Aramchol in patients with nonalcoholic steatohepatitis: a randomized, double-blind, placebo-controlled phase 2b trial

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ABSTRACT

Nonalcoholic steatohepatitis, a chronic liver disease without an approved therapy, is associated with lipotoxicity and insulin resistance and is a major cause of cirrhosis and hepatocellular carcinoma. Aramchol, a partial inhibitor of hepatic stearoyl-CoA desaturase (SCD1) improved steatohepatitis and fibrosis in rodents and reduced steatosis in an early clinical trial. ARREST, a 52-week, double-blind, placebo-controlled, phase 2b trial randomized 247 non-alcoholic steatohepatitis (NASH) patients (101, 98, 48 in aramchol 400mg, 600mg, placebo, respectively; NCT 02279524). The primary endpoint was a decrease in hepatic triglycerides by magnetic resonance spectroscopy at 52 weeks with a dose of 600 mg of aramchol. Key secondary endpoints included liver histology and ALT. Aramchol 600 mg produced a placebo-corrected decrease in liver triglycerides without meeting the prespecified significance (-3.1, 95%CI -6.4 to 0.2, p=0.06), precluding further formal statistical analysis. NASH resolution without worsening fibrosis was achieved in 16.7% (13/78) of aramchol 600 mg versus 5% (2/40) of the placebo arm (OR=4.74, 95% CI:0.1-22.7) and fibrosis improvement by ≥1 stage without worsening NASH in 29.5% vs 17.5% (OR=1.88, 95% CI:0.7-5.0), respectively. The placebo-corrected decrease in ALT for 600 mg was -29.1 IU/L, (95% CI:-41.6 to -16.5). Early termination due to adverse events (AE) was <5% and aramchol 600 mg and 400 mg were safe, well tolerated, without imbalance in serious or severe AE between arms. Although the primary endpoint of a reduction in liver fat did not meet the prespecified significance level with aramchol 600mg, the observed safety and changes in liver histology and enzymes provide a rationale for SCD1 modulation as a promising therapy for NASH and fibrosis and is being evaluated in an ongoing phase 3 program.

Nonalcoholic fatty liver disease (NAFLD) is an increasingly common condition in the general population with a prevalence ranging from 13% in Africa to 32% in Latin America and the Middle-East, largely driven by rising rates of obesity and type 2 diabetes[1]. Nonalcoholic steatohepatitis (NASH), the progressive form of NAFLD, is characterized by liver fat accumulation coexisting with liver cell injury (hepatocyte ballooning) and hepatic inflammation. NASH leads to fibrosis progression and is a leading cause of cirrhosis, end-stage liver disease and liver transplantation. NASH is associated with overweight, obesity, type 2 diabetes (which are clinical features of the metabolic syndrome) and occurs in a context defined by insulin resistance and adipose tissue dysfunction[2]. Currently, there are no approved therapies for NASH. Ongoing late-phase clinical trials are designed to test histological improvement, such as resolution of steatohepatitis or fibrosis regression, while long-term outcome trials will evaluate whether these histological surrogates will result in less progression to cirrhosis and liver related morbidity and mortality.

Patients with NASH have increased de novo lipogenesis and lipotoxic species generated by the increased flux of fatty acids in the liver are a major contributor to hepatic inflammation and liver cell death associated with steatohepatitis[3]. Several agents in development specifically inhibit key enzymes of lipogenesis such as acetyl-co-A or fatty acid synthase. Stearoyl-CoA desaturase 1 (SCD1) catalyzes the rate-limiting step in the biosynthesis of monounsaturated fatty acids[4]. In rodents, down regulation of SCD1 reduced body adiposity, increased energy expenditure, and up-regulated expression of several genes encoding enzymes of fatty acid beta-oxidation in liver[5]. Reduction of SCD1 is also known to elevate adenosine monophosphate-activated protein kinase (AMPK) activity and enhance insulin sensitivity[6]. In hepatic stellate cells direct SCD1 depletion downregulates their fibrogenic phenotype[7]. Several small molecule complete SCD1 inhibitors have

been discontinued because of skin and lachrymal gland toxicity[8].

3β-arachidyl amido cholanoic acid (aramchol) is an oral, liver-targeted, fatty acid-bile acid conjugate[9] which partially inhibits hepatic SCD1 protein expression and reduces liver triglycerides [10,11] and fibrosis in animal models of steatohepatitis or fibrosis[12,13]. In hepatic stellate cells, aramchol down-regulates SCD1 and interferes with Wnt signaling to reduce cell proliferation, collagen and fibronectin production and α-smooth-muscle actin expression[7]. Direct SCD1 depletion using siRNA phenocopies the inhibitory effects of aramchol on HSC fibrogenesis[7]. In a 12-week phase 2a trial aramchol at 300mg daily significantly reduced liver fat content as measured by MR spectroscopy (MRS) vs. placebo in a dose dependent manner[14]. Aramchol was shown to be safe and well tolerated.

The results of the phase 2a study led to the initiation of a global phase 2b study to evaluate the effect of **AR**amchol for the **RE**solution of **ST**eatohepatitis (ARREST) in patients with NASH confirmed by liver biopsy. Here we report the safety and efficacy results of 52 weeks of treatment with 400 mg and 600 mg doses of aramchol in patients with NASH.

RESULTS

Study population

Between April 29th, 2015, and February 27th, 2017, 247 patients with NASH were randomized in a ratio of 2:2:1 to receive aramchol 400mg (n=101), aramchol 600mg (n=98) or placebo (n=48), once daily. The leading recruiting countries were Mexico (68 patients, 27% of study population) and the USA (64 patients, 26%). Thirty-two subjects (13%) were recruited in Israel (for the full list of countries, please see Methods). Figure 1 shows the disposition of the patients in the trial including reasons for trial discontinuation. The majority of study patients (219/247; 88.7%) completed 65 weeks in the study: 90/101 (89%), 88/98 (90%) and 41/48 (85%) in the aramchol 400mg, 600mg and placebo arms, respectively.

Baseline demographics and disease characteristics were balanced across study arms (Table 1). Mean age was 54.4 years, 160/247 (65%) of trial participants were females, 156/247 (63%) White, 78/247 (32%) Hispanic and Latin. As per inclusion criteria, all patients were overweight or obese with a mean body mass index (BMI) of 32.7 kg/m^2 (median 32.8 kg/m2; min 25 kg/m2; max 42.7 kg/m^2). Drug-treated type 2 diabetes was present in 170/247 (69%) of participants, hypertension in 135/247 (55%) and dyslipidemia in 132/247 (53.4%) of them. Normal alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were seen in 107/247 (43.3%) and 138/247 (55.9%) of patients, respectively. At baseline, mean hemoglobin A1c (HbA1c) was similar across treatment arms (6.6%, 6.7% and 6.5% in the 400mg, 600mg and placebo arms, respectively). Most patients had histologically significant or advanced fibrosis with stage 2 and 3 (149/247, 60%) and active steatohepatitis (nonalcoholic fatty liver disease activity score [NAS] ≥5) in 173/247 (70%). Seven patients had fibrosis stage 0. Median NAS was 5.0 and median grades of steatosis, ballooning and inflammation were 2.0, 1.0 and 2.0, respectively. Baseline histological parameters were comparable between study arms except for a higher proportion of subjects with fibrosis stage 3 in

the 400mg arm (Table 1). Mean baseline values for liver fat were comparable across study arms 27.3% ±11.8%, 30.2% ±12.4% and 27.5% ±9.3% in the aramchol 400mg, 600mg and placebo arms, respectively.

Efficacy Analyses

Hepatic fat reduction by imaging

214 subjects had paired MRS and were included in the FAS_{MRI} analysis set (n=90, 83 and 41 in the aramchol 400mg, 600mg and placebo arms, respectively (Figure 1). The primary endpoint analysis was performed in the FAS_{MRI} data set as prespecified. Hepatic triglyceride (%) measured by MRS was reduced in the aramchol 600mg [-3.2, 95% CI -5.2 to -1.2] vs. placebo [-0.1, 95% CI -2.8 to 2.6] with a mixed model for repeated measures (MMRM) difference between groups of -3.1 (95% CI -6.4 to 0.2, p=0.0655) (Table 2). Therefore, no further formal hierarchical statistical comparisons were performed. As prespecified, the remaining statistical comparisons of predefined key secondary and exploratory endpoints report the effects of aramchol with 95% confidence limits with nominal p-values. Hepatic fat was reduced in the aramchol 400 mg arm [-3.41; 95% CI -5.3 to -1.5] with an MMRM difference of -3.32 (95% CI 6.6 to -0.1; nominal p=0.045) (Table 2).

Liver histology

198 subjects had paired liver biopsies (n=80, 78 and 40 in the aramchol 400mg, 600mg and placebo arms, respectively; Figure 1) and were included in the pre-defined FAS_{Biopsy} analysis set. There was no statistical evidence of an imbalance across arms in the proportion of subjects without a pair of biopsies (21/101 (20.8%), 20/98 (20.4%) and 8/48 (16.7%) in the aramchol 400mg, 600mg and placebo arms, respectively). The effect of aramchol on key prespecified histological endpoints

with corresponding nominal p values are shown in Table 2. NASH resolution without worsening of fibrosis was achieved in 16.7% (13/78) of the 600 mg arm vs. 5% (2/40) in the placebo arm (OR 4.74; 95% CI: 0.1-22.7; p=0.051; Figure 2). Improvement in fibrosis by one stage or more without worsening of steatohepatitis was observed in 29.5% (23/78) of the 600 mg arm vs. 17.5% (7/40) for the placebo arm (OR 1.88; CI: 0.7-5.0; p=0.21; Figure 2).

Biochemical and metabolic changes

Results for the prespecified key secondary endpoint of mean change from baseline in ALT (LSM) are shown in Table 3. ALT was reduced in the aramchol 600mg (-17.29±3.7 IU/L, 95% CI -24.6 to -10.0) vs. placebo (+11.77±5.2 IU/L, 95%CI: 1.4 to 22.1) with an MMRM difference between groups of -29.06 (95% CI -41.6 to -16.5; p< 0.0001). ALT was reduced in the in aramchol 400mg (-12.0±3.6 IU/L, 95% CI -19.1 to -4.8) with an MMRM difference between groups of -23.76 (95%CI: -36.2 to -11.3; p=0.0002).

Prespecified exploratory endpoints

At week 52, both doses of aramchol showed a decrease in HbA1c while placebo patients showed an increase, despite no notable changes in anti-diabetic medications in any of the three arms. HbA1c (%) was reduced in the in aramchol 600mg (-0.13±0.08, 95% CI -0.3 to 0.02) vs. placebo (+0.32, 95%CI: 0.1 to 0.5) with an MMRM difference between groups of -0.45±0.13 (95%CI: -0.7 to -0.2; p=0.0008). HbA1c was reduced in the in aramchol 400mg (-0.04±0.08, 95%CI: -0.2 to 0.1) an MMRM difference between groups of -0.36±0.13 (95% CI -0.6 to -0.1; p=0.0061). There was a numerical reduction in fasting serum glucose in the 600mg and 400mg arms vs. placebo but without changes in HOMA- IR. At Week 52, there were no discernible changes for other biochemical parameters including lipid parameters. Mean body weight did not change significantly: placebo-subtracted differences were -1.15 kg in the 400 mg arm and -0.41 kg in

the 600 mg arm (Table 3).

The FIB-4 and NAFLD Fibrosis Score (NFS) scores, clinical and laboratory parameter-based scores associated with liver fibrosis in NASH, decreased at week 52 in the aramchol arms while placebo patients showed an increase. For FIB-4, placebo-subtracted differences were -0.27 in the 600 mg arm (95%CI: -0.5 to -0.1; p=0.008) and -0.21 in the 400 mg arm (95%CI: -0.4 to -0.02; p=0.033, respectively). For NFS, placebo-subtracted differences were -0.27 in the 600 mg arm (95%CI: -0.5 to -0.01; p=0.038) and -0.35 in the 400 mg arm (95% CI -0.6 to -0.1; p= 0.0080). There were no significant changes in fatty liver index (FLI), a marker of steatosis, fibrinogen, CRP, and adiponectin were not different between treatment arms.

Safety and tolerability

Aramchol was safe and well tolerated (Table 4). No deaths occurred during the study. (Table 4). Serious adverse events (SAEs) were reported in 8.9% (9/101), 9.2% (9/98) and (6/48) 12.5% patients in the 400mg, 600mg and placebo arms, respectively. No clustering of event types was noted in the active-treatment arms. The overall incidence of early termination was low and slightly higher in the placebo than the two active-treatment arms (10.9% (11/101), 10.2% (10/98) and 14.6% (7/48) in the 400mg, 600mg and placebo arms, respectively). The leading causes for early termination were consent withdrawal and AEs. The incidence of early termination due to AEs was low and similar across study arms: (3%, 4.1% and 4.2% of subjects in the 400mg, 600mg and placebo arms, respectively. AEs were mainly mild and reversible. Headache was the most commonly reported AE in all study arms (13.9%, 15.3% and 12.5% in the 400mg, 600mg and placebo arms, respectively). A higher incidence of urinary tract infections (UTI) was noted in both aramchol arms, 14.9%, 13.3% and 6.3% in the 400mg, 600mg and placebo arms, respectively

(p=0.13 and p=0.20 for 400mg and 600mg vs. placebo).

These were mostly single and mild events occurring in post-menopausal diabetic women. A numerical increase in pruritus was noted in the 600 mg arm, 11.2% compared to 6.9% and 6.3% in the 400 mg and placebo arms (p=0.34 for 600 mg vs. placebo). Pruritus events were mostly mild; none was severe and none leading to treatment discontinuation.

Post-hoc analyses

Post-hoc analyses for several proposed definitions of response[15,16] were performed to better understand the anti-steatotic effect of aramchol. The response rate for a ≥5% absolute reduction in liver fat content was 47.0% (39/83) for the 600 mg arm, 36.7% (33/90) for the 400 mg arm, and 24.4% (10/41) for the placebo arm (Table 2). Results for a 30% relative reduction were: 30.1% (25/83) in the 600mg arm, 25.6% (23/90) in the 400 mg arm and 14.6% (6/41) in the placebo arm.

Several additional post-hoc histological endpoints were analyzed to further characterize the effects of aramchol. Progression to cirrhosis occurred in only one patient (1.3%) in the 600 mg arm, six patients (7.5%) in the 400 mg arm and three patients (7.5%) in the placebo arm. Hepatocyte ballooning improved by one grade or more in the 600 mg arm in 64% (50/78) of patients vs. 45% (18/40) in the placebo arm (OR 2.38 CI: 1.1 to 5.2; p=0.032) but not in the 400 mg arm (50% (40/80) of patients, OR 1.5 CI: 0.7 to 3.2; p=0.3). A larger proportion of subjects no longer had hepatocyte ballooning (grade 0) in the aramchol 600 mg arm vs. placebo [50% (39/78) vs. 35% (14/40), OR 2.3 CI: 1.0 to 5.2; p=0.0484] but not in the 400 mg arm (37.5% (30/80) OR 1.4 CI: 0.6 to 3.2; p=0.43).

Reductions in AST were documented in the aramchol 600mg arm (-10.83±2.5 IU/L, 95% CI -15.7

to -6.0) vs. placebo (+6.68±3.5 IU/L, 95% CI -0.2 to 13.6) with an MMRM difference between groups of -17.5 (95% CI -25.9 to -9.1; p< 0.0001). AST was also reduced in the aramchol 400mg arm (-7.21±2.4 IU/L, 95% CI -12.0 to -2.4), an MMRM difference between groups of -13.88 95% (CI -22.2 to -5.6; p=0.0011). Moreover 29% of patients in the 600 mg arm normalized ALT by the end of treatment vs. 21.9% in the 400 mg arm and 13.3% in the placebo arm. AST was normal at the end of treatment in 22.6% of patients in the 600 mg arm, 18.8% in the 400 mg arm and only 4.4% in the placebo arm. There were no meaningful changes in the enhanced liver fibrosis (ELF) score vs placebo (-0.049 in the 600 mg arm and -0.016 in the 400 mg arm; p=0.67 and 0.89 respectively).

DISCUSSION

This 52-week international, randomized, placebo-controlled trial demonstrated the anti-steatotic potency of aramchol, building on a previous, smaller, lower dose, phase 2 study[14] and confirmed its tolerability and safety in patients with NASH. In the principal analysis aramchol at the dose of 600 mg daily produced a placebo-corrected decrease in liver triglycerides without meeting the prespecified significance (p=0.06), while for aramchol 400 mg daily the nominal p-value vs placebo was 0.045 reflecting the similarity in magnitude of treatment and heterogeneity of the study population.

The relevance of hepatic fat reduction as a predictor of histological improvement in NASH trials is a topic of great interest. Recent studies report that a 5% decrease in absolute liver fat content or a 30% relative fat reduction, measured by MRI-based methods, are associated with overall improvement in liver histology in several clinical trials[16-19]. This might suggest that a responder analysis based on these thresholds could be more suitable to detect clinically meaningful anti-steatotic effects. A post-hoc responder analysis based on this cutoff demonstrated a stepwise increase in response from placebo to 400 mg to 600 mg aramchol. When considering the proportion of patients with a 30% or more reduction in liver fat, results for aramchol were slightly lower than that of pegbelfermin, a pegylated human fibroblast growth-factor 21 analogue[20] and of firsocostat, an acetyl-CoA carboxylase inhibitor[21]. Conversely, obeticholic acid, the only drug with confirmed histological efficacy in a phase 3 trial to date, reported an absolute reduction in liver fat of similar magnitude as aramchol[16]. Whether compounds with stronger effect on steatosis such as aldafermin[22] or resmetirom[18] result in more marked histological improvement remains to be demonstrated in larger studies. These agents each have different mechanisms of action and the comparability of the clinical relevance of a specific change in liver fat measured by MRI on resolution of steatohepatitis and fibrosis improvement remains to be fully established while

accounting for duration of exposure to the drug.

Improvements in key histological features such as resolution of steatohepatitis and improvement in fibrosis are considered likely surrogates of clinical events and therefore are being used as regulatory endpoints for conditional approval in NASH[23,24]. A notable finding of this study is that resolution of steatohepatitis without worsening of fibrosis was achieved more frequently in the 600 mg arm than in the placebo arm. The low placebo rate noted in this trial is similar (6-12%) to that in some other trials (6-12%[18,25,26]). In contrast, higher placebo responses were occasionally documented[27,28], this heterogeneity in the placebo response possibly reflecting varying lifestyle choices, alcohol use, cross talk between liver disease and comorbid disease states, concomitant therapies and differences in biopsy interpretation. Other histological endpoints such as ballooning and fibrosis also favored the 600 mg dose. These findings may be relevant because ballooning is the hallmark of the steatohepatitic process and disease activity whereas fibrosis is the best histological marker of prognosis[29]. Fibrosis improvement by one stage or more was numerically higher in the 600mg arm than in the placebo arm, without reaching statistical significance. This trial was not powered for histological endpoints, which were the key secondary endpoints. Nonetheless, liver biopsy data suggest that key histological features related to disease progression may improve over a 52-week treatment. The numerical pattern of response for the 400 and 600 mg arms for both histological endpoints suggests that aramchol may improve NASH. Also, these results argue that while the dose of 400 mg may be sufficient for fat reduction and improvement in ALT and HbA1c, a higher dose may be needed for histological improvement. The current ongoing phase 3 trial (NCT04104321) is adequately powered to detect differences of the magnitude observed in this study and patients are receiving a different regimen (aramchol 300mg BID) to achieve higher exposure.

Some biochemical parameters suggestive of histological improvement were also affected by

the study drug. There was an early, dose-related reduction in ALT, that was maintained throughout the treatment period. Aminotransferase reduction has been observed each time histology improved in placebo-controlled trials of NASH[18,25,26,30,31]. The mean absolute change of -17 IU/L in the 600 mg arm is similar to the mean value that independently predicted histological improvement in a smaller phase 2 trial of obeticholic acid[32]. Gamma-glutamyl transferase declined in a pattern similar to ALT.

Two well-validated serum fibrosis markers, FIB-4 and NFS, although not ELF, were also reduced in the high dose aramchol arm vs. placebo. However, AST and ALT levels are part of the FIB4 and NFS and changes in the short term may reflect changes in these parameters and not changes in fibrogenesis. Despite this, improvements in FIB4 have been associated with improved liver histology both in the contexts of clinical trials and clinical practice[33,34]. The utility of FIB4, NFS and other biomarkers as surrogates of histological response are currently under active investigation in the fully powered phase 3 trial of aramchol for NASH.

Several studies have documented a reduction in SCD1 activity, without complete inhibition, upon aramchol administration both in vitro[9] and in vivo[11]. In the methionine-choline deficient model of steatohepatitis, aramchol reduced SCD1 protein content, liver monounsaturated fatty acid concentration and the desaturation index[13]. Multiple lines of evidence suggest that modulating SCD-1 activity is an attractive pharmacological target in metabolic diseases associated with obesity, including NAFLD[35]. In humans, obesity is associated with increased surrogates of SCD1 activity, such as desaturation indexes or palmitoleate concentrations, both in plasma and in adipose tissue[36]. In rodents, SCD1 genetic inactivation results in resistance to diet-induced weight gain, fat accumulation and dyslipidaemia[37]. Specifically, SCD1 is strongly induced in the liver upon high carbohydrate feeding and controls a rate-limiting step of hepatic de novo lipogenesis. Liver specific inhibition of SCD1 consequently protects against high carbohydrate diet-induced adiposity and

steatosis, reduces lipogenesis, hepatic triglyceride secretion and white adipose tissue weight. In addition to controlling the rate of lipogenesis and triglyceride synthesis and excretion, changes in SCD1 activity also modulate fatty acid disposal thus further promoting liver fat loss[37]. Inactivation of SCD1 activity in rodents results in upregulation of lipid oxidation genes including, carnitine palmitoyl 1, a major regulator of mitochondrial oxidation of fatty acids[37]. SCD1 activity also promotes AMPK activation which in turn down-regulates Acetyl-CoA Carboxylase activity[6]. Thus, SCD1 inhibition promotes both fatty acid disposal and reduces triglyceride synthesis.

In the current trial, aramchol induced an improvement in HbA1c levels. This change is clinically relevant as study participants had either type 2 diabetes or pre-diabetes[38]. Because type 2 diabetes is a major comorbidity associated with more severe forms of NASH and higher potential for disease progression, optimal control of their diabetes and other metabolic comorbidities is essential. Drugs that contribute to the control of these comorbidities or, at a minimum are neutral, are highly anticipated. Despite no changes in insulin levels, HbA1c improvement induced by aramchol without hypoglycemic episodes is supported by experimental data in rodents demonstrating both *in vitro* and *in vivo* an increase in AMPK activity with subsequent reduction in gluconeogenesis[39]. Other data linked SCD1 inactivation with an improved insulin sensitivity. Whole-body SCD1 knock-out rodents display improved insulin signalling[40] and increased GLUT-4 and GLUT-2 expression in skeletal muscle and hepatocytes[41] mainly mediated through a reduction in palmitoleate and oleate and in ceramide synthesis[42]. Larger studies in humans are however necessary to confirm a beneficial effect of aramchol on glycemic regulation and insulin sensitivity.

Although largely metabolically beneficial, SCD1 inactivation can theoretically also result in inflammatory tissue damage. Accumulation of SCD1 substrates such as palmitate and stearate can induce apoptosis[43] and endoplasmic reticulum stress[44] thus contributing to lipotoxic liver injury.

Mice treated with aramchol were protected from oxidative stress by an increased glutathione and glutathione/glutathione disulphide ratio and displayed less inflammation and also less fibrosis[13]. Differences between genetically-driven total suppression of SCD1 activity and partial pharmacological inhibition as induced by aramchol could explain the observed differences in the overall net effects. Similar data of reduced inflammation and prevention of fibrosis onset have been reported with other preclinical SCD1 inhibitor compounds[45]. Other fibrosis models have confirmed an antifibrotic potency of aramchol that parallels SCD1 inhibition in hepatic stellate cells[7]. Direct SCD1 depletion using siRNA results in down-regulation of fibrogenesis i.e., reduction of collagen 1A1 and alpha smooth muscle actin production by hepatic stellate cells[7]. Importantly, the ARREST study is the first biopsy-based clinical trial of aramchol and demonstrates no adverse impact on liver cell injury or inflammation. These findings support the safe use of this agent in a phase 3 trial.

Several attempts to develop small molecule SCD1 inhibitors for the treatment of metabolic diseases have failed due to severe skin and lachrymal gland toxicity in animals[8]. Owing to its different molecular structure which possibly targets tissue distribution preferentially to the liver and only partial SCD1 inhibition no particular side effects were noted with aramchol. There were a few cases of uncomplicated lower UTI in post-menopausal women. While there is no apparent explanation for this finding, given the inclusion criteria of pre-diabetes or type 2 diabetes and the occurrence of UTI mainly in post-menopausal women, this event is not considered atypical for the study population. There was a small numerical increase in mostly single and mild pruritus events in the 600 mg arm that did not lead to treatment discontinuation.

This study has several limitations. Histological outcomes were only key secondary endpoints, and the trial was not powered to show histological benefit. Patients from Israel did not have week 52 biopsy assessments because of restrictions imposed by the Ministry of Health. Strengths of trial include centralized assessment of biochemical parameters and blinded central

review of liver biopsies by an expert hepatopathologist. A strength of this study is the inclusion of a large proportion of Hispanic patients, who have a higher prevalence of a disease-associated variant (I1 48M) of the PNPLA3 gene, which is associated with higher risk of progression to NASH[46]. In addition, the population studied in ARREST was an enriched targeted population where all patients were overweight or obese and had pre-diabetes or type 2 diabetes.

In conclusion, in this randomized, placebo-controlled, global trial of aramchol, a partial SCD1 inhibitor, the reduction in liver fat did not meet the prespecified primary endpoint for statistical significance. However, the totality of the data based on prespecified key secondary endpoints, exploratory analyses and post-hoc analyses suggest a potential for improving liver histology in patients with type 2 diabetes or prediabetes with histologically confirmed steatohepatitis and with high disease activity and pre-cirrhotic stages of fibrosis. These are corroborated by the observed biochemical improvement in liver enzymes. This will be further tested in an ongoing large, international phase 3 trial (NCT 04104321).

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Competing Interests Statement

V.R. and R.L. are Galmed consultants and Investigators in the Galmed- sponsored study described in the article; S.F. and A.J.S are Galmed consultants, T.G., M.H., R.O. and L.H. are current or former Galmed employees, D. BB. was responsible for central lab services for the Galmed- sponsored study described in the article; K.L. was responsible for histological analysis services for the Galmed- sponsored study described in the article; S.K. is the Galmed statistician; L.L.de-G., R.S., F.P., F.F., J. F-F., M.A., and A.L.F were Investigators in the Galmed- sponsored study described in the article. The ARREST investigator study group members were investigators or sub-investigators in the Galmed- sponsored study described in the article.

TABLES

Table 1: Demographic and Baseline Characteristics

	Placebo N*=48	Aramchol 400 N*=101	Aramchol 600 N*=98
Demographics			
Age (yrs.)	54.4±10.3	53.9±10.9	54.9±9.8
Sex			
Male	23 (48%)	36 (36%)	28 (29%)
Race/ Ethnicity			
White	30 (63%)	63 (62%)	63 (64%)
Hispanic/Latin/Latin American	16 (33%)	33 (33%)	29 (30%)
Other	2 (4%)	5 (5%)	6 (6%)
Comorbidities			
Hypertension	24 (50%)	53 (52.5%)	58 (59.2%)
Dyslipidemia	30 (62.5%)	57 (56.4 %)	45 (45.9%)
Drug-treated type 2 diabetes (%)	72.9	68.3	67.3
Metabolic Factors			
BMI (kg/m^2)	32.6±4.9	32.4±4.5	33±4.2
Weight (Kg)	88.6±18.2	88.1±17.4	86.9±15.5
Waist Circumference (cm)	107.5±12.1	108.7±13.8	107.6±11.2
Glycemic Parameters			
Serum glucose (mmol/L)	6.55±1.9	6.56±1.5	6.94±2.4
Hemoglobin A1c (%)	6.53±1.0	6.56±0.9	6.65±1.0
HOMA-IR (Units)	10.0±8.7	9.1±6.5	9.6±6.5

	Placebo N*=48	Aramchol 400 N*=101	Aramchol 600 N*=98
Lipids			
Cholesterol (mmol/L)	4.93±1.4	4.64±1.1	4.88±1.1
HDL Cholesterol (mmol/L)	1.18±0.3	1.17±0.3	1.21±0.3
LDL (Direct) (mmol/L)	3.09±1.1	2.86±1.0	3.04±0.9
Triglycerides (mmol/L)	1.93±1.4	1.98±1.0	1.92±1.6
Liver Enzymes			
ALT (IU/L)	67.0±47.2	67.7±48.2	55.7±37.8
AST (IU/L)	47.6±29.9	50.9±39.9	42.0±25.6
GGT (IU/L)	62.9±45.0	60.6±56.3	68.2±91.8
Alkaline phosphatase (IU/L)	88.9±27.3	85.5±30.4	84.2±28.9
Total Bilirubin (µmol/L)	9.52±5.3	9.21±5.3	9.23±5.9
Chemistries			
Albumin (g/L)	45.35±2.5	45.75±2.6	45.41±2.8
Creatinine (µmol/L)	71.8±13.8	68.2±14.8	66.1±15.3
eGFR(MDRD) (ml/Mn/Sa)	89.0±17.5	92.0±21.0	93.2±21.1
Hematology and Coagulation			
International normalized ratio (INR)	1.06±0.1	1.05±0.1	1.04±0.1
Prothrombin time (PT) (Secs)	10.9±0.8	10.9±0.9	10.8±0.9
Hemoglobin (G/L)	143.4±16.0	142.3±13.3	141.9±13.0
Hematocrit (L/L)	0.46±0.1	0.46±0.1	0.46±0.0
Platelets (10e9/L)	224.1±55.5	236.1±70.6	234.2±67.5
White blood cells (10e9/L)	6.29±1.7	6.81±1.7	6.79±1.9

	Placebo N*=48	Aramchol 400 N*=101	Aramchol 600 N*=98
Concomitant medication use			
Lipid modifying agents	22 (45.8%)	44 (43.6%)	34 (34.7%)
Anti-hyperglycemic drugs	35 (72.9%)	69 (68.3%)	66 (67.3%)
Vitamin E (NOS)	0	1 (1.0%)	2 (2.0%)
MRS Evaluations			
Liver Fat-MRS %*	27.5% ± 9.3	27.3% ± 11.8	30.2% ± 12.4
Biopsy Evaluations			
NAS score, median	5.0 (2.0)	5.0 (1.0)	5.0 (1.0)
Steatosis score, median	1.5 (1.0)	1.0 (1.0)	2.0 (1.0)
Ballooning score, median	1.0 (1.0)	1.0 (1.0)	1.0 (1.0)
Inflammation score, median	2.0 (0.0)	2.0 (0.0)	2.0 (0.0)
Fibrosis stage, median	1.5 (2.0)	2.0 (2.0)	2.0 (2.0)
Fibrosis stage 2, %	16.7	18.8	22.4
Fibrosis stage 3, %	33.3	47.5	36.7

Data are n (%) or mean ± SD. HOMA-IR=Homeostatic Model Assessment of Insulin Resistance. ALT=alanine aminotransferase. AST=aspartate aminotransferase. GGT= Gamma-glutamyl transpeptidase. eGFR= Estimated glomerular filtration rate. * FAS_{MRS}. NAS=nonalcoholic fatty liver disease activity score.

Table 2: Change in MRS and histology-based endpoints after 52 weeks of treatment

				Difference as Compared to Placebo		Odds Ratio and 95% Confidence Interval	
Primary outcome	**Placebo**	**Aramchol 400mg**	**Aramchol 600mg**	**Aramchol 400mg**	**Aramchol 600mg**	**Aramchol 400mg**	**Aramchol 600mg**
Number of patients with paired MRI evaluations	41	90	83				
Absolute % Change from Baseline in Mean Liver Fat	-0.09%±1.38%	-3.41%±0.96%	-3.18%±1.01%	-3.32%±1.65% P=0.045	-3.09%±1.67% P=0.066		
% of MRS Responders*	24.4%	36.7%	47.0%			2.20 (0.89-5.46) P=0.088	2.77 (1.12-6.89) P=0.028
Changes from baseline in histopathological parameters							
Number of patients with paired biopsies	40	80	78				
NASH Resolution Without Worsening of Fibrosis	5.0%	7.5%	16.7%			1.79 (0.33-9.62) P=0.50	4.74 (0.99-22.66) P=0.051
Fibrosis Improvement Without Worsening of NASH	17.5%	21.3%	29.5%			1.11 (0.40-3.05) P=0.84	1.88 (0.7-5.04) P=0.21
≥2 points improvement in NAS contributed by at least two of: steatosis, inflammation, ballooning Without Worsening of Fibrosis	17.5%	20.0%	25.6%			1.36 (0.49-3.80) P=0.56	1.68 (0.62-4.57) P=0.31
≥2 points improvement in SAF activity score Without Worsening of Fibrosis	25.0%	25.0%	35.9%			1.08 (0.44-2.63) P=0.86	1.84 (0.78-4.35) P=0.16

Data is presented as % of subjects meeting endpoint or as mixed model derived least squares means ± standard error; p values beyond the primary endpoint are nominal; * Post-Hoc analysis: responder is defined according to ≥5% absolute improvement from baseline

Table 3: Changes from Baseline to Week 52 in liver and disease related parameters

	Placebo	Aramchol 400mg	Aramchol 600mg
Liver enzymes			
Number of patients	47	100	98
ALT (U/L) Change from Baseline to Week 52	11.77±5.24	-12.00±3.62	-17.29±3.72
Difference as Compared to Placebo		-23.76±6.32	-29.06±6.37
p-value		p=0.0002	p<0.0001
AST (U/L) Change from Baseline to Week 52	6.68±3.50	-7.21±2.42	-10.83±2.49
Difference as Compared to Placebo		-13.88±4.21	-17.50±4.24
p-value		p=0.0011	p<0.0001
Alkaline phosphatase (U/L) Change from Baseline to Week 52	11.64±4.55	-3.41±3.15	-3.76±3.24
Difference as Compared to Placebo		-15.06±5.52	-15.40±5.57
p-value		p=0.0068	p=0.0061
γ-glutamyl transpeptidase (U/L) Change from Baseline to Week 52	+66.03±22.74	-1.23±15.71	-15.18±16.18
Difference as Compared to Placebo		-67.25±27.62	-81.21±27.89
p-value		p=0.016	p=0.0040
Total Bilirubin (µmol/L) Change from Baseline to Week 52	+0.50±0.45	-0.17±0.32	-0.31±0.32
Difference as Compared to Placebo		-0.67±0.55	-0.81±0.55
p-value		p=0.22	p=0.14
Lipids			
Number of patients	47	100	98
Total cholesterol (mmol/L) Change from Baseline to Week 52	+0.08±0.11	+0.11±0.07	+0.11±0.08
Difference as Compared to Placebo		0.02±0.13	0.03±0.13
p-value		p=0.85	p=0.84
LDL cholesterol (mmol/L) Change from Baseline to Week 52	0.24±0.10	0.26±0.07	0.18±0.07
Difference as Compared to Placebo			

	Placebo	Aramchol 400mg	Aramchol 600mg
p-value		0.02±0.12 p=0.85	-0.06±0.12 p=0.62
HDL cholesterol (mmol/L) Change from Baseline to Week 52	-0.02±0.03	-0.04±0.02	-0.02±0.02
Difference as Compared to Placebo		-0.02±0.03	0.004±0.030
p-value		p=0.49	p=0.89
Triglycerides (mmol/L) Change from Baseline to Week 52	+0.08±0.13	+0.04±0.09	0.16±0.09
Difference as Compared to Placebo		-0.04±0.16	+0.07±0.16
p-value		p=0.78	p=0.64
Metabolic factors			
Glucose (mmol/L)	N=47	N=99	N=96
Number of patients			
Change from Baseline to Week 52	+0.54±0.26	+0.10±0.18	+0.01±0.18
Difference as Compared to Placebo		-0.44±0.31	-0.53±0.31
p-value		p=0.16	p=0.094
Hemoglobin A1c (%)	N=47	N=98	N=96
Number of patients			
Change from Baseline to Week 52	+0.32±0.11	-0.04±0.08	-0.13±0.08
Difference as Compared to Placebo		-0.36±0.13	-0.45±0.13
p-value		p=0.0061	p=0.0008
Weight (kg)	N=47	N=99	N=98
Number of patients			
Change from Baseline to Week 52	-0.11±0.59	-1.26±0.41	-0.52±0.42
Difference as Compared to Placebo		-1.15±0.71	-0.41±0.71
p-value		p=0.11	p=0.56)
Waist Circumference (cm)	N=46	N=98	N=98
Number of patients			
Change from Baseline to Week 52	-1.81±1.30	-2.23±0.89	-0.63±0.90
Difference as Compared to Placebo			

	Placebo	Aramchol 400mg	Aramchol 600mg
p-value		-0.41±1.55 p=0.79	1.19±1.56 p=0.45
Biomarkers			
Fibrosis 4 (FIB4) Number of patients	N=46	N=100	N=95
Change from Baseline to Week 52	+0.17±0.08	-0.05±0.06	-0.10±0.06
Difference as Compared to Placebo p-value		-0.21±0.10 p=0.033	-0.27±0.10 p=0.008
NFS Number of patients	N=42	N=91	N=89
Change from Baseline to Week 52	+0.23±0.11	-0.12±0.08	-0.04±0.08
Difference as Compared to Placebo p-value		-0.35±0.13 p=0.0080	-0.27±0.13 p=0.038
FLI Number of patients	N=44	N=95	N=95
Change from Baseline to Week 52			
Difference as Compared to Placebo p-value	0.59±1.51	-2.01±1.04	-1.07±1.06
		-2.60±1.80 p=0.15	-1.66±1.80 p=0.36

Results of baseline adjusted Mixed Models for Repeated Measurements (MMRM) Least Squares Means (LSM) ± standard error of absolute changes from baseline by treatment group; When there were no repeated measures, analysis of baseline adjusted covariance was used; Two-sided nominal p–values beyond the primary endpoint testing the between active groups and placebo contrasts

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Table 4: Safety and tolerability data

	Placebo (N=48)	Aramchol 400 (N=101)	Aramchol 600 (N=98)
Overall treatment withdrawal rate	7 (14.6%)	11 (10.9%)	10 (10.2%)
Treatment withdrawal due to adverse event	2 (4.2%)	3 (3%)	4 (4.1%)
Participants with serious adverse event	6 (12.5%)	9 (8.9%)	9 (9.2%)
Participants with severe adverse event	5 (10.4%)	7 (6.9%)	6 (6.1%)
Participants with any adverse event	33 (68.8%)	75 (74.3%)	77 (78.6%)
Gastrointestinal disorders			
Constipation	6 (12.5%)	5 (5%)	8 (8.2%)
Nausea	6 (12.5%)	10 (9.9%)	9 (9.2%)
Nervous system disorders			
Headache	6 (12.5%)	14 (13.9%)	15 (15.3%)
Skin disorders			
Pruritus	3 (6.3%)	7 (6.9%)	11 (11.2%)
Infections			
Urinary tract infection	3 (6.3%)	15 (14.9%)	13 (13.3%)

AEs with an incidence ≥10% in any treatment arm are presented by system organ class and preferred term. No deaths were reported during the study.

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FIGURE LEGENDS

Figure 1. Patient disposition in the trial

Figure 2. Analyses of biopsy derived endpoints used the baseline adjusted logistic regression testing the aramchol to placebo contrast (A) Proportion of patients with NASH resolution without worsening of fibrosis; (B) Proportion of patients with fibrosis improvement without worsening of NASH. Repeated measures analysis of covariance absolute change from baseline in (C) Alanine aminotransferase (ALT) (U/L), (D) Aspartate transaminase (AST) and (E) HbA1c (%), model adjusted means (±SE) of absolute change from baseline during treatment with aramchol 400mg (ALT and AST: N=100, N=98 and N=47 for aramchol 400mg, aramchol 600mg and placebo, respectively; HbA1c: N=98, N=96 and N=47) for up to 52 weeks. Two-sided nominal p-values beyond the primary endpoint

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PATIENTS AND METHODS

Study design and participants

This multicenter, randomized, double-blind, placebo-controlled, Phase 2b study was conducted at 57 centers in 11 countries (USA, Mexico, Israel, France, Germany, Italy, Chile, Lithuania, Georgia, Romania and Hong Kong). Eligible patients were adults, aged 18 to 75 years, with histological evidence of steatohepatitis, a nonalcoholic fatty liver disease (NAFLD) activity score (NAS) $\geq$4 (with at least grade 1 for hepatocyte ballooning and lobular inflammation and steatosis) on a diagnostic liver biopsy centrally read and obtained within <6 months from randomization; overweight or obesity (body mass index [BMI] 25kg/m^2-40kg/m^2) or increased waist circumference (88cm - 200cm for women, and 102cm - 200cm for men); known T2DM or pre-diabetes according to the criteria of the American Diabetes Association[1] or glycated hemoglobin (HbA1c) > 5.7%; liver fat content ≥5.5% on Screening MRS; and normal synthetic liver function (serum albumin >3.2g/dl, INR 0.8-1.2, conjugated bilirubin < 35 µmol/L). Patients with diabetes or pre-diabetes were included because they are at high risk for advanced disease or disease progression. Patients were excluded for: other acute or chronic liver disease; cirrhosis (fibrosis stage 4); daily alcohol intake >20 g/day for women and >30 g/day for men; drug or alcohol abuse or dependence in the last 5 years; bariatric surgery within 5 years of liver biopsy; weight loss>5% in the 6 months prior to randomization; uncontrolled arterial hypertension; uncontrolled hypothyroidism; diabetes mellitus other than T2DM; treatment with anti-diabetic medications, unless started prior to biopsy (6-12 months depending on drug) and stable; treatment with pre-defined disallowed medications that may cause or treat nonalcoholic steatohepatitis (NASH). A complete list of inclusion and exclusion criteria is provided in Supplementary appendix 2. All patients

provided written informed consent prior to any study-related activities. The study protocol was approved by the Ethics Committees (EC) at participating centers or by a national EC in accordance with local laws and regulations. Institutional Review Board (IRB) or Ethics ECs that reviewed and approved this study included: Schulman Associates IRB in the US, CPP lle-de-France VI - Pitié Salpêtrière in France and Comite de Etica en Investigación de Chirurgie & Medical and Comité de Investigación de Chirurgie & Medical in Mexico. The study was conducted in compliance with Good Clinical Practice guidelines and was registered online (clinicalTrials.gov No. NCT02279524).

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Randomization and blinding

Eligible patients were randomly assigned in a 2:2:1 ratio (48 blocks), to receive either daily aramchol 400mg, aramchol 600mg or placebo, orally, for 52 weeks. The randomization ratio was 2:2:1 stratified by country. The randomization list was generated prior to the study initiation, using a computer-generated randomization list, and done using an interactive web response system. Treatment assignments were masked to patients, investigators, site personnel, Sponsor, and central readers of biopsy and MRS data. Aramchol and matching placebo were of identical appearance.

Dose selection

Dose selection was based on clinical pharmacology considerations and corroborating evidence from the Phase 2a study. Aramchol is a biopharmaceutics classification system Class IV compound with low solubility and low permeability. Data from Phase 1 pharmacokinetics studies in healthy volunteers evaluating single doses up to 900mg aramchol and repeat daily doses of 600mg once daily showed sub-proportional increases in exposure with dose where once daily doses of >600 mg are not expected to result in higher exposures. None of the studies raised safety concerns and dose response data in the Phase 2a suggested that a higher

dose may result in better efficacy.

Procedures and assessments

Following randomization, patients were evaluated at 9 scheduled visits: weeks 2, 4, 8, 12, 24, 32, 40, 52 (end of treatment) and 65 (follow-up). Body weight and waist circumference were measured at Screening, Baseline, Week 24, Termination/Early Termination and at Week 65. During study visits, subjects were counseled on the importance of diet and exercise in proper weight management and asked if any change took place in their lifestyle between visits. Blood samples were obtained at these visits for routine biochemical and hematology tests and measured centrally (Clinical Research Laboratory (CRL)). Based on CRL cut-offs, normal alanine aminotransferase (ALT) was <45 IU/L and normal AST<41IU/L.

Data was collected using the electronic data capture system DSG eCaseLink V8.0.

MRS Evaluation

Patients were required to perform two MRS scans, at Screening and at week 52. MRS evaluation was also recommended for patients with early study termination at week 24 or beyond. MRS scans were read centrally at the Tel Aviv Sourasky Medical Center (Tel Aviv, Israel) by a specialized radiologist masked to treatment allocation (DBB).

Liver Biopsy

Biopsies were performed at Screening (if not available within 6 months prior) and at week 52. In case of early study termination, a biopsy was recommended if patients completed at least 40 weeks in the study. Patients from Israel (n=24) were not allowed to undergo an end-of-study liver biopsy as per Israeli Ministry of Health regulatory restrictions at the time the study was submitted. Liver biopsies were centrally read by a single pathologist (CL) masked to treatment allocation. Analyses used the initial, baseline qualifying read and the end-of-treatment read for assessing histological changes. Steatohepatitis was diagnosed based on the presence of steatosis, inflammation, and ballooning. Biopsy specimens were graded according

to the NASH Clinical Research Network (CRN) scoring system[2] for steatosis (scored 0-3),

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inflammation (scored 0-3) and hepatocellular ballooning (scored 0-2). Fibrosis was evaluated using the NASH CRN fibrosis staging system (stages 1-4)[2]. Biopsies were also scored based on the steatosis, activity, and fibrosis (SAF) algorithm[3].

Outcomes

The primary endpoint of the study was the absolute change from Baseline to end of study in liver fat content assessed by MRS and measured as a triglyceride-to water-ratio (fat/water+fat, %). Key secondary endpoints were: proportion of subjects with NASH resolution at week 52 (no evidence of steatohepatitis with ballooning score of 0 and an inflammation score of 0 or 1) without worsening of fibrosis; proportion of subjects with $\geq$1 stage fibrosis improvement without worsening of NASH (defined by any increase in inflammation or ballooning grade); proportion of subjects with a $\geq$2 point NAS improvement (contributed by at least two of: steatosis, inflammation, ballooning) without worsening of fibrosis; proportion of subjects with a $\geq$2 point reduction in SAF activity score without worsening of fibrosis; baseline adjusted mean change from Baseline to Week 52/Termination in ALT (IU/L) levels.

Exploratory endpoints included anthropometric and glycemic parameters, potential biomarkers of NASH and fibrosis (FIB-4, NFS, FLI), markers of inflammation (fibrinogen, CRP) and adiponectin.

Safety and tolerability were evaluated based on treatment emergent serious adverse events (SAEs); adverse events (AEs); safety laboratory; vital signs; 12-Lead electrocardiograms (ECG); physical examinations and the proportion of patients who prematurely discontinued from the study. AEs were graded for severity. An independent data monitoring committee reviewed safety data during the study.

Several post-hoc analyses were performed to further describe the effects of aramchol regarding: liver de-fattening as measured by MRS (responder analyses), histological changes

(progression to cirrhosis and change in hepatocyte ballooning) and biochemical responses (change from baseline in aspartate aminotransferase [AST] and normalization of ALT and AST) as well as change from baseline in Enhanced Liver Fibrosis (ELF) score.

Statistical analysis

Sample size and power considerations

The planned sample size was 215 patients, 86 in each of the two active groups and 43 in the placebo group. Sample size calculation was based on an effect size of 0.6 for the primary endpoint between the active groups and placebo with a 5% significance level and 89% power. Based on an expected drop-out rate of 10%, the total sample size was 240.

Significance level and multiplicity adjustment

One primary endpoint and five secondary endpoints were pre-defined. The overall experiment-wise significance level was 5% using two-tailed tests with the hierarchical gate-keeping approach to control the overall Type-I error rate for multiple contrasts and multiple endpoints (refer to supplementary Table 2; Order of testing for Contrasts). According to the gate-keeping approach, the 1st contrast (600mg vs. placebo in the primary endpoint) was tested using a two-tailed 5% significance level. If the first contrast fails to reach statistical significance, all p-values reported, as per SAP, are nominal p-values.

Pre-defined Analyses sets:

Full Analysis Set (FAS): included all patients randomized and who had baseline and at least one post-baseline efficacy assessment. The FAS analysis set includes efficacy observations that were collected up to and including Week 52. FAS for MRS (FAS_{MRI}) included all subjects that had a paired MRS with pre- and post-treatment measurements. FAS for Liver Biopsy Data (FAS_{Biopsy}): included all patients randomized who underwent the baseline and week 52 biopsies.

Primary efficacy endpoint and principal statistical analysis

The primary endpoint of the study was the absolute % change from baseline to end of study in liver triglycerides to water ratio (fat/water+fat) as measured by MRS. The FAS was used as the primary analysis set for efficacy analysis and inference. The statistical model was a Mixed Model (SAS® MIXED procedure) with random intercept subcommand and REML estimation method was used and degrees of freedom were adjusted using the Kenward-Roger method. The model included the following covariates: treatment group, Country and Geographical Region (CGR), age, sex, Baseline liver fat and Baseline BMI.

Other endpoints analyses

Analyses of biopsy derived endpoints used the baseline adjusted logistic regression (SAS® LOGISTIC procedure) stratified by CGR using the STRATA subcommand with the following effects: treatment group, Baseline CRN Fibrosis Score and NAS score was used to test the between the active groups and placebo contrasts.

The statistical model used for the analyses of change in baseline for laboratory derived endpoints was a Mixed Model for Repeated Measures (MMRM) (SAS® MIXED procedure with REPEATED subcommand). The model included the following fixed effects: categorical week in trial by treatment interaction, CGR and Baseline value using the unstructured covariance structure and the REML estimation method and degrees of freedom were adjusted using the Kenward-Roger method. When there were no repeated measures, analysis of covariance was used (NFS, FLI, Adiponectin, hs-CRP and ELF)

Data Availability

The data that supports the findings of this study are owned by Galmed Research and Development Ltd. (“Galmed”) and contains potentially identifying or sensitive patient information as it includes, among others, human research participant data. Therefore, the data

are not publicly available due to patients' right of privacy and confidentiality as well as ethical and commercial limitations imposed on Galmed. Upon request, Galmed will consider sharing certain data sets, in accordance with applicable local laws as well as patient consent. The data sharing request should include the type of data requested, the reason the data is requested and the intended use of the data.

Role of the funding source

This study was sponsored by Galmed Pharmaceuticals. The protocol was written by a panel of experts and sponsor representatives. Authors participated actively in drafting and reviewing

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the manuscript. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

References for the Methods Section

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