

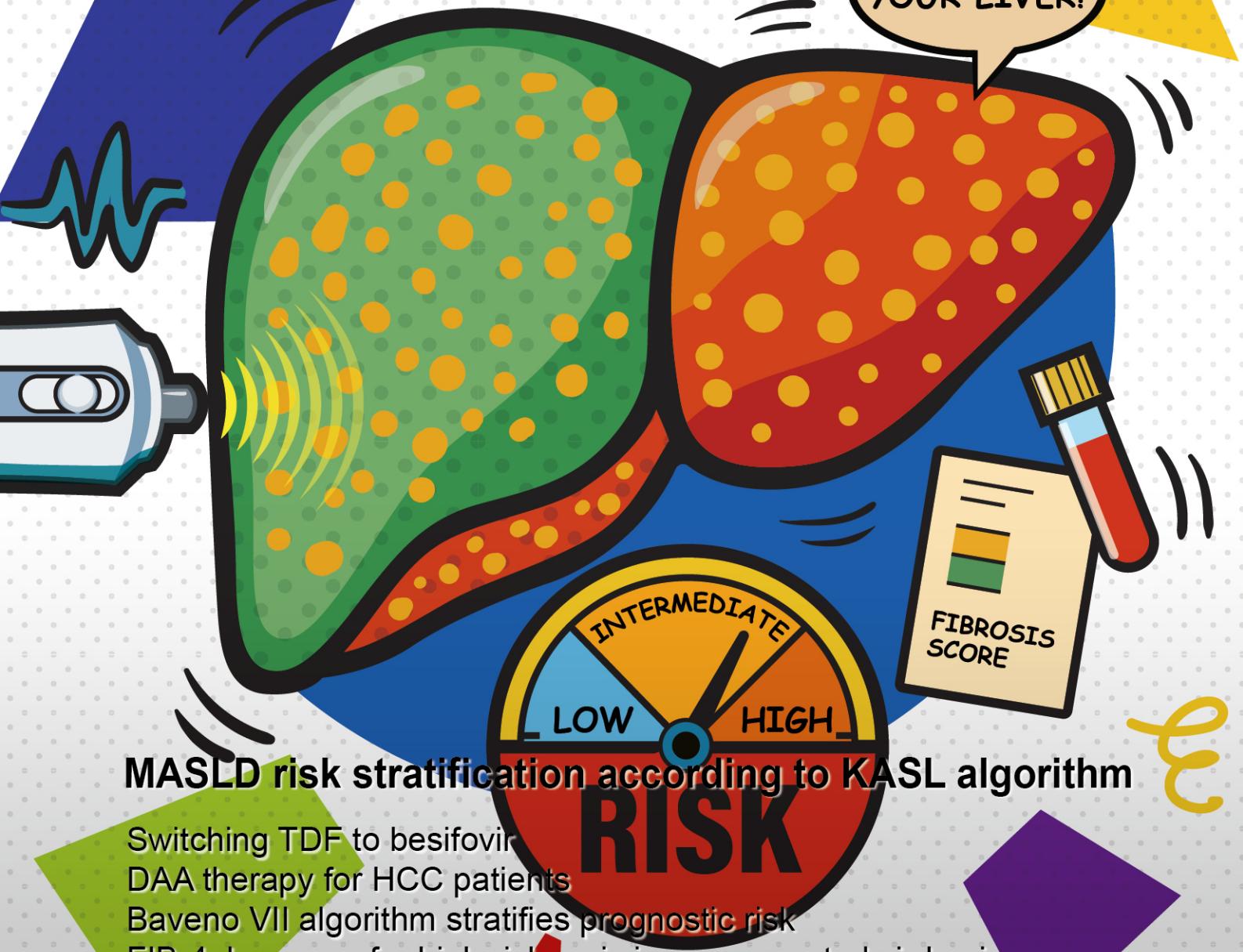
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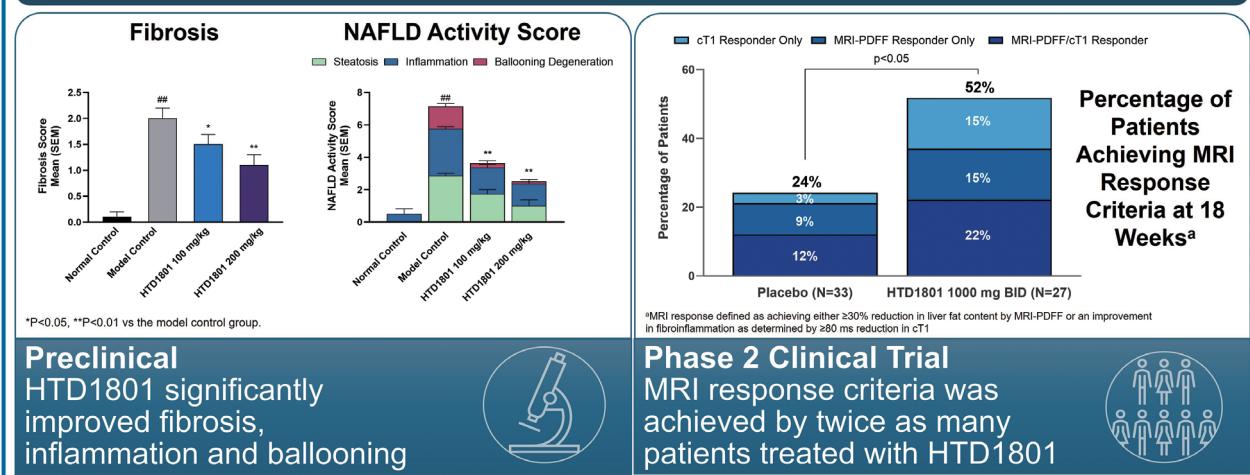
HTD1801 demonstrates promising potential for histologic improvements in metabolic dysfunction-associated steatohepatitis in both a preclinical and phase 2 study

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Graphical Abstract

Berberine ursodeoxycholate (HTD1801) demonstrated the potential to induce clinically relevant histologic improvements in patients with MASH



Study Highlights

- In a secondary analysis of a Phase 2 clinical trial in patients with presumed MASH and T2DM HTD1801 demonstrated the potential to induce clinically relevant improvements in noninvasive markers closely correlated with the histologic features of MASH and fibrosis.
- In a preclinical model of MASH in hamsters, HTD1801 improved fibrosis, inflammation and ballooning such that histologic features resembled normal controls.
- In the clinical trial, 52% of HTD1801-treated patients achieved MRI response criteria compared to 24% of placebo ($P<0.05$).
- Dose-dependent improvements were observed across biomarkers, with more HTD1801-treated patients achieving thresholds correlated with histologic improvement and lower disease activity.

Background/Aims: Berberine ursodeoxycholate (HTD1801) has been shown to significantly reduce liver fat content (LFC) in an 18-week, placebo-controlled Phase 2 study in patients with metabolic dysfunction-associated steatohepatitis (MASH) and type 2 diabetes mellitus. The purpose of this assessment was to establish proof of concept in liver histologic improvement with HTD1801 treatment based on preclinical and clinical evidence.

Methods: The efficacy of HTD1801 was evaluated in a preclinical MASH/dyslipidemia model (golden hamsters fed a high fat diet, eight/group) after six weeks of daily treatment. Additionally, in a secondary analysis of a Phase 2 clinical study, 100 patients with presumed MASH were evaluated by multiple noninvasive markers associated with MASH resolution and/or fibrosis improvement. These include magnetic resonance imaging proton density fat fraction (MRI-PDFF; $\geq 30\%$ LFC reduction), iron-corrected T1 (≥ 80 ms reduction), alanine aminotransferase (≥ 17 U/L reduction), weight loss ($\geq 5\%$ reduction), Fibrosis-4 index (shift to <1.3), and MASH resolution index (achieving ≥ -0.67).

Results: Preclinical findings in the MASH/dyslipidemia hamster model showed that HTD1801 significantly improved histologic fibrosis and the Nonalcoholic Fatty Liver Disease Activity Score to such a degree that improvements approximated the appearance of the normal controls. In the clinical study, 52% of HTD1801-treated patients achieved MRI response criteria compared to 24% of placebo ($P < 0.05$). Dose-dependent improvements were observed across biomarkers, with more HTD1801-treated patients achieving response criteria associated with improvements in the histologic features of MASH.

Conclusions: These findings suggest that HTD1801 has strong potential to produce histological improvements in patients with MASH. (*Clin Mol Hepatol 2025;31:1071-1083*)

Keywords: Noninvasive biomarkers; Liver histology; Metabolic dysfunction-associated steatohepatitis

INTRODUCTION

The complex and interrelated pathophysiology underlying metabolic liver diseases calls for treatments that address multiple targets. Metabolic dysfunction-associated steatohepatitis (MASH), a severe form of metabolic dysfunction-associated steatotic liver disease (MASLD), is the hepatic manifestation of metabolic syndrome and is characterized by dysregulation of glucose, lipid and bile acid metabolism, and progressive fibrosis.¹ The disease burden of MASLD is gradually increasing and parallels the obesity and type 2 diabetes (T2DM) epidemics.¹ In patients with MASLD, con-

comitant cardiometabolic risk factors such as T2DM drastically increase the risk of both cardiovascular and liver-related mortality.²

Liver histology has been used as the surrogate endpoint to assess treatment response in late-stage clinical trials. However, there are well-reported limitations and associated risks with the invasive procedure.³ Additionally, many non-invasive tests have repeatedly been shown to predict major adverse liver outcomes as good as, if not better than, histologic fibrosis staging.^{4,5}

Promising imaging biomarkers include the use of magnetic resonance imaging proton density fat fraction (MRI-

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Abbreviations:

ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; BID, twice daily; cT1, iron-corrected T1; FIB-4, fibrosis-4 index; HbA1c, glycated hemoglobin; HTD1801, berberine ursodeoxycholate; LDL-C, low-density lipoprotein cholesterol; LFC, liver fat content; MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; MRI, magnetic resonance imaging; MRI-PDFF, MRI-proton density fat fraction; NAS, Nonalcoholic Fatty Liver Disease Activity Score; SEM, standard error of means; T2DM, type 2 diabetes mellitus

PDFF) and iron-corrected T1 (cT1) mapping. MRI-PDFF is currently the gold standard for quantifying hepatic steatosis.¹ In a meta-analysis of seven clinical studies (n=346), a ≥30% relative decline in liver fat content (LFC) by MRI-PDFF was strongly associated with histologic response including a ≥2-point improvement in the Nonalcoholic Fatty Liver Disease Activity Score (NAS) with ≥1-point improvement in lobular inflammation or ballooning (odds ratio 6.98, P<0.001) and MASH resolution (odds ratio 5.45, P<0.01).⁶

Liver cT1 has been shown to correlate with fibroinflammatory activity present in histology and has high diagnostic accuracy in identifying patients with MASH across the disease spectrum.⁷ In a cross-sectional investigation of 264 patients, it was estimated that an approximately 80 ms reduction in cT1 corresponded to a ≥2-point decrease in NAS with no worsening of fibrosis.⁸ In a follow-up analysis of data pooled from three clinical trials (n=150), a cT1 reduction of 80 ms was associated with a 5-fold increase in the odds of achieving MASH resolution.⁹

In addition to imaging, other noninvasive laboratory-based markers and weight loss have been associated with a histologic response in patients with MASH. A secondary analysis of the FLINT study found that a decrease in alanine aminotransferase (ALT) of ≥17 U/L was significantly associated with ≥2-point improvement in NAS with no worsening of fibrosis (odds ratio 11.0, P<0.001).¹⁰ Similarly, in a prospective evaluation on the impact of lifestyle changes on the histologic features of MASH, a reduction in body weight of ≥5% was associated with higher rates of resolution of steatohepatitis (58% vs. 10%, P<0.001) and ≥2-point improvement in NAS (82% vs. 32%, P<0.001).¹¹⁻¹³ Lastly, the fibrosis-4 index (FIB-4) was developed to predict the severity of liver fibrosis in lieu of performing a liver biopsy using routine laboratory assessments and, in an analysis of 541 adults with MASLD, was shown to be superior to other noninvasive markers of fibrosis.^{12,13} A FIB-4 of ≤1.3 has been used to reliably exclude patients with advanced fibrosis with a negative predictive value of 88–95%.^{13,14}

Composite scores based on a combination of imaging and serum biomarkers have also been developed to better predict an improvement in histologic response. In a multi-variable analysis of 108 patients, a ≥30% decrease in MRI-PDFF and a ≥17 U/L decrease in ALT was a predictor of achieving a ≥2-point improvement in NAS with no worsening of fibrosis (adjusted odds ratio 11.3, P<0.01) and MASH

resolution (adjusted odds ratio 7.4, P<0.01).¹⁵ Loomba et al.¹⁶ used the strong association of ALT and MRI-PDFF with MASH resolution to develop a more robust noninvasive score, the MASH resolution index, which combines these with serum aspartate aminotransferase (AST). The MASH resolution index outperformed the individual ALT or MRI-PDFF response criteria in predicting MASH resolution with an area under the receiver-operating characteristic curve of 0.83 compared to 0.58 and 0.76, respectively.¹⁶

Berberine ursodeoxycholate (HTD1801) is a gut-liver anti-inflammatory metabolic modulator, a new molecular entity composed of an ionic salt of ursodeoxycholic acid and berberine that has the potential to offer a comprehensive benefit to diseases driven by insulin resistance and inflammation. With its unique dual mechanism of action (adenosine monophosphate kinase activation and NLRP3 inflammasome inhibition), HTD1801 was designed on the premise that it would have the benefits of both active moieties, but with improved and novel pharmacological effects resulting from its unique molecular structure. As a result, HTD1801 has the potential to become a novel treatment option for multiple chronic metabolic and liver diseases. The safety and efficacy of HTD1801 have been evaluated in Phase 1 and Phase 2 clinical studies globally and have demonstrated beneficial effects on glycemic parameters, lipid metabolism, markers of liver injury and cholestasis, body weight, and noninvasive markers of liver fibrosis.¹⁷⁻²⁰

In an 18-week study of HTD1801 in patients with presumed MASH and T2DM, patients treated with HTD1801 had significant reductions in LFC per MRI-PDFF, glycated hemoglobin (HbA1c), lipids, and body weight.¹⁸ While liver biopsies were not conducted in this study, several different non-invasive techniques have been evaluated to estimate the potential of HTD1801 on histologic improvements in patients with MASH. Noninvasive tests were selected based on a review of the most commonly used noninvasive biomarkers and treatment response criteria reported to be associated with histological improvements of MASH (steatosis, inflammation and fibrosis) and whose outcomes were also assessed in the present Phase 2 clinical study.

The purpose of this manuscript is to establish proof of concept of liver histologic improvement following treatment with HTD1801 based on histologic evaluation in a preclinical model of metabolic disease and assessment of noninvasive biomarkers associated with histologic improvements

in patients with MASH.

MATERIALS AND METHODS

Preclinical study: hamster model of MASH and dyslipidemia

Male Syrian golden hamsters ($n=32$; Vital River Laboratories, Beijing, China) were utilized in a preliminary evaluation of histologic and biochemical efficacy of HTD1801. Hamsters received a high fat diet containing 1.25% cholesterol and 20% grease (2.79% soybean oil and 17.27% cocoa butter) for 2 weeks prior to treatment administration to induce MASH/dyslipidemia and maintained this diet through Week 6. After a 2-week high fat diet feeding period, prior to dosing and grouping, 4 hamsters were sacrificed for histological evaluation. The remaining hamsters were randomly stratified by total cholesterol level prior to treatment into the following groups: normal control ($n=8$), model control ($n=8$), and HTD1801 (high dose: 200 mg/kg, $n=8$ and low dose: 100 mg/kg, $n=8$). Hamsters designated to the normal control group received normal chow throughout the study period. Food intake was monitored for all groups throughout the study period. Hamsters received daily treatment for 6 weeks. Blood was drawn by orbital vein for assessment of biochemical parameters (AST, ALT, total bilirubin, low-density lipoprotein cholesterol [LDL-C], total cholesterol) at Day 0 and Week 2, 4 and 6. Livers were resected for analysis at Week 6 after the animals were sacrificed. Livers were sectioned (paraffin embedded blocks into 4 μ m thickness sections) and stained with hematoxylin and eosin and Masson's trichrome for evaluation of the NAS and the Nonalcoholic Steatohepatitis Clinical Research Network fibrosis score by a pathologist blinded to group assignment.²¹ The liver index was calculated as liver weight (g) divided by body weight (kg). All animal experiments were conducted with Laboratory Animal Management and Ethics Committee of Zhejiang University of Traditional Chinese Medicine approvals.

For statistical testing of biochemical parameters in the preclinical hamster model, an analysis of variance (ANOVA) was used to evaluate differences between groups. Only for biochemical parameters, the Grubbs test was used to determine the outliers for each group with a confi-

dence interval of 97.5%.²² No more than one outlier was removed from each group. For histologic parameters in the preclinical hamster model, a non-parametric Kruskal-Wallis test was used. P -values less than 0.05 were considered to be significant. SPSS 22.0 software (IBM Co., Armonk, NY, USA) was used for statistical analyses and PRISM 9.5 (GraphPad Software, San Diego, CA, USA) was used for plotting data.

Phase 2 study in MASH

This secondary analysis used data from a Phase 2, randomized, double-blind, placebo-controlled study of the efficacy and safety of HTD1801 in patients with presumed MASH and T2DM. The primary results, including the study design and patient disposition were previously published by Harrison et al.¹⁸ (NCT03656744). In brief, 100 patients with presumed MASH based on an LFC $\geq 10\%$, cT1 ≥ 830 ms, AST ≥ 20 U/L, and a documented clinical diagnosis of T2DM (at least 6 months prior to randomization) were randomized 1:1:1 using permuted blocks without additional stratification factors and treated orally with placebo ($n=33$), HTD1801 500 mg BID ($n=33$), and HTD1801 1,000 mg BID ($n=34$) for 18 weeks. HTD1801 and placebo were provided as matching white tablets (supplied by HighTide Therapeutics Inc.). All patients, study personnel, and the sponsor were blinded to treatment assignment. MRIs were performed at baseline and at Week 18. Biochemistry and body weight were assessed at baseline and every visit throughout the study.

For this analysis, MRI response was defined as achieving either $\geq 30\%$ reduction in LFC by MRI-PDFF or an improvement in fibroinflammation as determined by ≥ 80 ms reduction in cT1 at 18 weeks. MRI-PDFF data was collected prospectively for evaluation of the primary endpoint. A cT1 segmented analysis (Perspectum, Oxford, UK) was evaluated following completion of the study in patients who had been randomized to HTD1801 1,000 mg BID or placebo only.

FIB-4 was derived from a patient's age, AST, ALT, and platelet count using the formula developed by Sterling et al.¹² with a shift in FIB-4 from > 1.3 at baseline to ≤ 1.3 at Week 18 representing an improvement in fibrosis stage.^{13,14}

The MASH resolution index, a composite score comprised of MRI-PDFF, ALT, and AST, was calculated using

the equation published by Loomba et al.¹⁶ For this analysis, a histologic improvement was defined as achieving a MASH resolution index of ≥ -0.67 .

The clinical study was conducted in accordance with the Declaration of Helsinki 2013, approved by the institutional review boards and written informed consent was obtained from all participants.

Statistical analysis for the comparisons between active treatment groups and placebo were tested using an estimation based on a Cochran-Mantel-Haenszel model. Analyses were performed using SAS V9.4.

RESULTS

Preclinical study: hamster model of MASH and dyslipidemia

Macroscopic observations and histological evaluation prior to dosing for golden hamsters fed a high fat diet for 2 weeks are presented in Supplementary Figure 1. Following dosing, the model control had marked elevation in aminotransferases, total bilirubin and lipids (Fig. 1) compared with normal controls. Each of the markers of liver inflammation and hepatocellular damage (AST, ALT) were improved with HTD1801 treatment by Week 2, with significant mean improvements in the HTD1801 high dose group to a level resembling the normal controls at Week 6 (Table 1). Furthermore, significant reductions with HTD1801 were also observed in key cardiometabolic parameters (LDL-C and total cholesterol) with mean LDL-C approaching the same values as the normal controls. Additionally, a clear dose-dependent effect is observed with greater reductions across each biochemical parameter with the higher dose of HTD1801 approximating the level of the normal control (Fig. 1).

Improvements in HTD1801 could be readily viewed in resected hamster livers without the aid of a microscope. As shown in Figure 2A, HTD1801-treated livers most closely resembled the normal control group macroscopically. The liver index in the model control increased significantly compared with the normal control (mean [standard error of means, SEM]: 51.9 g/kg [2.8] vs. 27.9 g/kg [1.1]; $P < 0.01$). Significant reductions in the liver index were observed with HTD1801 100 mg/kg (33.5 g/kg [0.9]; $P < 0.01$) and HTD1801

200 mg/kg (30.2 g/kg [0.6]; $P < 0.01$) compared to the model control.

Following hematoxylin and eosin staining (Fig. 2B), the structure of the liver in the normal control group was clear, the central vein of the liver was intact, the hepatic cords were arranged neatly, and the hepatic cells were normal in shape. In contrast, for the model control group, the boundary of hepatic lobules was unclear, hepatocytes were arranged mottly, cord structures disappeared, and a large number of hepatocytes showed swelling. Overall, the morphology in the HTD1801 groups improved compared to the model control group. In the HTD1801 200 mg/kg group, the hepatic lobular structure and cord structure were clear, and there was no obvious swelling of hepatocytes.

The model controls developed significant fibrosis, steatosis, inflammation, and ballooning compared to the normal controls (Fig. 2C). Treatment with both doses of HTD1801 resulted in significant reductions in fibrosis (by approximately 1 stage) as well as improvements in each component of the NAS with almost no residual ballooning. A clear dose-dependent effect was observed with HTD1801 resulting in greater histologic improvements at the highest dose across each histologic parameter.

Phase 2 study: study overview in MASH

A total of 100 patients were randomized and received at least one dose of study drug (placebo n=33, HTD1801 500 mg BID n=33, and HTD1801 1,000 mg BID n=34).¹⁸ Overall, 88 patients (87%) completed the study, 32 patients (97%) in the placebo group, 29 patients (85%) in the HTD1801 500 mg BID group and 27 patients (79%) in the HTD1801 1,000 mg BID group. The most common reason for study discontinuation was due to adverse events (6 patients), primarily gastrointestinal adverse events, followed by lost to follow-up.¹⁸ Demographic characteristics were generally well balanced across all three treatment groups (Supplementary Table 1). The median age overall was 57 years (range: 26 to 75 years), and 72% were female. Most patients were White (91%) and not Hispanic or Latino (62%). Median baseline weight and body mass index were similar across treatment groups. Baseline values for HbA1c, ALT, γ -glutamyl transferase, LDL-C, triglycerides, and LFC were similar across treatment groups. As described in Harrison et al.,¹⁸ the primary endpoint was

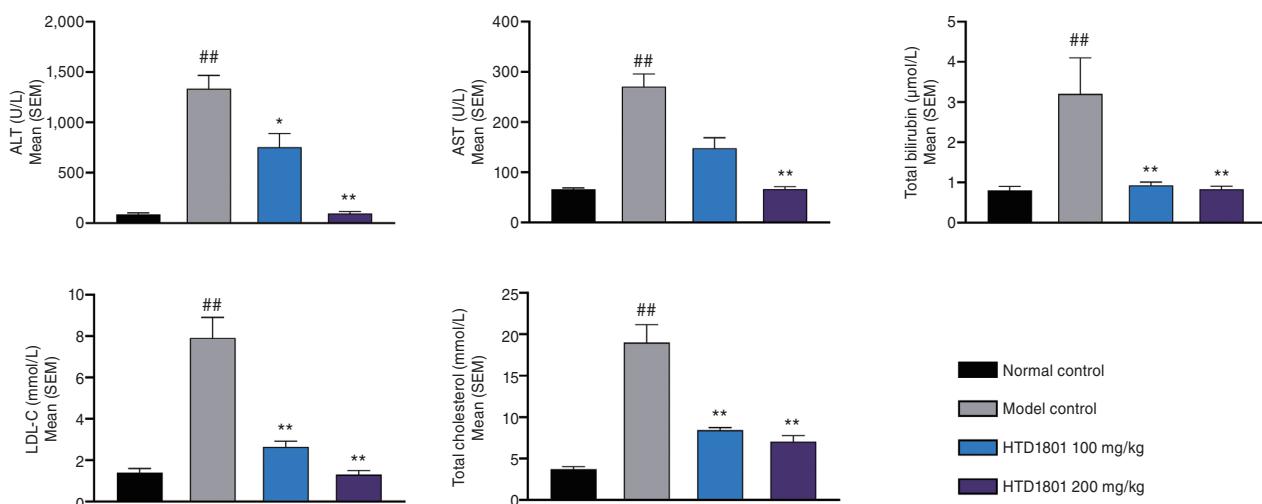


Figure 1. Liver biochemistry and lipids after 6 weeks of treatment in hamsters fed with a high fat diet. Outliers were determined using the Grubbs test and excluded from the analysis. An analysis of variance was used to evaluate differences between groups. *P<0.05, **P<0.01 vs. the model control group. #P<0.01 vs. the normal control group. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HTD1801, berberine ursodeoxycholate; LDL-C, low-density lipoprotein-cholesterol; SEM, standard error of the mean.

Table 1. ALT and AST over time - preclinical hamster model of MASH/dyslipidemia

Mean±SEM	Normal control	Model control	HTD1801 (100 mg/kg)	HTD1801 (200 mg/kg)
ALT (mmol/L)				
Baseline	85.8±5.0	1,423.6±284.9 [†]	1,419.8±220.7	1,421.6±217.3
Week 2	93.3±8.1	1,160.0±242.1 [†]	165.0±30.5**	233.3±53.4**
Week 4	147.6±46.1	959.9±114.8 [†]	489.4±125.4*	165.3±35.6**
Week 6	86.1±15.4	1,334.7±133.0 [†]	754.9±134.8*	96.6±17.5**
AST (mmol/L)				
Baseline	52.6±3.7	413.6±105.0 [†]	366.2±80.2	305.3±64.5
Week 2	48.1±2.9	312.4±57.6 [†]	61.9±13.4**	81.7±7.6**
Week 4	49.3±6.7	204.5±27.8 [†]	117.2±16.8*	73.1±9.6**
Week 6	66.0±2.9	270.60±25.3 [†]	147.7±21.0	66.4±4.6**

Outliers were determined using the Grubbs test and excluded from the analysis. An analysis of variance (ANOVA) was used to evaluate differences between groups.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HTD1801, berberine ursodeoxycholate; MASH, metabolic dysfunction-associated steatohepatitis; SEM, standard error of means.

*P<0.05, **P<0.01 compared with the model control. [†]P<0.05, [‡]P<0.01 compared with the normal control.

achieved with a significant reduction in LFC (via MRI-PDFF) with HTD1801 1,000 mg BID compared to placebo (-4.8% vs. -2.0%, P=0.011). The beneficial effect of HTD1801 on liver fat was accompanied by improvements in liver biochemistry and weight (Supplementary Table 2).

As shown in Supplementary Table 3, treatment with HTD1801 was generally safe and well tolerated. More patients in the HTD1801 1,000 mg BID group experienced a treatment emergent adverse event during the study com-

pared to placebo (26 [76%] vs. 20 [61%]). The most common adverse events were diarrhea and nausea which were generally mild to moderate in severity. During the study, 3 patients (3%) experienced serious adverse events of decreased oxygen saturation (1), myocardial infarction (1), and bladder transitional cell carcinoma (1) in the HTD1801 500 mg BID, 1,000 mg BID, and placebo groups, respectively. All serious adverse events were considered not related to study drug by the Investigator.

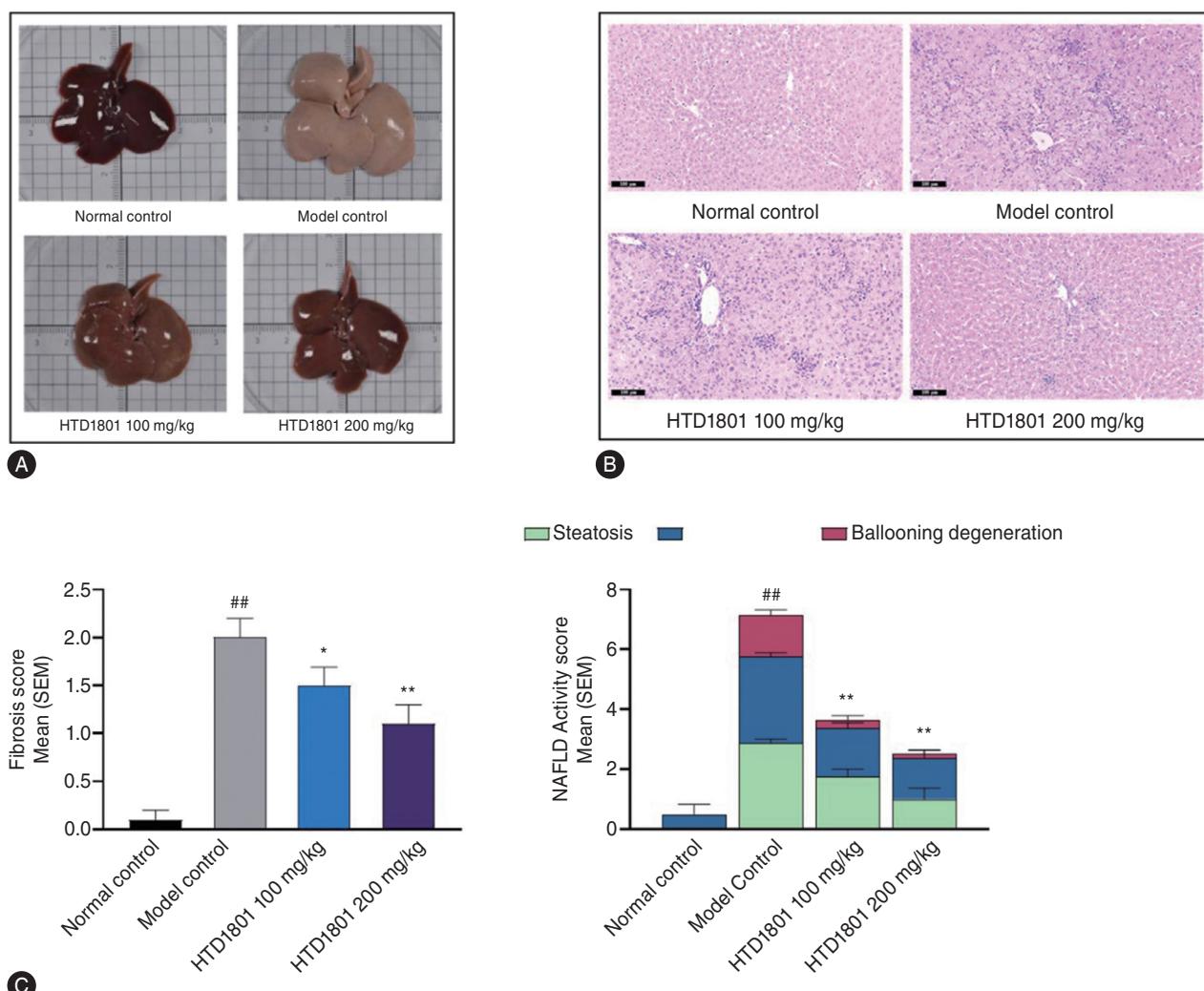


Figure 2. Macroscopic and histologic evaluation of hamster livers after 6 weeks of treatment. Golden hamster liver tissues: (A) macroscopic observations (representative photos); (B) hematoxylin and eosin staining where the nucleus is blue, and the cytoplasm and intercellular substance were pink; (C) change in fibrosis score and Nonalcoholic Fatty Liver Disease Activity Score. Fibrosis scores were based on the Nonalcoholic Steatohepatitis Clinical Research Network staging criteria. Analysis was performed with a non-parametric Kruskal-Wallis test. * $P<0.05$, ** $P<0.01$ vs. the model control group. # $P<0.01$ vs. the normal control group. HTD1801, berberine ursodeoxycholate; NAFLD, nonalcoholic fatty liver disease; SEM, standard error of the mean.

Phase 2 study: MRI response criteria

At baseline, patients receiving placebo and HTD1801 1,000 mg BID had elevated mean LFC by MRI-PDFF (20% and 19%, respectively) and cT1 (938 ms and 942 ms, respectively). As shown in Figure 3 and Table 2, after 18 weeks of treatment, the MRI response criteria (either MRI-PDFF or cT1) was achieved by 52% in the HTD1801 group and 24% in the placebo group ($P=0.03$). Of these patients, 22% in the HTD1801 group and 12% in the placebo group achieved both the MRI-PDFF and cT1 response criteria.

Phase 2 study: biochemical and anthropometric response criteria

In an analysis of biochemical and anthropometric response criteria, treatment with HTD1801 resulted in a larger proportion of patients achieving thresholds associated with a ≥ 2 -point improvement in NAS (Fig. 4A) or fibrosis (Fig. 4B) compared to placebo (Table 2). A clear dose response was observed in each criterion evaluated with HTD1801 treatment. For HTD1801 1,000 mg BID compared to placebo, a ≥ 17 U/L reduction in ALT was achieved by 12

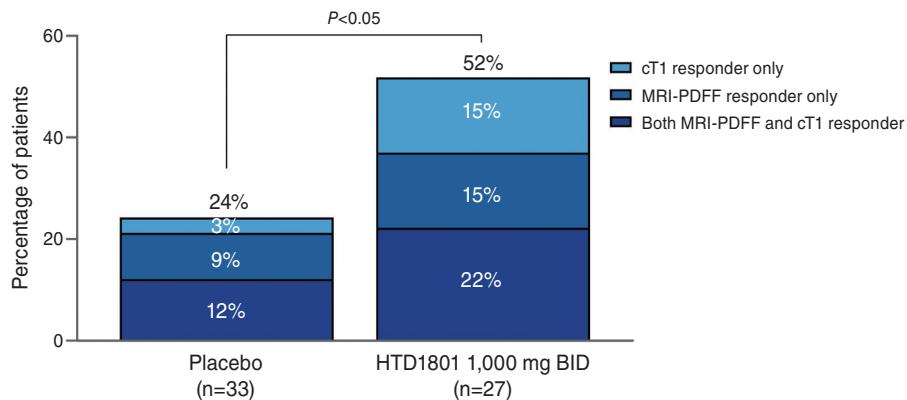


Figure 3. Percentage of patients achieving MRI response criteria. Percentage of patients achieving an MRI response defined as achieving either $\geq 30\%$ reduction in liver fat content by MRI-PDFF or an improvement in fibroinflammation as determined by ≥ 80 ms reduction in cT1 at 18 weeks. CMH model was used for statistical analysis. BID, twice daily; CMH, Cochran–Mantel–Haenszel; cT1, corrected T1; HTD1801, berberine ursodeoxycholate; MRI, magnetic resonance imaging; MRI-PDFF, MRI-proton density fat fraction.

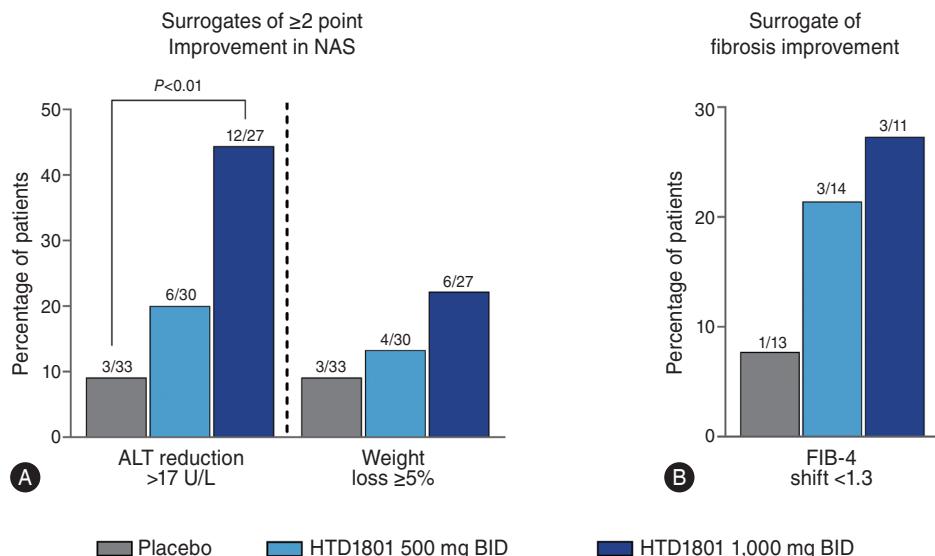


Figure 4. Percentage of patients achieving the biochemical and anthropometric response criteria. Percentage of patients achieving biochemical and anthropometric response criteria associated with a (A) ≥ 2 point improvement in NAS and (B) fibrosis improvement, which was limited to patients with a FIB-4 ≥ 1.3 at baseline. CMH model was used for statistical analysis. ALT, alanine aminotransferase; BID, twice daily; CMH, Cochran–Mantel–Haenszel; FIB-4, fibrosis-4 index; HTD1801, berberine ursodeoxycholate; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD Activity Score.

patients (44%) vs. 3 (9%); a $\geq 5\%$ reduction in body weight was achieved by 6 patients (22%) vs. 3 (9%); and a shift in FIB-4 to <1.3 was achieved by 3 patients (27%) vs. 1 (8%).

Phase 2 study: composite response criteria

More patients treated with HTD1801 compared to placebo achieved the composite response criteria of MRI-PDFF and ALT which is associated with achieving ≥ 2 -point improvement in NAS and MASH resolution. A dose-depen-

dent effect was observed, with 7% (n=2) and 33% (n=9) of patients in the HTD1801 500 mg BID and 1,000 mg BID group, respectively, achieving both a $\geq 30\%$ reduction in LFC and a ≥ 17 U/L reduction in ALT compared to none in the placebo group (Fig. 5A and Table 2).

Additionally, more than 3 times as many patients treated with HTD1801 1,000 mg BID (41%) than placebo (12%) achieved MASH resolution index ≥ -0.67 (Fig. 5B and Table 2).

Table 2. Proportion of patients achieving noninvasive biomarker response at week 18 - Phase 2 study in MASH

Proportion of patients achieving	Placebo (n=33)	HTD1801	
		500 mg BID (n=33)	1,000 mg BID (n=34)
≥30% reduction in MRI-PDFF only	33	NA*	27
Number (%)	3 (9.1)	NA*	4 (14.8)
≥80 ms reduction in cT1 only	33	NA*	27
Number (%)	1 (3.0)	NA*	4 (14.8)
Both MRI and cT1 response	33	NA*	27
Number (%)	4 (12.1)	NA*	6 (22.2)
MRI or cT1 response	33	NA*	27
Number (%)	8 (24.2)	NA*	14 (51.9)
95% CI†	-	NA*	1.12, 10.08
P-value†	-	NA*	0.029
≥17 U/L reduction in ALT	33	30	27
Number (%)	3 (9.1)	6 (20.0)	12 (44.4)
95% CI†	-	0.57, 11.05	1.96, 32.73
P-value†	-	0.220	0.002
≥5% reduction in body weight	33	30	27
Number (%)	3 (9.1)	4 (13.3)	6 (22.2)
95% CI†	-	0.31, 7.52	0.64, 12.73
P-value†	-	0.596	0.160
Shift in FIB-4 to <1.3	13	14	11
Number (%)	1 (7.7)	3 (21.4)	3 (27.3)
95% CI†	-	0.29, 36.31	0.39, 51.30
P-value†	-	0.325	0.209
≥30% reduction in MRI-PDFF and a ≥17 U/L reduction in ALT	33	30	27
Number (%)	0	2 (6.7)	9 (33.3)
95% CI†	-	NR	NR
P-value†	-	0.135	0.0004
MASH resolution index ≥−0.67	33	30	27
Number (%)	4 (12.1)	7 (23.3)	11 (40.7)
95% CI†	-	0.57, 8.47	1.36, 18.23
P-value†	-	0.246	0.012

ALT, alanine aminotransferase; BID, twice daily; CI, confidence interval; cT1, iron-corrected T1; FIB-4, fibrosis-4 index; HTD1801, berberine ursodeoxycholate; MASH, metabolic dysfunction-associated steatohepatitis; MRI, magnetic resonance imaging; MRI-PDFF, magnetic resonance imaging proton density fat fraction; NA, not applicable.

*A cT1 segmented analysis was evaluated following completion of the study in patients who had been randomized to HTD1801 1,000 mg BID or placebo only. †HTD1801 vs. placebo.

P-values are obtained using a Cochran–Mantel–Haenszel (CMH) model.

DISCUSSION

Using a preclinical model and secondary analysis of a Phase 2 clinical study with consistent findings across multiple noninvasive surrogates, HTD1801 demonstrated the potential to induce clinically relevant histologic improve-

ments in patients with MASH.

Preclinical findings in the MASH/dyslipidemia hamster model showed that HTD1801 significantly reduced ALT, AST, total bilirubin, LDL-C and total cholesterol after 6 weeks of treatment. Furthermore, HTD1801 improved histology including fibrosis and NAS to such a degree that the

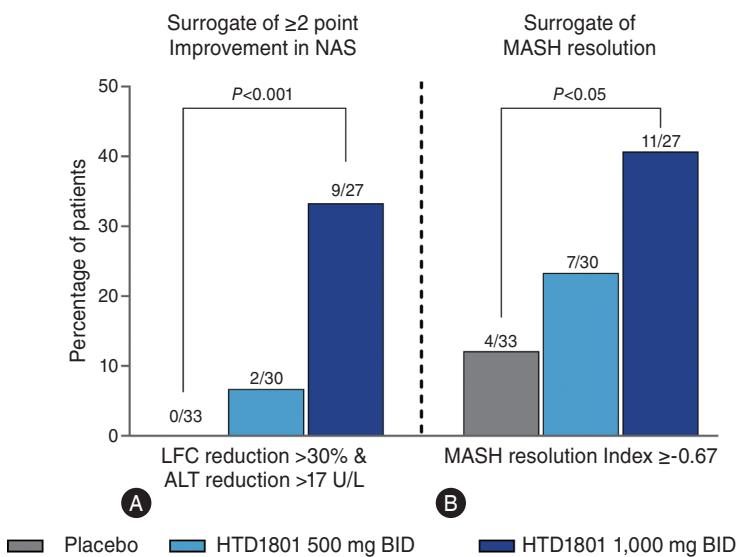


Figure 5. Percentage of patients achieving composite response criteria. Percentage of patients achieving composite response criteria associated with a (A) ≥ 2 point improvement in NAS and (B) MASH resolution. CMH model was used for statistical analysis. ALT, alanine aminotransferase; BID, twice daily; CMH, Cochran–Mantel–Haenszel; HTD1801, berberine ursodeoxycholate; LFC, liver fat content; MASH, metabolic dysfunction-associated steatohepatitis; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD Activity Score.

final histologic features were similar to control animals fed with normal chow. The improvements in liver size and steatosis were also obvious macroscopically.

This secondary analysis of a randomized, placebo-controlled Phase 2 study in patients with presumed MASH examined biomarkers and treatment response criteria that are associated with improvements across the spectrum of histologic features of MASH including steatosis (MRI-PDFF and weight loss), inflammation (cT1, MRI-PDFF, ALT, and weight loss) and fibrosis (cT1 and FIB-4). The MRI response criteria was achieved by twice as many patients treated with HTD1801 as placebo, with nearly half in the HTD1801 1,000 mg BID group achieving response criteria in both LFC and cT1. Dose-dependent improvements were observed across biomarkers, with more HTD1801-treated patients achieving thresholds correlated with histologic improvement and lower disease activity. Treatment with HTD1801 was generally safe and well tolerated. Mild to moderate diarrhea and nausea were the most frequently reported events and no serious adverse events were considered related to study drug.

HTD1801 is believed to work through multiple pathways that address the core aspects of MASH, including activation of adenosine monophosphate kinase, inhibition of the NLRP3 inflammasome, induction of the insulin receptor, and induction of the LDL receptor. The results on fibrosis

and metabolic control are consistent with adenosine monophosphate kinase activation and suppression of NLRP3 inflammasomes—addressing MASH fibrosis in two ways—improving the metabolic drivers of disease and potentially a direct anti-fibrotic effect through suppressing NLRP3, which is an inflammatory pathway reported to be tied to fibrogenesis.²³

In MASLD, biomarkers are generally developed for diagnostic, prognostic, monitoring, and response assessment purposes.²⁴ While numerous studies have confirmed the diagnostic and prognostic capabilities of various noninvasive tests, few have been validated as response biomarkers. This limitation arises because developing response biomarkers necessitates not only serial noninvasive tests paired with liver biopsies but also effective treatment between assessments. Among the proposed response biomarkers, an MRI-PDFF response of $\geq 30\%$ relative reduction has the most robust evidence base, validated in clinical trials such as the Phase 3 MAESTRO-NASH study for resmetirom.²⁵ However, it is crucial to understand that MRI-PDFF is a steatosis marker, with its association to improvements in inflammation and fibrosis being correlational. Conversely, while weight reduction has been linked to MASH resolution and fibrosis improvement in natural history or lifestyle intervention cohorts,^{11,26} the relationship between weight loss and histologic response may become

dissociated when a patient is undergoing effective drug treatment for MASH. Additionally, some biomarkers, such as liver stiffness measurement by vibration-controlled transient elastography, are influenced by weight changes, further complicating their use as response biomarkers.²⁷ Lastly, liver biopsies themselves are an imperfect reference standard for assessing treatment response in MASH, as imprecision in histologic scoring may result in dilution of treatment effect size and misvaluation of therapeutic effects.²⁸ Thus, consistency in response across multiple biomarkers would enhance confidence in identifying genuine therapeutic effects, as demonstrated in the current study.

Currently, the only pharmacological treatment to receive approval by the United States Food and Drug Administration for the treatment of MASH is resmetrirom, a small molecule thyroid hormone receptor beta agonist. In the pivotal Phase 3 study, treatment with resmetrirom resulted in histologic improvements in MASH and fibrosis. While histologic data are not yet available from clinical studies of HTD1801, treatment with HTD1801 may provide a broader metabolic benefit, including significant improvements in glycemic control and weight loss, which have not been shown with resmetrirom.^{18,20}

Limitations of this evaluation include the post hoc nature of the analysis of the clinical study, short treatment duration with HTD1801 (only 18 weeks), and limited number of biomarkers/imaging tools evaluated. Additionally, in this study the presumption of MASH was based on the combination of LFC $\geq 10\%$, cT1 ≥ 830 ms, and AST ≥ 20 U/L. Although these thresholds have been correlated with the presence of high-risk MASH and are higher than the clinical diagnostic criteria for MASLD, as liver biopsies were not performed, the presence of MASH was presumed.¹⁷ The strengths of this evaluation include the fact that the data are from a randomized, placebo-controlled, multi-site clinical study.

While these data are preliminary, it suggests that ongoing treatment with HTD1801 would be expected to result in improvements in the key histologic targets in patients with MASH. A Phase 2b study is currently ongoing to evaluate the histologic effects of HTD1801 in patients with MASH and T2DM or prediabetes (NCT05623189).

Authors' contribution

Conception and design: Di Bisceglie, Bai, Cheng, Yu, Liberman, Liu. Acquisition, analysis or interpretation of the

data: Wong, Neff, Di Bisceglie, Bai, Cheng, Yu, Liberman, Gunn. Drafting of the manuscript: Wong, Bai, Liberman. Critical revision of the manuscript: all authors. Final approval: all authors.

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Conflicts of Interest

Vincent Wong served as a consultant or advisory board member for AbbVie, AstraZeneca, Boehringer Ingelheim, Echosens, Eli Lilly, Gilead Sciences, Intercept, Inventiva, Merck, Novo Nordisk, Pfizer, Sagimet Biosciences, TARGET PharmaSolutions, and Visirna; and a speaker for Abbott, AbbVie, Echosens, Gilead Sciences, Novo Nordisk, and Unilab. He has received a research grant from Gilead Sciences, and is a co-founder of Illuminatio Medical Technology. Adrian M. Di Bisceglie has received consulting fees from HighTide Therapeutics, Inc. and Intercept Pharmaceuticals, Inc. Ru Bai, Junwei Cheng, Meng Yu, Alexander Liberman, and Liping Liu are employees and stock shareholders of HighTide Therapeutics, Inc.

SUPPLEMENTARY MATERIAL

Supplementary material is available at Clinical and Molecular Hepatology website (<http://www.e-cmh.org>).

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