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Randomised clinical study: GR-MD-02, a galectin-3 inhibitor, vs. placebo in patients having non-alcoholic steatohepatitis with advanced fibrosis

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SUMMARY

Background
Non-alcoholic steatohepatitis (NASH) and resultant liver fibrosis is a major health problem without approved pharmacotherapy. Pre-clinical results of GR-MD-02, a galectin-3 inhibitor, suggested potential efficacy in NASH with advanced fibrosis/cirrhosis and prompted initiation of a clinical development programme in NASH with advanced fibrosis.

Aim
To evaluate the safety, pharmacokinetics and exploratory pharmacodynamic markers of GR-MD-02 in subjects having NASH with bridging fibrosis.

Methods
The GT-020 study was a first-in-human, sequential dose-ranging, placebo controlled, double-blinded study with the primary objective to assess the safety, tolerability and dose limiting toxicity of GR-MD-02, in subjects with biopsy-proven NASH with advanced fibrosis (Brunt stage 3). The secondary objectives were to characterise first-dose and multiple-dose pharmacokinetic profiles and to evaluate changes in potential serum biomarkers and liver stiffness as assessed by FibroScan.

Results
GR-MD-02 single and three weekly repeated of 2, 4 and 8 mg/kg revealed no meaningful clinical differences in treatment emergent adverse events, vital signs, electrocardiographic findings or laboratory tests. Pharmokinetic parameters showed a dose-dependent relationship with evidence of drug accumulation following 8 mg/kg (~twofold).

Conclusions
GR-MD-02 doses were in the upper range of the targeted therapeutic dose determined from pre-clinical data and were safe and well tolerated with evidence of a pharmacodynamic effect. These results provide support for a Phase 2 development programme in advanced fibrosis due to NASH.

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INTRODUCTION

The galectin-3 protein has been implicated in the pathogenesis of liver fibrosis, including inflammation and fibrosis due to non-alcoholic steatohepatitis (NASH). Galectin-3 is a member of a family of proteins, containing 15 members (11 identified in humans), which have the property of binding avidly to galactose containing oligosaccharides associated with glycoproteins.[1] Mice that lack the galectin-3 gene have been shown to be resistant to liver fibrosis due to toxin administration,[2] lung fibrosis due to bleomycin toxicity[3] and kidney fibrosis due to ureteral ligation.[4] The effect of galectin-3 knockouts on NASH is more controversial. One group has shown that galectin-3 knockout mice are also resistant to fat accumulation, inflammation and fibrosis when fed a high-fat diet,[5] while another group of investigators has suggested that galectin-3 null mice may have an increased propensity to develop NASH.[6, 7] A recent study has further clarified the role of galectin-3, showing that while galectin-3 null animals develop greater liver steatosis, there is decreased inflammation, liver cell injury and fibrosis.[8] Taken together, these data suggest that galectin-3 plays a critical role in fibrogenesis in a number of organs, and in liver due to several aetiologies.

GR-MD-02 (galactoarabino-rhamnogalaturonan) is a complex carbohydrate molecule derived from a natural plant compound which contains oligosaccharide chains containing galactose residues and binds to galectin-3. Studies were completed in a mouse NASH model that showed that GR-MD-02 consistently reduced the activity of NASH [NAFLD (non-alcoholic fatty liver disease) activity score], reduced or eliminated fibrosis as measured by liver collagen, and reduced the expression of galectin-3 in liver macrophages.[9] Studies were also completed in rats treated with thioacetamide in which animals developed advanced fibrosis that replaced over 25% of the liver with collagen and had all the histopathological characteristics of cirrhosis. Treatment of cirrhotic rats with 4 weekly doses GR-MD-02 resulted in a reduction of collagen to below 10%, reversal of cirrhosis, and reduced portal hypertension.[10] These results in two preclinical models of liver fibrosis, along with regulatory toxicologic, pharmacology and pharmaceutical development evaluations, formed the basis of an IND (115459) with the U.S. Food and Drug Administration which also received fast track designation for GR-MD-02 in the treatment of NASH with advanced fibrosis.

Reported herein are the results from a first-in-human Phase 1 study of GR-MD-02 in subjects with biopsy-proven NASH with bridging fibrosis.

MATERIALS AND METHODS

Trial design and objectives

The GT-020 trial was a first-in-human, multiple-dose, randomised, double-blinded, dose-ranging study to assess in sequential fashion, the safety, tolerability, and dose limiting toxicity of GR-MD-02, in subjects with biopsy-proven NASH with advanced fibrosis. The secondary objectives were to characterise the first-dose and multiple-dose pharmacokinetic profile, evaluate changes in potential serum biomarkers, and in the third cohort to evaluate changes in FibroScan, a measure of liver stiffness as a surrogate for change in fibrosis.

Dose escalation was achieved in three sequential cohorts at eight study sites in the US, including Brooke Army Medical Center, Fort Sam Houston, TX; University of Indiana School of Medicine, Indianapolis, IN; The Texas Liver Institute, University of Texas Health Science Center, San Antonio, TX; Cedars-Sinai Medical Center, Los Angeles, CA; Icahn School of Medicine at Mount Sinai, New York NY; Virginia Commonwealth University Medical School, Richmond, VA; Saint Louis School of Medicine, St. Louis, MO.

In cohort 1, six subjects were randomised (predetermined algorithm held by nonblinded data group at CRO) to an initial IV infusion of GR-MD-02 at a dose of 2 mg/kg and two patients were randomised to matching placebo. In cohort 2, eight subjects were randomised to an initial IV infusion of GR-MD-02 at a dose of 4 mg/kg and two patients were randomised to matching placebo. In cohort 3, seven subjects were randomised to an initial IV infusion of GR-MD-02 at a dose of 8 mg/kg and six patients were randomised to matching placebo. Following the initial administration, all patients received three additional doses for a total four doses of GR-MD-02 (Figure 1). The number of subjects included in the trial were selected as is typical for Phase 1 trials for initial safety evaluation and to obtain sufficient samples for pharmacokinetic analysis of GR-MD-02.

As shown in Figure 1, the protocol was amended following the first and second cohorts. Following the first cohort, the dosing interval after the first dose was decreased by a week since the pharmacokinetic profile indicated that a 3-week washout period was not needed prior to administering the next dose. In cohort 3, more time points for biomarker analysis were added to the protocol for cohort 3.

The study protocol, protocol amendments and the informed consent form were reviewed and approved by

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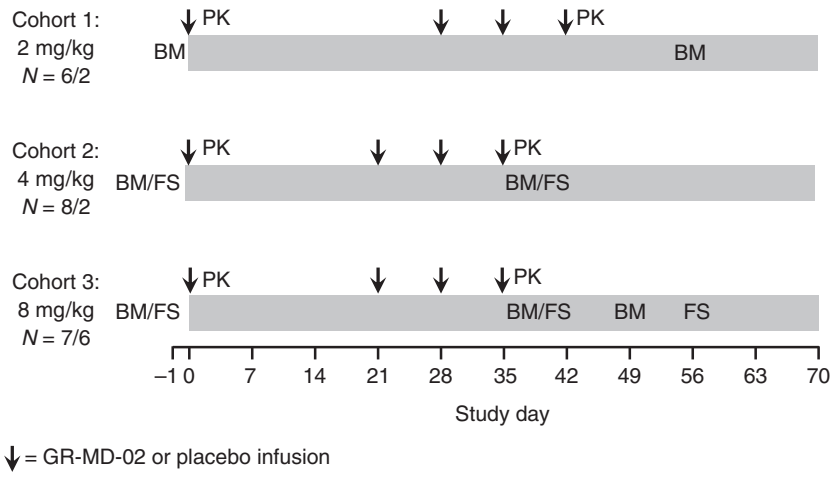

Figure 1 | Study time course. The study patients were divided into three sequential cohorts with escalating doses given to subsequent cohorts. Doses of placebo or active drug (GR-MD-02) were administered at the times indicated by arrows. Blood was obtained for pharmacokinetic studies of GR-MD-02 disposition following the first and last infusions in each cohort (PK) and biomarker studies at other time points as shown (BM). FibroScan studies of liver stiffness were also obtained at specific time points (FS). Patient numbers (*N*) indicate the number who completed the study and received active drug or placebo, respectively.

an Institutional Review Board constituted in accordance with the International Conference on Harmonization guidelines for Good Clinical Practice and relevant U.S. Food and Drug Administration requirements prior to the initiation of any study procedures. This trial was designed and monitored in accordance with Sponsor procedures, which comply with the principles of good clinical practice as required by the major regulatory authorities, all applicable national, state and local regulations and in accordance with the Declaration of Helsinki. Written informed consent was obtained from each subject prior to enrolment in the study and before any protocol-related procedures were performed.

An independent data and safety monitoring board, consisting of three hepatologist physicians experienced in clinical trial conduct, met after each dose level and at the end of the trial to evaluate the safety of participants and ensure that subjects were not exposed to any undue risk. Details of the trial were registered at clinicaltrials.gov: (https://clinicaltrials.gov/ct2/show/NCT01899859?term=GR-MD-02&rank=3). The CONSORT 2010 requirements are met in this manuscript.[11]

Inclusion and exclusion criteria

Subjects were included in the study if they had a liver biopsy within 12 months from screening that showed a definitive diagnosis of NASH with bridging hepatic fibrosis (Brunt stage 3[12]), were ≥18 years of age and <75 years old, agreed to contraception during the trial, and signed an informed consent.

Exclusion criteria for subjects included pregnancy, lactation, history of significant alcohol consumption, use of drugs historically associated with NAFLD, planned or history of bariatric surgery within the last 5 years, history of liver decompensation (defined by development of ascites, encephalopathy or variceal haemorrhage), evidence of other liver disease (viral hepatitis, primary sclerosing cholangitis, primary biliary cirrhosis, autoimmune chronic hepatitis, Wilson's disease, alpha-1 anti-trypsin deficiency, haemochromatosis, drug-induced liver injury, bile duct obstruction, liver cancer), participation in an investigational new drug trial in the 30 days before randomisation, known allergies to the study drug or any of its excipients, recent surgery or significant trauma, substance abuse, concurrent infection or other serious medical or psychiatric illness.

Laboratory parameters which excluded subjects included a platelet count <100 000/mm^3, serum albumin <3.5 g/dL, an INR >1.1, direct bilirubin >1.3 mg/dL, serum alanine aminotransferase (ALT) >300 U/L, serum creatinine ≥1.5 mg/dL, or positive HIV serology.

Subjects who were using vitamin E or omega-3 fatty acids were allowed to continue their use. Because of potential metabolic interaction with CYP3A4, subjects were excluded if they required use of drugs with a narrow therapeutic window metabolised by CYP3A4 such as fast acting

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opioids (alfentanil and fentanyl), immunosuppressive drugs (ciclosporin, sirolimus and tacrolimus), some cardiovascular agents (ergotamine, quinidine and dihydroergotamine), and select psychotropic agents (pimozide).

Investigational drug

GR-MD-02 is a soluble and physiologically compatible polysaccharide (galactoarabino-rhamnogalacturonate) composed of alternating α-(1,2)-L-rhamnosyl-α-(1,4)-D-galacturonosyl backbone with side branches composed of mainly galactose and arabinose oligosaccharides.

GR-MD-02 for infusion was prepared at the appropriate patient dose (based on lean body weight) in 100 mL of normal saline, stored at 2–8 °C and infused within 24 h of preparation. Placebo was 100 mL normal saline administered in a manner identical to active drug. All solutions for administration were prepared by an unblinded pharmacist. The prepared infusion bag was delivered in a covered sheath to prevent unblinding of the subject and blinded site personnel due to the light yellow tint that may be visible at higher GR-MD-02 doses. Subjects, Principal Investigators and health care providers were blinded throughout the study period.

Subjects who met the inclusion/exclusion criteria were randomly assigned to receive GR-MD-02 or placebo, at the following dose levels: Cohort 1: 2.0 mg/kg lean body mass; Cohort 2: 4.0 mg/kg lean body mass; Cohort 3: 8.0 mg/kg lean body mass. GR-MD-02 was administered as an IV infusion through a peripheral vein over 60 min. Lean body mass was used for calculation of all doses because it was anticipated that many subjects would be obese and GR-MD-02 is distributed primarily in aqueous compartments. Lean body mass was calculated as follows:

$$\text{Male lean body mass} = 1.1 \times (\text{weight in kg}) - 128 \times (\text{weight in kg/height in cm})^2$$

$$\text{Female lean body mass} = 1.07 \times (\text{weight in kg}) - 148 \times (\text{weight in kg/height in cm})^2$$

Pharmacokinetic blood sampling

Plasma samples were collected for PK after study drug dose 1 and dose 4. Sampling was taken predose, 0.5, 1, 4, 8, 121, 24, 72, 144, 240 (first dose only) and 336 h after the end of the first and fourth infusions.

Bioanalytical methods

GR-MD-02 plasma levels were measured using by an ELISA assay that was developed and validated by Galectin Therapeutics and then transferred, validated and performed by PPD Bioanalytical Lab (Richmond, VA, USA). The lower limit of quantitation was 1.2 μg/mL.

Pharmacokinetic analysis

Individual pharmacokinetic parameters were determined using 'noncompartmental' analysis following the first (week 1) and last (week 6 or 7) dose.[13, 14] For the pharmacokinetic analysis, GR-MD-02 plasma concentrations reported as below quantitative levels were imputed as zero (0) prior to the start of the intravenous infusion and were imputed as missing following the end of the intravenous infusion. Since concentrations were generally below quantitative levels after 72 h following administration and the terminal exponential half-life is ~1 day with a 7-day dosing interval, data were analysed as two separate single doses [i.e. AUC_τ and AUC (area under the curve) were assumed to be identical].

The maximum plasma concentration (C_{max}) and the time at which C_{max} occurred (T_{max}) were determined from visual inspection of the individual plasma concentration–time profiles. Area under the plasma concentration–time curve from time zero (0) until the last quantifiable concentration (AUC_t) was determined by the linear trapezoidal rule. The remaining area was determined by dividing the observed concentration at the time of the last quantifiable concentration by the terminal exponential rate constant (λ_z); AUC was the sum of these two areas. The percent extrapolated of AUC extrapolated (AUC % ext) was determined as $(1-(AUC_t/AUC)) \times 100$. The terminal exponential rate constant (λ_z) was determined from linear regression analysis of data points during the terminal exponential phase of the plasma concentration–time data. Terminal exponential half-life ($t_{1/2,z}$) was calculated from the relationship: $t_{1/2,z} = \ln 2/\lambda_z$. The steady-state ($V_{ss}$) and terminal ($V_z$) volumes of distribution and total clearance (CL) were obtained via standard equations (1, 2). The accumulation ratio upon multiple dosing was obtained as the ratio of AUC following the last dose to the AUC following the first dose.[15] Data analyses were performed using WinNonlin Phoenix version 6.4 (Cetera, Princeton, NJ).

Subject assessments

Safety testing. Vitals signs (heart rate, oral temperature, systolic and diastolic blood pressure and respiration rate) were measured at various times over 24 h following infusion of study drug. Serial electrocardiograms (ECGs) were performed during the 24 h following the first and

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fourth infusions and during the 6 h following the second and third infusions.

Laboratory testing was performed prior to the first dose and at intervals during the study to assess safety included complete blood count with differential, platelet count, prothrombin time, partial thromboplastin time, haptoglobin, reticulocyte count, a serum chemistry panel [fasting glucose, phosphorus, total protein, creatinine, blood urea nitrogen (BUN), albumin, creatine kinase (CK), lactate dehydrogenase (LDH), amylase and electrolytes (sodium, potassium, calcium, magnesium, bicarbonate, chloride), serum bilirubin (total, direct and indirect), ALT, aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma glutamyl transpeptidase (GGTP), lipid profile and urinalysis].

Serum biomarkers. There are currently no validated serum biomarkers for the evaluation of potential therapeutic changes over time in NASH or fibrosis. Therefore, a panel of serum tests was evaluated to explore potential biomarkers for use in future studies. For each exploratory biomarker, there was some evidence to suggest that these biomarkers may be involved in the pathogenesis of NASH or fibrosis from human and/or animal studies.

Serum biomarkers were evaluated at various time points (Figure 1) including the FibroTest (FibroSure) score, the enhanced liver fibrosis (ELF) score, ALT, AST, GGTP, galectin-3, alpha-2 macroglobulin, apolipoprotein A1, CD40 Ligand, cytokeratin 18 (M65), cytokeratin 18 (M30), endothelin 1, hyaluronic acid (HA), interferon-gamma, interleukin-6, interleukin-8, IP-10 (CXCL10), matrix metalloproteinase-1, 3 and 9, transforming growth factor beta (TGF-beta 1), tumour necrosis factor-alpha (TNF-alpha), vascular endothelial growth factor (VEGF), haptoglobin, tissue inhibitor of metalloproteinase 1 (TIMP-1), osteopontin, lumican, ferritin and C-reactive protein. All serum biomarkers and safety laboratory testing were performed using validated assays at PPD Central Lab (Highland Heights, KY, USA). Mouse galectin-3 serum levels were measured using a validated assay by Stelic Institute, Tokyo, Japan.

Plasma levels of total CoQ, its oxidised form CoQox and its reduced form CoQred were measured by high-performance liquid chromatography (HPLC/EC) at the University of Cincinnati as previously described.[16] Analysis was conducted without knowledge of the randomisation assignments. Total CoQ was corrected for cholesterol level (CoQTC) to account for potential variation in lipid profiles associated with NASH and obesity.

Fibroscan. Evaluation of liver stiffness was performed using Fibroscan 502 Touch with setup and instructions on use provided by the manufacturer. All but one site had two probes available for use, the M and XL probes, which were used depending on the determination by the instrument. Examinations were performed on subjects following an 8 h fast.

Statistical analysis

The primary analysis set for all safety, pharmacokinetic and efficacy analyses was defined as all enrolled subjects who received at least one dose of study drug. Continuous variables are summarised by presenting the mean, s.d., median, minimum and maximum, and categorical variables by presenting the number of subjects and percentage for each category. Summary statistics are presented by randomised GR-MD-02 dose (2, 4 and 8 mg/kg) and combined placebo.

For the analysis of serum biomarkers, data from cohort 3 only were utilised in which the number of subjects allowed statistical analysis. Change in serum biomarkers were evaluated by fitting a linear mixed effects model to the data. Model fitting was performed by first evaluating the functional relationship over time by producing individual scatter (spaghetti) plots over time (data not shown) as well as plots of mean values. Based on these plots a piecewise linear mixed effects model (random intercept) was fit to the data. This was done by creating a variable for time (T) where T = Time–21 if measurement time post randomisation was greater than 21 days and $T = 0$ otherwise. In addition to the variable T, the linear mixed effects model included Treatment and Time (per week increase) as well as two two-way interactions as fixed effects: (i) treatment × time and (ii) treatment × T. Test of the null hypothesis of no treatment group differences in the changes in the serum biomarker was performed using the likelihood ratio (LR) chi-square on two degrees of freedom. If the LR test was statistically significant, tests of the null hypotheses of no treatment group difference in the changes in the serum biomarker before or after day 21 were performed. This was accomplished by tests of linear combination of linear mixed effects model parameter estimates.

For CoQ measurements a two-sample t-test was used to evaluate differences within groups before and after treatment regimens (day 0 and day 38 were the only time points with CoQ analysis performed).

Analyses were performed using SAS for Windows statistical software (SAS, Cary, NC, USA). P-value less than

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0.05 was considered statistically significant. No adjustment for multiplicity was performed as all analyses were considered exploratory.

RESULTS

Study subjects

Thirty-one subjects were enrolled and 30 subjects completed the study between July 2013 and December 2014 and the trial ended based on successful completion of predetermined number of subjects. All subjects had a documented history of NASH and liver biopsies with histological evidence of NASH and fibrosis, with 30 subjects with Brunt stage 3 fibrosis and 1 subject with Brunt stage 4 on post-enrolment analysis. Demographic characteristics were comparable across the treatment groups, although the 8 mg/kg treatment group were predominately older (range: 60–69) (Table 1). One third of subjects were of Hispanic/Latino origin.

The most frequently reported conditions other than NASH included hypertension (20 subjects), gastro-oesophageal reflux disease (18 subjects), obesity (18 subjects), type 2 diabetes (14 subjects) and hyperlipidaemia (13 subjects). There were no discernible differences in concomitant medications between treatment groups.

A total of 26 subjects had at least one protocol deviation during the study. The most common were classified as minor deviations and involved laboratory or other study procedures such as missed laboratory tests or samples drawn out of window. There were eight major protocol deviations including five which related to safety inclusion criteria that were reviewed at the time of occurrence by the medical monitor and determined to be inconsequential and three which related to liver biopsies in three subjects that occurred longer than 12 months prior to enrolment (16, 32 and 47 days out of window). Overall, the minor and major deviations were clinically judged to have no effect on the data or conclusions.

Safety evaluation

A total of 17 (81%) subjects who received GR-MD-02 experienced at least one AE compared to 7 (70%) subjects who received placebo (Table 2). A female partner of a male subject in the 4 mg/kg dose group experienced a spontaneous abortion at week 10 of gestation, which was recorded as a treatment-emergent serious adverse event, grade 5 (fetal death). The estimated date of conception was approximately 38 days after the last dose of study drug. Both the site investigator and the DSMB considered this event to be unrelated to study drug.

One subject discontinued study drug (4 mg/kg) due to QTc prolongation, as determined by automated ECG readings, which was considered possibly related to the study drug. However, a blinded manual read of the ECGs by an experienced cardiologist showed no prolongation of the baseline QT/QTC interval and no significant change with study drug administration compared to baseline. Because of the several repeated borderline prolonged QTc intervals in this subject and multiple concomitant medications that could affect the QTc interval, the subject was withdrawn from the study.

Most treatment-emergent AEs (TEAE) occurred in one or two subjects and were considered unrelated to study drug. The most common (occurring in >2 subjects) reported AEs were headache (five subjects), dizziness (three subjects) and diarrhoea (three subjects). The

Table 1 | Summary of demographic characteristics – intention-to-treat (ITT) subjects

Demographic characteristic	Placebo (N = 10)	2 mg/kg (N = 6)	4 mg/kg (N = 8)	8 mg/kg (N = 7)
Age (years)				
Mean (s.d.)	49 (12)	54 (10)	54 (14)	64 (3)
Median (range)	45 (31–64)	55 (40–64)	57 (34–69)	63 (60–69)
Gender *n* (%)				
Male	5 (50)	4 (67)	3 (38)	5 (71)
Female	5 (50)	2 (33)	5 (62)	2 (29)
Weight (kg)				
Mean (s.d.)	111 (21)	97 (14)	101 (14)	90 (21)
Median (range)	110 (90–155)	95 (81–116)	99 (84–125)	90 (57–125)
BMI (kg/m^2)				
Mean (s.d.)	40 (8.6)	37 (3.9)	35 (2.6)	33 (6.1)
Median (range)	39 (29–54)	38 (31–41)	35 (32–39)	35 (22–40)

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Table 2 | Overview of treatment emergent adverse events – intention-to-treat (ITT) subjects

	Placebo (*N* = 10)		2 mg/kg (*N* = 6)		4 mg/kg (*N* = 8)		8 mg/kg (*N* = 7)	
	No. of subjects	No. of events	No. of subjects	No. of events	No. of subjects	No. of events	No. of subjects	No. of events
TEAE, *n* (%)	7 (70)	29	5 (83)	15	7 (88)	30	5 (71)	11
TESAE, *n* (%)	0 (0)	0	0 (0)	0	1 (13)	1	0 (0)	0
TEAE leading to discontinuation, *n* (%)	0 (0)	0	0 (0)	0	1 (13)	1	0 (0)	0
Relationship with study drug, *n* (%)								
Unrelated	3 (30)	23	5 (83)	15	3 (38)	18	4 (57)	10
Possible	4 (40)	6	0 (0)	0	4 (50)	12	1 (14)	1
NCI-CTCAE intensity grade, *n* (%)								
1 – Mild	4 (40)	23	5 (83)	15	4 (50)	25	3 (43)	8
2 – Moderate	2 (20)	5	0 (0)	0	2 (25)	4	2 (29)	3
3 – Severe	1 (10)	1	0 (0)	0	0 (0)	0	0 (0)	0
5 – Death	0 (0)	0	0 (0)	0	1 (13)	1	0 (0)	0

TEAE, treatment emergent adverse event; TESAE, treatment emergent serious adverse event; NCI-CTCAE, National Cancer Institute Common Technology Criteria for Adverse Events, ver. 4.03; Percentages are based on the total number of subjects in each treatment group (*n*). Treatment with placebo and ascending doses of GR-MD-02.

incidence and type of TEAE were not appreciably different between treatment groups.

The incidence of AEs that were considered related or possibly related to study drug are summarised in Table 3. At the highest dose (8 mg/kg), the only AE that was considered possibly related was an occurrence of headache. There were no infusion-related reactions reported in the study.

A total of eight (26%) subjects experienced an AE that was considered moderate and one subject had an AE that was considered severe in intensity (Table 4). Both events were considered possibly related to the study drug of moderate or severe intensity, headache and gout were present in subjects who received placebo.

The overall incidence of AEs was similar between treatment groups. The majority of AEs were judged to be mild in severity and unrelated to the study drug. There was no discernible trend for increased incidence or severity of AEs with increasing dose of GR-MD-02 nor were there any apparent differences in the incidence, type or severity of AEs between subjects treated with placebo or active drug.

Clinical and laboratory safety evaluation

There were no differences between treatment groups for means and mean changes from baseline in vital signs. A review of individual ECG parameters and mean changes from baseline did not reveal any differences between treatment groups. Specifically, none of the study subjects had a HR, PR, QRS, QT, QTcB, QTcF or QTcH measurement that was considered clinically significant by the investigators.

There were no notable differences between treatment groups in mean and mean changes from baseline for WBC, neutrophils, lymphocytes, monocytes, eosinophils, basophils, RBC, haemoglobin, haematocrit, platelets, prothrombin time or activated partial thromboplastin time. Additionally, there were no differences between treatment groups in means and mean changes from baseline for electrolytes, measures of renal function, lipid parameters, or fasting glucose or urinalysis parameters.

Drug pharmacokinetics

Mean GR-MD-02 plasma concentration–time profiles following the first and last dose of 2, 4 and 8 mg/kg of GR-MD-02 are illustrated in Figure 2. GR-MD-02 PK parameters following the first dose (week 1) and the last dose (week 6 or 7) for each dose level are provided in Table 5.

Following the first dose, peak concentrations were observed at a median time of 1.6–2.0 h after the start of the infusion and the geometric mean terminal exponential half-life was approximately 18–20 h. The geometric mean steady state and terminal volume of distribution for the 2 mg/kg dose were 5.3 and 5.7 L, respectively, and the geometric mean total clearance was 200 mL/h. The geometric mean steady-state and terminal volume of distribution for the 4 mg/kg dose were 6.4 and 6.7 L, respectively, and the geometric mean total clearance was 237 mL/h. The geometric mean steady state and terminal

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Table 3 | Summary of related or possibly related treatment emergent adverse events by system organ class and preferred term – intention-to-treat (ITT) subjects

System organ class Preferred term, *n* (%)	Placebo (*N* = 10)	2 mg/kg (*N* = 6)	4 mg/kg (*N* = 8)	8 mg/kg (*N* = 7)
All body systems	4 (40)	0 (0)	4 (50)	1 (14)
Gastrointestinal disorders	1 (10)	0 (0)	0 (0)	0 (0)
Constipation	1 (10)	0 (0)	0 (0)	0 (0)
General disorders and administration site conditions	0 (0)	0 (0)	2 (25)	0 (0)
Local swelling	0 (0)	0 (0)	1 (13)	0 (0)
Oedema peripheral	0 (0)	0 (0)	1 (13)	0 (0)
Investigations	0 (0)	0 (0)	2 (25)	0 (0)
Electrocardiogram change	0 (0)	0 (0)	1 (13)	0 (0)
Electrocardiogram QT prolonged	0 (0)	0 (0)	1 (13)	0 (0)
Metabolism and nutrition disorders	1 (10)	0 (0)	0 (0)	0 (0)
Gout	1 (10)	0 (0)	0 (0)	0 (0)
Musculoskeletal and connective tissue disorders	0 (0)	0 (0)	1 (13)	0 (0)
Arthralgia	0 (0)	0 (0)	1 (13)	0 (0)
Joint swelling	0 (0)	0 (0)	1 (13)	0 (0)
Pain in extremity	0 (0)	0 (0)	1 (13)	0 (0)
Nervous system disorders	2 (20)	0 (0)	1 (13)	1 (14)
Dizziness	1 (10)	0 (0)	1 (13)	0 (0)
Headache	1 (10)	0 (0)	0 (0)	1 (14)
Paresthesia	1 (10)	0 (0)	0 (0)	0 (0)
Renal and urinary disorders	1 (10)	0 (0)	0 (0)	0 (0)
Urine odor abnormal	1 (10)	0 (0)	0 (0)	0 (0)
Respiratory, thoracic and mediastinal disorders	0 (0)	0 (0)	1 (13)	0 (0)
Throat tightness	0 (0)	0 (0)	1 (13)	0 (0)
Skin and subcut tissue	0 (0)	0 (0)	1 (13)	0 (0)
Nail discoloration	0 (0)	0 (0)	1 (13)	0 (0)

Treatment with placebo and ascending doses of GR-MD-02.

volumes of distribution for the 8 mg/kg dose were 3.9 and 4.2 L, respectively, and the geometric mean total clearance was 160 mL/h.

Following the last dose of multiple dosing with weekly administration, GR-MD-02 PK parameters were generally similar to those observed following the first dose for 2 and 4 mg/kg. The results indicate that upon IV administration, systemic exposure (C_{max} and *AUC*) appeared to increase in proportion to dose. GR-MD-02 primary PK parameters (V_{ss}, V_z and CL) were generally similar between first and last dose and between 2 and 4 mg/kg; V_{ss} was ~5.6 L, V_z was ~6.0 L and CL was ~211 mL/h. The $t_{1/2,z}$ was ~20 h. Upon multiple dosing no significant accumulation was observed (RAUC was ~1).

Following 8 mg/kg, drug concentration *AUC* appeared to increase in proportion to the dose following the first dose, whereas C_{max} appeared to increase ~65% more than expected. Following the last dose, both C_{max} and *AUC* both appeared to increase upon multiple dosing (C_{max} ~70% and *AUC* ~90%). GR-MD-02 primary PK parameters (V_{ss}, V_z and CL) appeared to decrease ~50% following multiple dosing; V_{ss} was ~1.9 L, V_z was ~2.0 L and CL was ~83 mL/h. The decrease in the volume of distribution is consistent with plasma volume vs. blood volume as observed for the other doses. The decrease in CL suggested a saturation of a metabolic and/or transport mechanism(s). Since both CL and the volume of distribution showed a similar decrease, the $t_{1/2,z}$ was similar following the first and last dose (~18 h). Upon multiple dosing, a twofold increase in accumulation was observed (relative area under the curve was ~1.9).

Cell injury, cell death and inflammatory biomarkers

Analysis of serum biomarkers of cell injury (AST, ALT and AST:ALT ratio), cell death and inflammation showed a broad range of baseline values for subjects in each group and fluctuations within individuals over time in each treatment group, including placebo. When the values of the change from baseline to end of study were compared for placebo and the third GR-MD-02 treatment cohort, there were no statistically significant differences in AST, ALT, CK-18 (both M30 and M65

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Table 4 | Summary of moderate or severe treatment emergent adverse events – intention-to-treat (ITT) subjects

System organ class Preferred term Severity *N* (%)	Placebo (*N* = 10)	2 mg/kg (*N* = 6)	4 mg/kg (*N* = 8)	8 mg/kg (*N* = 7)
All body systems				
Moderate	2 (20)	0 (0)	2 (25)	2 (29)
Severe	1 (10)	0 (0)	0 (0)	0 (0)
Moderate				
Infections and Infestations	2 (20)	0 (0)	1 (13)	0 (0)
Escherichia urinary tract infection	1 (10)	0 (0)	0 (0)	0 (0)
Pharyngitis, streptococcal	1 (10)	0 (0)	0 (0)	0 (0)
Upper respiratory tract infection	0 (0)	0 (0)	1 (13)	0 (0)
Musculoskeletal and connective tissue disorders	1 (10)	0 (0)	1 (13)	2 (29)
Arthralgia	1 (10)	0 (0)	1 (13)	0 (0)
Back pain	0 (0)	0 (0)	0 (0)	1 (14)
Bursitis	1 (10)	0 (0)	0 (0)	0 (0)
Myalgia	0 (0)	0 (0)	0 (0)	1 (14)
Neoplasms benign, malignant and unspecified	0 (0)	0 (0)	0 (0)	1 (14)
Basal cell carcinoma	0 (0)	0 (0)	0 (0)	1 (14)
Nervous system disorders	1 (10)	0 (0)	0 (0)	0 (0)
Headache	1 (10)	0 (0)	0 (0)	0 (0)
Surgical and medical procedures	0 (0)	0 (0)	1 (13)	0 (0)
Endodontic procedure	0 (0)	0 (0)	1 (13)	0 (0)
Severe				
Metabolism and nutrition disorders	1 (10)	0 (0)	0 (0)	0 (0)
Gout	1 (10)	0 (0)	0 (0)	0 (0)

Treatment with placebo and ascending doses of GR-MD-02.

antibodies), IL-6, IL-8, TNF-α, endothelin-1, IP-10, VEGF or CD40-ligand (data not shown). The lack of changes in ALT and AST as a marker of the potential for drug-induced liver injury are encouraging.

Fibrosis-associated biomarkers

A number of potential serum tests to evaluate the state of liver fibrosis were evaluated including the composite scores of FibroTest (FibroSURE in the US) and ELF as well as a number of exploratory fibrosis markers.

FibroTest is a biomarker score that is calculated from the age and gender of the patient and the results of five blood tests including alpha-2-macroglobulin, haptoglobin, apolipoprotein A1, GGTP and total bilirubin. Because of the few data points in Cohort 1 and 2, statistical analysis was not possible, but there were no obvious differences. In cohort 3, there was a statistically significant reduction in FibroTest scores in patients treated with 8 mg/kg of GR-MD-02 as compared to placebo (LR = 10.4; $P = 0.0055$) (Figure 3). The reduction was evident at 21 days following the first dose ($P < 0.0001$) with no differences at subsequent time points from day 21 scores. Of note, the FibroTest scores were significantly higher at baseline for the treated group vs. placebo ($P = 0.001$).

Alpha-2 macroglobulin (A2M) levels, a component of the FibroTest, followed a similar pattern. There was a statistically significant reduction in A2M patients treated with 8 mg/kg of GR-MD-02 as compared to placebo (LR = 20.4; $P < 0.0001$) (Figure 4). The reduction was evident at 21 days following the first dose ($P < 0.0001$) with no differences at subsequent time points in levels from day 21. There was no significant difference in baseline A2M levels between placebo and treated subjects. The other components of the score, apolipoprotein A1, haptoglobin, GGTP and bilirubin were not different between any of the groups.

Evaluation of the ELF score showed no difference between any of the groups, as did the components of the ELF score, HA, TIMP-1 and P3NP (data not shown). Other exploratory fibrosis biomarkers also did not show differences between groups including osteopontin, lumican, TGF-β and metal metalloproteinases 1, 3 and 9 (data not shown).

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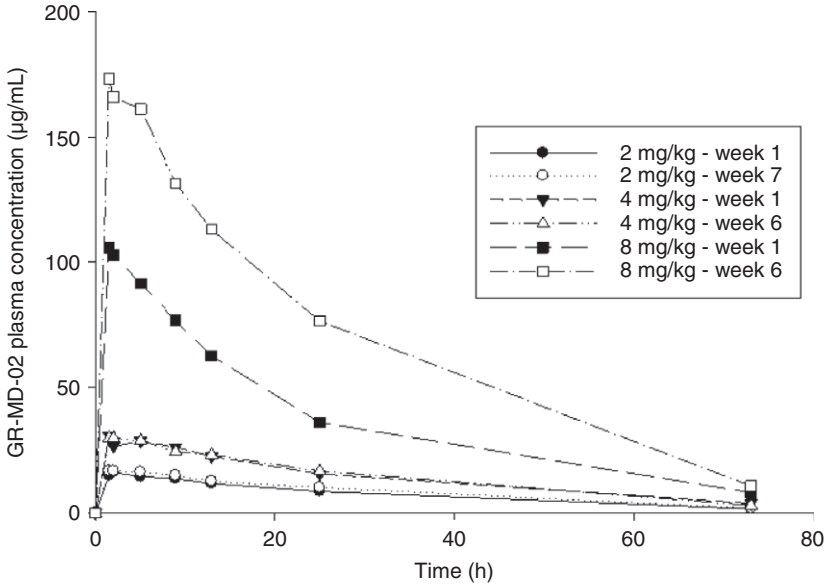

Figure 2 | Mean GR-MD-02 plasma concentration–time profiles on weeks 1 and week 6 (4 and 8 mg/kg) or week 7 (2 mg/kg) upon multiple dose IV Administration of 2, 4 or 8 mg/kg. Doses of GR-MD-02 were based on lean body weight and administered as a 60 min IV infusion.

FibroScan

FibroScan, a non-invasive ultrasound-based measure of liver stiffness, uses an electromechanical vibrator and pulse-echo ultrasound to evaluate the shear wave velocity in liver tissue. The stiffness of the liver as assessed by FibroScan been shown to correlate with the degree of liver fibrosis as assessed by liver biopsy, including patients with NASH.[17]

While there were several FibroScans performed on subjects in cohort 2, there were too few done for comparative analysis. In cohort 3 subjects, evaluable FS evaluations were obtained at baseline, day 38 and day 63 in five subjects administered GR-MD-02 and three subjects administered placebo. Five subjects in cohort 3 were not available for FS analysis (three placebo and two active) because of unavailability of the instrument at the site (one placebo and one active), unavailability of the appropriate instrument probe (one active; site only had M probe where examination called for XL probe), a technically inadequate baseline scan (one placebo, confirmed with Echosens technical evaluation), and the day 63 scan not being performed (one placebo).

As shown in Figure 5, there was essentially no change in the liver stiffness for the three placebo subjects, with all FibroScan scores within 20% of baseline. In contrast, three of the five subjects treated with GR-MD-02 had a reduction in liver stiffness at 68 days that was below 20% of the baseline value, with two subjects having a reduction of 50% from baseline. Comparison of change in FibroScan scores for placebo vs. GR-MD-02 was not statistically significant possibly due of the low number of subjects, but the reduction in FS scores for three of the five subjects suggests there may be a signal for reduced liver stiffness following four doses of 8 mg/kg.

CoQ measurements

Measurement of Co-enzyme Q10 (CoQ10), a vital component in the mitochondrial electron respiratory chain, may relate to mitochondrial dysfunction and oxidative stress which play an important role in the pathophysiology of NASH and liver fibrosis

No significant change was seen in CoQ metabolites in the 2 and 4 mg/kg GR-MD-02 cohorts in comparison to placebo (data not shown). However, in the subjects who received four doses of the 8 mg/kg dose of GR-MD-02 (placebo $n = 6$; active $n = 7$), there was a statistically significant percentage decrease in CoQTC at 38 days from their baseline values compared to placebo ($P = 0.023$) (Figure 6). There was no difference between the groups in baseline values. The remaining CoQ metabolites also trended in the expected physiological direction for an improving liver physiology, although they did not reach statistical significance.

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Table 5 | Summary of GR-MD-02 pharmacokinetic parameters following single dose (week 1) and weekly multiple dose (week 6 or week 7) IV administration of 2, 4 or 8 mg/kg

Dose (mg/kg)	Week	Statistical parameter	C_{max} (μg/mL)	T_{max} (h)	AUC_t (h*μg/mL)	AUC (h*μg/mL)	AUC %Ext	$T_{1/2,z}$ (h)	V_{ss} (mL)	V_z (mL)	CL (mL/h)	R_{AUC}
2	1	*N*	6	6	6	6	6	6	6	6	6	NA
		Geometric mean	16.3	2.1	525.3	571.7	7.75	19.88	5293	5723	199.6	
		CV% geometric mean	14.47	46.56	14.16	14.24	30.53	10.92	23.11	22.56	21.38	
2	7	*N*	6	6	6	6	6	6	6	6	6	6
		Geometric mean	17.7	3.06	594.3	645.4	7.856	20.45	4782	5215	176.8	1.129
		CV% geometric mean	14.93	59.51	23.49	23.97	13.8	3.966	17.01	16.96	16.11	28.02
4	1	*N*	8	8	8	8	8	8	8	8	8	NA
		Geometric mean	30.17	2.666	927.7	1039	9.527	19.75	6380	6746	236.8	
		CV% geometric mean	26.05	77.35	18.42	19.02	49.87	23.08	18.95	18.73	201	
4	6	*N*	7	7	7	7	7	7	7	7	7	7
		Geometric mean	31.37	2.382	947.9	1075	10.29	19.46	6037	6489	231.2	1.005
		CV% geometric mean	11.56	55.16	33.03	23.59	47.85	17.65	24.22	24.07	33.7	23.38
8	1	*N*	7	7	7	7	7	7	7	7	7	NA
		Geometric mean	106.9	1.708	2435	2679	6.883	18.37	3904	4233	159.7	
		CV% Geometric mean	42.60	14.59	39.52	36.26	93.00	20.31	49.95	47.40	43.18	
8	6	*N*	7	7	7	7	7	7	7	7	7	7
		Geometric mean	182.1	2.140	4902	5134	2.69	16.79	1893	2019	83.36	1.916
		CV% geometric mean	26.76	46.40	32.72	29.82	200.7	21.19	48.23	53.23	39.71	45.63

C_{max}, maximum plasma concentration; T_{max}, time corresponding to C_{max}; AUC_t, area under the plasma concentration–time profile from time zero up to the last quantifiable plasma concentration; *AUC*, area under the plasma concentration–time profile from time zero to infinity; $AUC_{\%Ext}$, percent *AUC* obtained upon extrapolated; $t_{½,z}$, terminal exponential half-life; V_{ss}, steady-state volume of distribution; V_z, terminal volume of distribution; CL, total clearance; R_{AUC}, accumulation ratio based on *AUC*; NA, not applicable.

Galectin-3 serum levels

Serum galectin-3 levels in all cohorts were within the range of reference values reported by the assay manufacturer (5.4–26.2 ng/mL). In cohort 3, the mean baseline serum levels for galectin-3 were 17.1 ± 3.5 ng/mL and there was no difference between placebo and treated subjects ($P = 0.72$). Since there were no subjects in this study that did not have NASH, whether these levels are elevated over normal individuals cannot be determined. It has been reported that galectin-3 levels are not altered in NASH,[18] but are elevated in obesity and diabetes.[19]

Since we have shown in animal models that galectin-3 is elevated in liver tissue in NASH,[9] we examined tissue and serum from the dose–response experiments previously published to determine the correlation between liver and serum galectin-3 in normal vs. NASH animals. While the galectin-3-stained area was markedly increased in NASH vs. normal animals (Figure 7a), the serum

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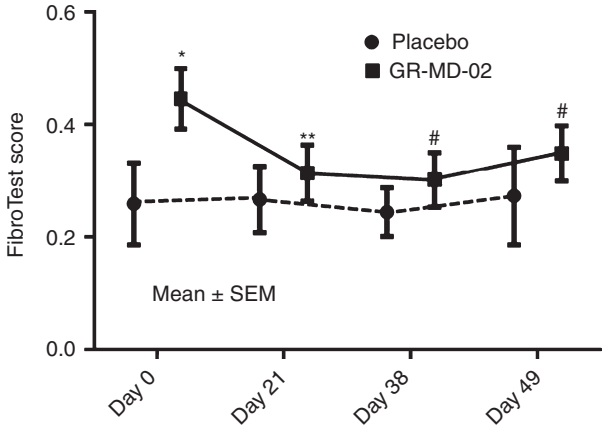

Figure 3 | FibroTest scores: cohort 3. Placebo (n = 6); GR-MD-02 (n = 7). *Difference from day 0 placebo, $P < 0.001$; **Difference from day 0 GR-MD-02, $P < 0.0001$; #No difference from day 21, GR-MD-02.

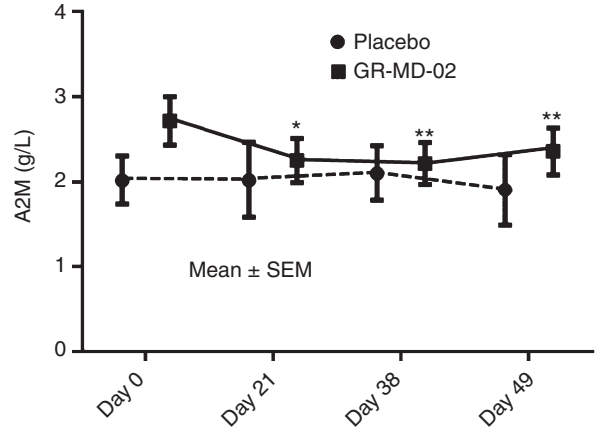

Figure 4 | Alpha-2 macroglobulin levels: Cohort 3. Placebo (n = 60; GR-MD-02 (n = 7). *Difference from day 0 GR-MD-02, $P < 0.001$; **No difference from day 21 GR-MD-02.

levels in these same animals were not different (Figure 7b).

DISCUSSION

This study was a first-in-human trial of multiple doses of GR-MD-02 in subjects with a definitive histopathological diagnosis of NASH with advanced hepatic fibrosis. Doses of GR-MD-02 up to 8 mg/kg were safe, with no clinically meaningful differences between placebo and treatment groups in the type, incidence or severity of AEs. Additionally, there were no notable differences in any safety laboratory parameters.

Plasma levels of GR-MD-02 and pharmacokinetic parameters are of interest because the drug is a large, complex polysaccharide which is a novel type of drug compound. The systemic exposure of GR-MD-02 increased in proportion to the dose at all three doses examined following the first dose, although C_{max} of the 8 mg/kg dose appeared to increase ~65% more than expected following the first dose and ~180% following the last dose. At doses of 2 and 4 mg/kg, there were no changes in the pharmacokinetic parameters after four doses, however, there was an approximately twofold increase in systemic exposure at the 8 mg/kg dose based on *AUC*.

The distribution, metabolism and elimination of GR-MD-02 are only partially characterised at this time. The pharmacokinetic observations in human subjects in this study are similar to those found in multiple animal species. Elimination studies in animals indicate little renal excretion with a moderate degree of biliary excretion. The primary cells targeted by GR-MD-02 are reticuloendothelial cells including resident liver macrophages, the primary immune cells expressing high amounts of galectin-3 (data not shown). The results after multiple doses of the high dose in this study are suggestive of possible saturation of a metabolic mechanism, possibly macrophage metabolism, or a transport mechanism which may be biliary. Future human studies will require additional pharmacokinetic monitoring to fully characterise the potential for accumulating systemic exposure with prolonged treatment.

Pre-clinical, dose-ranging studies in a mouse NASH model demonstrated that doses between 10 and 30 mg/kg gave optimal efficacy results on both inflammation and fibrosis.[9] The systemic exposure in humans (*AUC*) from this study of the 8 mg/kg dose (after last dose) was equivalent to approximately 25 mg/kg in mice. Therefore, the 8 mg/kg dose was equivalent to the upper range of the targeted therapeutic dose determined from effective doses in NASH animal models (*AUC*).

The primary endpoints of this phase 1 study were safety and pharmacokinetics, and these objectives were met. However, a variety of potential disease-related pharmacodynamic biomarkers were also explored to evaluate for potential drug effects. These evaluations must be considered as preliminary because of the small number of subjects in the study and the exploratory nature of the analyses. Despite these caveats, the data suggest that there may be an effect at the high dose of GR-MD-02.

The discovery and validation of biomarkers in NASH and liver fibrosis remains challenging.[20] A notable

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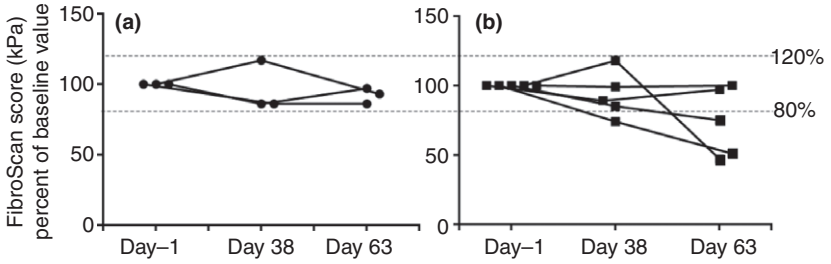

Figure 5 | FibroScan scores: mean percent change from baseline in cohort 3. FibroScan evaluations were obtained at baseline, day 38 and day 63 in three subjects administered placebo (a) and five subjects administered GR-MD-02 in a dose of 8 mg/kg (b). The values for each patient and study day are presented as a percent of the value at day −1 (baseline).

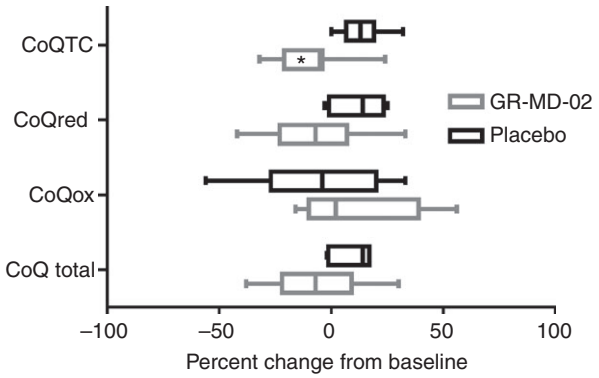

Figure 6 | Analysis of CoQ metabolic products in subjects receiving 8 mg/kg GR-MD-02 as compared to placebo. Boxplots indicate median, 25th and 75th percentile, and range of values. Percent change in CoQTC, CoQox, CoQred, and total CoQ from day 0 to after four infusions on day 38 of either placebo (n = 6) or 8 mg/kg GR-MD-02 (n = 7). *P = 0.023 by t-test.

observation from the biomarker analysis was the marked variability in the majority of measurements between dosing groups and placebo and between baseline and following treatment. This variability and the small subject numbers precluded reliable analysis of many biomarkers, including markers of liver injury, apoptosis and inflammation which showed no differences.

Among the fibrosis-associated markers evaluated, the FibroTest score decreased at 21 days following the first dose in patients treated with 8 mg/kg, and most of this effect seemed related to a reduction in alpha 2 macroglobulin levels. These results should be viewed cautiously since the baseline FibroTest mean value for the treated subjects was higher than for placebo, although there was no difference in baseline values for alpha 2 macroglobulin. Multiple studies have demonstrated the utility of FibroTest[21–24] and alpha-2 macroglobulin[25, 26] in predicting the degree of liver fibrosis in patients with multiple liver diseases, including NASH. Alpha 2 macroglobulin is a large serum glycoprotein that functions as a proteinase inhibitor by entrapping many proteinases.[27] While the molecule may be involved in liver fibrosis, the mechanism of the reduction in this study is uncertain since there are many sources of the protein and serum levels do not indicate the tissue origin and may not reflect the tissue levels.

Mitochondrial dysfunction and oxidative stress may play a role in the pathophysiology of NASH and liver fibrosis. Co-enzyme Q10 (CoQ10) is a vital component in the mitochondrial electron respiratory chain, contributing to the transport of electrons across complex III involving the Qo and Qi sites within the inner mitochondrial membrane to enable generation of ATP. Further, the relative ratio of the oxidised form (ubiquinone) to the reduced form (ubiquinol) may result from an anti-oxidant imbalance, which could contribute to the progression of liver injury. Earlier studies have established that CoQ plasma levels in humans are not changed by obesity alone,[28] but given the pathophysiological role of mitochondrial dysfunction and oxidative stress in NASH, plasma levels of CoQ have been correlated with experimental NASH in mice found to reflect the spectrum of NAFLD and NASH histological phenotype.[16] We therefore, evaluated whether oxidised and reduced CoQ levels, critical components of mitochondrial transport, were altered by treatment with GR-MD-02. At the 8 mg/kg dose, there was a significant reduction in the oxidised to reduced ratio of CoQ in the liver or systemically. Interestingly we found that the plasma CoQTC was decreased in the treatment group. We speculate that the drug may result in an increased utilisation of CoQ from the systemic circulation into the liver resulting in a

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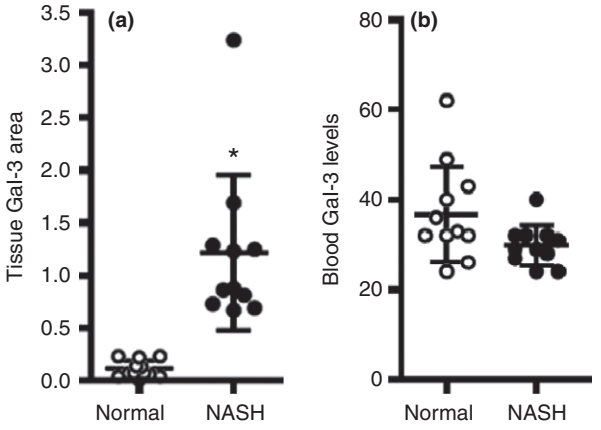

Figure 7 | Galectin-3 levels in serum and liver tissue of NASH mouse model. A mouse model of NASH was used to compare liver and blood levels of galectin-3 in normal mice and those with NASH. The samples used for this experiment were taken from previously published experiments.[9] (a) Area of tissue immunostained for galectin-3 (%) in normal and NASH animals, as described.[9] *$P < 0.001$. (b) Serum galectin-3 levels (ng/mL) in normal and NASH animals.

lower plasma CoQTC level. The significance of these findings will require further study.

In addition to the exploratory serum tests, we also utilised a physical measurement of liver stiffness using FibroScan in the third cohort of patients receiving 8 mg/kg of GR-MD-02. These data showed that there was essentially no change in the liver stiffness for three placebo subjects, while three of the five subjects treated with GR-MD-02 had more than a 20% reduction in liver stiffness at 68 days compared to the baseline value, with two subjects having a reduction of ~50% from baseline. The number of subjects having FibroScans was small and the results were not statistically significant, likely due to the small numbers, but the changes suggest there may be a signal for reduced liver stiffness with GR-MD-02 treatment.

In conclusion, this first-in-human, Phase 1 clinical trial in patients with histologically confirmed NASH and advanced fibrosis demonstrated that GR-MD-02 was safe and well tolerated. Moreover, PK parameters were defined which will guide further clinical trials of this drug. In sum, these results provide the basis for progression of GR-MD-02 into Phase 2 efficacy clinical trials.

AUTHORSHIP

Guarantor of the article: Peter Trabler.

Author contributions: Drs Harrison, Marri, Chalasani, Lawitz, Noureddin, Schiano, Siddiqui, Sanyal and Neuschwander-Tetri were investigators who conducted the clinical trial. Dr Traber designed the clinical trial. Drs Traber, Irish, Kohli, Aronstein, Thompson, Miles and Xanthakos collected and analysed the data. Dr Traber was the primary author with principle support from Drs Harrison, Chalasani, Kohli, Irish, Aronstein and Thompson. All authors reviewed the manuscript and approved the final version.

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