The autotaxin inhibitor BLD-0409 improves disease hallmarks in the CDAA-HFD-induced non-obese and biopsy-confirmed rat model of NASH with progressive fibrosis

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Background & Aim

Non-alcoholic steatohepatitis (NASH) predisposes to development of fibrosis ultimately progressing to cirrhosis.

In preclinical drug discovery, animal models of advanced NASH with fibrotic disease progression is highly warranted for exploring novel drug candidates for NASH.

The present study aimed to evaluate an autotaxin inhibitor (BLD-0409, cudetaxestat) in the choline-deficient L-amino-acid defined high-fat diet (CDAA-HFD)-induced non-obese and biopsy-confirmed rat model of advanced NASH with advanced fibrosis.

Methods

Sprague-Dawley rats (SPD RjHan:SD) were fed chow or choline-deficient high-fat diet (CDAA-HFD: L-amino acid diet with 45 kcal-% fat with 0.1% methionine, no added choline, 1% cholesterol) for 36 days before treatment start. 2 weeks prior to treatment, all animals underwent liver biopsy for histological confirmation of fibrosis (Ishak score 1-2), supplemented with Ishak score 0. CDAA-HFD rats were randomized to treatment based on %-area of PSR staining and body weight. CDAA-HFD rats (n=15-16 per group) received (PO) vehicle or BLD-0409 (5 mg/kg) for 8 weeks. Vehicle-dosed chow-fed controls served as healthy controls. Within-subject comparisons (pre- vs. post-treatment) were performed for NAFLD Activity Score (NAS) and fibrosis score (Ishak). Terminal quantitative endpoints included plasma/liver biochemistry and liver histomorphometry and liver RNA sequencing.

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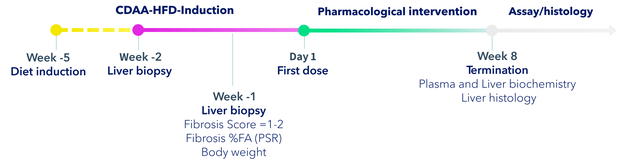
1 Study Outline



Group no.	Group	Name	Number of animals	Administration route	Dosing frequency	Dosing concentration
1	Chow	**Chow**	10	PO	QD	0
2	CDAA-HFD Vehicle	**Vehicle**	14	PO	QD	0
3	CDAA-HFD Vehicle BLD-0409	**BLD-0409**	14	PO	QD	5mg/kg

2 Metabolic and biochemical parameters

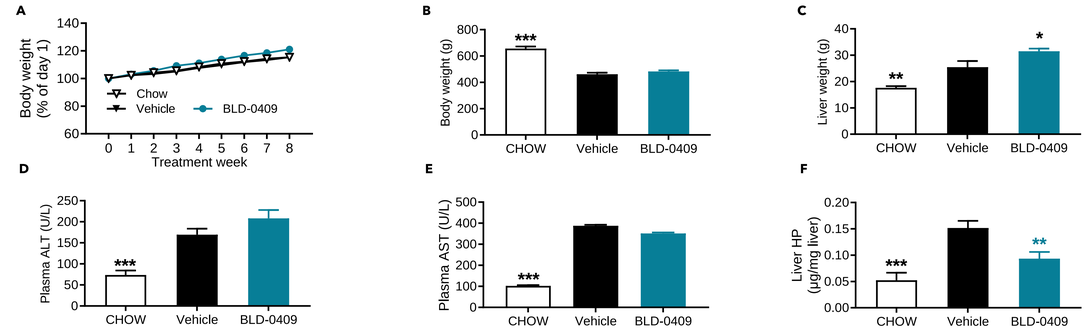


Figure 1. BLD-0409 increase hepatomegaly and reduce liver Hydroxyprolines in CDAA-HFD rats. **(A)** Body weight change relative to baseline (day 1). **(B)** Terminal body weight (g). **(C)** Terminal liver weight. **(D)** Terminal plasma alanine aminotransferase (ALT). **(E)** Terminal plasma aspartate aminotransferase (AST). **(F)** Terminal liver hydroxyproline(HP).*p<0,05, **p<0.01, ***p<0.001 compared to corresponding CDAA-HFD vehicle control (Dunnett's test one-factor linear model).

3 NAFLD activity score and fibrosis score (Ishak)

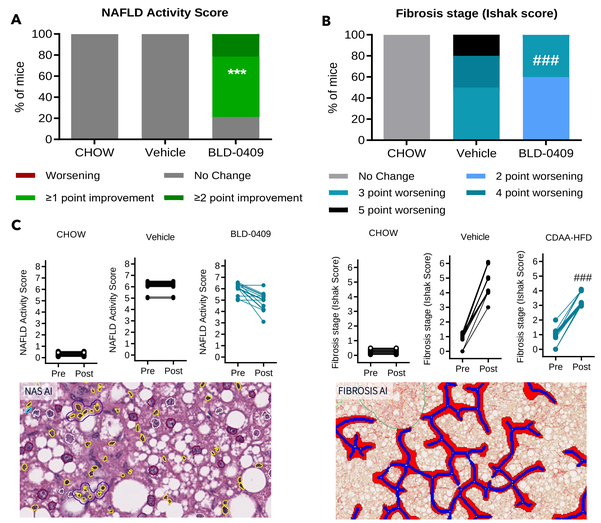


Figure 2. BLD-0409 improves liver histopathological scores in CDAA-HFD rats.
Histopathological scores were determined by Gubra Histopathological Objective Scoring Technique (GHOST) deep learning-based image analysis. **(A)** NAFLD Activity Score (NAS). **(B)** Fibrosis stage (Ishak score). **(C)** Comparison of individual pre-post NAS and individual pre-post Fibrosis stage (ishak score). Bottom panels: Representative HE and PSR photomicrographs used for GHOST evaluation. ***p<0.01 with 1 point improvement, ###p<0.001 with a prevention in fibrosis worsening compared to corresponding CDAA-HFD vehicle group (One-sided Fisher's exact test with Bonferroni correction). Bottom panels: Representative HE and PSR photomicrographs used for GHOST evaluation.

4 Histological markers of steatosis, inflammation and fibrosis

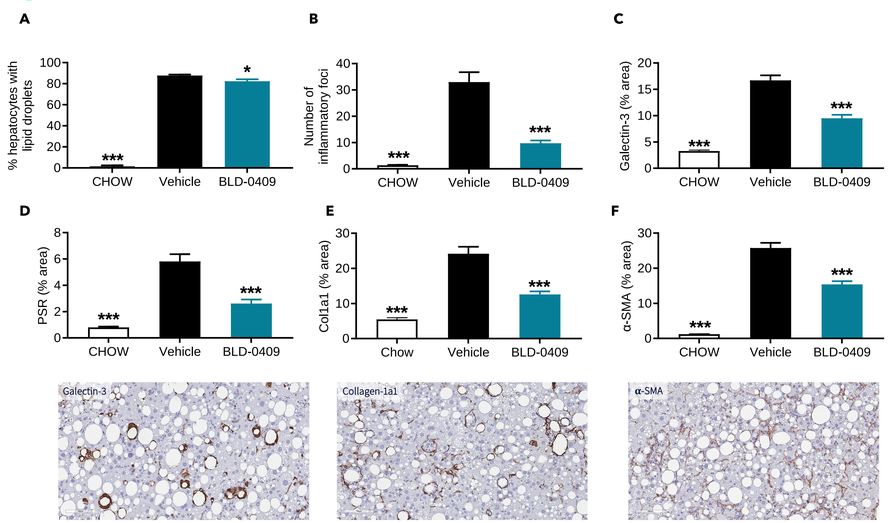


Figure 3. BLD-0409 improves quantitative liver histological markers in CDAA-HFD rats.
Histomorphometric assessments were performed by GHOST deep learning-based image analysis on scoring-associated variables **(A)** % hepatocytes with lipid droplets. **(B)** Number of inflammatory foci. **(C)** % area of galectin-3. **(D)** % area of PSR. **(E)** % area of collagen-1a1. **(F)** % area of alpha-smooth muscle actin (α-SMA) as marker for stellate cell activation. Mean ± SEM. *p<0.05, ***p<0.001 to corresponding CDAA-HFD vehicle group (Dunnett's test one-factor linear model). Bottom panels: Representative galectin-3, collagen 1a1 and α-SMA photomicrographs (scale bar, 100 µm).

Conclusion

- BLD-0409 reduces body weight and liver hydroxyproline levels while increasing liver weight in CDAA-HFD rats.
- BLD-0409 promotes ≥1-point significant improvement in NAFLD Activity Score.
- BLD-0409 improves fibrosis scores (Ishak) and quantitative fibrosis histology.
- BLD-0409 also reduces quantitative histological markers of steatosis and inflammation.
- Collectively, BLD-049 improves hallmarks of NASH and fibrosis in the CDAA-HFD-induced non-obese and biopsy-confirmed rat model of NASH with progressive fibrosis.