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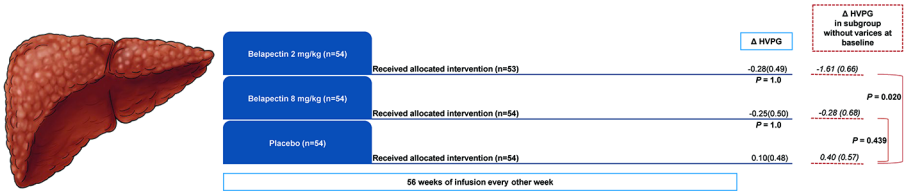
Change in Hepatic Vein Pressure Gradient With Galectin-3 Inhibitor in Patients With NASH Cirrhosis



Gastroenterology

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

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Disclosures

Dr. 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. Dr. Chalasani receives research grant support from Intercept, Lilly, Exact Sciences, and Galectin Therapeutics where his institution receives the funding. Over the last decade, Dr. Chalasani has served as a paid consultant to more than 35 pharmaceutical companies, and these outside activities have regularly been disclosed to his institutional authorities.

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Mary Rinella reports consulting for the following companies: Intercept, Gilead, NGM, BMS, Enanta, Novartis, Genfit, Immuron, Cymabay, Merck, Gelesis, Metacrine, Viking, Madrigal, Allergan, Thetis, Fractyl, 3vBio. She has received independent research funding from Novartis. She has no stock ownership in any company for whom she consults and is on no speakers bureaus.

Mazen Noureddin: MN has been on the advisory board or a speaker for Allergan, Gilead, Intercept, Pfizer, Novartis, Blade, EchoSens North America, OWL, Simply Speaking, and Abbott; MN has received research support from Allergan, BMS, Gilead, Galmed, Galectin, Genfit, Conatus, Enanta, Novartis, Shire and Zydus; MN is a minor shareholder or has stocks in Anaetos and Viking.

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Maxmillan Pyko: Has no financial conflicts of interests to declare.

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Arun Sanyal is President of Sanyal Biotechnology and has stock options in Genfit, Akarna, Tiziana, Indalo, Durect and Galmed. He has served as a consultant to Astra Zeneca, Nitto Denko, Enyo, Ardelyx, Conatus, Nimbus, Amarin, Salix, Tobira, Takeda, Jannsen, Gilead, Terns, Birdrock, Merck, Valeant, Boehringer-Ingelheim, Lilly, Hemoshear, Zafgen, Novartis, Novo Nordisk, Pfizer, Exhalenz and Genfit. He has been an unpaid consultant to Intercept, Echosens, Immuron, Galectin, Fractyl, Syntlogic, Affimune, 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, Astra Zeneca, Malinckrodt, Cumberland and Novartis. He receives royalties from Elsevier and UptoDate.

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Abstract

Background & Aims: 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. 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] ≥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 (-28) 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 mmHG vs 0.10 mmHG, *P=1.0)* or between the 8 mg/kg belapectin and placebo group (-0.25 mmHG vs 0.10 mmHG, *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.

KEY WORDS: NAFLD, carbohydrate-binding protein, inflammation, steatosis

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Introduction

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 etiology for liver transplantation in men and leading etiology 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 can easily traverse the intracellular and plasma membranes to translocate into the nucleus, 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 non-classical pathway and may serve as damage-associated molecular pattern candidate.[7] Previous studies have demonstrated 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 (ECM) 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 (anti-apoptotic, 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 which 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 as they are carbohydrates with no toxic metabolites.[12] Belapectin (GR-MD-02 , galactoarabino-rhamnogalacturonate) is a complex carbohydrate molecule derived from a natural plant compound which contains oligosaccharide chains containing galactose residues and binds to galectin-3, and a lesser extent, galectin-1. It has shown robust efficacy in preclinical models of NASH and liver fibrosis, and 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 galectin-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 below 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 Phase2b, multicenter, randomized, double-blind, placebo-controlled trial of beapectin in patients with NASH cirrhosis and portal hypertension. Two doses of 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 two 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 **(NCT02462967)**. This study was sponsored by Galectin Therapeutics and had an independent data safety monitoring board (DSMB) and a medical monitor associated with a clinical research organization. The review board approved the study at each participating center, and all subjects gave written informed consent. The data were analyzed independently and were reviewed by both the investigators and the DSMB.

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 two main inclusion criteria were hepatic venous pressure gradient (HVPG) ≥6 mm Hg and liver biopsy showing cirrhosis due to NASH.

Study Design

Eligible participants underwent an upper endoscopy within 2 months prior to 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 (a) large varices (>50% impingement on the lumen); (b) small varices (<25% impingement on lumen); and (c) medium varices were 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 prior to patient enrollment. Portal pressure was determined indirectly by the HVPG as previously described.[19] Briefly, using the transjugular approach, a balloon-tipped catheter was advanced into a hepatic vein under fluoroscopic guidance. The free hepatic venous pressure (FHVP) was measured with the balloon deflated, the wedged hepatic venous pressure (WHVP) was measured with the balloon inflated until the branch of hepatic vein was completely occluded. HVPG was obtained by subtracting the FHVP from the WHVP. All measurements were performed in triplicate and tracings were read independently by a single experienced investigator (GGT, Yale University). Liver histology was centrally read in a blinded fashion by a single experienced hepatopathologist (ZG, Inova).

Trial Endpoints

The primary endpoint was to evaluate the efficacy of belapectin in reducing HVPG as a measure of portal pressure compared to placebo after 12 months of treatment. Predefined secondary efficacy endpoints were (a) baseline-adjusted mean change in the collagen proportion area (CPA), (b) proportion of participants with ≥ 1 point change in fibrosis stage; (c) baseline adjusted mean change in liver stiffness; and (d) complications of cirrhosis, defined as

the development of any of the following: (i) esophageal variceal hemorrhage or portal hypertensive gastropathy hemorrhage (confirmed by endoscopy), (ii) clinically apparent ascites or spontaneous bacterial peritonitis, (iii) overt hepatic encephalopathy (d) an increase in Child-Turcotte-Pugh score ≥2 points, (iv) newly diagnosed varices in a subject without prior varices or progression from small to medium or large varices, (v) reaching a model for end-stage liver disease (MELD) score ≥15 as measured on 2 consecutive occasions, (vi) listing for a liver transplant or the performance of a liver transplant, or (vii) 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 mmHg) and clinically significant portal hypertension (HVPG ≥ 10 mmHg) (prespecified subgroups) as well as patients with no varices at baseline (posthoc analysis). Safety endpoints included the incidence of treatment-emergent adverse events (TEAE), serious adverse events (SAEs), and study discontinuations during the trial.

Statistical Analysis

Continuous variables were summarized as mean (SD or SE), 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 0.05 significance level. If analysis variables were not normally distributed, 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.

Sample size calculations were based on the comparison of the primary efficacy variable, change in HVPG from baseline, with the following key assumptions: a) true mean change in HVPG from baseline at 52 weeks in the placebo group, $\Delta p = 0$, b) true mean change in HVPG from baseline at 52 weeks in either belapectin dose group, $\Delta G = -2$ mm Hg, c) common SD for difference in HVPG, $\sigma = 3$ mm Hg, d) null hypothesis, H0: $\theta = G - \Delta p = 0$, e) Type I error, $\alpha = 0.05$ (2-sided significance test), f) Power = 80%, g) Statistical test = 2-sample *t*-test for mean difference, h) Randomization ratio = 1:1:1, and i) 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 subjects (n = 52 subjects per group) was required to achieve the power of 80% with a 2-sided type I error rate of 0.05.

Primary Efficacy Endpoint Analyses were conducted as an intention-to-treat (ITT) on the full-analysis set (FAS), which included all participants randomized. All subjects 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 subjects were summarized by the following HVPG categories (mild portal hypertension [MPH], i.e., ≥6 mm Hg to <10 mm Hg and clinically significant portal hypertension [CSPH], i.e., ≥10 mm Hg). The primary efficacy endpoint, change from baseline in HVPG at the end of treatment (EOT), was analyzed using analysis of covariance (ANCOVA) with baseline values taken as a covariate using the FAS at a significant level of 0.05 (2-sided). The treatment effect was evaluated as a contrast of each active treatment versus placebo and described using continuous summary with an estimate of mean difference along with a 95% confidence interval (CI). The subgroup analyses were conducted in the modified-intent-to-treat (mITT) set that included only subjects who were randomly assigned, received at least one infusion, and had at least one postbaseline efficacy assessment.

Results

Participant disposition

A total of 162 participants were randomly assigned to receive study drug (54 individuals each in the belapectin 2 mg/kg [hereafter referred to as GR2], belapectin 8 mg/kg, and placebo groups). Histological 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 **Figure 1**. All 162 randomly assigned participants were included in the FAS, and 161 participants (99.4%) were included in the mITT and the safety cohorts (**Figure 1)**. Eleven participants (6.8%) discontinued the study early with 151 participants (93.2%) completing the study. Two primary reasons for study discontinuation were AE (3 subjects) and lost to follow-up (3 participants). The proportion of participants completing the study was similar across the treatment groups (**Figure 1)**.

Efficacy

Hepatic Vein Pressure Gradient

The LS mean change in HVPG from baseline at EOT in each treatment arm was not significantly different among placebo (0.10 mmHg), belapectin 2 mg/kg (-0.28 mmHg) or belapectin 8 mg/kg (-0.25 mmHg) groups. Compared to placebo, LS mean change from baseline in HVPG were also not different for the two active treatment groups (-0.38 mmHg for belapectin 2 mg/kg and -0.35 mmHg for belapectin 8 mg/kg, P=1.0 for both comparisons) **(Table 3)**. Similarly, in the pre-planned separate analysis of the MPH and CSPH 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 to placebo (0.40 mmHg), the LS mean change with belapectin 2 mg/kg was significantly different (-1.61 mmHg, P=0.02) but not with belapectin 8 mg/kg (-0.28 mmHg, P=0.4) **(Table 3).**

The effects of belapectin 2 mg/kg and 8 mg/kg on HVPG when calculated as % change from baseline were as follows: (a) for the FAS, mean (s.d) percent change between EOT and BL was 6.15 ± 31% in the placebo group --1.74 ± 33% in the belapectin 2 mg/kg group (p=0.11 vs placebo), and -1.09 ± 22% in the belapectin 8 mg/kg group (p=0.42 vs. placebo); (b) and for the MPH subgroup, mean (s.d) percent change between EOT and BL was 26 ± 47% in the placebo group, -3.27 ± 32% in the belapectin 2 mg/kg group (p=0.021 vs placebo), and -2.04 ± 25% in the belapectin 8 mg/kg group (p=0.027 vs. placebo); and (c) for the subgroup without varices at baseline, mean (s.d) percent change between EOT and BL was 11.7 ± 33.7% in the placebo group, -8.46 ± 26% in the belapectin 2 mg/kg group (p=0.011 vs placebo), and 0.6 ± 25% in the belapectin 8 mg/kg group (p=0.42 vs. placebo).

There was a significant interaction between baseline varices status and the treatment response (P=0.037).

Histology

There was no statistically significant difference between the two belapectin groups and the placebo group for the change from baseline in CPA, ≥ 1 stage improvement in fibrosis or NAFLD activity score at the end of treatment (EOT) **(Table 4).** While there were no significant differences among treatment groups in the lobular inflammation or steatosis, for hepatocyte ballooning there was a statistically significant difference for the belapectin 2 mg/kg group [OR: 2.42 (95% CI: 1.09, 5.37, P=0.030)] and a trend towards significance in the belapectin 8 mg/kg group [OR: 1.98 (95% CI: 0.90, 4.34, P=0.089)] compared with the placebo group. Histological

changes associated with two belapectin groups in the subgroups of patients with MPH or no varices at baseline are shown in **Supplemental Table 1.**

Liver-Related Clinical Outcomes

At least one 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>0.05). 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>0.05) **(Table 5)**.

In subjects without varices at baseline, there was no statistically significant difference between the belapectin groups and placebo group in the number of subjects with at least 1 complication of cirrhosis at Year 1 (P>0.05). 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=0.032) and borderline significance for belapectin 8 mg/kg (4% vs. 18% placebo, P=0.12) **(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 two treatment groups and the placebo-treated patients for any of these endpoints. The changes in the overall score or in the six domains (abdominal symptoms, fatigue, systemic symptoms, activity, emotional function, and worry), as assessed by the Chronic Liver Disease Questionnaire (CLD-Q), were not different between the treatment groups.

Safety and Tolerability

A very high proportion of patients in each treatment group reported at least one treatment-emergent adverse event (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 **(Supplemental Table 2)**. The system organ classes with the highest incidence of TEAEs were infections and infestations, gastrointestinal disorders, and musculoskeletal and connective tissue disorders **(Supplemental Table 3)**. The proportions of patients with at least one ≥ grade

3 AEs or at least one treatment-emergent serious adverse event (SAE) were comparable among three treatment groups **(Supplemental Table 2)**.

Treatment-emergent AEs considered related to study treatment were reported in 13 (24%) among the placebo, 19 (36%) belapectin 2 mg/kg, and 23 (42.5%) belapectin 8 mg/kg treated patients. Treatment-emergent AEs leading to study drug discontinuation were reported in 3 participants, all receiving belapectin at 8 mg/kg dose **(Supplemental 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 number of participants with at least one serious adverse event (SAE) were 5 (10%), 12 (22%), and 8 (15.5%) in the belapectin 2 mg/kg, belapectin 8 mg/kg, and placebo groups respectively **(Supplemental Table 2)**. During the study period, one individual in the belapectin 2 mg/kg group died due to fatal TEAE of pulmonary embolism, immediately following 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 ECG 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 three treatment groups.

Pharmacokinetics

Mean plasma concentrations of belapectin at 2-hour post-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 the AUCs for belapectin 2 mg/kg were tightly clustered with median level of 2665.5 mg*h/L (10^{th} - 90^{th} percentile: 2004-3785 mg*h/L) whereas they were widely dispersed for belapectin 8 mg/kg with median level of 10954 mg*h/L (10^{th} - 90^{th} percentile: 8088-14,847 mg*h/L). Further details of the pharmacokinetics and their interpretation are described in **Supplemental Material 4.**

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 non-selective 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 HCV patients.[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 histological 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 varices 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 histological 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 varices 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 phase3 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 with changes in steatosis and inflammation.[23-25] The significant benefit of belapectin on ballooning in isolation we observed in this study while unusual is biologically plausible because of the previously reported role of galectin 3 in macrophage activation,[26] migration,[26] and cell survival.[9] However, we note that galectin-3 is believed to be important in the hepatic stellate activation and yet we did not observe a significant effect on αSMA staining, a marker of hepatic fibrogenesis.

In the subgroup of participants without varices at baseline, there was no dose-response with belapectin as it showed positive effects at 2 mg/kg dose but not at 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 its primary efficacy endpoint of a statistically significant improvement in kidney function at a dose of 1.5 mg/m^2, but not at 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 **(Supplemental Material 4).** 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] Due to the interrelated dose PK and subject 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 revealed a potential therapeutic window with significant clinical benefit in HVPG at the range of 3,000 to 12,000 AUC $_{0\text{-}240}$. The preclinical studies in mice and the drug PK in the phase 1 study (patients with advance fibrosis, not cirrhotics) demonstrated that the relationship of AUC to dose was different in the cirrhosis patients **(see ref 15 and also Supplemental Material 4)**. By comparison of the AUC from normal mice to predicted AUC from experiments in NASH mice, the higher AUC observed in cirrhotic patients 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 iNOs activities were higher at the predicted high AUC in the NASH model as compared to the optimal efficacy at about 10-30 mg/Kg which correspond to ~ 2-6 mg/kg in human patients with non-cirrhotic NASH [12]. When 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 **(Supplemental Material 4)**. Considering the optimal window of AUC-240 for achieving meaningful clinical benefit, an upper dose of 4 mg/kg (GR4) is recommended for future studies. The PK analysis for belapectin 4 mg/kg predicts a mean AUC-240 value of 6,275 with a range of 3,056 to 10,302 mg*h/L for 90% of the cirrhotic population.

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 one TEAE. There were a higher number of study drug discontinuations due to an AE in the belapectin 8 mg/kg group; however, only one of three 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. 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,[29] casting doubt on the utility of 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 (a) the duration therapy was not sufficiently long; (b) our study population included patients with established cirrhosis and portal hypertension, a group in whom fibrosis reversal may not possible; and (c) the doses we chose were not appropriate, especially in 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 in the incidence of complications of cirrhosis. However, in a subgroup of patients without varices at baseline, belapectin administered at 2 mg/kg dose administered every two weeks for 12 months was associated with a significant effect on HVPG and the development of new varices. A phase3 study to evaluate to safety and efficacy of

belapectin in patients with NASH cirrhosis without esophageal varices is currently being initiated.

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Figure Legends

Figure 1. Study disposition with eligible subjects randomly assigned (1:1:1) to receive one of the 3 treatment assignments before the first infusion and 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.

Table 1: Inclusion and Exclusion Criteria

Inclusion Criteria

A subject was eligible for inclusion if he/she met all of the following criteria:

1. Had an HVPG measurement ≥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 pathological 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 subject with at least a 5-year history of obesity (BMI ≥30) 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 ≥18 years of age and ≤75 years of age at the time of screening.
4. Had absence of hepatocellular carcinoma by valid imaging (liver ultrasound, triple phase computed tomography of liver, or magnetic resonance imaging of liver) within 6 months prior to 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 prior to the initiation of any study-specific procedures.
6. Was not pregnant and had a negative serum pregnancy test result prior to randomization. If a fertile man or woman participating in heterosexual relations, must agree to use effective means of contraception (ie, 2 effective methods of contraception, one of which must be a physical barrier method). Effective forms of contraception included condom, hormonal methods (birth control pills, injections, or implants), diaphragm, cervical cap, or intrauterine device throughout his/her participation in this study and for 90 days after discontinuation of study treatment. Surgically sterile males and females 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 following the last dose of study drug. Female subjects could not begin a cycle of ova donation or harvest throughout the study period and for a period of 90 days following the last dose of study drug.
9. Prior to randomization, any subject on statins, angiotensin converting enzyme inhibitors angiotensin II receptor blockers, or β-1 selective adrenergic receptor inhibitors was to be on a stable dose for at least 2 months and all attempts were to be made to continue the subject on the same dose of the medication for the duration of study participation.

Exclusion Criteria

Subjects 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 included 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 (up to 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 porto-systemic 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's disease, alpha-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 ALT levels >10 × the upper limits of normal
    b. Serum AST levels >10 × the upper limits of normal
    c. Platelet count <60 000/mm3
    d. Serum albumin ≤2.8 g/dL
    e. International normalized ratio (INR) ≥1.7
    f. Direct bilirubin ≥2.0 mg/dL
    g. Alpha fetoprotein >200 ng/mL
6. Had a MELD score ≥15 or Child-Turcotte-Pugh Class B or C.
7. Had an estimated creatinine clearance of <50 mL/minute. Glomerular filtration rate was estimated using the Cockcroft-Gault equation:
    - Males: CrCl (mL/min) = ([140 – age] × weight) / (SCr × 72)
    - Females: CrCl (mL/min) = ([140 – age] × weight) / (SCr × 72)] × 0.85
    - Where CrCl is creatinine clearance, age is in years, weight is in kg, 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 human immunodeficiency virus (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 prior to 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 prior to screening.
    - Significant alcohol consumption is defined as more than 20 g per day in females and more than 30 g per day in males. On average, a standard drink in the United States is considered to be 14 g of alcohol, equivalent to 12 fl oz or 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 (AUDIT) resulted in exclusion.

16. Had a positive urine screen 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 prior to 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 prior to randomization.
18. Had a history of clinically significant hematologic, renal, hepatic, pulmonary, neurological, psychiatric, gastrointestinal, systemic inflammatory, metabolic, or endocrine disorder or any other condition that, in the opinion of the investigator, rendered the subject a poor candidate for inclusion into the study.
19. Had concurrent infection including diagnoses 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 prior to enrollment; and adequately treated in situ cervical cancer that had not recurred for at least 1 year prior to screening.
21. Participated in an investigational new drug study within 30 days prior to 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.

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

	Belapectin 2 mg/kg (n=54)	Belapectin 8 mg/kg (n=54)	Placebo (n=54)
Age (years)	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/m2)	35.7 (6.5)	34.4 (5.7)	34.6 (7.1)
Type2 Diabetes (%)	59	67	59
Statin use (%)	40	43	30
AST (U/L)	48 (23)	49 (25)	52 (48)
ALT (U/L)	42 (21)	51 (40)	48 (38)
T. Bilirubin (mg/dL)	0.76 (0.45)	0.67 (0.33)	0.75 (0.47)
Albumin (g/dL)	4.2 (0.4)	4.2 (0.4)	4.2 (0.4)
INR	1.05 (0.10)	1.05 (0.077)	1.06 (0.11)
Platelet Count ($x10^3/mm^3$)	131 (55)	121 (49)	115 (45)
Child-Turcotte-Pugh Class A (%)	98	100	100
MELD	7.3 (1.53)	6.9 (1.03)	7.4 (1.73)
HVPG (mmHg)	12.4 (4.3)	12.7 (4.2)	11.6 (4.0)
MPH (≥6 to <10 mmHG) (%)	30	30	39
CSPH (≥10 mm Hg) (%)	69	70	61
HVPG in patients without baseline varices (mmHg)	8.22 (0.97)	7.78 (1.25)	7.79 (1.34)
ELF	10.73 (1.26)	10.64 (1.16)	10.81 (1.1)
Collagen proportionate area (%)	9.88 (5.88)	12.72 (4.2)	11.63 (6.12)
α-SMA staining at baseline (%)	13.6 (10.39)	15.4 (11.2)	13.6 (10.55)
Galectin-3 staining (%)	14.8 (8.9)	14.67 (7.4)	14.03 (6.67)
MBT $cPDR_{30}$	692 (399)	702 (322)	635 (308)
Liver stiffness measurement (kPa)	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)			
-Baseline	26 (9.2)	25 (8.9)	24 (11)
- End of treatment	24 (15)	24 (10.2)	24 (9.7)
NAFLD activity score	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	4.59 (1.26)	4.74 (1.2)	4.88 (1.2)

¶Values are reported as mean (SD) unless specified otherwise

Abbreviations: AST: Aspartate aminotransferase; **ALT:** Alanine aminotransferase; **INR:** International Normalized Ratio; **MELD:** Model for End stage Liver Disease; **HVPG:** Hepatic venous pressure gradient; **EOT:** End of treatment; **MPH:** Mild portal hypertension; **CSPH:** Clinically significant portal hypertension; **ELF:** Enhanced liver fibrosis panel; **NAFLD:** Nonalcoholic fatty liver disease; **MBT**: ^{13}Methacetin breath test; **$_cPDR_{30}$:** Cumulative percentage dose recovery at 30 minutes; **CLD-Q:** Chronic liver disease questionnaire

Table 3. Primary endpoint: Change in hepatic vein pressure gradient at end of treatment from baseline

	Belapectin 2mg/kg	Belapectin 8mg/kg	Placebo
Full Analysis Set	**n=54**	**n=54**	**n=54**
LS Mean (SE) change from baseline [1]	–0.28 (0.49)	–0.25 (0.50)	0.10 (0.48)
LS Mean difference from placebo (95% CI)	–0.38 (–1.73, 0.98)	–0.35 (–1.72, 1.02)	
Adjusted P-value [2]	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**	**n=16**	**n=21**
LS Mean (SE) change from baseline [1]	-0.03 (0.74)	-0.21 (0.66)	1.46 (0.61)
LS Mean difference from placebo (95% CI)	-1.49(-3.43,0.45)	-1.67(-3.48,0.15)	
Adjusted P-value [2]	0.258	0.142	
CSPH subgroup	**n=38**	**n=38**	**n=33**
LS Mean (SE) change from baseline [1]	-0.50 (0.62)	-0.21 (0.68)	-0.66 (0.66)
LS Mean difference (95% CI)	0.16(-1.65, 1.96)	0.45(-1.45, 2.34)	
Adjusted P-value [2]	1.0	1.0	
Subgroup with varices at baseline	**n=28**	**n=31**	**n=21**
LS Mean (SE) change from baseline [1]	0.81 (0.62)	-0.27 (0.59)	-0.32 (0.70)
LS Mean difference (95% CI)	1.13(-0.72,2.97)	0.04(-1.77,1.85)	
Adjusted P-value [2]	0.230	0.963	
Subgroup without varices at baseline	**n=25**	**n=23**	**n=33**
LS Mean (SE) change from baseline [1]	-1.61 (0.66)	-0.28 (0.68)	0.40 (0.57)
LS Mean difference (95% CI)	-2.00(-3.69,-0.32)	-0.68(-2.41,1.05)	
Adjusted P-value [2]	0.020	0.439	

[1] It is least square mean as an ANCOVA model is used with baseline score as covariate and treatment group as factors. Treatment comparison was made between the two doses of GR-MD-02 and placebo. [2] Bonferoni-Holm procedure is used to control the type I error for multiple comparisons.

Abbreviations: **LS Mean:** Least square mean; **SE:** Standard error; **CI:** Confidence intervals; **HVPG:** Hepatic venous pressure gradient; **EOT:** End of treatment; **MPH:** Mild portal hypertension; **CSPH:** Clinically significant portal hypertension

Table 4. Histological changes at the end of treatment as compared to baseline in the study cohort

	Belapectin 2mg/kg (n=46)	Belapectin 8mg/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¶ (%)	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

¶When assessed by the NASH CRN Scoring System, 3 patients in belapectin 2 mg/kg, 2 in belapectin 8 mg/kg, and 1 in placebo group had one stage improvement in fibrosis.

Abbreviations: CPA: Collagen proportional area; **NAS:** NAFLD Activity Score; **SD:** Standard deviation

Table 5. Complications of Cirrhosis during the study period

	Belapectin 2 mg/kg (n=54)	Belapectin 8 mg/kg (n=54)	Placebo (n=54)
Development of at least one 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

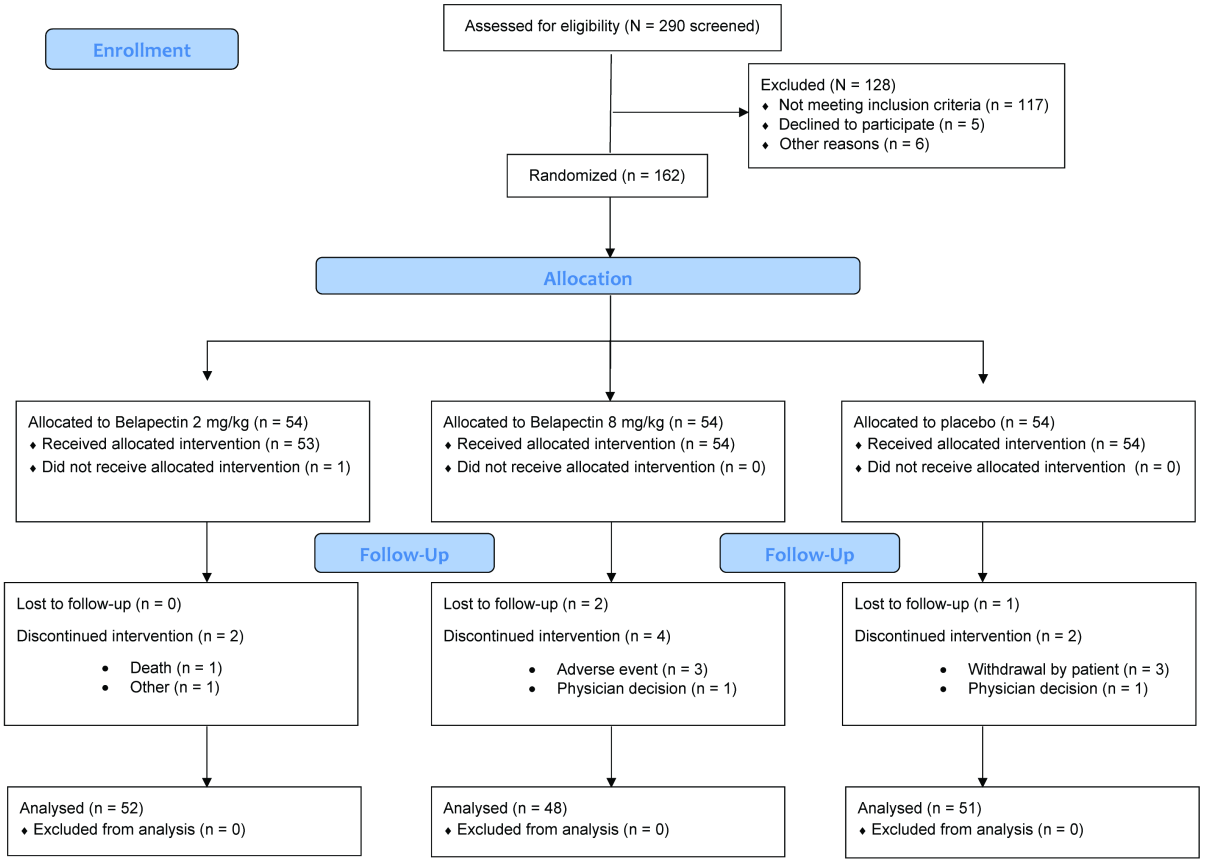
Abbreviations: CTP: Child-Turcotte-Pugh score; MELD: Model for Endstage Liver Disease; OLT: Orthotopic Liver Transplantation

Table 6: Selected Secondary and Exploratory Efficacy Endpoints: Mean (SD) changes at the end of treatment from baseline

	Belapectin 2mg/kg (n=54)	**Belapectin 8mg/kg (n=54)**	**Placebo (n=54)**
ELF– mean change between EOT & BL	0.49 (0.83)	10.99 (0.92)	11.20 (0.63)
Fibrotest – mean change between EOT & BL	0.02 (0.02)	0.01 (0.02)	0.03 (0.02)
α-SMA staining (%) –mean change between EOT & BL	2.5 (12.72)	4.4 (12.2)	1.3 (9.68)
Galectin-3 staining (%) - mean change between EOT & BL	1.17 (12)	0.93 (8.1)	0.36 (7.9)
LSM (kPa) - mean change between EOT & BL	-1.3 (12.5)	-2.34 (10.8)	-0.47 (18.6)
MBT $cPDR_{30}$ - mean change between EOT & BL	-40 (258)	-27 (242)	-45.4 (279)
CLD-Q - mean change between EOT & BL Overall score Abdominal Fatigue Systemic symptoms Activity Emotional function Worry	 0.33 (0.9) 0.28 (1.3) 0.32 (1.25) 0.20 (0.82) -0.23 (1.66) 0.36 (1.07) 0.57 (1.2)	 -0.03 (0.85) 0.06 (1.4) -0.03 (2.2) -0.17 (0.86) -0.08 (1.2) - 0.02 (0.9) -0.03 (1.21)	 0.06 (0.8) 0.13 (1.38) 0.03 (0.9) 0.05 (0.89) 0.03 (1.25) 0.0 (0.9) 0.11 (1.15)

Abbreviations: ELF: Enhanced liver fibrosis panel; SMA: Smooth muscle actin; LSM: Liver stiffness measurement; **MBT**: ^{13}Methacetin breath test; **$_{C}PDR_{30}$:** Cumulative percentage dose recovery at 30 minutes; **CLD-Q:** Chronic liver disease questionnaire

Figure 1. Study disposition with eligible subjects randomly assigned (1:1:1) to receive one of the 3 treatment assignments before the first infusion and 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.



Supplemental Table 1. Histological changes at the end of treatment as compared to baseline in two subgroups

	GR- 2 (n=54)	GR- 8 (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

Abbreviations: CPA: Collagen proportional area; **NAS:** NAFLD Activity Score; **SD:** Standard deviation

Supplemental Table 2. Adverse events and study drug discontinuations during the study period

	GR-2 (n=53) n (%)	GR-8 (n=54) n (%)	Placebo (n=54) n (%)	Total (N=161) n (%)
Treatment emergent adverse events (TEAEs)	509	383	431	1323
Participants with at least one TEAE	52(98.1)	48 (88.9)	51 (94.4)	151 (93.8)
Patients with at least one grade ≥3 adverse event , n(%)	11 (20.8)	11 (20.4)	22 (20.5)	33 (20.5)
Patients with at least one SAE¶	5 (10)	12 (14)	8 (15)	25 (15.5)
Study drug discontinuation due to an AE, n(%)	0	3	0	3*
Death**	1	0	0	1

¶Two treatment emergent SAEs were deemed as possibly related to study drug by the site investigator (one instance of hyponatremia and another episode of transient ischemic attack, both in GR-8 group). Sponsor's DSMB adjudicated these two as well as other SAEs are unrelated to the study drug

*Spasmodic cough (probably related to study drug), two patients with esophageal variceal bleeding (unrelated to study drug)

** One death occurred in an individual receiving GR-2 who developed pulmonary embolism following surgical repair of hernia. Adjudicated as unrelated to study drug

Supplemental Table 3. Treatment-Emergent Adverse Events (>10% Subject Incidence in Any Treatment Group) by System Organ Class and Preferred Term (Safety Set). At each level of subject summarization, a subject was counted once if the subject reported 1 or more events. Percentages are based on the number of subjects in each treatment group (N).

System Organ Class	GR-2 (n=53)	GR-8 (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)

The total number of adverse events counts all treatment-emergent adverse events for participants. Adverse events were coded using Medical Dictionary for Regulatory Activities, Version 18.0.

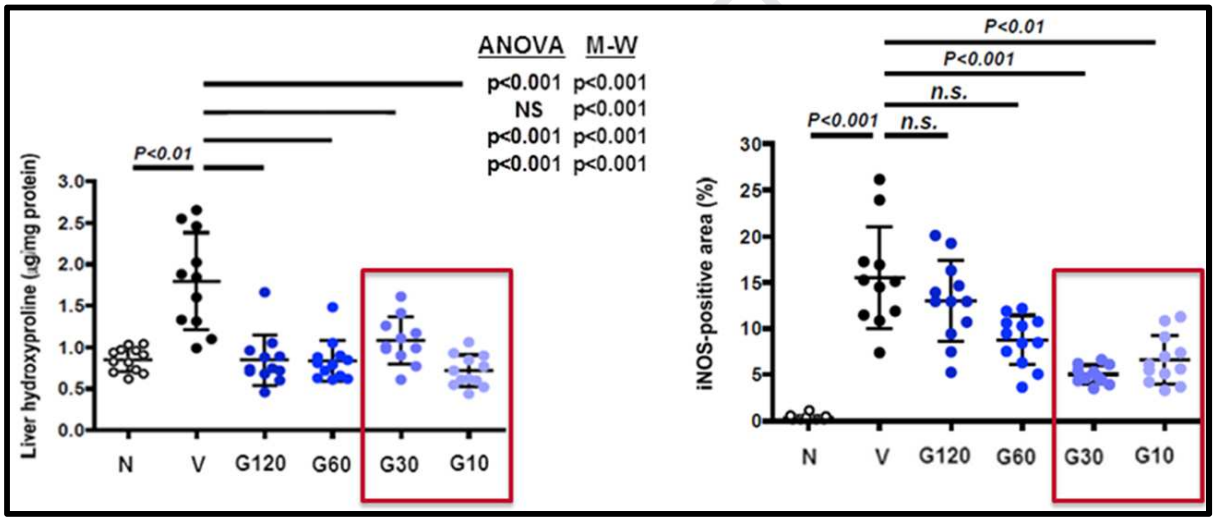
Supplemental Material 4:

Supplemental Discussion on why GR2, but not GR8 had some efficacy in a cirrhotic population with portal hypertension

Our explanation 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 optimal therapeutic window 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 iNOS activity. See Figure 1 below. Exact mechanism on how a higher dose actually leads to lower efficacy is unknown.

Figure 1: GR-MD-02 dose response effect on liver hydroxyproline and iNOS expression identifies a potential therapeutic window (Ref 15; Traber PG, Zomer E. Therapy of experimental NASH and fibrosis with galectin inhibitors. PLoS ONE 8(12):383481)



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

3) Somewhat unexpectedly, the PK of GR2 and GR8 in this trial turned out to be different from our Phase1 study. Our phase1 study included NASH with bridging fibrosis whereas the current study obviously includes cirrhotics with portal hypertension. This makes us believe that cirrhosis with portal HTN significantly alters the pharmacokinetics of GR-MD-02. Below tables demonstrate that GR-B has significantly longer T ½ and AUC 0-240 in cirrhotics with portal hypertension, as compared to our Phase1 study which enrolled NASH patients with bridging fibrosis.

Phase I, Summary of GR-MD-02 Plasma PK parameters

Weekly Dose (x doses)	Cmax $_{(0-240)}$ µg/mL	T1/2 H	AUC_{0-240} µg*h/mL
2 mg/kg (x1)	16.3	19.9	**573**
2 mg/kg (x4)	17.7	20.5	**645**
4 mg/kg (x1)	30	19.8	**1039**
4 mg/kg (x4)	31	19.5	**1075**
8 mg/kg (x1)	99.5	18.2	**2449**
8 mg/kg (x4)	169.9	18.4	**4909**

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 Bi-Weekly Doses	$C_{MAX\ (0-240)}$ Mean µg/mL	T1/2 H	AUC_{0-240} Mean µg*h/mL
2 mg/kg	34.32	>24	**3414**
8 mg/kg	128.13	>24	**11835**

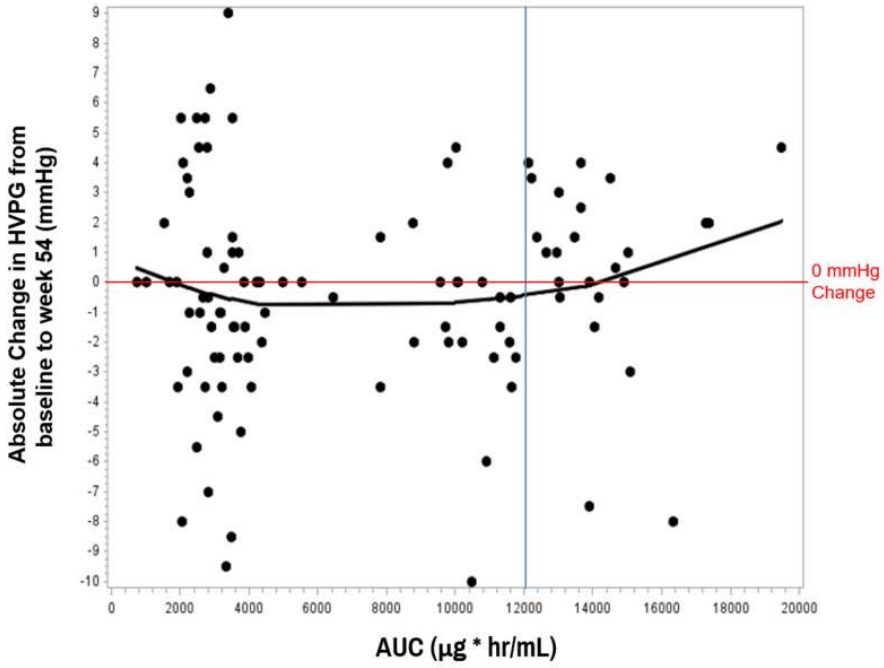
Abbreviations: AUC=area under the curve, GR2=2 mg/kg GR-MD-02 treatment group, GR8=8 mg/kg GR-MD-02 treatment group, HVPG=hepatic venous pressure gradient, PLB=placebo

4) As we described in our results section, The total drug exposure as assessed by the area under the concentration (AUC) curve for serial GR-MD-02 levels showed the AUCs for GR2 were tightly clustered with median level of 2665.5 mg*h/L (10^{th} - 90^{th} percentile: 2004-3785 mg*h/L) whereas they were widely dispersed for GR8 with median level of 10,954 mg*h/L (10^{th} - 90^{th} 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-D4 was plotted against the change in HVPG for each subject. The curve fit shows three regions

including a negative slope in the lower AUC region (<3000) indicating that as AUC is decreasing the change in HVPG is increasing, while in a flat slope in the mid AUC region (3000 – 12000) indicating a steady relationship between AUC and change in HVPG (below the zero line). In the upper AUC region (>12000) there is an increasing slope indicating that the change in HVPH is going up or worsening in this range. **See Figure 2.**

Figure 2: Change in HVPG Versus AUC (µg*hr./mL)¶



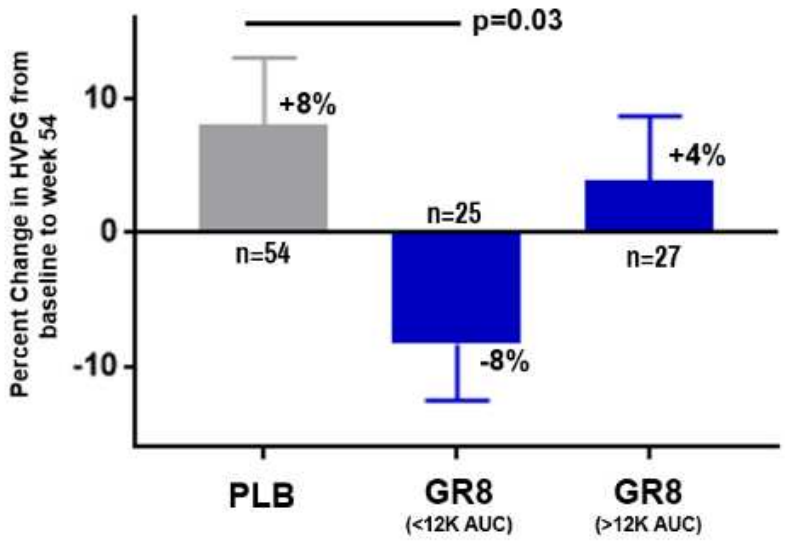
¶The AUC-D4 of all patients in the two treatment groups (less three high outliers in the GR8 group) were plotted against the change in HVPG from baseline to end of study for each subject. Loess regression analysis was used for fitting a curve between the two variables. The red horizontal line is zero change in HVPG.

Abbreviations: AUC=area under the curve, HVPG=hepatic venous pressure gradient.

6) Since the AUCs in GR-8 group were high and widely spread, in a post-hoc analysis we sub-divided into GR8 group into two subgroups based on an AUC cut off 12,000 µg*hr./mL. This AUC cut off was chosen because it appeared to be the infliction point based on above figure which examined the relationship between AUC and response to HVPG.

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

   Figure 3: Percent change in HVPG in GR8 group when stratified according AUC



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.

Lay Summary: In a clinical trial of patients with NASH and cirrhosis, belapectin was not associated with reductions in HVPG or fibrosis. However, it was safe and showed some benefit for a subgroup of patients with no varices when the trial began.