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

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Nonalcoholic steatohepatitis (NASH), 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 patients with NASH ($n = 101$, $n = 98$ and $n = 48$ in the Aramchol 400 mg, 600 mg and placebo arms, respectively; NCT02279524). The primary end point was a decrease in hepatic triglycerides by magnetic resonance spectroscopy at 52 weeks with a dose of 600 mg of Aramchol. Key secondary end points included liver histology and alanine aminotransferase (ALT). Aramchol 600 mg produced a placebo-corrected decrease in liver triglycerides without meeting the prespecified significance (−3.1, 95% confidence interval (CI) −6.4 to 0.2, $P = 0.066$), precluding further formal statistical analysis. NASH resolution without worsening fibrosis was achieved in 16.7% (13 out of 78) of Aramchol 600 mg versus 5% (2 out of 40) of the placebo arm (odds ratio (OR) = 4.74, 95% CI = 0.99 to 22.7) and fibrosis improvement by ≥1 stage without worsening NASH in 29.5% versus 17.5% (OR = 1.88, 95% CI = 0.7 to 5.0), respectively. The placebo-corrected decrease in ALT for 600 mg was −29.1 IU l^{-1} (95% CI = −41.6 to −16.5). Early termination due to adverse events (AEs) was <5%, and Aramchol 600 and 400 mg were safe, well tolerated and without imbalance in serious or severe AEs between arms. Although the primary end point of a reduction in liver fat did not meet the prespecified significance level with Aramchol 600 mg, 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 are 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, which is largely driven by rising rates of obesity and type 2 diabetes (T2D)[1]. 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, T2D (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 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; 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 coenzyme A (acetyl-CoA) or fatty acid synthase. SCD1 catalyzes the rate-limiting step in the biosynthesis of monounsaturated fatty acids[4]. In rodents, downregulation of SCD1 reduced body adiposity, increased energy expenditure and upregulated expression of several genes encoding enzymes of fatty acid beta-oxidation in the liver[5]. Reduction of SCD1 is also known to elevate 5′ adenosine monophosphate-activated protein kinase (AMPK) activity and enhance insulin sensitivity[6]. In hepatic stellate cells (HSCs), direct

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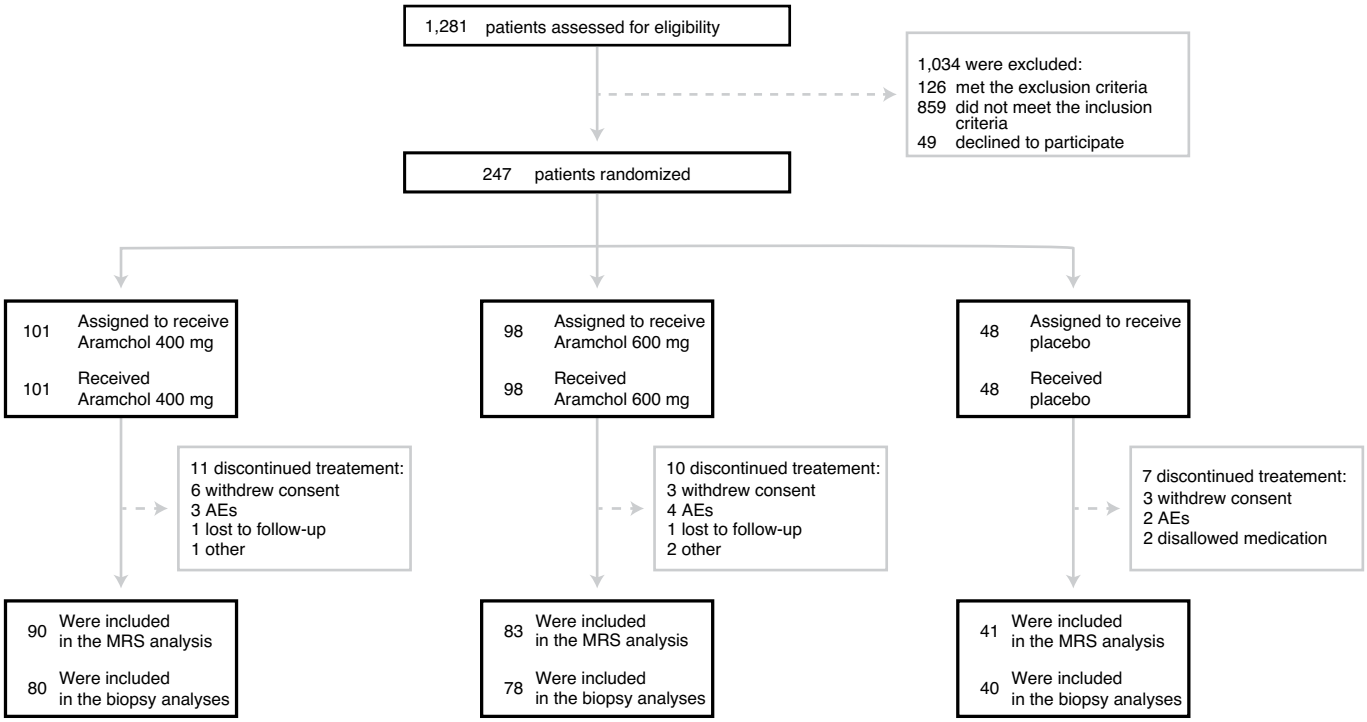

Fig. 1 | Trial patient disposition. Patient disposition, including reasons for trial discontinuation.

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] that partially inhibits hepatic SCD1 protein expression and reduces liver triglycerides[10,11] and fibrosis in animal models of steatohepatitis or fibrosis[12,13]. In HSCs, Aramchol downregulates 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 small interfering RNA (siRNA) phenocopies the inhibitory effects of Aramchol on HSC fibrogenesis[7]. In a 12-week phase 2a trial, Aramchol at 300 mg daily markedly reduced liver fat content as measured by magnetic resonance spectroscopy (MRS) versus placebo in a dose-dependent manner[14]. Aramchol was 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 Aramchol for the REsolution of STeatohepatitis (ARREST) in patients with NASH confirmed by liver biopsy. In this article, we report the safety and efficacy results of 52 weeks of treatment with 400 and 600-mg doses of Aramchol in patients with NASH.

Results

Study population. Between 29 April 2015 and 27 February 2017, 247 patients with NASH were randomized at a ratio of 2:2:1 to receive Aramchol 400 mg ($n=101$), Aramchol 600 mg ($n=98$) 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 the Methods). Figure 1 shows the disposition of patients in the trial including reasons for trial discontinuation. Most study patients (219 out of 247; 88.7%) completed 65 weeks in the study: 90 out of 101 (89%), 88 out of 98 (90%) and 41/48 (85%) in the Aramchol 400 mg, 600 mg and placebo arms, respectively.

Baseline demographics and disease characteristics were balanced across study arms (Table 1). Mean age was 54.4 years, 160 out of 247 (65%) of trial participants were females, 156 out of 247 (63%) were White and 78 out of 247 (32%) Hispanic and Latin. As per the 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 m^{-2}; minimum 25 kg m^{-2}; maximum 42.7 kg m^{-2}). Drug-treated T2D was present in 170 out of 247 (69%) of participants, hypertension in 135 out of 247 (55%) and dyslipidemia in 132 out of 247 (53.4%). Normal ALT and aspartate aminotransferase (AST) were seen in 107 out of 247 (43.3%) and 138 out of 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 400 mg, 600 mg and placebo arms, respectively). Most patients had histologically significant or advanced fibrosis with stage 2 and 3 (149 out of 247, 60%) and active steatohepatitis (NAFLD activity score (NAS) ≥5) in 173 out of 247 (70%). Seven patients had stage 0 fibrosis. The 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 patients with stage 3 fibrosis in the 400-mg 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 400 mg, 600 mg and placebo arms, respectively).

Efficacy analyses. *Hepatic fat reduction by imaging.* A total of 214 patients had paired MRS and were included in the full analysis set (FAS) magnetic resonance imaging (MRI) analysis set ($n=90$, $n=83$ and $n=41$ in the Aramchol 400 mg, 600 mg and placebo arms, respectively (Fig. 1)). The analysis of the primary end point was performed in the FAS MRI dataset as prespecified. Hepatic triglyceride (%) measured by MRS was reduced in the Aramchol 600 mg (−3.2, 95% CI = −5.2 to −1.2) versus 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, $P=0.066$) (Table 2). Therefore, no further formal hierarchical statistical comparisons

Table 1 | Demographic and baseline characteristics

	Placebo $n=48$	Aramchol 400 mg $n=101$	Aramchol 600 mg $n=98$
Demographics			
Age, years	54.4 ± 10.3	53.9 ± 10.9	54.9 ± 9.8
Sex, *n* (%)			
Male	23 (48)	36 (36)	28 (29)
Ethnicity, *n* (%)			
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, *n* (%)	24 (50)	53 (52.5)	58 (59.2)
Dyslipidemia, *n* (%)	30 (62.5)	57 (56.4)	45 (45.9)
Drug-treated T2D (%)	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^{-1}	6.55 ± 1.9	6.56 ± 1.5	6.94 ± 2.4
HbA1c, %	6.53 ± 1.0	6.56 ± 0.9	6.65 ± 1.0
HOMA-IR, U	10.0 ± 8.7	9.1 ± 6.5	9.6 ± 6.5
Lipids			
Cholesterol, mmol l^{-1}	4.93 ± 1.4	4.64 ± 1.1	4.88 ± 1.1
High-density lipoprotein cholesterol, mmol l^{-1}	1.18 ± 0.3	1.17 ± 0.3	1.21 ± 0.3
Low-density lipoprotein direct, mmol l^{-1}	3.09 ± 1.1	2.86 ± 1.0	3.04 ± 0.9
Triglycerides, mmol l^{-1}	1.93 ± 1.4	1.98 ± 1.0	1.92 ± 1.6
Liver enzymes			
ALT, IU l^{-1}	67.0 ± 47.2	67.7 ± 48.2	55.7 ± 37.8
AST, IU l^{-1}	47.6 ± 29.9	50.9 ± 39.9	42.0 ± 25.6
γ-Glutamyltransferase, IU l^{-1}	62.9 ± 45.0	60.6 ± 56.3	68.2 ± 91.8
Alkaline phosphatase, IU l^{-1}	88.9 ± 27.3	85.5 ± 30.4	84.2 ± 28.9
Total bilirubin, μmol l^{-1}	9.52 ± 5.3	9.21 ± 5.3	9.23 ± 5.9
Chemistry			
Albumin, g l^{-1}	45.35 ± 2.5	45.75 ± 2.6	45.41 ± 2.8
Creatinine, μmol l^{-1}	71.8 ± 13.8	68.2 ± 14.8	66.1 ± 15.3
Estimated glomerular filtration rate (modification of diet in renal disease), ml Mn Sa^{-1}	89.0 ± 17.5	92.0 ± 21.0	93.2 ± 21.1
Hematology and coagulation			
International normalized ratio	1.06 ± 0.1	1.05 ± 0.1	1.04 ± 0.1
Prothrombin time, s	10.9 ± 0.8	10.9 ± 0.9	10.8 ± 0.9
Hemoglobin, G L^{-1}	143.4 ± 16.0	142.3 ± 13.3	141.9 ± 13.0
Hematocrit, l l^{-1}	0.46 ± 0.1	0.46 ± 0.1	0.46 ± 0
Platelets, 10^9 l^{-1}	224.1 ± 55.5	236.1 ± 70.6	234.2 ± 67.5
White blood cells, 10^9 l^{-1}	6.29 ± 1.7	6.81 ± 1.7	6.79 ± 1.9
Concomitant medication use			
Lipid-modifying agents, *n* (%)	22 (45.8)	44 (43.6)	34 (34.7)
Antihyperglycemic drugs, *n* (%)	35 (72.9)	69 (68.3)	66 (67.3)
Vitamin E (NOS), *n* (%)	0	1 (1.0)	2 (2.0)

Continued

Table 1 | Demographic and baseline characteristics (continued)

	Placebo $n=48$	Aramchol 400 mg $n=101$	Aramchol 600 mg $n=98$
MRS evaluations			
Liver fat MRS %[a]	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)	2.0 (0)	2.0 (0)
Fibrosis stage, median	1.5 (2.0)	2.0 (2.0)	2.0 (2.0)
Stage 2 fibrosis, %	16.7	18.8	22.4
Stage 3 fibrosis, %	33.3	47.5	36.7

Data are *n* (%) or mean ± s.d. [a]FAS MRI.

Table 2 | Change in MRS and histology-based end points after 52 weeks of treatment

				Difference when compared to placebo		OR and 95% CI	
	Placebo	**Aramchol 400 mg**	**Aramchol 600 mg**	**Aramchol 400 mg**	**Aramchol 600 mg**	**Aramchol 400 mg**	**Aramchol 600 mg**
Primary outcome							
Number of patients with paired MRI evaluations	41	90	83				
Absolute percentage 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% $P=0.066$		
Percentage of MRS responders[a]	24.4	36.7	47.0			2.20 (0.89 to 5.46) $P=0.088$	2.77 (1.12 to 6.89) $P=0.028$
Changes in histopathological parameters from baseline							
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 to 9.62) $P=0.50$	4.74 (0.99 to 22.66) $P=0.051$
Fibrosis improvement without worsening of NASH, %	17.5	21.3	29.5			1.11 (0.40 to 3.05) $P=0.84$	1.88 (0.7 to 5.04) $P=0.21$
Two or more 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 to 3.80) $P=0.56$	1.68 (0.62 to 4.57) $P=0.31$
Two or more points improvement in SAF activity score without worsening of fibrosis, %	25.0	25.0	35.9			1.08 (0.44 to 2.63) $P=0.86$	1.84 (0.78 to 4.35) $P=0.16$

Data is presented as the percentage of patients meeting the end point or as mixed model-derived least squares mean ± s.e.m.; *P* values beyond the primary end point are nominal. [a]Posthoc analysis: responder is defined according to ≥5% absolute improvement from baseline.

were performed. As prespecified, the remaining statistical comparisons of predefined key secondary and exploratory end points 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. A total of 198 patients had paired liver biopsies ($n=80$, $n=78$ and $n=40$ in the Aramchol 400 mg, 600 mg and placebo arms, respectively; Fig. 1) and were included in the predefined FAS biopsy analysis set. There was no statistical evidence of an imbalance across arms in the proportion of patients without a pair of biopsies (21 out of 101 (20.8%), 20 out of 98 (20.4%) and 8/48

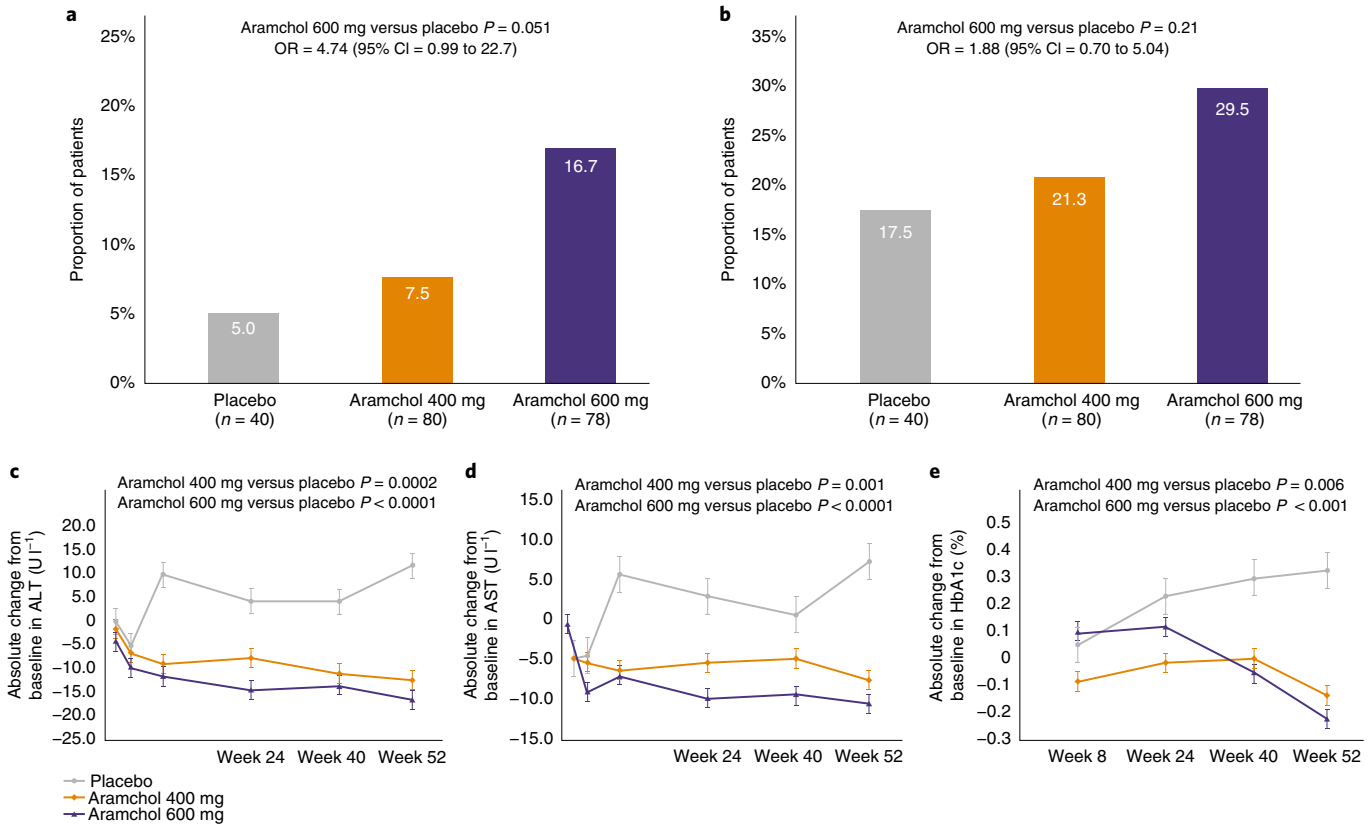

Fig. 2 | Histological and biochemical efficacy results. a, Analyses of biopsy-derived end points used the baseline-adjusted logistic regression to test the Aramchol to placebo contrast. The proportion of patients with NASH resolution without worsening of fibrosis is shown. **b**, Proportion of patients with fibrosis improvement without worsening of NASH. **c**–**e**, Repeated-measures ANCOVA absolute change from baseline in ALT ($U l^{-1}$) (**c**), AST (**d**) and HbA1c (%) (**e**); model-adjusted means (±s.e.m.) of absolute change from baseline during treatment (ALT and AST: $n = 100$, $n = 98$ and $n = 47$ for Aramchol 400 mg, Aramchol 600 mg and placebo, respectively, and HbA1c: $n = 98$, $n = 96$ and $n = 47$) for up to 52 weeks. Two-sided nominal P values beyond the primary end point.

(16.7%) in the Aramchol 400 mg, 600 mg and placebo arms, respectively). The effect of Aramchol on key prespecified histological end points with corresponding nominal P values are shown in Table 2. NASH resolution without worsening of fibrosis was achieved in 16.7% (13 out of 78) of the 600-mg arm versus 5% (2 out of 40) in the placebo arm (OR = 4.74; 95% CI = 0.99 to 22.7; $P = 0.051$; Fig. 2). Improvement in fibrosis by 1 stage or more without worsening of steatohepatitis was observed in 29.5% (23 out of 78) of the 600-mg arm versus 17.5% (7 out of 40) for the placebo arm (OR = 1.88; 95% CI = 0.7 to 5.0; $P = 0.21$; Fig. 2).

Biochemical and metabolic changes. The results for the prespecified key secondary end point of mean change from baseline in ALT (least squares means (LSM)) are shown in Table 3. ALT was reduced in the Aramchol 600 mg arm ($-17.29 \pm 3.7\ IU l^{-1}$, 95% CI = −24.6 to −10.0) versus placebo ($+11.77 \pm 5.2\ IU l^{-1}$, 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 Aramchol 400 mg arm ($-12.0 \pm 3.6\ IU l^{-1}$, 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 end points. At week 52, both doses of Aramchol showed a decrease in HbA1c while patients who received the placebo showed an increase, despite no notable changes in antidiabetic medications in any of the three arms. HbA1c (%) was reduced in the in Aramchol 600 mg (-0.13 ± 0.08, 95% CI = −0.3 to 0.02) versus 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 400-mg arm (-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 600 and 400 mg arms versus placebo but without changes in the homeostatic model assessment of insulin resistance (HOMA-IR). At week 52, there were no discernible changes for other biochemical parameters including lipid parameters. Mean body weight did not change markedly: placebo-subtracted differences were −1.15 kg in the 400-mg arm and −0.41 kg in the 600-mg arm (Table 3).

The FIB4 score and NAFLD fibrosis score (NFS), clinical and laboratory parameter-based scores associated with liver fibrosis in NASH, decreased at week 52 in the Aramchol arms while patients who received the placebo showed an increase. For FIB4, 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 the 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, C-reactive protein (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 AEs were reported in 8.9% (9 out of 101), 9.2% (9 out of 98) and (6 out of 48)

Table 3 | Changes from baseline to week 52 in liver- and disease-related parameters

	Placebo	Aramchol 400 mg	Aramchol 600 mg
Liver enzymes			
Number of patients	47	100	98
ALT (U l^{-1}) change from baseline to week 52	11.77 ± 5.24	−12.00 ± 3.62	−17.29 ± 3.72
Difference when compared to placebo		−23.76 ± 6.32	−29.06 ± 6.37
P		*P* = 0.0002	*P* < 0.0001
AST (U l^{-1}) change from baseline to week 52	6.68 ± 3.50	−7.21 ± 2.42	−10.83 ± 2.49
Difference when compared to placebo		−13.88 ± 4.21	−17.50 ± 4.24
P		*P* = 0.0011	*P* < 0.0001
Alkaline phosphatase (U l^{-1}) change from baseline to week 52	11.64 ± 4.55	−3.41 ± 3.15	−3.76 ± 3.24
Difference when compared to placebo		−15.06 ± 5.52	−15.40 ± 5.57
P		*P* = 0.0068	*P* = 0.0061
γ-glutamyl transpeptidase (U l^{-1}) change from baseline to week 52	+66.03 ± 22.74	−1.23 ± 15.71	−15.18 ± 16.18
Difference when compared to placebo		−67.25 ± 27.62	−81.21 ± 27.89
P		*P* = 0.016	*P* = 0.0040
Total bilirubin (μmol l^{-1}) change from baseline to week 52	+0.50 ± 0.45	−0.17 ± 0.32	−0.31 ± 0.32
Difference when compared to placebo		−0.67 ± 0.55	−0.81 ± 0.55
P		*P* = 0.22	*P* = 0.14
Lipids			
Number of patients	47	100	98
Total cholesterol (mmol l^{-1}) change from baseline to week 52	+0.08 ± 0.11	+0.11 ± 0.07	+0.11 ± 0.08
Difference when compared to placebo		0.02 ± 0.13	0.03 ± 0.13
P		*P* = 0.85	*P* = 0.84
LDL cholesterol (mmol l^{-1}) change from baseline to week 52	0.24 ± 0.10	0.26 ± 0.07	0.18 ± 0.07
Difference when compared to placebo		0.02 ± 0.12	−0.06 ± 0.12
P		*P* = 0.85	*P* = 0.62
HDL cholesterol (mmol l^{-1}) change from baseline to week 52	−0.02 ± 0.03	−0.04 ± 0.02	−0.02 ± 0.02
Difference when compared to placebo		−0.02 ± 0.03	0.004 ± 0.030
P		*P* = 0.49	*P* = 0.89
Triglycerides (mmol l^{-1}) change from baseline to week 52	+0.08 ± 0.13	+0.04 ± 0.09	0.16 ± 0.09
Difference when compared to placebo		−0.04 ± 0.16	+0.07 ± 0.16
P		*P* = 0.78	*P* = 0.64
Metabolic factors			
Number of patients	*n* = 47	*n* = 99	*n* = 96
Glucose, mmol l^{-1} change from baseline to week 52	+0.54 ± 0.26	+0.10 ± 0.18	+0.01 ± 0.18
Difference when compared to placebo		−0.44 ± 0.31	−0.53 ± 0.31
P		*P* = 0.16	*P* = 0.094
Number of patients	*n* = 47	*n* = 98	*n* = 96
HbA1c (%) change from baseline to week 52	+0.32 ± 0.11	−0.04 ± 0.08	−0.13 ± 0.08
Difference when compared to placebo		−0.36 ± 0.13	−0.45 ± 0.13
P		*P* = 0.0061	*P* = 0.0008
Number of patients	*n* = 47	*n* = 99	*n* = 98
Weight (kg) change from baseline to week 52	−0.11 ± 0.59	−1.26 ± 0.41	−0.52 ± 0.42
Difference when compared to placebo		−1.15 ± 0.71	−0.41 ± 0.71
P		*P* = 0.11	*P* = 0.56
Number of patients	*n* = 46	*n* = 98	*n* = 98
Waist circumference (cm) change from baseline to week 52	−1.81 ± 1.30	−2.23 ± 0.89	−0.63 ± 0.90
Difference when compared to placebo		−0.41 ± 1.55	1.19 ± 1.56

Continued

Table 3 | Changes from baseline to week 52 in liver- and disease-related parameters (continued)

	Placebo	Aramchol 400 mg	Aramchol 600 mg
P		*P* = 0.79	*P* = 0.45
Biomarkers			
Number of patients	*n* = 46	*n* = 100	*n* = 95
FIB4 change from baseline to week 52	+0.17 ± 0.08	−0.05 ± 0.06	−0.10 ± 0.06
Difference when compared to placebo		−0.21 ± 0.10	−0.27 ± 0.10
P		*P* = 0.033	*P* = 0.008
Number of patients	*n* = 42	*n* = 91	*n* = 89
NFS change from baseline to week 52	+0.23 ± 0.11	−0.12 ± 0.08	−0.04 ± 0.08
Difference when compared to placebo		−0.35 ± 0.13	−0.27 ± 0.13
P		*P* = 0.0080	*P* = 0.038
Number of patients	*n* = 44	*n* = 95	*n* = 95
FLI change from baseline to week 52	0.59 ± 1.51	−2.01 ± 1.04	−1.07 ± 1.06
Difference when compared to placebo		−2.60 ± 1.80	−1.66 ± 1.80
P		*P* = 0.15	*P* = 0.36

Results of baseline-adjusted MMRM LSM ± s.e.m. 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 end point was used when testing between active groups and placebo contrasts.

12.5% patients in the 400 mg, 600 mg 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 out of 101), 10.2% (10 out of 98) and 14.6% (7 out of 48) in the 400 mg, 600 mg 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 patients in the 400 mg, 600 mg 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 400 mg, 600 mg and placebo arms, respectively). A higher incidence of urinary tract infections (UTIs) was noted in both Aramchol arms, 14.9%, 13.3% and 6.3% in the 400 mg, 600 mg and placebo arms, respectively ($P = 0.13$ and $P = 0.20$ for 400 mg and 600 mg versus placebo).

These were mostly single and mild events occurring in postmenopausal women with diabetes. 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 versus placebo). Pruritus events were mostly mild; none was severe and none led to treatment discontinuation.

Posthoc analyses. Posthoc 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 out of 83) for the 600-mg arm, 36.7% (33 out of 90) for the 400-mg arm and 24.4% (10 out of 41) for the placebo arm (Table 2). Results for a 30% relative reduction were: 30.1% (25 out of 83) in the 600-mg arm; 25.6% (23 out of 90) in the 400-mg arm and 14.6% (6 out of 41) in the placebo arm.

Several additional posthoc histological end points were analyzed to further characterize the effects of Aramchol. Progression to cirrhosis occurred in only one patient (1.3%) in the 600-mg arm, 6 patients (7.5%) in the 400-mg arm and 3 patients (7.5%) in the placebo arm. Hepatocyte ballooning improved by 1 grade or more in the 600-mg arm in 64% (50 out of 78) of patients versus 45% (18 out of 40) in the placebo arm (OR = 2.38, 95% CI = 1.1 to 5.2; $P = 0.032$) but not in the 400-mg arm (50% (40 out of 80) of patients, OR = 1.5, 95% CI = 0.7 to 3.2; $P = 0.3$). A larger proportion of patients no longer had hepatocyte ballooning (grade 0) in the Aramchol 600-mg arm versus placebo (50% (39 out of 78) versus 35% (14 out of 40), OR = 2.3, 95% CI = 1.0 to 5.2; $P = 0.0484$) but not in the 400-mg arm (37.5% (30 out of 80) OR = 1.4, 95% CI = 0.6 to 3.2; $P = 0.43$).

Reductions in AST were documented in the Aramchol 600-mg arm (−10.83 ± 2.5 IU l^{-1}, 95% CI = −15.7 to −6.0) versus placebo (+6.68 ± 3.5 IU l^{-1}, 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 400-mg arm (−7.21 ± 2.4 IU l^{-1}, 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 versus 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 versus 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 ($P = 0.066$), while for Aramchol 400 mg daily, the nominal *P* value versus 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 reported 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 posthoc responder analysis based on this cutoff demonstrated a stepwise increase in response from placebo to 400 mg to 600 mg of 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,

Table 4 | Safety and tolerability data

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

a pegylated human fibroblast growth-factor 21 analog[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 is 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 end points 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 other trials[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 end points, 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 1 stage or more was numerically higher in the 600 mg arm than in the placebo arm, without reaching statistical significance. This trial was not powered for histological end points, which were the key secondary end points. 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 end points suggests that Aramchol may improve NASH. Also, these results argue that while the 400-mg dose 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 300 mg twice daily) 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, which was maintained throughout the treatment period. Aminotransferase reduction was observed each time histology improved in placebo-controlled trials of NASH[18,25,26,30,31]. The mean absolute change of $-17\,IU\,l^{-1}$ 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]. γ-Glutamyltransferase declined in a pattern similar to ALT.

Two well-validated serum fibrosis markers, FIB4 and NFS, although not ELF, were also reduced in the high-dose Aramchol arm versus 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, on 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 SCD1 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 adipose tissue[36]. In rodents, SCD1 genetic inactivation results in resistance to diet-induced weight gain, fat accumulation and dyslipidemia[37]. Specifically, SCD1 is strongly induced in the liver on 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 and 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 downregulates 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 since study participants had either T2D or prediabetes[38]. Because T2D is a major comorbidity associated with more severe forms of NASH and higher potential for disease progression, optimal control of 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, the 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 improved insulin sensitivity. Whole-body SCD1 knockout rodents display improved insulin signaling[40] and increased GLUT4 and GLUT2 expression in skeletal muscle and hepatocytes[41] mainly mediated through a reduction in palmitoleate and oleate and in ceramide synthesis[42]. However, larger studies in humans are 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 disulfide ratio and displayed less inflammation and 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 HSCs[7]. Direct SCD1 depletion using siRNA results in downregulation of fibrogenesis, that is, reduction of collagen 1A1 and alpha smooth muscle actin production by HSCs[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 postmenopausal women. While there is no apparent explanation for this finding, given the inclusion criteria of prediabetes or T2D and the occurrence of UTI mainly in postmenopausal 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 end points 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 the 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 (I148M) of the *PNPLA3* gene, which is associated with a 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 prediabetes or T2D.

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 end point for statistical significance. However, the totality of the data based on prespecified key secondary end points, exploratory analyses and posthoc analyses suggest a potential for improving liver histology in patients with T2D or prediabetes with histologically confirmed steatohepatitis and with high disease activity and precirrhotic 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 (NCT04104321).

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-021-01495-3.

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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 18–75 years, with histological evidence of steatohepatitis, an NAS ≥ 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 (BMI = 25–40 kg m^{-2}) or increased waist circumference (88–200 cm for women and 102–200 cm for men); known T2D or prediabetes according to the criteria of the American Diabetes Association[38,47] or HbA1c > 5.7%; liver fat content ≥5.5% on screening MRS; and normal synthetic liver function (serum albumin >3.2 g dl^{-1}, international normalized ratio 0.8–1.2 and conjugated bilirubin <35 μmol l^{-1}). Patients with diabetes or prediabetes 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 (stage 4 fibrosis), daily alcohol intake >20 g day^{-1} for women and >30 g day^{-1} 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 before randomization, uncontrolled arterial hypertension, uncontrolled hypothyroidism, diabetes mellitus other than T2D, treatment with antidiabetic medications, unless started before biopsy (6–12 months depending on drug) and stable and treatment with predefined disallowed medications that may cause or treat NASH. A complete list of inclusion and exclusion criteria is provided in the Supplementary Methods. All patients provided written informed consent before any study-related activities. The study protocol was approved by the ethics committees at participating centers or by a national ethics committee in accordance with local laws and regulations. Institutional review board or ethics committees that reviewed and approved this study included: Schulman Associates institutional review board in the US; CPP Ile-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 (NCT02279524).

Randomization and blinding. Eligible patients were randomly assigned in a 2:2:1 ratio (48 blocks) to receive either daily Aramchol 400 mg, Aramchol 600 mg or placebo orally for 52 weeks. The randomization ratio was 2:2:1 stratified by country. The randomization list was generated before 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 900 mg Aramchol and repeat daily doses of 600 mg once daily showed subproportional increases in exposure with dose where once daily doses of >600 mg were 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, patients 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). Based on clinical research laboratory cutoffs, normal ALT was <45 IU L^{-1} and normal AST was <41 IU l^{-1}.

Data were collected using the electronic data capture system eCaseLink v.8.0 (DSG).

MRS evaluation. Patients were required to undergo 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 by a specialized radiologist masked to treatment allocation (D.B.B.).

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 (C.L.) 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[2,47,48] for steatosis (scored 0–3), inflammation (scored 0–3) and hepatocellular ballooning (scored 0–2). Fibrosis was evaluated using the NASH CRN fibrosis staging system (stages 1–4)[2,48]. Biopsies were also scored based on the steatosis, activity and fibrosis (SAF) algorithm[3,49].

Outcomes. The primary end point 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 end points were: proportion of individuals 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 individuals with ≥1 stage fibrosis improvement without worsening of NASH (defined by any increase in inflammation or ballooning grade); proportion of individuals with a ≥2 point NAS improvement (contributed by at least two of steatosis, inflammation, ballooning) without worsening of fibrosis; proportion of individuals with a ≥2 point reduction in SAF activity score without worsening of fibrosis; and baseline-adjusted mean change from baseline to week 52/termination in ALT (IU l^{-1}) levels.

Exploratory end points included anthropometric and glycemic parameters, potential biomarkers of NASH and fibrosis (FIB4, NFS, FLI), markers of inflammation (fibrinogen, CRP) and adiponectin.

Safety and tolerability were evaluated based on treatment-emergent serious AEs; AEs; safety laboratory; vital signs; 12-lead electrocardiogram; 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 posthoc 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 AST and normalization of ALT and AST) as well as change from baseline in 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 end point between the active groups and placebo with a 5% significance level and 89% power. Based on an expected dropout rate of 10%, the total sample size was 240.

Significance level and multiplicity adjustment. One primary end point and five secondary end points were predefined. The overall experiment-wise significance level was 5% using two-tailed tests with the hierarchical gatekeeping approach to control the overall type I error rate for multiple contrasts and multiple end points (Supplementary Table 1; order of testing for contrasts). According to the gatekeeping approach, the first contrast (600 mg versus placebo in the primary end point) was tested using a two-tailed 5% significance level. If the first contrast failed to reach statistical significance, all P values reported, as per SAP, were nominal P values.

Predefined analyses sets. FAS included all patients randomized and who had baseline and at least one postbaseline efficacy assessment. The FAS analysis set included efficacy observations that were collected up to and including week 52. FAS for MRS (FAS MRI) included all patients that had a paired MRS with pretreatment and posttreatment measurements. FAS for the liver biopsy data (FAS biopsy) included all patients randomized who underwent the baseline and week 52 biopsies.

Primary efficacy end point and principal statistical analysis. The primary end point of the study was the absolute percentage change from baseline to end of study in liver triglycerides to water ratio (fat/water + fat) as measured by MRS. 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; restricted maximum likelihood (REML) estimation was used and d.f. 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 end point analyses. Analyses of the biopsy-derived end points 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, to test the contrasts between the active groups and contrasts with placebo.

The statistical model used for the analyses of change from baseline for laboratory-derived end points was an 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 REML estimation method; and d.f. were adjusted using the Kenward–Roger method. When there were no repeated measures, analysis of covariance (ANCOVA) was used (NFS, FLI, adiponectin, high-sensitivity CRP and ELF).

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data supporting the findings of this study are owned by Galmed Research and Development Ltd. (Galmed) and contains potentially identifying or sensitive patient information since it includes, among others, human research participant data. Therefore, data are not publicly available due to patients' right of privacy and confidentiality as well as ethical and commercial limitations imposed on Galmed. On request, Galmed will consider sharing certain datasets in accordance with applicable local laws as well as patient consent. Data sharing requests should include the type of data requested, the reason the data is requested and the intended use of the data.

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Author contributions

V.R. designed the study, was responsible for patient recruitment and treatment, data analysis and interpretation, is a member of the study advisory committee and wrote the manuscript. L.d.G. was a principal investigator responsible for patient recruitment and treatment and is a member of the study advisory committee. R.S., F.P., F.F., A.L.F. and J.F-F. recruited and treated patients. M.A. recruited and treated patients and is a member of the study advisory committee. D.B.B. performed the Central MRI Reading. K.L. performed the Central Pathology Reading. T.G. helped with the data analysis and interpretation and manuscript writing. S.K. helped with the statistical analysis plan, data analysis and interpretation. R.O. helped with study design, data review and interpretation. M.H. helped with study planning and design. L.H. helped with the data analysis and interpretation. R.L. helped with patient recruitment and treatment and is a member of the study advisory committee. S.F. helped with data review and interpretation. A.J.S. was the US lead principal investigator, helped with study design, data analysis and interpretation, supported the setup of the MRS Central Reading and is a member of the study advisory committee. Members of the ARREST investigator study group helped in patient recruitment and treatment.

Competing interests

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.B.B. was responsible for central lab services for the Galmed-sponsored study described in the article. K.L. was responsible for the histological analysis services for the Galmed-sponsored study described in the article. S.K. is the Galmed statistician. L.d.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.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-021-01495-3.

Correspondence and requests for materials should be addressed to V. Ratziu.

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☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	DSG eCaseLink system V8.0
Data analysis	SAS® version 9.4

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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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 is 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 shall include the type of data requested, the reason the data is requested and the intended use of the data.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	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.
Data exclusions	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 (FASMRI) included all subjects that had a paired MRS with pre- and post-treatment measurements. FAS for Liver Biopsy Data (FASBiopsy): included all patients randomized who underwent the baseline and week 52 biopsies.
Replication	This clinical study utilized a uniform protocol for all study sites, to ensure reproducibility of results across stues. It was a phase 2b study. A phase 3 study was initiated and is currently ongoing with the aim of verifying the effect of aramchol in NASH.
Randomization	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.
Blinding	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.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	Eligible patients were adults, aged 18 to 75 years, with histological evidence of steatohepatitis, a nonalcoholic fatty liver disease (NAFLD) activity score (NAS) >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/m2- 40kg/m2) 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 Association1 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).
Recruitment	Subjects were recruited by Investigators from their patient populations based on protocol inclusion/exclusion criteria at

Recruitment	participating sites. The predefined eligibility criteria (provided in full in the supplementary materials) were designed to minimize biases.
Ethics oversight	Institutional Review Board (IRB) or Ethics Committees (EC) that reviewed and approved this study in accordance with local laws and regulations included: Schulman Associates IRB in the US, CPP Ile-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 Ethics Committees (EC) at participating centers or by a national EC in accordance with local laws and regulations.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	No. NCT02279524
Study protocol	Submitted with the article
Data collection	The study period was 29 April 2015 to 22 May 2018. Following randomization, patients were evaluated at 9 scheduled visits in the study sites: 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.
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 >1 stage fibrosis improvement without worsening of NASH (defined by any increase in inflammation or ballooning grade); proportion of subjects with a >2 point NAS improvement (contributed by at least two of: steatosis, inflammation, ballooning) without worsening of fibrosis; proportion of subjects with a >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.