Preclinical Evaluation of the Calpain Inhibitor BLD-3051 as a Therapeutic Strategy for Liver Fibrosis

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INTRODUCTION

Calpains are a family of non-lysosomal intracellular calcium-dependent cysteine proteases that perform limited proteolytic cleavage of its substrates which in turn modulate a variety of signaling pathways controlling phenotypic effects like cell proliferation, migration, differentiation, and apoptosis. Elevated dimeric calpain activity levels are implicated in a variety of disease pathologies including fibrotic diseases. Additionally, calpain knock-outs and small molecule dimeric calpain inhibitors are efficacious in animal models of fibrosis. We have generated calpain inhibitors with significantly improved potency, selectivity, and ADMET properties that may represent important new anti-fibrotic agents.

AIM

Described the properties of BLD-3051, an orally bioavailable small molecule dimeric calpain inhibitor, and characterize its activity in a therapeutic model of liver fibrosis.

METHODS

C57BL/6 mice (8 weeks old) were fed a choline-deficient, L-amino acid, high fat diet (CDAHFD) for the entire 10-week study duration. Oral gavage dosing of BLD-3051 (60, 200 mg/kg daily (QD) and 30, 100 mg/kg twice daily (BID)) was initiated on Week 5 of CDAHFD and continued until termination. Endpoint analyses included body and liver weights and liver enzyme panel. Anti-fibrotic efficacy was evaluated by histopathology using fibrosis, inflammation, and steatosis scores as well as hydroxyproline levels. RNA sequencing of liver tissues was performed on Illumina platform. Differential expression and pathway analysis were performed with Omics Playground®, CLC Genomic Workbench®, and IPA® software. 1-way ANOVA statistics with multiple comparisons testing was used. P values < 0.05 are denoted with an asterisk.

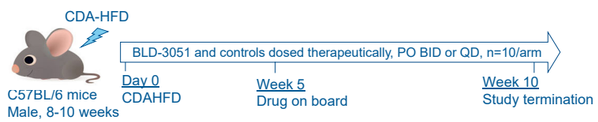
PRECLINICAL PROFILE

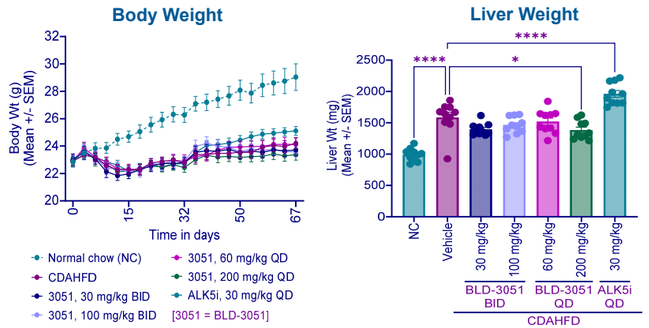
- Biochemical inhibition – IC_{50} values of 20-60 nM for calpains 1,2,9 with slow dissociative behavior ($t_{1/2}$ ~ 2.3h)
- Selectivity over cysteine cathepsins (e.g., Cat K)
- Cellular substrate cleavage assays - low μM IC_{50} values for inhibition of ILK and spectrin cleavage
- Preclinical PK consistent with QD or BID dosing with a favorable liver to plasma ratio

Preclinical Pharmacokinetics			
Species	PO Dose mg/kg	% F	$T_{1/2}$ (h)
Mouse	10	22	2.4
Rat	10	45	5.0
Dog	10	31	2.0
Monkey	10	29	4.3

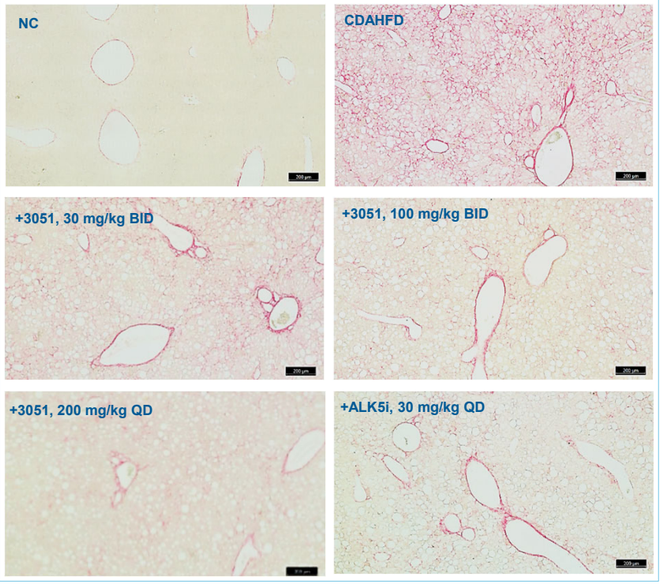
RESULTS

CDAHFD Induced Model of Liver Fibrosis

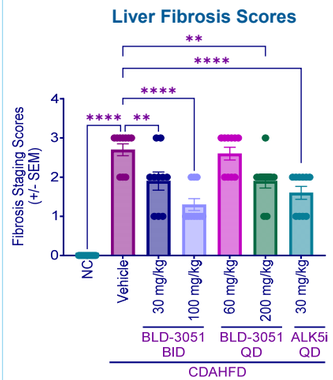




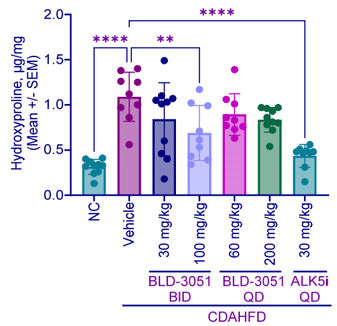
Representative Images Of Fibrosis (PSR Stain)



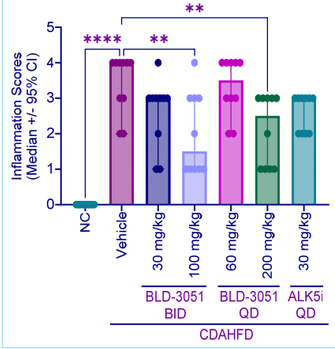
Liver Fibrosis Scores



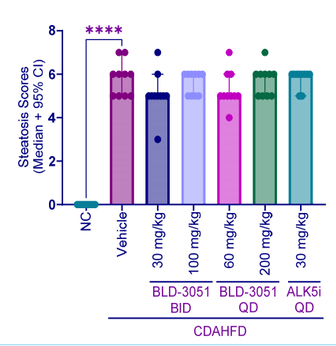
Hydroxyproline Levels



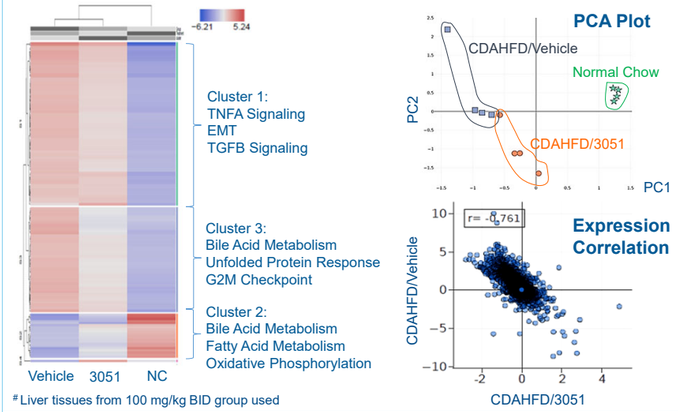
Inflammation Scores



Steatosis Scores



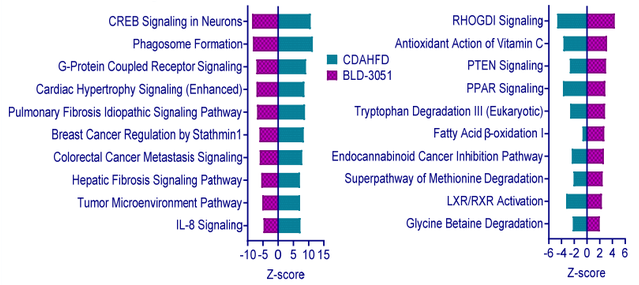
Transcriptomic Profiling of Fibrotic Livers Treated With BLD-3051#



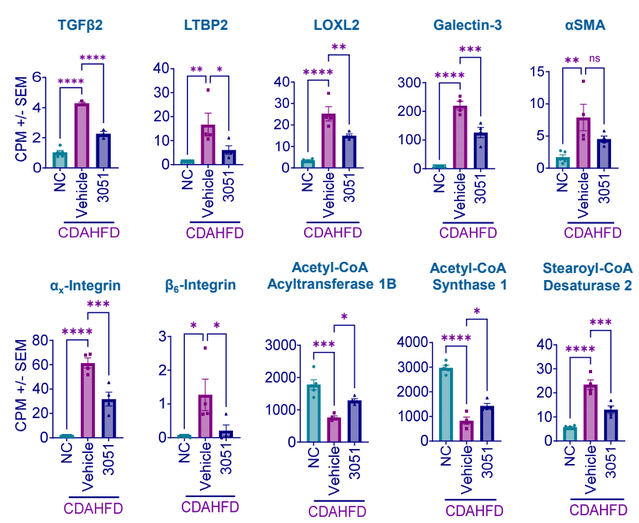
Liver tissues from 100 mg/kg BID group used

PATHWAY AND TARGET GENE ANALYSIS

Top Molecular Pathways Decreased or Increased by BLD-3051 Administration in CDAHFD Mice



BLD-3051 Modulates Key Regulators and Mediators of Liver Fibrosis



CONCLUSIONS

- BLD-3051 reduced liver fibrosis scores and hydroxyproline levels in a dose-dependent manner with BID dosing being more efficacious
- BLD-3051 modulated gene expression of key liver fibrosis pathways, consistent with an antifibrotic effect
- BLD-3051 also demonstrated efficacy in preclinical models of lung and cardiac fibrosis (data not shown) and is currently in preclinical development.

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