes interfere with ActRII signaling cascades in adipose tissue to reduce fat storage
- ActRII ligand signaling in adipocytes promotes a fat storage phenotype through changes in adipocyte metabolic pathways. The impact of taldefgrobep to prevent ActRII ligand signaling offers a potential mechanism leading to the fat loss observed in animal models
- In an obese mouse model, taldefgrobep demonstrated significant reductions in fat mass and body weight while increasing lean mass as monotherapy and in combination with a GLP-1 receptor agonist
- These data support development of taldefgrobep as a monotherapy and in combination with GLP-1 receptor agonists; a phase 2 clinical trial evaluating taldefgrobep in overweight and obesity is planned

Presented at Keystone Symposia:
Obesity and Adipose Tissue
February 23-26, 2025 | Banff, Alberta, Canada

DISCLOSURES: DP, **CB**, **EHM**, **NN**, **BL**, **CS**, **VG**, **CJ**, **CS**, **PA**, **BC**, and **VC** are employed by and/or hold stock/stock options in Biohaven Pharmaceuticals. **A** and **SJL** have nothing to disclose.

ACKNOWLEDGMENTS: This study is funded by Biohaven Pharmaceuticals. Medical writing and editorial support were provided by James Banigan, PhD, and Dena McWain of Apollo Medical Communications, part of Helios Global Group, and funded by Biohaven Pharmaceuticals.

REFERENCES:
1. WHO. Accessed January 15, 2025. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. **2.** Panuganti KK, et al. In: *StatPearls*. StatPearls Publishing; 2023. **3.** Shuster A, et al. *Br J Radiol*. 2012;85(1009):1-10. **4.** Pi-Sunyer X, et al. *N Engl J Med*. 2015;373(1):11-22. **5.** Wilding JPH, et al. *N Engl J Med*. 2021;384(11):989-1002. **6.** Locatelli JC, et al. *Diabetes Care*. 2024;47(10):1718-1730. **7.** Wilding JPH, et al. *J Endocr Soc*. 2021;5(suppl 1):A16-A17. **8.** Magdasy S, et al. *Nat Metab*. 2022;4(2):190-202. **9.** Barbato DL, et al. *Cell Death Differ*. 2014;21(1):113-123. **10.** Lee SJ, et al. *J Gerontol A Biol Sci Med Sci*. 2023;78(suppl 1):32-37. **11.** Suh J, et al. *J Bone Metab*. 2020;27(3):151-165. **12.** Ackerman P, et al. Presented at: ObesityWeek 2023; Oct 14-17, 2023; Dallas, TX. Poster 211. **13.** Bechtold C, et al. Presented at: American Diabetes Association 84th Scientific Sessions; Jun 21-24, 2024; Orlando, FL. Poster 2053-LB. **14.** McKinnon KM. *Curr Protoc Immunol*. 2018;120:5.1.1-5.1.11.

To download a copy of this poster, scan QR code.

biohaven