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CLINICAL—LIVER

Effects of Belapectin, an Inhibitor of Galectin-3, in Patients With Nonalcoholic Steatohepatitis With Cirrhosis and Portal Hypertension

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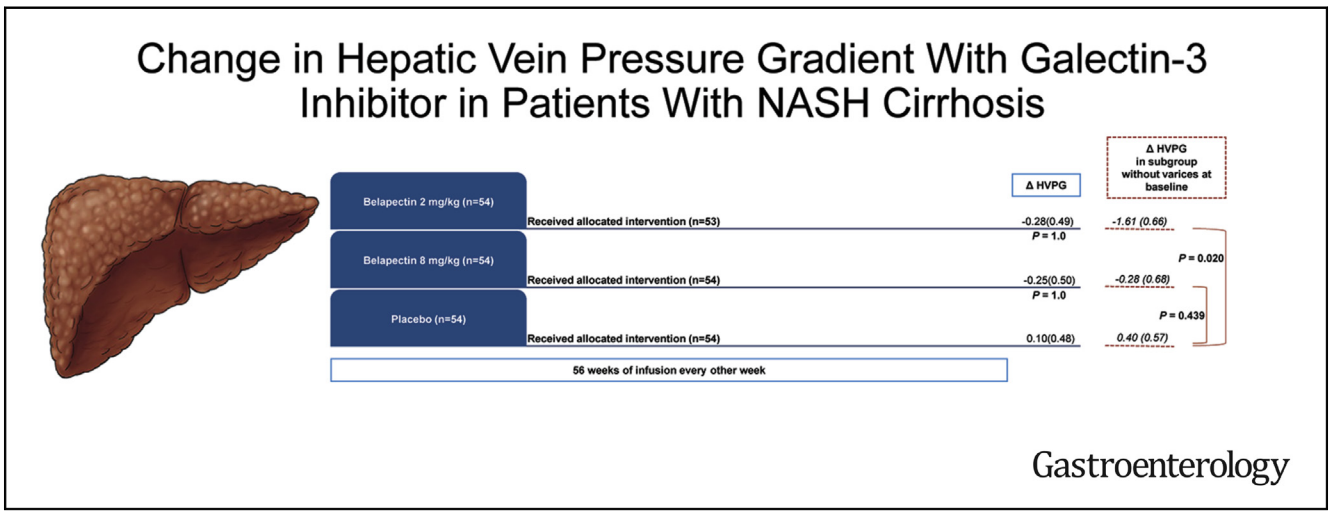

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BACKGROUND & AIMS: Increased levels of galectin 3 have been associated with nonalcoholic steatohepatitis (NASH) and contribute to toxin-induced liver fibrosis in mice. GR-MD-02 (belapectin) is an inhibitor of galectin 3 that reduces liver fibrosis and portal hypertension in rats and was safe and well tolerated in phase 1 studies. We performed a phase 2b, randomized trial of the safety and efficacy of GR-MD-02 in patients with NASH, cirrhosis, and portal hypertension. **METHODS:** Patients with NASH, cirrhosis, and portal hypertension (hepatic venous pressure gradient [HVPG] $\geq$ 6 mm Hg) from 36 centers were randomly assigned, in a double-blind manner, to groups that received biweekly infusions of belapectin 2 mg/kg (n = 54), 8 mg/kg (n = 54), or placebo (n = 54) for 52 weeks. The primary endpoint was change in HVPG (Δ HVPG) at the end of the 52-week period compared with baseline. Secondary endpoints included changes in liver histology and development of liver-related outcomes. **RESULTS:** We found no significant difference in ΔHVPG between the 2 mg/kg belapectin group and placebo group (–0.28 mm HG vs 0.10 mm HG, $P = 1.0$) or between the 8 mg/kg belapectin and placebo group (–0.25 mm HG vs 0.10 mm HG, $P = 1.0$). Belapectin had no significant effect on fibrosis or nonalcoholic fatty liver disease activity score, and liver-related outcomes did not differ significantly among groups. In an analysis of a subgroup of patients without esophageal varices at baseline (n = 81), 2 mg/kg belapectin was associated with a reduction in HVPG at 52 weeks compared with baseline ($P = .02$) and reduced development of new varices ($P = .03$). Belapectin (2 mg/kg) was well tolerated and produced no safety signals. **CONCLUSIONS:** In a phase 2b study of 162 patients with NASH, cirrhosis, and portal hypertension, 1 year of biweekly infusion of belapectin was safe but not associated with significant reduction in HVPG or fibrosis compared with placebo. However, in a subgroup analysis of patients without esophageal varices, 2 mg/kg belapectin did reduce HVPG and development of varices. ClinicalTrials.gov number: NCT02462967

Keywords: NAFLD; Carbohydrate-Binding Protein; Inflammation; Steatosis.

WHAT YOU NEED TO KNOW

BACKGROUND & CONTEXT

Increased levels of galectin 3 have been associated with nonalcoholic steatohepatitis (NASH) and contributes to toxin-induced liver fibrosis in mice. GR-MD-02 (belapectin) is an inhibitor of galectin 3 that reduces liver fibrosis and portal hypertension in rats and was safe and well tolerated in phase 1 studies.

NEW FINDINGS

In a study of patients with NASH cirrhosis, and portal hypertension, 1 year of biweekly infusion of belapectin was safe but not associated with significant reductions in hepatic venous pressure gradient (HVPG) or fibrosis, compared with placebo. However, in patients without esophageal varices, belapectin reduced HVPG and development of varices.

LIMITATIONS

This was a phase 2 trial of 162 patients.

IMPLICATIONS FOR PATIENT CARE

Belapectin might be developed to reduce HVPG and prevent varices in select patients with NASH-induced cirrhosis.

CLINICAL LIVER

Nonalcoholic steatohepatitis (NASH) is a common chronic liver disease that can progress to cirrhosis, liver failure, and liver cancer.[1] It currently is the second most common cause of liver transplant in men and the leading cause in women in the United States.[2,3] Importantly, patients with NASH cirrhosis are at significant risk for complications related to portal hypertension such as variceal bleeding, ascites with bacterial peritonitis and hepatic encephalopathy, resulting in significant morbidity and mortality.[1] Portal hypertension is the main predictor of hepatic decompensation (development of ascites, variceal hemorrhage, or encephalopathy) which, in turn, is the strongest predictor of death in cirrhosis.[4] In both compensated and decompensated cirrhosis, a decrease in portal pressure (assessed by hepatic venous pressure gradient [HVPG]) is associated with lower rates of decompensation and death.[5] There are currently no medical therapies approved for the treatment of NASH cirrhosis or reversal of portal hypertension. This therapeutic area represents an area of significant unmet medical need.

Galectins are carbohydrate-binding proteins belonging to the family of non-integrin β-galactoside-binding lectins.[6] They are mainly cytosolic proteins, but they can easily traverse the intracellular and plasma membranes to translocate into the nucleus and mitochondria and be externalized.[7] They are known to be stored in the cytoplasm when cells are in a quiescent state, but, upon tissue injury, cytosolic galectins could be actively secreted by activated cells through a nonclassical pathway and may serve as damage-associated molecular pattern candidates.[7] Previous studies have shown that galectins are markedly increased in inflammation, fibrosis, and cancer and are involved in their pathogenesis.[8,9] Galectin-3 (Gal-3) is the most prominent galectin secreted in the disease state, mainly secreted by macrophages. It binds to the cell surface and extracellular matrix glycans and affects a variety of physiologic and pathologic processes, including cell apoptosis, adhesion, migration, angiogenesis, and inflammatory responses.[8,9] Gal-3, through its intracellular effects (antiapoptotic, macrophage differentiation) and extracellular functions (chemokinetic/chemotactic factor), is relevant to the physiopathology of hepatic fibrosis from various chronic liver diseases.[8–11]

Galectin inhibitors are a new class of agents that target both secreted and membrane-associated galectins by virtue of their high molecular weight.[12] They have the strongest binding affinity to Gal-3 and disrupt its function.[12] These drugs have low toxicity potential because they are carbohydrates with no toxic metabolites.[12] Belapectin (galactoarabino-rhamnogalacturonate [GR-MD-02]) is a complex carbohydrate molecule derived from a natural plant compound that contains oligosaccharide chains containing galactose residues and binds to Gal-3, and a lesser extent, galectin-1. It has shown robust efficacy in preclinical models of NASH and liver fibrosis, and it was safe and well tolerated in phase 1 human studies. For example, Gal-3–deficient mice are protected from diet-induced NASH or fibrosis.[13,14] In dietary-induced mouse NASH models, belapectin consistently reduced the disease activity, reduced or eliminated fibrosis as measured by liver collagen, and reduced the expression of Gal-3 in liver macrophages.[15] The belapectin treatment of rats with advanced fibrosis and cirrhosis induced by thioacetamide resulted in a reduction of collagen to less than 10%, a reversal of cirrhosis, and reduced portal hypertension.[16] A phase 1 study has shown that belapectin is safe and well tolerated at single and multiple doses of 2, 4, and 8 mg/kg in patients with well-characterized NASH and advanced fibrosis but not cirrhosis.[17] Here, we report the results of a phase 2b, multicenter, randomized, double-blind, placebo-controlled trial of belapectin in patients with NASH cirrhosis and portal hypertension. Two doses of

Abbreviations used in this paper: **AE, adverse event; AUC, area under the curve; CI, confidence interval; EOT, end of treatment; FAS, full-analysis set; Gal-3, galectin 3; GR-MD-02, galactoarabino-rhamnogalacturonate; HVPG, hepatic venous pressure gradient; ITT, intention to treat; LS, least squares; MPH, mild portal hypertension; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SAE, serious adverse event; SD, standard deviation; TEAE, treatment-emergent adverse events.**

Most current article

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Table 1. Inclusion and Exclusion Criteria

Inclusion Criteria
A participant was eligible for inclusion if he/she met all of the following criteria:
1. Had an HVPG measurement $\geq$6 mm Hg.
2. Had a liver biopsy with cirrhosis (Ishak stage 5 or 6) presumably due to NASH. A liver biopsy diagnosis of cirrhosis presumably due to NASH included the following categories:
a) Cirrhosis with a definitive pathologic diagnosis of NASH (presence of fat, ballooning degeneration, and inflammation);
b) Cirrhosis wherein the biopsy contained either fat (>5%) or ballooning hepatocytes with no evidence of viral hepatitis or other liver disease; or
c) Cirrhosis with no evidence of viral hepatitis or other liver disease in a participant with at least a 5-year history of obesity (body mass index $\geq$ 30 kg/m^2) or at least a 5-year history of diabetes mellitus (as defined by diagnosis by a physician and treatment with at least 1 antidiabetic medication).
3. Was $\geq$18 years of age and $\leq$75 years of age at the time of screening.
4. Had absence of hepatocellular carcinoma by valid imaging (liver ultrasonography, triple phase computed tomography of liver, or magnetic resonance imaging of liver) within 6 months before randomization. If there was not such test available, then it was to be performed as part of standard of care.
5. Was willing and able to provide written informed consent before the initiation of any study-specific procedures.
6. Was not pregnant and had a negative serum pregnancy test result before randomization. If the participant was a fertile man or woman participating in heterosexual relations, he/she needed to agree to use effective means of contraception (ie, 2 effective methods of contraception, 1 of which must be a physical barrier method) throughout his/her participation in this study and for 90 days after discontinuation of study treatment. Effective forms of contraception included condom, hormonal methods (birth control pills, injections, or implants), diaphragm, cervical cap, or intrauterine device. Surgically sterile men and women were not required to use contraception provided they had been considered surgically sterile for at least 6 months. Surgical sterility included history of vasectomy, hysterectomy, bilateral salpingo-oophorectomy, or bilateral tubal ligation. Postmenopausal women who were amenorrheic for at least 2 years at the time of screening were considered sterile.
7. If a lactating woman, agreed to discontinue nursing before the start of study treatment and refrain from nursing until 90 days after the last dose of study treatment.
8. If a man, agreed to refrain from sperm donation throughout the study period and for a period of 90 days after the last dose of study drug. Female participants could not begin a cycle of ova donation or harvest throughout the study period and for a period of 90 days after the last dose of study drug.
9. Before randomization, any participant taking statins, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, or β-1 selective adrenergic receptor inhibitors was receiving a stable dose for at least 2 months, and all attempts were to be made to continue the participant on the same dose of the medication for the duration of study participation.
Exclusion Criteria
Participants meeting any of the following criteria were excluded from the study:
1. Had a history of hepatic decompensation, including any episode of variceal bleeding, ascites not controlled by medication, or overt hepatic encephalopathy (defined by the clinical judgment of the principal investigator, but including the presence of lethargy, disorientation, inappropriate behavior, and the presence of asterixis).
2. Had a presence of medium or large varices or varices with red signs regardless of size based on endoscopy.
a. Small varices were defined by veins that occupied <25% of the distal one third of the esophageal lumen when insufflated. Veins that completely flattened upon insufflation of the esophagus were not conserved varices. Any varices larger than that were medium ($\leq$50%) or large (>50%).
b. Red signs included red wale markings (dilated venules oriented longitudinally on the variceal surface), cherry red spots (small, red, spotty dilated venules usually approximately 2 mm in diameter on the variceal surface) or hematocystic spots (large, round, crimson red projection >3 mm that looked like a blood blister on the variceal surface).
3. Had a prior transjugular portosystemic shunt procedure.
4. Had evidence of other forms of chronic liver disease, including viral hepatitis B or C, primary biliary cirrhosis, primary sclerosing cholangitis, Wilson disease, α-1 antitrypsin deficiency, alcoholic hepatitis, hemochromatosis, liver cancer, history of biliary diversion, or autoimmune hepatitis.
5. Had any of the following laboratory values:
a. Serum alanine aminotransferase levels $>$ 10 $\times$ the upper limits of normal
b. Serum aspartate aminotransferase levels $>$ 10 $\times$ the upper limits of normal
c. Platelet count $<$ 60,000/mm^3
d. Serum albumin $\leq$ 2.8 g/dL
e. International normalized ratio $\geq$ 1.7
f. Direct bilirubin $\geq$ 2.0 mg/dL
g. Alpha fetoprotein $>$ 200 ng/mL
6. Had a Model for End-Stage Liver Disease score $\geq$1 5 or Child–Turcotte–Pugh CLASS B or C.

Table 1. Continued

Exclusion Criteria
7. Had an estimated creatinine clearance (CrCl) of <50 mL/minute. Glomerular filtration rate was estimated using the Cockcroft–Gault equation: • Men: CrCl (mL/min) = ([140 – age] × weight) / (SCr × 72) • Females: CrCl (mL/min) = ([140 – age] × weight) / (SCr × 72)] × 0.85 where age is in years, weight is in kilograms, and SCr is serum creatinine in mg/dL.
8. Was unwilling or unable to safely undergo HVPG or liver biopsy.
9. Had known positivity for HIV infection or a positive HIV test result at screening.
10. Had a history of major surgery within 8 weeks of randomization, significant traumatic injury within 6 months, or anticipation of need for major surgical procedure during the course of the study.
11. Had a history of a solid organ transplant requiring current immunosuppression therapy.
12. Had used nonselective β-adrenergic inhibitors within 6 weeks before randomization.
13. Had planned or anticipated variceal ligation therapy during the study.
14. Had weight reduction surgery within the past 3 years or planned to undergo weight reduction surgery during the study.
15. Had current, significant alcohol consumption or a history of significant alcohol consumption for a period of more than 3 consecutive months any time within 1 year before screening. • *Significant alcohol consumption* is defined as more than 20 g per day in women and more than 30 g per day in men. On average, a standard drink in the United States is considered to be 14 g of alcohol, equivalent to 12 fluid ounces (fl oz) of regular beer (5% alcohol), 5 fl oz of table wine (12% alcohol), or 1.5 fl oz of 80-proof spirits (40% alcohol). A score of ≥8 on the Alcohol Use Disorders Identification Test resulted in exclusion.
16. Had a positive urine screening test result for amphetamines, cocaine, or nonprescription opiates (heroin, morphine) at screening.
17. Had clinically significant and uncontrolled cardiovascular disease (eg, uncontrolled hypertension, myocardial infarction within 6 months before randomization, unstable angina), New York Heart Association grade II or greater congestive heart failure, serious cardiac arrhythmia requiring devise/ablation, or grade II or greater peripheral vascular disease within 12 months before randomization.
18. Had a history of clinically significant hematologic, renal, hepatic, pulmonary, neurologic, psychiatric, gastrointestinal, systemic inflammatory, metabolic, or endocrine disorder or any other condition that, in the opinion of the investigator, rendered the participant a poor candidate for inclusion into the study.
19. Had concurrent infection, including diagnosis of fever of unknown origin at the time of randomization.
20. Had a history of malignancy, except for the following: adequately treated nonmetastatic basal cell skin cancer; any other type of skin cancer, except melanoma, that had been adequately treated and had not recurred for at least 1 year before enrollment; and adequately treated in situ cervical cancer that had not recurred for at least 1 year before screening.
21. Participated in an investigational new drug study within 30 days before randomization (including follow-up visits) or at any time during the current study.
22. Had a clinically significant medical or psychiatric condition considered high risk for participation in an investigational study.
23. Failed to give informed consent.
24. Had known allergies to the study drug or any of its excipients.
25. Had previously received GR-MD-02 within 6 months of randomization.
26. Was an employee or family member of the investigator or study center personnel.

belapectin (2 mg/kg and 8 mg/kg) and matching placebo were administered biweekly as infusions for 52 weeks.

Methods

Trial Oversight

This randomized, double-blind, placebo-controlled trial comparing 2 doses of belapectin to placebo in patients with NASH cirrhosis and portal hypertension meeting predefined eligibility criteria was conducted throughout 36 centers in the United States (ClinicalTrials.gov NCT02462967). This study was sponsored by Galectin Therapeutics and had an independent data safety monitoring board and a medical monitor associated with a clinical research organization. The review board approved the study at each participating center, and all participants gave written informed consent. The data were analyzed independently and were reviewed by both the investigators and the data safety monitoring board.

Patients

Patients were assigned to study treatment only if they met all of the inclusion criteria and none of the exclusion criteria. The inclusion and exclusion criteria are listed in Table 1. The 2 main inclusion criteria were HVPG ≥6 mm Hg and liver biopsy showing cirrhosis due to NASH.

Study Design

Eligible participants underwent an upper endoscopy within 2 months before randomization and within 14 to 28 days after the final dose of study drug, and the size of esophageal varices, if present, was classified as (1) large varices (>50% impingement on the lumen), (2) small varices (<25% impingement on lumen) or (c) medium varices (intermediate between small and large varices). Participants with medium or large varices or varices with red signs at baseline, regardless of size, were excluded from study participation. Participants without varices or with small varices were advanced to HVPG measurement and transjugular liver biopsy. Consistent with the American

Association for the Study of Liver Disease practice guidelines,[18] participants with small varices did not receive prophylaxis with nonselective β-adrenergic inhibitors or variceal ligation therapy during the clinical trial.

All HVPG measurements were performed and pressure tracings recorded according to standard operating procedure provided in the study manual to all sites. Each study site had provided an acceptable sample HVPG tracing before patient enrollment. Portal pressure was determined indirectly by the HVPG as previously described.[19] Briefly, by using the transjugular approach, a balloon-tipped catheter was advanced into a hepatic vein under fluoroscopic guidance.The free hepatic venous pressure was measured with the balloon deflated, and the wedged hepatic venous pressure was measured with the balloon inflated until the branch of hepatic vein was completely occluded. HVPG was obtained by subtracting the free hepatic venous pressure from the wedged hepatic venous pressure. All measurements were performed in triplicate, and tracings were read independently by a single experienced investigator (Guadalupe Garcia-Tsau, Yale University). Liver histology was centrally read in a blinded fashion by a single experienced hepatopathologist (Zachary Goodman, Inova).

Trial Endpoints

The primary endpoint was to evaluate the efficacy of belapectin in reducing HVPG as a measure of portal pressure compared with placebo after 12 months of treatment. Predefined secondary efficacy endpoints were (1) baseline-adjusted mean change in the collagen proportion area, (2) proportion of participants with $\geq$ 1 point change in fibrosis stage, (3) baseline adjusted mean change in liver stiffness, and (4) complications of cirrhosis, defined as the development of any of the following: esophageal variceal hemorrhage or portal hypertensive gastropathy hemorrhage (confirmed by endoscopy), clinically apparent ascites or spontaneous bacterial peritonitis, overt hepatic encephalopathy, an increase in Child-Turcotte-Pugh score $\geq$2 points, newly diagnosed varices in a participant without prior varices or progression from small to medium or large varices, reaching a model for end-stage liver disease score $\geq$15 as measured on 2 consecutive occasions, listing for a liver transplant or the performance of a liver transplant, or liver-related mortality. Fibrosis staging was assessed primarily by Ishak Scoring System[20] and secondarily by the NASH CRN Scoring System.[21] The efficacy was also assessed separately in patients with mild portal hypertension (HVPG, 6-9 mm Hg) and clinically significant portal hypertension (HVPG $\geq$ 10 mm Hg) (prespecified subgroups) and those with no varices at baseline (post hoc analysis). Safety endpoints included the incidence of treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), and study discontinuations during the trial.

Statistical Analysis

Continuous variables were summarized as mean (standard deviation [SD] or standard error) and median (interquartile range), and categorical variables were summarized as frequencies and percentages. Unless otherwise specified, all statistical tests and confidence intervals (CI) were 2-sided and conducted at the .05 significance level. If analysis variables were not normally distributed, a Poisson regression model (or the negative binomial) was applied for counts data. No imputation was applied for missing data unless otherwise specified. All analyses were conducted using SAS software, version 9.3 (Cary, NC).

Sample size calculations were based on the comparison of the primary efficacy variable, change in HVPG from baseline, with the following key assumptions: (1) true mean change in HVPG from baseline at 52 weeks in the placebo group, $\Delta p = 0$; (2) true mean change in HVPG from baseline at 52 weeks in either belapectin dose group, $\Delta G = -2$ mm Hg; (3) common SD for difference in HVPG, $\sigma = 3$ mm Hg; (4) null hypothesis, H0: $\theta = G - \Delta p = 0$; (5) type I error, $\alpha = .05$ (2-sided significance test); (6) power = 80%; (7) statistical test: 2-sample *t*-test for mean difference; (8) randomization ratio = 1:1:1; and (9) drop-out rate of 25%. For a mean difference of 2 mm Hg and accounting for a 25% dropout rate, the total sample size of 156 participants (n = 52 participants per group) was required to achieve the power of 80% with a 2-sided type I error rate of .05.

Primary efficacy endpoint analyses were conducted as an intention-to-treat (ITT) on the full-analysis set (FAS), which included all participants randomly assigned. All particpants in the FAS were analyzed according to the treatment they were randomly assigned to receive. The HVPG was summarized for the FAS by visit and treatment group, for all scheduled visits, along with the change from baseline descriptively. In addition, the number and percentage of participants were summarized by the following HVPG categories (mild portal hypertension [MPH], ie, $\geq$6 mm Hg to <10 mm Hg, and clinically significant portal hypertension, ie, $\geq$10 mm Hg). The primary efficacy endpoint, change from baseline in HVPG at the end of treatment (EOT), was analyzed by using analysis of covariance, with baseline values taken as a covariate by using the FAS at a significant level of .05 (2-sided). The treatment effect was evaluated as a contrast of each active treatment vs placebo and described by using continuous summary with an estimate of mean difference along with a 95% CI. The subgroup analyses were conducted in the modified-ITT set, which included only participants who were randomly assigned, received at least 1 infusion, and had at least 1 postbaseline efficacy assessment.

Results

Participant Disposition

A total of 162 participants were randomly assigned to receive the study drug (54 individuals each in the belapectin 2 mg/kg, belapectin 8 mg/kg, and placebo groups). Histologic NASH cirrhosis definition was based on eligibility criterion 2a in 95 patients, 2b in 51 patients, and 2c in 15 patients (see Table 1 for the criteria). Select demographics, baseline characteristics, liver biochemistries, and severity of liver disease were evenly matched and are reported in Table 2. The study flow is shown in Supplementary Figure 1. All 162 randomly assigned participants were included in the FAS, and 161 participants (99.4%) were included in the modified-ITT and safety cohorts (Supplementary Figure 1). Eleven participants (6.8%) discontinued the study early, and 151 participants

Table 2. Selected clinical characteristics of the study cohort (N = 162)[¶]

Characteristics	Belapectin 2 mg/kg (n = 54)	Belapectin 8 mg/kg (n = 54)	Placebo (n = 54)
Age, *y*, mean (SD)	59.2 (7.5)	57.1 (9.3)	58.4 (8.5)
Females, %	63	80	67
Non-Hispanic White, %	85	74	85
Body mass index, *kg/m*, mean (SD)[2]	35.7 (6.5)	34.4 (5.7)	34.6 (7.1)
Type 2 diabetes, %	59	67	59
Statin use, %	40	43	30
AST, *U/L*, mean (SD)	48 (23)	49 (25)	52 (48)
ALT, *U/L*, mean (SD)	42 (21)	51 (40)	48 (38)
Total Bilirubin, *mg/dL*, mean (SD)	0.76 (0.45)	0.67 (0.33)	0.75 (0.47)
Albumin, *g/dL*, mean (SD)	4.2 (0.4)	4.2 (0.4)	4.2 (0.4)
INR, mean (SD)	1.05 (0.10)	1.05 (0.077)	1.06 (0.11)
Platelet count, $\times 10^3/mm^3$, mean (SD)	131 (55)	121 (49)	115 (45)
Child–Turcotte–Pugh class A, %	98	100	100
MELD, mean (SD)	7.3 (1.53)	6.9 (1.03)	7.4 (1.73)
HVPG, *mm Hg*, mean (SD)	12.4 (4.3)	12.7 (4.2)	11.6 (4.0)
MPH ($\geq$6 to $<$10 mm Hg), %	30	30	39
CSPH ($\geq$10 mm Hg), %	69	70	61
HVPG in patients without baseline varices, *mm Hg*, mean (SD)	8.22 (0.97)	7.78 (1.25)	7.79 (1.34)
ELF, mean (SD)	10.73 (1.26)	10.64 (1.16)	10.81 (1.1)
Collagen proportionate area, %, mean (SD)	9.88 (5.88)	12.72 (4.2)	11.63 (6.12)
α-SMA staining at baseline, %, mean (SD)	13.6 (10.39)	15.4 (11.2)	13.6 (10.55)
Galectin-3 staining, %, mean (SD)	14.8 (8.9)	14.67 (7.4)	14.03 (6.67)
MBT $cPDR_{30}$, mean (SD)	692 (399)	702 (322)	635 (308)
Liver stiffness measurement, *kPa*, mean (SD)	32.4 (17.7)	29.3 (14.9)	29.9 (17.8)
Esophageal varices, %			
None	48	43	61
Small	52	57	39
Liver histology			
Biopsy length, *mm*, mean (SD)			
Baseline	26 (9.2)	25 (8.9)	24 (11)
End of treatment	24 (15)	24 (10.2)	24 (9.7)
NAFLD activity score, mean (SD)	4.3 (1.3)	4.2 (1.6)	4.2 (1.5)
Cirrhosis, %	98	100	100
$>$80% compliance, %	98	93	94
CLD-Q overall score, mean (SD)	4.59 (1.26)	4.74 (1.2)	4.88 (1.2)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CLD-Q, chronic liver disease questionnaire; $_{C}PDR_{30}$, cumulative percentage dose recovery at 30 minutes; CSPH, clinically significant portal hypertension; ELF, enhanced liver fibrosis panel; INR, international normalized ratio; MBT, ^{13}methacetin breath test; MELD, Model for End-Stage Liver Disease; MPH, mild portal hypertension; NAFLD, nonalcoholic fatty liver disease; SMA, smooth muscle actin.

(93.2%) completed the study. Two primary reasons for study discontinuation were adverse events (AEs) (3 participants) and loss to follow-up (3 participants). The proportion of participants completing the study was similar across the treatment groups (Supplementary Figure 1).

Efficacy

Hepatic Vein Pressure Gradient. The least-squares (LS) mean change in HVPG from baseline at EOT in each treatment arm was not significantly different among placebo (0.10 mm Hg), belapectin 2 mg/kg (–0.28 mm Hg), or belapectin 8 mg/kg (–0.25 mm Hg) groups. Compared with placebo, LS mean change from baseline in HVPG were also not different for the 2 active treatment groups (–0.38 mm Hg for belapectin 2 mg/kg and –0.35 mm Hg for belapectin 8 mg/kg; $P = 1.0$ for both comparisons) (Table 3). Similarly, in the preplanned separate analysis of the MPH and clinically significant portal hypertension subgroups there were no significant differences in LS mean changes between the treatment groups and the placebo group (Table 3). However, in the subgroup of patients without varices at baseline, compared with placebo (0.40 mm Hg), the LS mean change with belapectin 2 mg/kg was significantly different (–1.61 mm Hg, $P = .02$) but not with belapectin 8 mg/kg (–0.28 mm Hg, $P = .4$) (Table 3).

The effects of belapectin 2 mg/kg and 8 mg/kg on HVPG when calculated as percent change from baseline were as follows. For the FAS, the mean (SD) percent change between EOT and baseline was 6.15% ± 31% in the placebo group, –1.74% ± 33% in the belapectin 2 mg/kg group, ($P = .11$ vs placebo), and –1.09% ± 22% in the belapectin 8 mg/kg group ($P = .42$ vs placebo). For the MPH subgroup, the mean (SD) percent change between EOT and baseline was 26% ± 47% in

Table 3. Primary Endpoint: Change in Hepatic Vein Pressure Gradient at end of Treatment From Baseline

	Belapectin 2 mg/kg	Belapectin 8 mg/kg	Placebo
Full analysis set, n	54	54	54
LS mean (SE) change from baseline[a]	–0.28 (0.49)	–0.25 (0.50)	0.10 (0.48)
LS mean difference from placebo (95% CI)	–0.38 (–1.73 to 0.98)	–0.35 (–1.72 to 1.02)	—
Adjusted *P* value[b]	1.0	1.0	—
Number of patients with decrease in HVPG at EOT			
≥10% from baseline, %	13	13	7
≥20% from baseline, %	9	9	7
MPH subgroup, n	16	16	21
LS mean (SE) change from baseline[a]	–0.03 (0.74)	–0.21 (0.66)	1.46 (0.61)
LS mean difference from placebo (95% CI)	–1.49 (–3.43 to 0.45)	–1.67 (–3.48 to 0.15)	—
Adjusted *P* value[b]	0.258	0.142	—
CSPH subgroup, n	38	38	33
LS mean (SE) change from baseline[a]	–0.50 (0.62)	–0.21 (0.68)	–0.66 (0.66)
LS mean difference (95% CI)	0.16 (–1.65 to 1.96)	0.45 (–1.45 to 2.34)	—
Adjusted *P* value[b]	1.0	1.0	—
Subgroup with varices at baseline, n	28	31	21
LS mean (SE) change from baseline[a]	0.81 (0.62)	–0.27 (0.59)	–0.32 (0.70)
LS mean difference (95% CI)	1.13 (–0.72 to 2.97)	0.04 (–1.77 to 1.85)	—
Adjusted *P* value[b]	0.230	0.963	—
Subgroup without varices at baseline, n	25	23	33
LS mean (SE) change from baseline[a]	–1.61 (0.66)	–0.28 (0.68)	0.40 (0.57)
LS mean difference (95% CI)	–2.00 (–3.69 to –0.32)	–0.68 (–2.41 to 1.05)	—
Adjusted *P* value[b]	0.020	0.439	

CSPH, clinically significant portal hypertension; SE, standard error.
[a]It is LS mean because an ANCOVA model is used with baseline score as covariate and treatment group as factors. Treatment comparison was made between the 2 doses of GR-MD-02 and placebo.
[b]Bonferroni–Holm procedure is used to control the type I error for multiple comparisons.

the placebo group, –3.27% ± 32% in the belapectin 2 mg/kg group ($P = .021$ vs placebo), and –2.04% ± 25% in the belapectin 8 mg/kg group ($P = .027$ vs placebo). For the subgroup without varices at baseline, the mean (SD) percent change between EOT and baseline was 11.7% ± 33.7% in the placebo group, –8.46% ± 26% in the belapectin 2 mg/kg group ($P = .011$ vs placebo), and 0.6% ± 25% in the belapectin 8 mg/kg group ($P = .42$ vs placebo).

There was a significant interaction between baseline varices status and the treatment response ($P = .037$).

Histology. There was no statistically significant difference between the 2 belapectin groups and the placebo group for the change from baseline in collagen proportion area, ≥1 stage improvement in fibrosis or nonalcoholic fatty liver disease (NAFLD) activity score at the EOT (Table 4). Although there were no significant differences among treatment groups in lobular inflammation or steatosis, for hepatocyte ballooning there was a statistically significant difference for the belapectin 2 mg/kg group (odds ratio, 2.42; 95% CI, 1.09–5.37; $P = .030$)] and a trend toward significance in the belapectin 8 mg/kg group (odds ratio, 1.98; 95% CI, 0.90–4.34; $P = .089$) compared with the placebo group. Histologic changes associated with 2 belapectin groups in the subgroups of patients with MPH or no varices at baseline are shown in Supplementary Table 1.

Liver-Related Clinical Outcomes. At least 1 complication of cirrhosis at year 1 was reported for 10 participants (18.5%), 11 participants (20.4%), and 12 participants (22.2%) in the belapectin 2 mg/kg, belapectin 28 mg/kg, and placebo groups, respectively ($P > .05$). The median time to complications of cirrhosis was 367 days, 379 days, and 371 days in the belapectin 2 mg/kg, belapectin 2 mg/kg, and placebo groups, respectively ($P > .05$) (Table 5).

In participants without varices at baseline, there was no statistically significant difference between the belapectin groups and placebo group in the number of participants with at least 1 complication of cirrhosis at year 1 ($P > .05$). In this subgroup of patients without varices at baseline, there was a favorable treatment effect on the development of new varices, which was statistically significant for belapectin 2 mg/kg (0% vs 18% placebo, $P = .032$) and of borderline significance for belapectin 8 mg/kg (4% vs 18% placebo, $P = .12$) (Table 5).

Secondary and Exploratory Efficacy Endpoints

The effect of GR-MD-02 on various secondary and exploratory efficacy endpoints is shown in Table 6. There were no statistical differences between the 2 treatment groups and the placebo-treated patients for any of these endpoints. The changes in the overall score or in the 6 domains (abdominal symptoms, fatigue, systemic symptoms, activity, emotional function, and worry) assessed by the Chronic Liver Disease Questionnaire were not different between the treatment groups.

Table 4. Histologic Changes at the EOT Compared With Baseline in the Study Cohort

	Belapectin 2 mg/kg (n = 46)	Belapectin 8 mg/kg (n = 41)	Placebo (n = 45)
CPA, mean change from baseline	1.2 ± 5.5	0.1 ± 5.7	1.3 ± 8.2
1 stage improvement in fibrosis by Ishak Score, %[a]	31.5	24.1	25.9
NAS, change from baseline, mean ± SD	0.1 ± 1.4	0.2 ± 1.2	0.4 ± 1.3
Steatosis, change from baseline, mean ± SD	0.0 ± 0.4	–0.0 ± 0.5	0.2 ± 0.6
Inflammation, change from baseline, mean ± SD	0.1 ± 0.9	0.2 ± 0.8	0.1 ± 0.8
Ballooning, change from baseline, mean ± SD	–0.1 ± 0.7	0.1 ± 0.7	0.3 ± 0.7

CPA, collagen proportional area; NAS, Nonalcoholic Fatty Acid Liver Activity Score.
[a]When assessed by the NASH CRN Scoring System, 3 patients in the belapectin 2 mg/kg group, 2 in the belapectin 8 mg/kg group, and 1 in the placebo group had 1-stage improvement in fibrosis.

Safety and Tolerability

A very high proportion of patients in each treatment group reported at least 1 TEAE (placebo, 94%; belapectin 2 mg/kg, 98.1%; and belapectin 2 mg/kg, 89%). The majority of the TEAEs were grade 1 or grade 2 in severity (Supplementary Table 2). The system organ classes with the highest incidence of TEAEs were infections and infestations, gastrointestinal disorders, and musculoskeletal and connective tissue disorders (Supplementary Table 3). The proportions of patients with at least 1 ≥ grade 3 AE or at least 1 treatment-emergent SAE were comparable among the 3 treatment groups (Supplementary Table 2).

TEAEs considered related to study treatment were reported in 13 (24%) placebo-, 19 (36%) belapectin 2 mg/kg–, and 23 (42.5%) belapectin 8 mg/kg–treated patients. TEAEs leading to study drug discontinuation were reported in 3 participants, all receiving belapectin at 8 mg/kg dose (Supplementary Table 2). These included 1 participant with a spasmodic cough (adjudicated as probably related to the study drug) and 2 participants with esophageal variceal bleeding (adjudicated as unrelated to the study drug). The numbers of participants with at least 1 SAE were 5 (10%), 12 (22%), and 8 (15.5%) in the belapectin 2 mg/kg, belapectin 8 mg/kg, and placebo groups, respectively (Supplementary Table 2). During the study period, 1 individual in the belapectin 2 mg/kg group died of fatal TEAE of pulmonary embolism, immediately after a surgical procedure. No fatal TEAEs were reported in the belapectin 2 mg/kg or placebo groups. No apparent treatment-related or dose-related trends were observed in the clinical laboratory, vital sign, physical examination, or 12-lead electrocardiography results.

No treatment-related or dose-related trends were observed in the clinical laboratory, vital sign, physical examination, or 12-lead electrocardiography results. There were no reported cases of drug-induced liver injury during the trial in any individuals across the 3 treatment groups.

Pharmacokinetics

The mean plasma concentrations of belapectin at 2 hours after infusion were similar at visit 1 through visit 4, ranging between 18,050 ng/mL and 21,110 ng/mL for belapectin 2 mg/kg and between 75,420 ng/mL and 95,880 ng/mL for belapectin 2 mg/kg, indicating that belapectin did not accumulate in plasma after multiple doses. The total drug exposure as assessed by the area under the concentration (AUC) curve for serial belapectin levels showed that

Table 5. Complications of Cirrhosis During the Study Period

Histologic change	Belapectin 2 mg/kg (n = 54)	Belapectin 8 mg/kg (n = 54)	Placebo (n = 54)
Development of at least 1 complication of cirrhosis, %	18.5	20	22
Median days to first complications of cirrhosis	367	379	371
Individual cirrhosis complications, n			
Portal hypertension related bleeding (varices or gastropathy)	1	3	0
Clinically apparent ascites	2	1	1
Spontaneous bacterial peritonitis	0	0	0
Overt hepatic encephalopathy	3	3	1
Change in CTP score ≥2	2	0	3
Newly diagnosed varices in those without prior varices	0	1	6
Progression from small to medium or large varices	4	6	3
MELD score ≥15/eligibility for OLT	1	0	2
Liver-related mortality	0	0	0

CTP, Child–Turcotte–Pugh score; MELD, Model for Endstage Liver Disease; OLT, orthotopic liver transplantation.

Table 6. Selected Secondary and Exploratory Efficacy Endpoints: Mean (SD) Changes at the End of Treatment From Baseline

Endpoints	Belapectin 2 mg/kg (n = 54)	Belapectin 8 mg/kg (n = 54)	Placebo (n = 54)
ELF, mean change between EOT and BL	0.49 (0.83)	0.50 (0.78)	0.37 (0.63)
Fibrotest, mean change between EOT and BL	0.02 (0.02)	0.01 (0.02)	0.03 (0.02)
α-SMA staining, %, mean change between EOT and BL	2.5 (12.72)	4.4 (12.2)	1.3 (9.68)
Galectin-3 staining, %, mean change between EOT and BL	1.17 (12)	0.93 (8.1)	0.36 (7.9)
LSM, *kPa*, mean change between EOT and BL	–1.3 (12.5)	–2.34 (10.8)	–0.47 (18.6)
MBT $cPDR_{30}$, mean change between EOT and BL	–40 (258)	–27 (242)	–45.4 (279)
CLD-Q, mean change between EOT and BL			
Overall score	0.33 (0.9)	–0.03 (0.85)	0.06 (0.8)
Abdominal	0.28 (1.3)	0.06 (1.4)	0.13 (1.38)
Fatigue	0.32 (1.25)	–0.03 (2.2)	0.03 (0.9)
Systemic symptoms	0.20 (0.82)	–0.17 (0.86)	0.05 (0.89)
Activity	–0.23 (1.66)	–0.08 (1.2)	0.03 (1.25)
Emotional function	0.36 (1.07)	–0.02 (0.9)	0.0 (0.9)
Worry	0.57 (1.2)	–0.03 (1.21)	0.11 (1.15)

BL, baseline; CLD-Q, chronic liver disease questionnaire; $_{C}PDR_{30}$, cumulative percentage dose recovery at 30 minutes; ELF, enhanced liver fibrosis panel; LSM, liver stiffness measurement; MBT, ^{13}methacetin breath test; SMA, smooth muscle actin.

the AUCs for belapectin 2 mg/kg were tightly clustered, with median level of 2665.5 mg·h/L (10th–90th percentile, 2004–3785 mg·h/L), whereas they were widely dispersed for belapectin 8 mg/kg, with a median level of 10,954 mg·h/L (10th–90th percentile, 8088–14,847 mg·h/L). Further details of the pharmacokinetics and their interpretation are described in the supplementary materials.

Discussion

Patients with cirrhosis due to NASH represent a challenging problem due to the lack of effective therapies. The current clinical approach is to assess the severity of portal hypertension for prognostication with an upper endoscopy and offer primary prophylaxis with a nonselective beta-blocker or endoscopic band ligation in patients with high-risk varices (medium to large size or any size varices with red wale marks).[18] Importantly, lowering portal pressure in patients with clinically significant portal hypertension and no or small varices has been recently shown to decrease the risk of decompensation in a recent study that comprised mostly patients with HCV.[22] Therefore, any therapeutic agent that can prevent the progression of portal hypertension or reverse fibrosis with a resultant decrease in portal hypertension is very desirable.

In the current study, belapectin at either dose did not meet either the primary endpoint of reduction in HVPG or the clinically important secondary endpoints of fibrosis improvement or the incidence of complications of cirrhosis. Interestingly and somewhat unexpectedly, belapectin was associated with an improvement in hepatocyte ballooning. The significance of such improvement in hepatocyte ballooning in the absence of improvement of other histologic components, especially inflammation, is unknown.

Our post-hoc analysis suggests that there may be benefits from belapectin in select patients with NASH cirrhosis. In a subgroup of patients with NASH cirrhosis without varices at baseline, belapectin 2 mg/kg had a significant favorable effect on HVPG and was associated with a significantly lower incidence of varix development. These effects are intriguing because belapectin 2 mg/kg was not associated with demonstrable changes in liver fibrosis. This raises the possibility that either our sample size in this subgroup was too small to detect the histologic changes associated with belapectin 2 mg/kg treatment, or the favorable effects of belapectin 2 mg/kg on the development of new varices and HVPG are due to mechanisms other than directly improving liver fibrosis. It is noteworthy that there was no central reading of the endoscopic findings, and this could make the estimation of the rate of varix development less reliable. Nonetheless, if this observation can be reproduced in a subsequent study, then belapectin may have a role in the management of patients with NASH cirrhosis and portal hypertension but no varices. In fact, the sponsor and the investigators are planning to initiate a phase 3 study of belapectin in this population.

Hepatocyte ballooning is considered fundamental to the pathogenesis of disease progression in NASH. Many other agents have improved hepatocyte ballooning in NASH but, virtually in all instances, this improvement accompanied changes in steatosis and inflammation.[23–25] The significant benefit of belapectin on ballooning in isolation we observed in this study, although unusual, is biologically plausible because of the previously reported role of galectin 3 in macrophage activation,[26] migration,[26] and cell survival.[9] However, Gal-3 is believed to be important in hepatic stellate activation, and yet we did not observe a significant effect on α–smooth muscle actin staining, a marker of hepatic fibrogenesis.

In the subgroup of participants without varices at baseline, there was no dose response with belapectin because it showed positive effects at the 2 mg/kg dose but not at the 8 mg/kg dose. This observation is somewhat consistent with GCS-100, another galectin 3 inhibitor, in

patients with chronic renal disease. In a multicenter, randomized, blinded, placebo-controlled, phase 2 study in advanced chronic kidney disease, patients met the primary efficacy endpoint of a statistically significant improvement in kidney function at a dose of 1.5 mg/m^2, but not at a 30-mg/m^2 dose.[27]

In our population pharmacokinetic modeling, we observed that the total drug exposure, as assessed by the AUC for serial belapectin levels showed that the AUCs for belapectin 2 mg/kg were tightly clustered, whereas they were widely dispersed in the belapectin 2 mg/kg (supplementary materials). The overall drug exposure in many patients was more than double the expected level based on the phase 1 study, which was conducted in patients with advanced fibrosis but not cirrhosis.[17] Because of the interrelated dose pharmacokinetics and participant liver impairment due to the cirrhotic state itself, a further correlation analysis of the primary endpoint of HVPG was conducted against the individual calculated AUC_{240}. This analysis showed a potential therapeutic window with significant clinical benefit in HVPG at the range of 3,000 to 12,000 AUC_{0-240}. The preclinical studies in mice and the drug pharmacokinetics in the phase 1 study (patients with advance fibrosis but not those with cirrhosis) showed that the relationship of AUC to dose was different in the patients with cirrhosis (see Traber and Zomer[15] and the supplementary materials). By comparison of the AUC from normal mice to the predicted AUC from experiments in NASH mice, the higher AUC observed in patients with cirrhosis may explain the lower efficacy of GR-MD-02 through reduction in effect on anti-inflammatory pathways, as observed in the preclinical experiments. Both NAFLD score and inducible nitric oxide synthase activities were higher at the predicted high AUC in the NASH model compared with the optimal efficacy at approximately 10–30 mg/kg, which correspond to approximately 2–6 mg/kg in human patients with noncirrhotic NASH (Supplementary Table 4).[12] When the belapectin 8 mg/kg group was subdivided based on an AUC (12,000 mg·h/L) deemed optimal for a therapeutic response from a post hoc review of the current study data, belapectin 8 mg/kg group with AUCs within the therapeutic range had an HVPG response similar to that of belapectin 2 mg/kg group (Supplementary Figure 5, supplementary materials). Considering the optimal window of AUC_{240} for achieving meaningful clinical benefit, an upper dose of 4 mg/kg is recommended for future studies. The PK analysis for belapectin 4 mg/kg predicts a mean AUC_{240} value of 6275 mg·h/L, with a range of 3056–10,302 mg·h/L, for 90% of the population with cirrhosis.

From a safety and tolerability standpoint, belapectin was safe and well tolerated without a specific safety signal. As expected, the study population is AE prone, and more than 90% of the participants had at least 1 TEAE. There were more drug discontinuations due to an AE in the belapectin 8 mg/kg group; however, only 1 of 3 such instances was deemed related to the study drug (spasmodic cough).

It is disappointing that belapectin did not exhibit robust efficacy related to endpoints such as improvement in fibrosis, although it significantly improved fibrosis in preclinical models.[15,16] It is well recognized that small animal model systems do not reliably translate well into human clinical trials. For example, it was estimated that the average rate of successful translation from animal models to human cancer clinical trials is less than 10%.[28] In an elegant study, Teufel et al[29] have shown that there is little overlap in the hepatic gene expression between 9 different mouse models of NAFLD and patients with different stages of NAFLD, casting doubt on the utility of using a mouse model for developing novel therapeutics for human NASH and advanced fibrosis. Some of the reasons why we may not have seen an improvement in fibrosis with belapectin include the facts that (1) the duration therapy was not sufficiently long; (2) our study population included patients with established cirrhosis and portal hypertension, a group in whom fibrosis reversal may not possible; and (3) the doses we chose were not appropriate, especially in the population with portal hypertension.

In summary, in this randomized, double-blind, placebo-controlled study of patients with NASH cirrhosis and portal hypertension, belapectin was not associated with significant changes in HVPG, liver histology, or the incidence of complications of cirrhosis. However, in a subgroup of patients without varices at baseline, belapectin at the 2-mg/kg dose administered every 2 weeks for 12 months was associated with a significant effect on HVPG and the development of new varices. A phase 3 study to evaluate to safety and efficacy of belapectin in patients with NASH cirrhosis without esophageal varices is currently being initiated.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://doi.org/10.1053/j.gastro.2019.11.296.

References

1. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology 2018;67:328–357.
2. Parikh ND, Marrero WJ, Wang J, et al. Projected increase in obesity and non-alcoholic-steatohepatitis-related liver transplantation waitlist additions in the United States. Hepatology 2019;70:487–495.
3. Noureddin M, Vipani A, Bresee C, et al. NASH leading cause of liver transplant in women: updated analysis of indications for liver transplant and ethnic and gender variances. Am J Gastroenterol 2018;113:1649–1659.
4. Ripoll C, Groszmann R, Garcia-Tsao G, et al. Hepatic venous pressure gradient predicts clinical decompensation in patients with compensated cirrhosis. Gastroenterology 2007;133:481–488.
5. Turco L, Villanueva C, La Mura V, et al. Lowering portal pressure improves outcomes of patients with cirrhosis,

with or without ascites: a meta-analysis. Clin Gastroenterol Hepatol 2020;18:313–327.
6. Barondes SH, Castronovo V, Cooper DN, et al. Galectins: a family of animal β-galactoside-binding lectins. Cell 1994;76:597–598.
7. Davidson PJ, Li SY, Lohse AG, et al. Transport of galectin-3 between the nucleus and cytoplasm. I. Conditions and signals for nuclear import. Glycobiology 2006;16:602–611.
8. Ochieng J, Fridman R, Nangia-Makker P, et al. Galectin-3 is a novel substrate for human matrix metalloproteinases-2 and -9. Biochemistry 1994; 33:14109–14114.
9. Yang RY, Hsu DK, Liu FT. Expression of galectin-3 modulates T-cell growth and apoptosis. Proc Natl Acad Sci U S A 1996;93:6737–6742.
10. Jeng KC, Frigeri LG, Liu FT. An endogenous lectin, galectin-3 (εBP / Mac-2), potentiates IL-1 production by human monocytes. Immunol Lett 1994;42:113–116.
11. Sano H, Hsu DK, Yu L, et al. Human galectin-3 is a novel chemoattractant for monocytes and macrophages. J Immunol 2000;165:2156–2164.
12. Tellez-Sanz R, Garcia-Fuentes L, Vargas-Berenguel A. Human galectin-3 selective and high affinity inhibitors. Present state and future perspectives. Curr Med Chem 2013;20:2979–2990.
13. Iacobini C, Menini S, Ricci C, et al. Galectin-3 ablation protects mice from diet-induced NASH: a major scavenging role for galectin-3 in liver. J Hepatol 2011;54:975–983.
14. Jeftic I, Jovicic N, Pantic J, et al. Galectin-3 ablation enhances liver steatosis, but attenuates inflammation and IL-33-dependent fibrosis in obesogenic mouse model of nonalcoholic steatohepatitis. Mol Med 2015; 21:453–465.
15. Traber PG, Zomer E. Therapy of experimental NASH and fibrosis with galectin inhibitors. PLoS One 2013;8(12): e83481.
16. Traber PG, Chou H, Zomer E, et al. Regression of fibrosis and reversal of cirrhosis in rats by galectin inhibitors in thioacetamide-induced liver disease. PLoS One 2013; 8(10):e75361.
17. Harrison SA, Marri SR, Chalasani N, et al. Randomised clinical study: GR-MD-02, a galectin-3 inhibitor, vs. placebo in patients having non-alcoholic steatohepatitis with advanced fibrosis. Aliment Pharmacol Ther 2016; 44(11–12):1183–1198.
18. Garcia-Tsao G, Abraldes JG, Berzigotti A, et al. Portal hypertensive bleeding in cirrhosis: risk stratification, diagnosis, and management: 2016 practice guidance by the American Association for the study of liver diseases. Hepatology 2017;65:310–335.
19. Groszmann RJ, Wongcharatrawee S. The hepatic venous pressure gradient: anything worth doing should be done right. Hepatology 2004;39:280–282.
20. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696–699.
21. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.
22. Villanueva C, Albillos A, Genesca J, et al. β blockers to prevent decompensation of cirrhosis in patients with clinically significant portal hypertension (PREDESCI): a randomised, double-blind, placebo-controlled, multicentre trial. Lancet 2019;393(10181):1597–1608.
23. Harrison SA, Rinella ME, Abdelmalek MF, et al. NGM282 for treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 2018;391(10126):1174–1185.
24. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet 2015;385(9972):956–965.
25. Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 2010;362:1675–1685.
26. Goetz JG, Joshi B, Lajoie P, et al. Concerted regulation of focal adhesion dynamics by galectin-3 and tyrosine-phosphorylated caveolin-1. J Cell Biol 2008;180:1261–1275.
27. La Jolla Pharmaceutical. Results from phase 2 study of GCS-100 in chronic kidney disease being presented at American Society of Nephrology Kidney Week. http://lajollapharmaceutical.com/2014/11/results-from-phase-2-study-of-gcs-100-in-chronic-kidney-disease-being-presented-at-american-society-of-nephrology-kidney-week/. Accessed July 28, 2019.
28. Mak IW, Evaniew N, Ghert M. Lost in translation: animal models and clinical trials in cancer treatment. Am J Transl Res 2014;6:114–118.
29. Teufel A, Itzel T, Erhart W, et al. Comparison of gene expression patterns between mouse models of nonalcoholic fatty liver disease and liver tissues from patients. Gastroenterology 2016;151:513–525.

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Conflicts of interest

These authors disclose the following: Naga Chalasani has ongoing consulting activities (or had in preceding 12 months) with NuSirt, AbbVie, Afimmune (DS Biopharma), Allergan (Tobira), Madrigal, Shire, Foresite Labs, Coherus, Siemens, and Genentech (these consulting activities are generally in the areas of nonalcoholic fatty liver disease and drug hepatotoxicity); receives research grant support from Intercept, Lilly, Exact Sciences, and Galectin Therapeutics, where his institution receives the funding; and over the last decade, has served as a paid consultant to more than 35 pharmaceutical companies, and these outside activities have regularly been disclosed to his institutional authorities. Guadalupe Garcia-Tsao has ongoing consulting activities (or had in preceding 12 months) with BioVie, Boehringer Ingelheim, Bristol-Myers Squibb, Conatus, Cook, Enterome, Galectin, Intercept and has received research grant support from Intercept. Naim Alkhouri has received research funding from Albireo, Akero, Allergan, Boehringer Ingelheim, Bristol-Myers Squibb, Galmed, GENFIT, Gilead, Intercept, Madrigal, MedImmune, Novartis, Novo Nordisk, Pfizer, Poxel, and Zydus; has served as a speaker for AbbVie, Alexion, Allergan, Eisai, Exelixis, Gilead, Intercept, and Salix; and has served as a consultant for Allergan, Gilead, and Intercept. Manal F. Abdelmalek has ongoing consulting activities (or had in preceding 12 months) with Bristol-Myers Squibb, NGM Bio, TaiwanJ, Prometic, Inventiva, Novo-Nordisk, and Allergan (Tobira) (these consulting activities are generally in the areas of nonalcoholic fatty liver disease); receives research grant support from Intercept, Galectin Therapeutics, Allergan, Conatus, Gilead, Madrigal, Genfit, Novartis, NGM Bio, Bristol-Myers Squibb, Poxel, DURECT, Enyo, Inventiva, Novo Nordisk, and Celgene; and has served as a paid consultant to more than 20 pharmaceutical companies, and these outside activities have regularly been disclosed to her institutional authorities. Mary Rinella reports consulting for Intercept, Gilead, NGM, BMS, Enanta, Novartis, GENFIT, Immuron, CymaBay, Merck, Gelesis, Metacrine, Viking, Madrigal, Allergan, Thetis, Fractyl, and 3vBio and has received independent research funding from Novartis Mazen Noureddin has been on the advisory board or a speaker for Allergan, Gilead, Intercept, Pfizer, Novartis, Blade, Echosens North America, OWL, Simply Speaking, and Abbott; has received research support from Allergan, Bristol-Myers Squibb, Gilead, Galmed, Galectin, GENFIT, Conatus, Enanta, Novartis, Shire, and Zydus; and is a minor shareholder or has stocks in Anaetos and Viking. Raj Vuppalanchi has received consulting fees for serving on the Data Safety Monitoring Boards for Covance and Enanta and has received research grant support from Gilead Sciences, Zydus Discovery, and Intercept, where his institution receives the funding. 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Arun Sanyal is president of Sanyal Biotechnology and has stock options in GENFIT, Akarna, Tiziana, Indalo, DURECT and Galmed; has served as a consultant to AstraZeneca, Nitto Denko, Enyo, Ardelyx, Conatus, Nimbus, Amarin, Salix, Tobira, Takeda, Janssen, Gilead, Terns, Birdrock, Merck, Valeant, Boehringer Ingelheim, Lilly, HemoShear, Zafgen, Novartis, Novo Nordisk, Pfizer, Exalenz, and GENFIT; and has been an unpaid consultant to Intercept, Echosens, Immuron, Galectin, Fractyl, Synlogic, Afimmune, ChemomAb, Zydus, Nordic Bioscience, Albireo, ProSciento, Surrozen, and Bristol-Myers Squibb; his institution has received grant support from Gilead, Salix, Tobira, Bristol Myers, Shire, Intercept, Merck, AstraZeneca, Mallinckrodt, Cumberland, and Novartis; and receives royalties from Elsevier and *UptoDate*. Adam Allgood, Harold Shlevin, Rex Horton, and Eliezer Zomer are the employees of Galectin Therapeutics, Inc. 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Supplementary Material

Discussion of Why GR2, but Not GR8, Had Some Efficacy in a Population With Cirrhosis With Portal Hypertension

Our explanation for why GR2, but not GR8, had some efficacy in our study population can be supported based on the following.

1) In a preclinical NASH model, the dose-response effect of GR-MD-02 had an inverted-shaped performance, with an indication that the optimal therapeutic window is at 10–30 μg/kg dose. Higher-than-optimal doses and out of this window had lesser efficacy, as assessed by the NAFLD activity score and inducible nitric oxide synthase (iNOS) activity (Supplementary Figure 2). The exact mechanism on how a higher dose actually leads to lower efficacy is unknown.

2) Based on preclinical NASH and thioacetamide (TAA) models[15,16] and nonhuman primate experiments and phase 1 human study,[17] before initiating the current study, our study clinical pharmacologists estimated that the optimal human therapeutic window corresponding to the described preclinical optimal window would range between 2 mg/kg and 8 mg/kg—hence, our choice for testing these 2 doses in our phase 2 study.

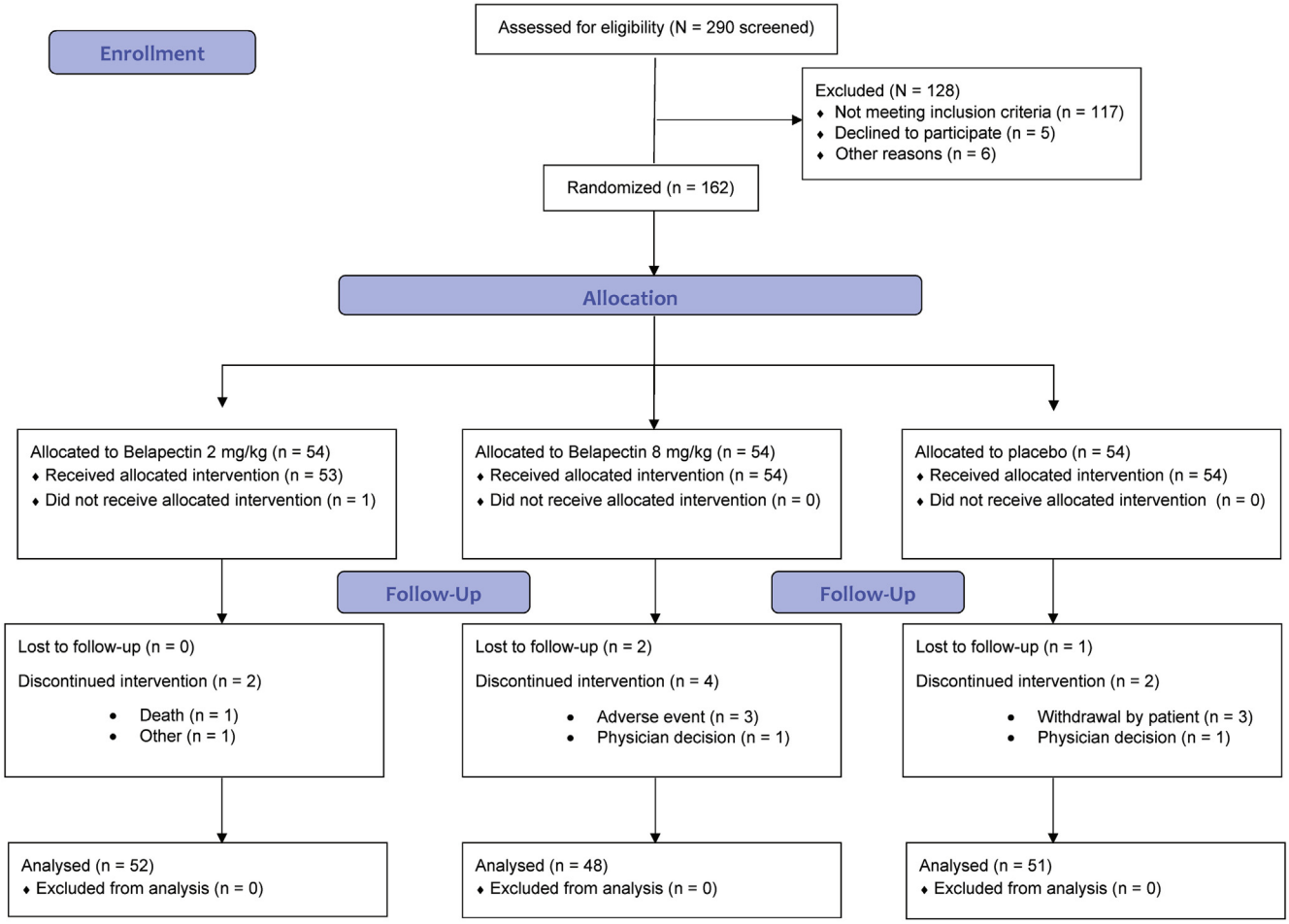
3) Somewhat unexpectedly, the pharmacokinetics (PK) of GR2 and GR8 in this trial turned out to differ from our phase1 study. Our phase1 study included NASH with bridging fibrosis, whereas the current study obviously includes individuals with cirrhosis with portal hypertension. This makes us believe that cirrhosis with portal hypertension significantly alters the PK of GR-MD-02. The following charts show that GR-B has significantly longer half-life ($T_{1/2}$) and $AUC_{0\text{-}240}$ in individuals with cirrhosis with portal hypertension compared with our phase1 study, which enrolled patients with NASH with bridging fibrosis.

4) As we described in our results section, the total drug exposure as assessed by the AUC curve for serial GR-MD-02 levels showed that the AUCs for GR2 were tightly clustered, with a median level of 2665.5 mg·h/L (10th–90th percentile, 2004-3785 mg·h/L), whereas they were widely dispersed for GR8, with median level of 10,954 mg·h/L (10th–90th percentile: 8088–14,847 mg·h/L).

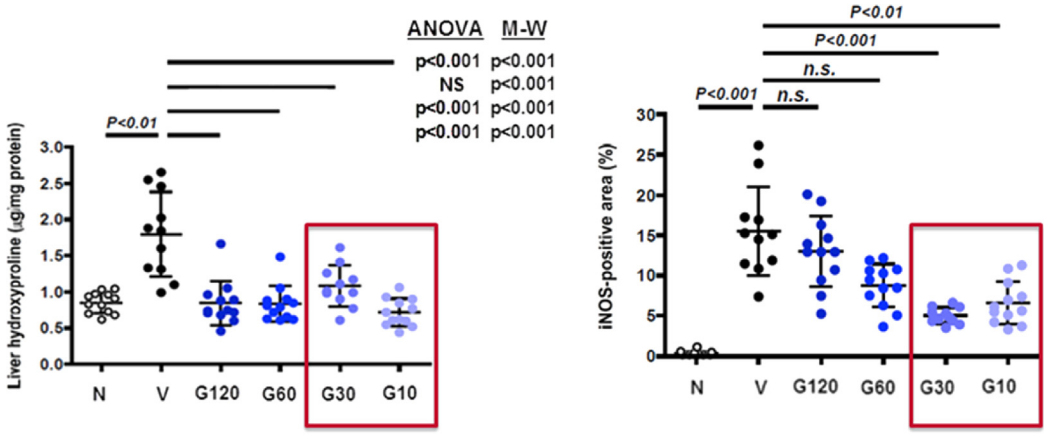
5) We observed an interesting relationship between AUC and change in HVPG. To better understand the relationship of AUC to therapeutic response, the individual AUC-Day 4 was plotted against the change in HVPG for each participant. The curve fit shows 3 regions, including a negative slope in the lower AUC region ($<$3000) indicating that as AUC is decreasing, the change in HVPG is increasing, whereas there is a flat slope in the mid-AUC region (3000–12,000), indicating a steady relationship between AUC and change in HVPG (below the zero line). In the upper AUC region ($>$12,000) there is an increasing slope, indicating that the change in HVPH is going up or worsening in this range (see Supplementary Figure 3).

6) Because the AUCs in the GR8 group were high and widely spread, in a post hoc analysis, we subdivided into GR8 group into tw2o subgroups based on an AUC cutoff of 12,000 μg·hr/mL. This AUC cutoff was chosen because it appeared to be the infliction point based on Supplementary Figure 3, which shows the relationship between AUC and response to HVPG.

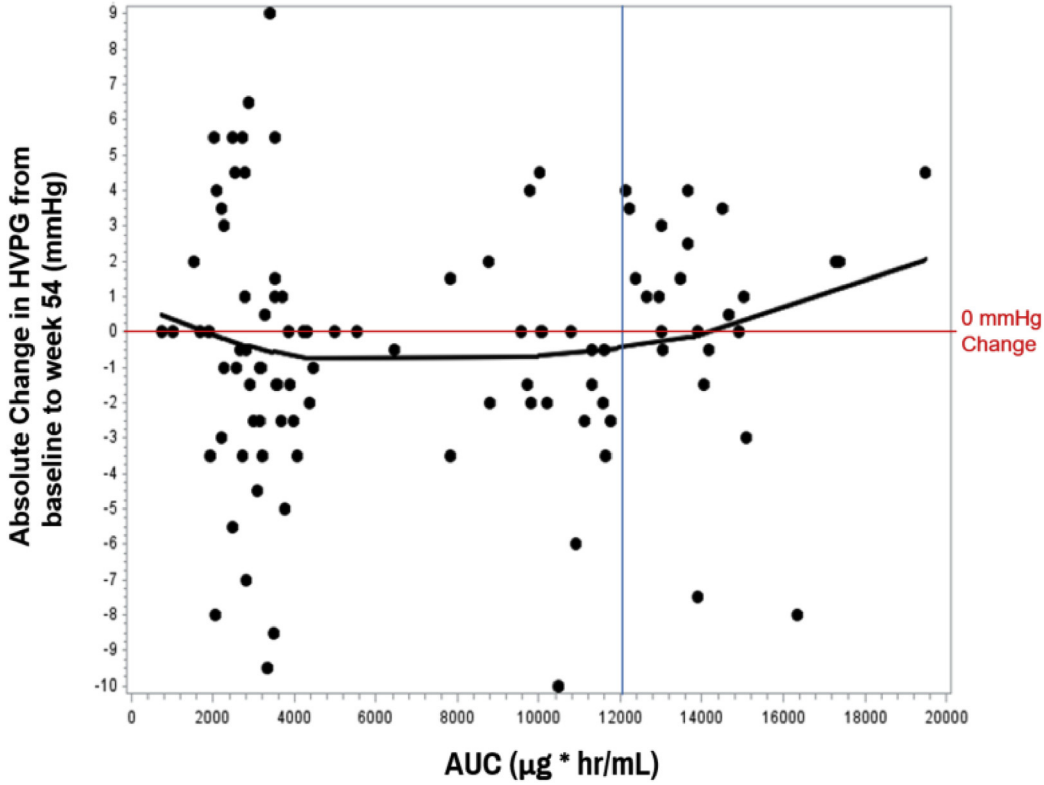
In the GR8 group, there were 25 patients with AUC $<$ 12,000 and 27 with AUC $>$ 12,000. The change in GR8 patients with AUC $<$ 12,000 was statistically significantly different from placebo (ie, similar to the GR2 group).



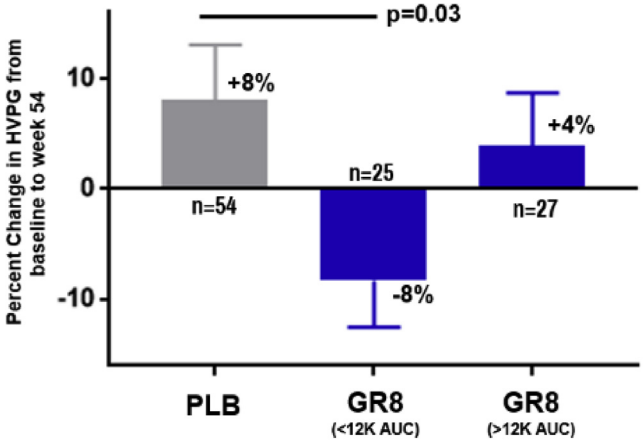
Supplementary Figure 1. Study disposition with eligible participants randomly assigned (1:1:1) to receive 1 of the 3 treatment assignments before the first infusion; doses were administered every other week over a 52-week period, for a total of 26 infusions. Safety and efficacy assessments were monitored during the treatment phase.



Supplementary Figure 2. The GR-MD-02 dose-response effect on liver hydroxyproline and inducible nitric oxide synthase expression identifies a potential therapeutic window.[15] ANOVA, analysis of variance; NS, not significant.



Supplementary Figure 3. Change in HVPG vs AUC (μg·h/mL). The AUC-D4 of all patients in the 2 treatment groups (fewer than 3 high outliers in the GR8 group) were plotted against the change in HVPG from baseline to the end of the study for each participant. Loess regression analysis was used for fitting a curve between the 3 variables. The red horizontal line is zero change in HVPG.



Supplementary Figure 4. Percent change in HVPG in the GR8 group when stratified according AUC. GR8, 8 mg/kg GR-MD-02 treatment group; PLB, placebo.

Supplementary Table 1. Histologic Changes at the End of Treatment Compared With Baseline in 2 Subgroups

Subgroup	GR2 (n = 54)	GR8 (n = 54)	Placebo (n = 54)
Subgroup with no varices at baseline (n = 77)			
CPA mean change from baseline	0.3 ± 4.8	–0.4 ± 3.9	–0.1 ± 6.1
1-stage improvement in fibrosis, %	16.0	26.1	18.2
NAS change from baseline, mean ± SD	0.2 ± 1.3	0.3 ± 1.0	0.1 ± 1.1
Steatosis change from baseline, mean ± SD	0.0 ± 0.3	0.0 ± 0.5	0.0 ± 0.6
Inflammation change from baseline, mean ± SD	0.3 ± 0.7	0.3 ± 1.0	-0.1 ± 0.9
Ballooning change from baseline, mean ± SD	–0.1 ± 0.7	0.2 ± 0.6	0.2 ± 0.6
Subgroup with mild portal hypertension at baseline (n = 51)			
CPA mean change from baseline	–0.1 ± 6.5	0.3 ± 2.5	0.7 ± 6.4
1 stage improvement in fibrosis, %	8	6	8
NAS change from baseline, mean ± SD	0.5 ± 1.2	0.3 ± 1.1	0.6 ± 1.5
Steatosis change from baseline, mean ± SD	0.1 ± 0.5	0.3 ± 1.1	0.1 ± 0.7
Inflammation change from baseline, mean ± SD	0.3 ± 0.6	0.0 ± 0.7	0.1 ± 0.9
Ballooning change from baseline, mean ± SD	0.1 ± 0.5	0.1 ± 0.5	0.5 ± 0.6

CPA, collagen proportional area; GR2, 2 mg/kg GR-MD-02 treatment group; GR8, 8 mg/kg GR-MD-02 treatment group. NAS, Nonalcoholic Fatty Liver Disease Activity Score.

Supplementary Table 2. Adverse Events and Study Drug Discontinuations During the Study Period

Events	GR2 (n = 53)	GR8 (n = 54)	Placebo (n = 54)	Total (N = 161)
TEAEs, n	509	383	431	1323
Participants with at least 1 TEAE, n (%)	52 (98.1)	48 (88.9)	51 (94.4)	151 (93.8)
Patients with at least 1 grade ≥3 adverse event, n (%)	11 (20.8)	11 (20.4)	22 (20.5)	33 (20.5)
Patients with at least 1 SAE, n (%)[a]	5 (10)	12 (14)	8 (15)	25 (15.5)
Study drug discontinuation due to an AE, n	0	3	0	3[c]
Death, n[b]	1	0	0	1

GR2, 2 mg/kg GR-MD-02 treatment group; GR8, 8 mg/kg GR-MD-02 treatment group.
[a]Two treatment-emergent SAEs were deemed as possibly related to study drug by the site investigator (1 instance of hyponatremia and 1 episode of transient ischemic attack, both in the GR8 group). The sponsor's data safety monitoring board adjudicated that these 2 and the other SAEs were unrelated to the study drug.
[b]One death occurred in an individual receiving GR2 who developed pulmonary embolism after surgical repair of hernia. This was adjudicated as unrelated to the study drug.
[c]Spasmodic cough (probably related to study drug) and 2 patients with esophageal variceal bleeding (unrelated to study drug).

Supplementary Table 3. Treatment-Emergent Adverse Events (>10% Participant Incidence in Any Treatment Group) by System Organ Class and Preferred Term (Safety Set)

System organ class	GR2 (n = 53)	GR8 (n = 54)	Placebo (n = 54)	Total (N = 161)
Infections and infestations				
Nasopharyngitis	14 (26.4)	5 (9.3)	8 (14.8)	27 (16.8)
Urinary tract infection	8 (15.1)	6 (11.1)	9 (16.7)	23 (14.3)
Sinusitis	6 (11.3)	7 (13.0)	4 (7.4)	17 (10.6)
Upper respiratory tract infection	8 (15.1)	4 (7.4)	5 (9.3)	17 (10.6)
Bronchitis	7 (13.2)	3 (5.6)	5 (9.3)	15 (9.3)
Gastrointestinal disorders				
Nausea	14 (26.4)	8 (14.8)	11 (20.4)	33 (20.5)
Diarrhea	12 (22.6)	8 (14.8)	11 (20.4)	31 (19.3)
Abdominal pain upper	8 (15.1)	8 (14.8)	13 (24.1)	29 (18.0)
Vomiting	7 (13.2)	7 (13.0)	5 (9.3)	19 (11.8)
Abdominal pain	5 (9.4)	3 (5.6)	7 (13.0)	15 (9.3)
Musculoskeletal and connective tissue disorders				
Arthralgia	6 (11.3)	9 (16.7)	1 (1.9)	16 (9.9)
Muscle spasms	8 (15.1)	3 (5.)	4 (7.4)	15 (9.3)
Pain in extremity	4 (7.5)	4 (7.4)	6 (11.1)	14 (8.7)
Back pain	3 (5.7)	7 (13.0)	2 (3.7)	12 (7.5)
General disorders and administration site conditions				
Fatigue	9 (17.0)	9 (16.7)	10 (18.5)	28 (17.4)
Peripheral edema	8 (15.1)	4 (7.4)	7 (13.0)	19 (11.8)
Skin and subcutaneous tissue disorders				
Rash	5 (9.4)	5 (9.3)	6 (11.1)	16 (9.9)
Injury, poisoning, and procedural complications				
Contusion	10 (18.9)	3 (5.6)	6 (11.1)	19 (11.8)
Nervous system disorders				
Headache	9 (17.0)	6 (11.1)	9 (16.7)	24 (14.9)
Dizziness	2 (3.8)	5 (9.3)	9 (16.7)	16 (9.9)

NOTE. At each level of participant summarization, a participant was counted once if the he/she reported 1 or more events. Percentages are based on the number of participants in each treatment group (n). The total number of AEs counts all TEAEs for participants. AEs were coded using Medical Dictionary for Regulatory Activities, version 18.0.
GR2, 2 mg/kg GR-MD-02 treatment group; GR8, 8 mg/kg GR-MD-02 treatment group.

Supplementary Table 4. Phase IIb: (NASH-CX) Mean of Cmax (μg/mL) and AUC_{0-240} (mg·h/L) of GR-MD-02, Calculated Using Population PK Analysis Set for All Plasma Samples

24 Biweekly Doses	$C_{max(0-240)}$, mean, μg/mL	$T_{1/2}$, h	AUC_{0-240}, mean, μg·h/mL
2 mg/kg	34.32	>24	3414
8 mg/kg	128.13	>24	11,835

Supplementary Table 5. Phase I: Summary of GR-MD-02 Plasma PK Parameters

Weekly dose (×doses)	$Cmax_{(0-240)}$, μg/mL	$T_{1/2}$, h	AUC_{0-240}, μg·h/mL
2 mg/kg (×1)	16.3	19.9	573
2 mg/kg (×4)	17.7	20.5	645
4 mg/kg (×1)	30	19.8	1039
4 mg/kg (×4)	31	19.5	1075
8 mg/kg (×1)	99.5	18.2	2449
8 mg/kg (×4)	169.9	18.4	4909