Norursodeoxycholic acid versus placebo in the treatment of non-alcoholic fatty liver disease: a double-blind, randomised, placebo-controlled, phase 2 dose-finding trial

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Summary

Background Norursodeoxycholic acid is an orally administered side chain-shortened homologue of ursodeoxycholic acid that undergoes hepatic enrichment with hepatoprotective, anti-inflammatory, and antifibrotic activity. We assessed the efficacy of two doses of norursodeoxycholic acid versus placebo for the treatment of non-alcoholic fatty liver disease.

Methods We did a multicentre, double-blind, placebo-controlled, randomised, phase 2 dose-finding clinical trial in tertiary referral hospitals and medical centres in Austria (n=6) and Germany (n=23) for patients with non-alcoholic fatty liver disease with or without diabetes. Patients with a clinical diagnosis of non-alcoholic fatty liver disease and serum alanine aminotransferase (ALT) concentrations of more than 0·8 times the upper limit of normal were randomly assigned (1:1:1) using a computer-generated central randomisation. Patients were randomly assigned to receive either norursodeoxycholic acid capsules at 500 mg per day or 1500 mg per day, or placebo, for 12 weeks with a subsequent 4-week follow-up period. All individuals involved in the trial were masked to treatment allocation. The primary efficacy endpoint was the mean relative percentage change in ALT concentrations between baseline and end of treatment assessed in the intention-to-treat population. This trial is registered with EudraCT, number 2013-004605-38.

Findings Between March 30, 2015, and Sept 20, 2016, of 198 individuals included in the analysis, 67 patients were randomly assigned to receive 500 mg norursodeoxycholic acid, 67 to 1500 mg norursodeoxycholic acid, and 64 to placebo. A dose-dependent reduction in serum ALT between baseline and end of treatment was observed with norursodeoxycholic acid versus placebo, with a significant effect in the 1500 mg group (mean change –27·8%, 95% repeated CI –34·7 to –14·4; p<0·0001). Serious adverse events (n=6) and treatment-emergent adverse events (n=314) were reported in a similar proportion of patients across groups. 112 treatment-emergent adverse events occurred in the 1500 mg group, 99 in the 500 mg group, and 103 in the placebo group. The most frequent adverse events were headache, gastrointestinal disorders, and infections (eg, diarrhoea, abdominal pain, or nasopharyngitis).

Interpretation Norursodeoxycholic acid at 1500 mg resulted in a significant reduction of serum ALT within 12 weeks of treatment when compared with placebo. Norursodeoxycholic acid was safe and well tolerated encouraging further studies.

Funding Dr Falk Pharma GmbH.

Introduction

The prevalence of non-alcoholic fatty liver disease (NAFLD) in the general population in Europe is 20–30%, with numbers increasing.[1,2] Insulin resistance, oxidative stress, and inflammation play a major role in the pathogenesis of NAFLD and its progression towards non-alcoholic steatohepatitis (NASH)[3,4] with development of advanced stages of fibrosis and cirrhosis, including its complications such as portal hypertension, liver failure, and hepatocellular carcinoma. Few options are available for the therapeutic management of NAFLD, and available treatments are poor. So far, dietary changes and lifestyle modifications, including weight reduction and increase in physical activity, are the mainstay of therapy.[5] To date, no established pharmacological therapy has been approved.[3] Ursodeoxycholic acid, a hydrophilic bile acid, showed few benefits in clinical trials[6–8] although it reduced endoplasmatic reticulum stress, insulin resistance, and fatty liver in diabetic mice.[9] Other bile acid-based strategies target the farnesoid X receptor (FXR), which plays an important role in hepatic glucose and lipid metabolism. These strategies have shown beneficial effects on liver enzymes and histology, although showed deteriorating HOMA index and increasing serum cholesterol profiles in patients with NASH.[10] However, a short-term pilot study in patients with diabetes and NASH showed improved insulin resistance after treatment with an FXR targeting strategy assessed by clamp studies.[11]

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See Online for appendix

Research in context

Evidence before this study

Non-alcoholic fatty liver disease (NAFLD) comprises a disease spectrum ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocellular carcinoma. Both excessive body-mass index and, specifically, visceral obesity are recognised risk factors for NAFLD. An even higher prevalence of NAFLD is observed in individuals with type 2 diabetes. Importantly, NAFLD itself is an independent risk factor for cardiovascular mortality. So far, no drug is approved for the treatment of NAFLD or NASH. Several studies in patients with NASH with pioglitazone, vitamin E (PIVENS trial), liraglutide (a glucagon-like peptide-1 analogue, LEAN trial), cenicriviroc (a dual antagonist of C-C chemokine receptor types 2 and 5, CENTAUR study), selonsertib (an apoptosis signal-regulating kinase-1 inhibitor), elafibranor (a PPARαδ agonist, GOLDEN-505 trial), or obeticholic acid (a farnesoid X receptor agonist, FLINT trial) showed promising results for improvements in the histological features of NASH, including fibrosis. Nevertheless, side-effects and unfavourable metabolic changes seen with some of the drugs might negatively affect patient compliance or long-term outcomes.

We searched for clinical trials on NAFLD or NASH in PubMed, including all publications in English from Jan 1, 1965, to June 1, 2018. We searched "fatty liver", "NAFLD", "NASH" or "steatohepatitis" and "bile acid" or "norursodeoxycholic acid". In 2017, norursodeoxycholic acid has already been shown to be safe, well tolerated, and effective in the treatment of patients with primary sclerosing cholangitis in a phase 2 clinical trial with a dose-dependent effect on liver enzymes and a significant reduction of serum alkaline phosphatase.

In contrast to ursodeoxycholic acid, in which clinical benefits in the treatment of NAFLD and NASH are limited, norursodeoxycholic acid, a side chain-shortened C23 homologue of the C24 bile acid ursodeoxycholic acid, showed promising results in preclinical studies such as a reduction of inflammation, fibrosis, and apoptosis. Norursodeoxycholic acid also improved steatohepatitis in a Nemo knock-out mouse model by downregulating lipogenic and apoptotic pathways. On the basis of these findings, this double-blind, randomised, placebo-controlled, dose-finding, proof-of-concept trial was done as the fourth human exposure study with norursodeoxycholic acid.

Added value of this study

This study resulted in a dose-dependent reduction of alanine aminotransferase with a significant effect at a daily dose of 1500 mg of norursodeoxycholic acid and therefore met the primary endpoint of reduction of serum alanine aminotransferase in patients with NAFLD. In addition, serum aspartate aminotransferase and γ-glutamyltransferase also decreased, whereas alkaline phosphatase and bilirubin concentrations remained stable. In a subset of patients, hepatic fat fraction was measured by MRI and MR-spectroscopy showing a reduction in hepatic fat in the 1500 mg group. While LDL concentrations increased slightly in the first 2 weeks of the trial, remaining stable thereafter, HDL concentrations also increased, while triglyceride concentrations were unchanged. Importantly, norursodeoxycholic acid was safe and well tolerated.

Implications of all the available evidence

Norursodeoxycholic acid seems a promising treatment option in patients with NAFLD or NASH. Long-term data for norursodeoxycholic acid, including histological assessment, are needed to further explore the clinical efficacy in patients with NAFLD or NASH.

24-norursodeoxycholic acid is a synthetic side chain-shortened C23 homologue of ursodeoxycholic acid;[12] notably, neither ursodeoxycholic acid nor norursodeoxycholic acid activate FXR.[9] In contrast to ursodeoxycholic acid, norursodeoxycholic acid is a poor substrate for acyl-coenzyme A synthetase and undergoes only minimal N-acyl amidation with taurine or glycine, which results in cholehepatic shunting leading to hepatic enrichment with norursodeoxycholic acid. It was also found to reduce hepatic steatosis, inflammation, fibrosis, and apoptosis in mouse models of NAFLD or NASH (eg, by downregulating lipogenic and apoptotic pathways).[9,13] Before the current study, phase 1 and 2 studies with norursodeoxycholic acid have shown its safety and efficacy in healthy volunteers and patients with primary sclerosing cholangitis.[14]

The primary objective of this study was to evaluate the efficacy of two doses of norursodeoxycholic acid versus placebo for the treatment of NAFLD with or without type 2 diabetes. The secondary objective was to study the safety and tolerability of norursodeoxycholic acid in the treatment of NAFLD.

Methods

Study design and participants

We did a double-blind, randomised, multicentre, placebo-controlled, comparative, exploratory phase 2 dose-finding trial (figure 1) in study centres in Austria (n=6) and Germany (n=23; see list of all centres in the appendix).

We included men and women aged between 18 and 75 years. NAFLD was defined by at least one of the following criteria: (1) hepatic steatosis on ultrasound (at least stage 1)[15] or other diagnostic imaging such as MRI, chemical shift index, or magnetic resonance spectroscopy (MRS; fat fraction >10%) within the past 4 weeks; (2) at least grade 1 (>10%) steatosis measured in transient elastography by controlled attenuation parameter within the past 4 weeks; or (3) diagnostic histological findings

compatible with NAFLD shown on previous biopsy within the past 5 years. Serum alanine aminotransferase (ALT) concentrations had to be more than 0·8 times the upper limit of normal (ULN) as considered by the central laboratory (women 28 units per L, men 40 units per L). Patients with type 2 diabetes had to be diagnosed by at least one of the American Diabetes Association criteria: random plasma glucose concentration more than 200 mg/dL (11·1 mmol/L), fasting plasma glucose more than 126 mg/dL (7·0 mmol/L), 2 h post-dose glucose more than 200 mg/dL during a 75 g oral glucose tolerance test, or glycated haemoglobin of 6·5% or greater.[16] Women of childbearing potential had to be using a highly effective method of birth control (ie, with a failure rate of <1% per year).

Key exclusion criteria included alcoholic liver disease or other concomitant liver diseases such as hepatitis B or C, primary biliary cholangitis, primary sclerosing cholangitis, Wilson's disease, haemochromatosis, or autoimmune hepatitis. Substantial alcohol consumption was defined as more than 20 g per day in women and more than 30 g per day in men. Patients with type 2 diabetes were excluded if prescribed current or intended therapy with insulin, glucagon-like peptide-1 receptor agonists, or DPP-4 inhibitors, any change in oral antidiabetic or statin treatment within the past 12 months before baseline, or with glycated haemoglobin of 9·5% or more. Other exclusion criteria included: any treatment with ursodeoxycholic acid, high-dose vitamin E (≥400 IU per day), vitamin C (≥500 mg per day), or other antioxidants within 8 weeks before baseline, the presence of cirrhosis of Child–Pugh class B or worse or hepatic decompensation, bilirubin more than twice the ULN unless due to Gilbert's syndrome, aspartate aminotransferase (AST) or ALT concentrations more than four times the ULN, any relevant infectious disease, abnormal renal function, a poorly controlled thyroid dysfunction, any active malignant disease, or history or treatment thereof in the past 5 years.

All patients gave written informed consent. Independent ethics committees responsible for the participating investigators in Hannover, Germany, and Vienna, Austria, approved the study protocol in both countries.

Randomisation and masking

Patients were randomly assigned to oral treatment with either 500 mg per day norursodeoxycholic acid, 1500 mg per day norursodeoxycholic acid, or placebo capsules for the treatment of NAFLD. At randomisation, patients were assigned to a consecutive ascending three-digit random number according to their sequential entrance into the trial. This randomisation number was determined by a computer-generated random code using randomly permuted blocks. The randomisation list was generated using an allocation ratio of 1:1:1. Central randomisation took place in blocks aiming for a well balanced group distribution at each recruitment time. A block size of three was used. A 4-week follow-up phase served to ensure thorough follow-up of safety parameters and indirect signs of efficacy such as rebound after the treatment period. All people involved in the conduct of the study (eg, patients, investigators, data collectors, statisticians, sponsor) were masked until database closure with a masked data review meeting done before unmasking.

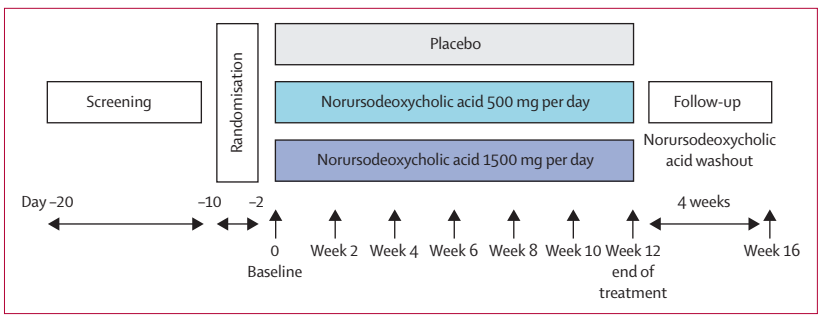

Figure 1: **Study design**

Procedures

Patients in all groups were monitored after randomisation at baseline; interim visits at weeks 2, 4, and 8, end of treatment at week 12; and the follow-up visit at week 16 (figure 1). Vital signs and weight were assessed at all visits. Electrocardiography was done at baseline, week 2, and end of treatment. Laboratory assessments (ie, blood count, liver enzymes, total and conjugated bilirubin, albumin, C-reactive protein, electrolytes, creatinine, cholesterol, and triglycerides) were done at all visits and blood coagulation at screening, baseline, and end of treatment, at a central laboratory (Spranger Laboratories, Ingoldstadt, Germany). Abdominal ultrasound examination and optional transient elastography including controlled attenuation parameter, MRI chemical shift index or MRS, and acoustic radiation force impulse were done at screening and at the end of treatment visit. Steatosis grade 1 in controlled attenuation parameter was defined as 238 db/m or more. NAFLD fibrosis score was calculated at baseline and end of treatment on the basis of published recommendations.[17] To address the influence of apoptosis and necrosis, soluble serum cytokeratin 18-M65 and caspase-cleaved fragment of cytokeratin 18-M30 concentrations were measured at baseline and end of treatment. Tolerability was classified as very good, good, satisfactory, or poor by an investigator and patient independently at end of treatment. Compliance was recorded by counting the investigational medicinal products returned (daily boxes and capsules). Adverse events such as any unfavourable and unintended signs, including an abnormal laboratory finding, symptom, or disease were recorded at each visit. Serious adverse events included death, life-threatening events, new

in-patient hospitalisation or prolongation of existing hospitalisation, events resulting in persistent or substantial disability or incapacity, or unintended pregnancy, congenital anomaly, or birth defect.

Outcomes

The primary objective of the trial was to evaluate the efficacy of 500 mg per day or 1500 mg per day norursodeoxycholic acid against placebo for the treatment of NAFLD. The primary efficacy variable was the mean relative percentage change in serum ALT concentrations between baseline and the end of treatment visit. This result was calculated for each patient as follows:

$$100 \times (ALT_{[end\ of\ treatment]} - ALT_{[baseline]}) / ALT_{[baseline]}$$

Secondary outcomes included the safety and tolerability of norursodeoxycholic acid. Secondary efficacy endpoints were analysed using descriptive statistics. They included the ALT to AST ratio at each study visit, and the absolute and relative percentage changes of the ALT to AST ratio from baseline to each visit up to end of treatment and from end of treatment to the follow-up visit. Further secondary objectives included the course of ALT, AST, alkaline phosphatase, γ-glutamyltransferase (GGT), and serum bilirubin concentrations; the absolute and relative percentage changes of ALT, AST, and serum bilirubin from baseline to each visit up to end of treatment and from end of treatment to the follow-up visit; and the change of hepatic steatosis on ultrasound from screening to end of treatment. Additional secondary outcomes comprised the therapeutic success (response categories 1 or 2 of physician's global assessment) and therapeutic benefit (response categories 1, 2, 3, or 4 of physician's global assessment) according to the physician's global assessment of efficacy at end of treatment.

The following additional secondary endpoints were optional and the assessment was dependent on availability of the measuring instrument: the change of hepatic steatosis measured by controlled attenuation parameter fibroscan between screening and end of treatment, the change of liver stiffness measured by fibroscan or acoustic radiation force impulse between screening and end of treatment, the proportion of patients with reduction of hepatic fat fraction (steatosis) measured by any MRI chemical shift index or MRS by at least 5% between screening and end of treatment.

Statistical analysis

The primary efficacy variable was analysed by the concept of hierarchically ordered hypotheses. The level of significance for each pair-wise comparison was set to 0·025 (one-sided).

The trial was done according to a two-stage group sequential test design with the possibility to adapt the sample size, to stop the trial, or to stop treatment groups following the interim analysis. For confirmatory hypothesis testing (primary analysis) within the interim and the final analysis, the inverse normal method of combining the p values of the normal approximation-test for comparing two rates was used.[18]

For estimating the treatment effect, the difference between the mean relative ALT percentage change from baseline in the respective treatment group and in the placebo group is provided together with the corresponding two-sided 95% repeated CI.

For sample size calculations, a standardised mean difference (effect size) of the mean relative changes of 0·5 was presumed. For example, an effect size of 0·5 is observed, if a mean relative change of –30% is observed in one group and –20% in the other group with an SD of 20%. Sample size calculation was done with ADDPLAN (version 6.0.1), considering the group-sequential adaptive study design. A power of 80% was targeted to detect the assumed treatment effect for at least the first of the hierarchically ordered hypotheses, which yielded a sample size of 65 patients per treatment group (significance level α of 0·025). The adaptive two-stage group sequential tests for the primary efficacy variable were done with the statistical software ADDPLAN14 (version 6.1.1). All remaining statistical analyses were done with SAS (version 9.4).

The null hypotheses were tested against the alternative hypotheses using the concept of hierarchically ordered hypotheses: (1) group A (norursodeoxycholic acid 1500 mg) versus group C (placebo):

$$H_{01}: \mu_A - \mu_C \geq 0, \text{alternative hypotheses} - H_{11}: \mu_A - \mu_C < 0$$

(2) group B (norursodeoxycholic acid 500 mg) versus group C (placebo):

$$H_{02}: \mu_B - \mu_C \geq 0, \text{alternative hypotheses} - H_{12}: \mu_B - \mu_C < 0$$

and for exploratory purposes (3) group A (norursodeoxycholic acid 1500 mg) versus group B (norursodeoxycholic acid 500 mg):

$$H_{03}: \mu_A - \mu_B \geq 0, \text{alternative hypotheses} - H_{13}: \mu_A - \mu_B < 0$$

The hypotheses H_{01} and H_{02} were tested in an a priori order with ordering given above—ie, H_{02} could only be rejected if the test of H_{01} was significant at the local significance level. Therefore, the planned sample size was 195 patients (1:1:1 randomisation) in the full analysis set if the trial was completed without modification following the interim analysis, which was to be done on the basis of 99 patients with a full analysis set. After interim analysis, no modifications to the original design were introduced. The full analysis set included all

randomly assigned patients who received at least one dose of the investigational medicinal product, referred to as the modified intention-to-treat principle.

Missing values of the primary efficacy variable and the secondary efficacy variables, except other variables related to efficacy, at end of treatment were replaced by the last measurement obtained since baseline visit (last observation carried forward).

The primary analysis was the modified intention-to-treat analysis. As a sensitivity analysis, the primary efficacy variable was also evaluated for the per-protocol set. This trial is registered with EudraCT, number 2013-004605-38.

Role of the funding source

Norursodeoxycholic acid and an identical placebo were provided by the funder. The funder was involved in designing the study and in writing the study protocol in collaboration with MT. Collection of data, analysis of data, and writing the study report were done by Gesellschaft für klinisches Monitoring, the contract research organisation, who were also involved in the interpretation of the data together with MT and the funder. MT, ST, and the funder had access to all data including the raw data. Writing of the first draft and submission of the final version of the manuscript were done by ST and MT.

Results

282 patients were screened between March 30, 2015, and Sept 20, 2016. 82 patients could not be randomly assigned, mainly because they did not comply with the inclusion or exclusion criteria (n=59; figure 2). Consequently, 200 patients were randomly assigned and 198 patients took at least one dose of the study medication or placebo. Therefore, both the safety analysis set and the full analysis set comprised 198 patients. On the basis of the decisions of a masked data review meeting done before database closure, 59 patients from the full analysis set were excluded from the per-protocol analysis set (appendix p 4), which finally comprised 139 patients. 185 patients completed the trial (n=60 for 1500 mg, n=64 for 500 mg, n=61 for placebo). The data presented subsequently are the data obtained in the modified intention-to-treat population (data for the per-protocol analysis not shown, see appendix p 4 for the primary outcome parameter).

The treatment groups were similar and well balanced with respect to demographic and other baseline characteristics (table 1). For 182 (92%) of 198 patients with established diagnosis, the median duration of NAFLD was 2·4 years (IQR 0·5–5·8) at baseline. Only 16 (8%) of all 198 patients were newly diagnosed with NAFLD (<4 weeks to baseline). On the basis of the NAFLD fibrosis score, most patients had no significant fibrosis at baseline (table 1). 20 patients had type 2 diabetes, with a slightly higher proportion in the placebo group (table 1).

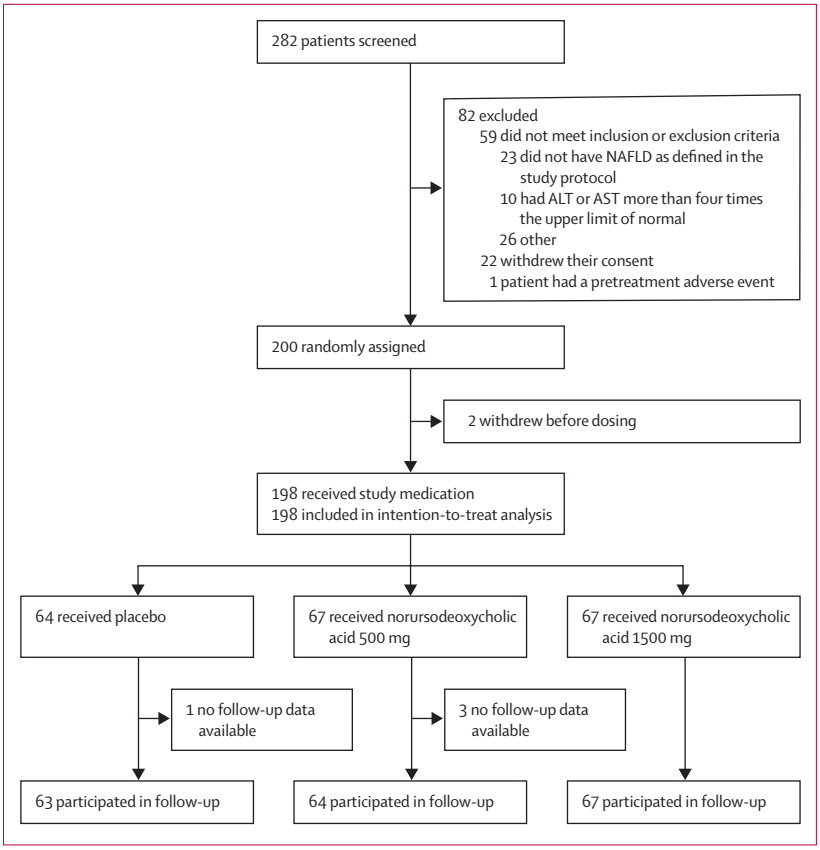

Figure 2: **Trial profile**

A dose-dependent effect of norursodeoxycholic acid versus placebo was observed in the modified intention-to-treat analysis set with respect to the relative change in ALT between baseline and end of treatment, showing a clear effect in the 1500 mg group. The difference between 1500 mg and placebo was significant (mean change –27·8%, 95% repeated CI –34·7 to –14·4, p<0·0001; figure 3).

ALT concentrations decreased with 1500 mg norursodeoxycholic acid by a mean of 17·4%, while with placebo the ALT concentrations increased by 10·4% (figure 3). Although ALT concentrations decreased by 4·2% with 500 mg norursodeoxycholic acid, the difference versus placebo was not significant (–14·6%, 95% repeated CI –20·7 to 3·59; p=0·0905). Mean ALT decreased in the 1500 mg group considerably more on than in the 500 mg group (p<0·0007). Significant differences compared with placebo were also seen in the 1500 mg norursodeoxycholic acid group in the per-protocol analysis (data not shown). Bodyweight change in individual patients did not influence the results of this trial, as shown by a multivariate regression analysis to adjust for individual weight changes (data not shown). In the follow-up visit, ALT values increased from end of treatment for patients given norursodeoxycholic acid (1500 mg: 71·0 units per L [SD 35·9], 500 mg:

	1500 mg group (N=67)	500 mg group (N=67)	Placebo group (N=64)
Sex			
Men	40 (60%)	45 (67%)	38 (60%)
Women	27 (40%)	22 (33%)	26 (41%)
Age (years)	48·9 (12·8)	44·9 (11·6)	48·8 (11·4)
Body-mass index (kg/m²)	29·5 (4·8)	30·6 (5·7)	30·5 (5·3)
Serum alanine aminotransferase (units per L)	78·6 (34·2)	80·3 (33·3)	77·4 (30·2)
Serum aspartate aminotransferase (units per L)	49·3 (25·1)	49·8 (27·4)	51·6 (23·5)
Serum γ-glutamyltransferase (units per L)	178·8 (205·6)	144·7 (160·0)	150·0 (140·8)
Serum alkaline phosphatase (units per L)	92·0 (36·6)	83·9 (27·6)	94·3 (32·1)
Serum bilirubin (mg/dL)	0·61 (0·32)	0·64 (0·40)	0·57 (0·47)
NAFLD diagnosis			
New	5 (8%)	7 (10%)	4 (6%)
Established	62 (93%)	60 (90%)	60 (94%)
NAFLD duration since diagnosis (years; median, IQR)	2·6 (0·6–6·5)	1·8 (0·3–4·4)	3·0 (0·6–5·9)
NAFLD fibrosis score category			
Not significant	51 (80%)	52 (79%)	40 (65%)
Intermediate	13 (20%)	13 (20%)	20 (32%)
Significant	0	1 (2%)	2 (3%)
Missing	3	1 (2%)	2 (3%)
Type 2 diabetes	4 (6%)	6 (9%)	10 (16%)
Hypertension	26 (39%)	21 (31%)	26 (41%)
Obesity	10 (15%)	5 (8%)	6 (9·%)

Data are n (%) or mean (SD) unless otherwise indicated. NAFLD=non-alcoholic fatty liver disease.

Table 1: **Baseline characteristics**

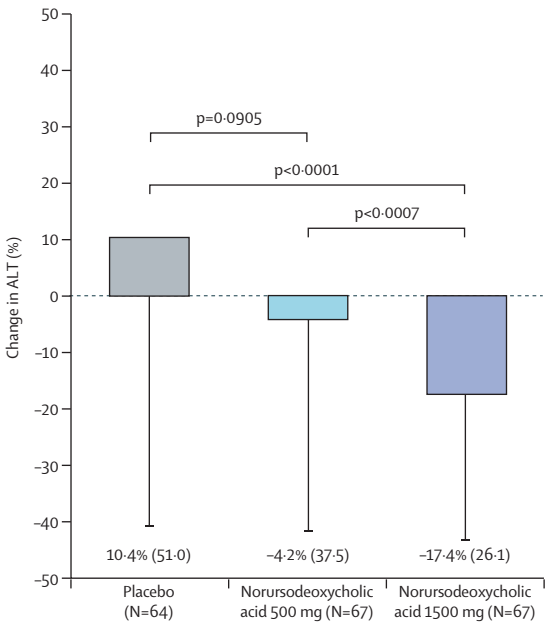

Figure 3: **Relative changes in ALT from baseline to end of treatment**
Values (%, SD) are the relative change of ALT between baseline and end of treatment. Error bars show repeated 95% CIs for difference in means. ALT=alanine aminotransferase.

79·8 units per L [40·5]), but decreased slightly for patients given placebo (79·7 units per L [35·5]).

In the subgroup analysis for men, the mean relative ALT decrease from baseline to end of treatment was highest in the 1500 mg group (–20·1%) and similar between 500 mg (–3·5%) and placebo (0·2%). For women, a more distinct dose-dependent effect was observed (–13·5% for 1500 mg, –5·8% for 500 mg, 25·4% for placebo). Further results of the subgroup analysis are shown in the appendix (p 4).

Patients with diabetes in the 1500 mg group had a similar mean relative decrease in ALT values (–21·1%) compared with patients without diabetes (–17·2%). By contrast, the ALT values of patients with diabetes also decreased in the placebo group (–9·3%), whereas they remained nearly unchanged (1·6%) in the 500 mg group. These results should be interpreted cautiously because of the low number of patients with diabetes in the trial (table 1).

For the liver enzyme parameters ALT, AST, glutamate dehydrogenase (GDH), and GGT, treatment effects of norursodeoxycholic acid were observed (figure 4, table 2). ALT had a clear dose-dependent decrease between baseline and treatment (mean change –17·2 units per L in the 1500 mg group, –7·0 units per L in the 500 mg group, +5·3 units per L in the placebo group), whereas a moderate dose-dependent reduction was observed for AST concentrations (mean change –8·7 units per L in the 1500 mg group, –3·8 units per L in the 500 mg group, –0·9 units per L in the placebo group). Mean GGT values had a clear dose-dependent effect with norursodeoxycholic acid (mean change from baseline to end of treatment –67·1 units per L in the 1500 mg group, –7·1 units per L in the 500 mg group, +2·9 units per L in the placebo group). No relevant differences were found during the trial for alkaline phosphatase values between 1500 or 500 mg per day norursodeoxycholic acid and placebo (table 2). At follow-up, most changes in aminotransferase and GGT levels regressed towards baseline values (table 2). Analogous to the results of the primary analysis, the mean absolute change of ALT from baseline to end of treatment differed considerably between groups, showing a clear decrease in the 1500 mg group, a moderate decrease in the 500 mg group, and a slight increase in the placebo group (table 2). A dose-dependent treatment effect of norursodeoxycholic acid was also observed for the outcome criteria of ALT of 0·8 times or less the ULN with respect to patients with an ALT of more than 0·8 times the ULN at baseline (mean change from baseline to end of treatment: 17·5% for 1500 mg, 14·8% for 500 mg, 5·2% for placebo; figure 5). A moderate dose dependency was also observed for the absolute change of AST, the ALT to AST ratio, and GDH concentrations, whereas for GGT, a clear and dose-dependent effect of treatment with norursodeoxycholic acid was seen throughout the trial (table 2). No relevant differences were observed throughout the trial for alkaline

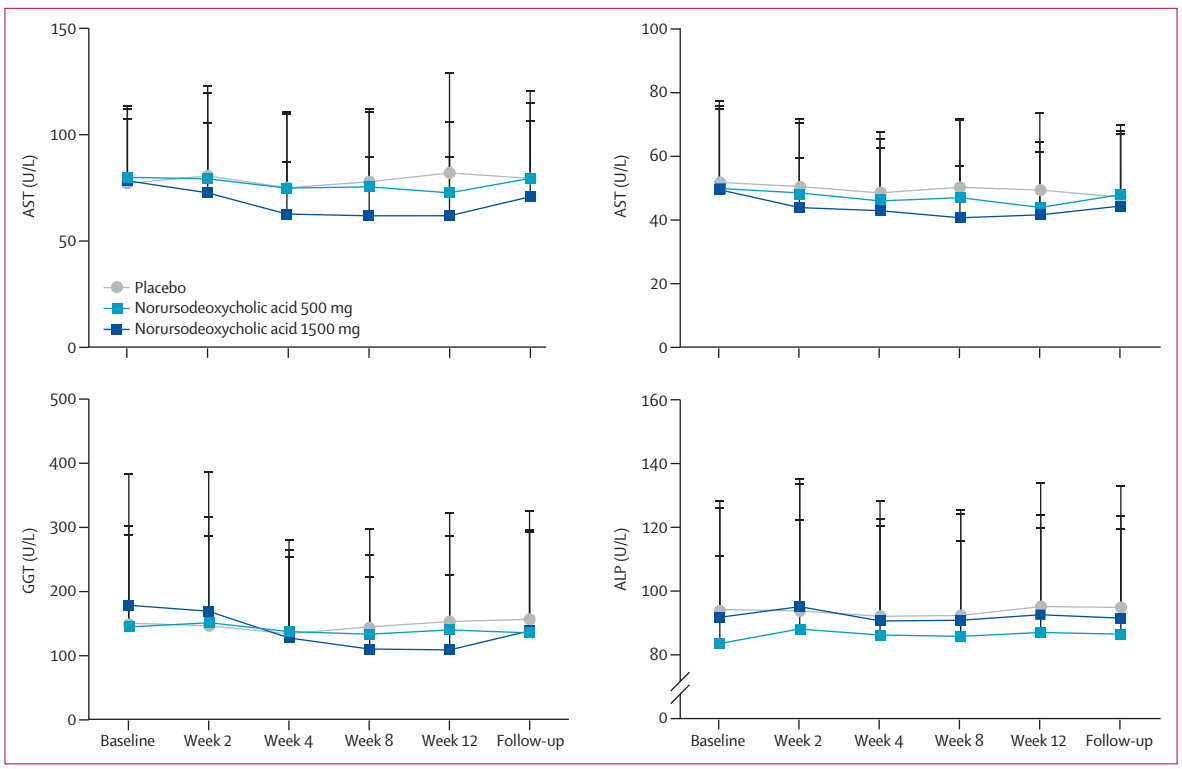

Figure 4: **Absolute changes in ALT, AST, GGT, and AP values from baseline to end of treatment and follow-up**
ALT=alanine aminotransferase. AST=aspartate aminotransferase. GGT=γ-glutamyltransferase. AP=alkaline phosphatase.

phosphatase and serum bilirubin values between the 1500 mg, 500 mg, and placebo groups (table 2). Both, ALT and AST values increased after the end of treatment with norursodeoxycholic acid. Similarly, mean values of GGT increased from end of treatment (111·7 units per L) to follow-up (138·8 units per L) in patients given 1500 mg (figure 4). No changes in mean serum bilirubin concentrations were observed at the follow-up visit (1500 mg: 0·55 mg/dL [SD 0.29], 500 mg: 0·66 mg/dL [0·36], placebo: 0·56 mg/dL [0·4]).

Fibroblast growth factor (FGF)19 remained unchanged in the 1500 mg group and decreased both in the 500 mg group (appendix p 4) and in the placebo group between baseline and end of treatment. FGF21, by contrast, decreased in the 1500 mg and 500 mg groups, yet increased in the placebo group over the same timeframe (appendix p 4).

Regarding lipid profiles and bodyweight, a moderate positive dose relationship was observed for triglycerides, showing slightly decreasing concentrations with the higher norursodeoxycholic acid dose. Triglycerides decreased between baseline and end of treatment by a mean of 14·6 mg/dL in the 1500 mg group, by 5·0 mg/dL in the 500 mg group, and even increased moderately by 9·5 mg/dL in the placebo group (table 2). Contrastingly, between baseline and end of treatment, LDL-cholesterol concentrations increased by a mean of 14·6 mg/dL in the 1500 mg group and by 9·0 mg/dL in the 500 mg group, while decreasing slightly by 3·4 mg/dL in the placebo group (figure 6). However, these changes occurred rapidly, within 2 weeks, and stayed stable during the remaining treatment period. At the end of the 4-week follow-up phase, a normalisation of the LDL, HDL, and triglyceride values was observed (table 2). Notably, mean HDL-cholesterol concentrations remained fairly stable in the 500 mg and placebo group, and even slightly increased in the 1500 mg group between baseline and end of treatment. The LDL to HDL ratio slightly increased in the 1500 mg group (2·79 to 3·01) and 500 mg group (2·89 to 3·09) between baseline and end of treatment, but decreased in the placebo group (2·91 to 2·78). No remarkable treatment effects were observed for plasma glucose (data not shown). No relevant changes in bodyweight were observed during the trial in any group (data not shown).

The proportion of patients with severe steatosis on ultrasound[14] was reduced from baseline to end of treatment in the 1500 mg group (baseline n=13 [20%], end of treatment n=7 [11%]) and the 500 mg group (baseline n=18 [27%], end of treatment n=11 [17%]), and remained nearly unchanged in the placebo group (baseline n=12 [19%], end of treatment n=11 [18%]).

Liver stiffness reflecting fibrosis stage was assessed by fibroscan or acoustic radiation force impulse, while controlled attenuation parameter fibroscan or MRI or MRS examinations were used to establish the grade of hepatic steatosis throughout the trial. Because of the

	1500 mg group		500 mg group		Placebo group	
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
Alanine aminotransferase (units per L)						
Baseline	63	78·6 (34·2)	66	80·3 (33·3)	63	77·4 (30·2)
End of treatment (LOCF)	67	61·8 (28·3)	67	72·8 (33·2)	64	82·3 (46·7)
End of treatment (LOCF) minus baseline	67	−17·2 (23·3)	67	−7·0 (27·4)	64	5·3 (41·0)
Follow-up	67	71·0 (35·9)	64	79·8 (40·5)	63	79·7 (35·5)
Aspartate aminotransferase (units per L)						
Baseline	63	49·3 (25·1)	66	49·8 (27·4)	63	51·6 (23·5)
End of treatment (LOCF)	67	40·8 (19·1)	67	45·6 (24·7)	64	50·4 (24·8)
End of treatment (LOCF) minus baseline	67	−8·7 (13·6)	67	−3·8 (14·3)	64	−0·9 (19·3)
Follow-up	67	44·1 (22·6)	64	47·0 (22·7)	63	47·9 (19·7)
Alanine aminotransferase to aspartate aminotransferase ratio						
Baseline	63	1·68 (0·53)	66	1·71 (0·50)	63	1·60 (0·56)
End of treatment (LOCF)	67	1·54 (0·34)	67	1·67 (0·48)	64	1·70 (0·57)
End of treatment (LOCF) minus baseline	67	−0·14 (0·42)	67	−0·05 (0·37)	64	0·10 (0·40)
Follow-up	67	1·65 (0·45)	64	1·71 (0·47)	63	1·74 (0·55)
γ-glutamyltransferase (units per L)						
Baseline	64	178·8 (205·6)	66	144·7 (160·0)	63	150·0 (140·8)
End of treatment (LOCF)	67	111·7 (116·3)	67	137·6 (144·8)	64	152·9 (166·2)
End of treatment (LOCF) minus baseline	64	−64·5 (132·6)	66	−7·7 (68·8)	63	3·1 (75·8)
Follow-up	67	138·8 (153·9)	64	136·5 (160·4)	63	157·8 (168·4)
Glutamate dehydrogenase (units per L)						
Baseline	65	11·6 (9·8)	67	12·5 (11·7)	62	13·3 (13·8)
End of treatment (LOCF)	61	7·8 (8·0)	63	10·3 (8·7)	61	14·2 (14·7)
End of treatment (LOCF) minus baseline	59	−3·8 (6·9)	63	−2·2 (9·1)	59	0·5 (15·7)
Follow-up	66	11·3 (11·1)	64	13·8 (17·8)	63	13·2 (12·9)
Alkaline phosphatase (units per L)						
Baseline	64	92·0 (36·6)	66	83·9 (27·6)	63	94·3 (32·1)
End of treatment (LOCF)	67	91·8 (32·1)	67	86·9 (32·2)	64	94·7 (38·0)
End of treatment (LOCF) minus baseline	64	0·0 (19·3)	66	3·3 (16·2)	63	1·6 (16·6)
Follow-up	67	89·8 (30·5)	64	83·8 (25·1)	63	96·1 (39·8)
Serum bilirubin (mg/dL)						
Baseline	61	0·61 (0·32)	65	0·64 (0·40)	62	0·57 (0·47)
End of treatment (LOCF)	67	0·57 (0·31)	67	0·62 (0·38)	64	0·58 (0·38)
End of treatment (LOCF) minus baseline	66	−0·03 (0·20)	67	−0·01 (0·22)	64	0·02 (0·24)
Follow-up	67	0·55 (0·29)	63	0·66 (0·36)	62	0·56 (0·40)
LDL-cholesterol (mg/dL)						
Baseline	65	119·5 (38·6)	67	124·6 (43·9)	62	121·8 (38·4)
End of treatment	61	131·6 (42·0)	63	134·4 (46·3)	61	120·9 (36·2)
End of treatment minus baseline	59	14·6 (19·7)	63	9·0 (18·3)	59	−3·4 (18·4)
Follow-up	67	115·2 (40·1)	64	124·4 (43·3)	63	122·8 (39·8)
HDL-cholesterol (mg/dL)						
Baseline	65	44·9 (12·2)	67	45·1 (11·9)	62	43·4 (10·7)
End of treatment	61	47·2 (11·7)	63	46·5 (12·8)	61	45·6 (11·5)
End of treatment minus baseline	59	2·8 (7·2)	63	1·4 (4·8)	59	1·5 (6·1)
Follow-up	67	45·0 (12·4)	64	45·1 (12·0)	63	44·7 (11·1)
Triglycerides (mg/dL)						
Baseline	65	220·4 (212·2)	67	182·8 (91·0)	62	190·4 (125·0)
End of treatment	61	212·7 (256·5)	63	175·2 (108·6)	61	195·8 (168·8)
End of treatment minus baseline	59	−14·6 (127·1)	63	−5·0 (83·5)	59	9·5 (112·8)
Follow-up	67	200·3 (173·4)	64	188·3 (130·6)	63	181·3 (117·3)

Data are N and mean (SD). LOCF=last observation carried forward.

***Table 2*: Liver enzyme values (full analysis set)**

limited number of patients with such examinations, no clear treatment effect could be observed using only one method. To analyse liver stiffness throughout the trial, results of fibroscan and acoustic radiation force impulse examinations were pooled—the worst liver fibrosis stage of a patient on a particular visit was used for the analysis. The proportions of patients with liver stiffness of stage 0 or 1 increased in the 1500 mg group throughout the trial (screening 56% [n=20], end of treatment 68% [n=27]), whereas it decreased in the placebo group (screening 73%, end of treatment 61% [n=22]) and in the 500 mg group (screening 63% [n=30], end of treatment 58% [n=28]; appendix p 4). By contrast, the proportion of patients with stage 4 liver stiffness decreased in all treatment groups from screening (19% [n=7] in the 1500 mg group, 13% [n=6] in the 500 mg group, 24% [n=8] in the placebo group) to end of treatment (15% [n=6] in the 1500 mg group, 10% [n=5] in the 500 mg group, 14% [n=5] in the placebo group; appendix p 4).

Results of hepatic fat fraction measured by MRS and MRI examinations were analysed; if the MRS values were missing, MRI-chemical shift index was considered for the analysis. The mean value of hepatic fat fraction decreased in the 1500 mg group from 21·3% at screening to 16·3% at end of treatment, equivalent to a relative reduction of 23·5%. In the placebo group (screening 17·0%, end of treatment 16·0%) and in the 500 mg group (screening 14·6%, end of treatment: 15·5%) the mean hepatic fat fraction remained unchanged over the study period. However, because of the low number of patients with measurements of hepatic fat fraction (at screening 1500 mg, n=8 [12%]; 500 mg, n=7 [10%]; placebo n=5 [8%]), these results should be interpreted cautiously.

Oral treatment with norursodeoxycholic acid was well tolerated in most patients as assessed by both investigators and patients. More adverse drug reactions and adverse events leading to study withdrawal were observed in the 1500 mg group than the 500 mg group and placebo (table 3). In particular, occurrence of skin and subcutaneous tissue disorders and nervous system disorders were observed in the norursodeoxycholic acid 1500 mg group. 314 treatment-emergent adverse events were documented (table 3). No patients died during the trial. Treatment-emergent serious adverse events occurred in one (1%) of 67 patients in the 1500 mg group (ventricular extrasystoles), in two (3%) of 67 patients in the 500 mg group (neck abscess and postmenopausal haemorrhage), and three (5%) of 64 patients in the placebo group (erysipelas, joint dislocation, and bladder cancer recurrence).

The incidence of treatment-emergent adverse events was similar between treatment groups. The most frequent adverse events were gastrointestinal disorders, infections, and infestations (table 3). Study drug withdrawal due to treatment-emergent adverse events occurred in six (9%) of 67 patients in the 1500 mg group (rash in three patients [4%], one of them generalised [1%], ventricular extrasystoles, hypoglycaemia, nausea, each one patient [1%]), in two (3%) of 67 patients in the 500 mg group (ventricular extrasystoles and hepatic enzyme increased) and in two (3%) of 64 patients taking placebo (headache and oral numbness). Vital signs remained fairly constant for all treatment groups. Tolerability of the study medication was described as very good or good by the investigators for 60 (90%) of 67 patients in the 1500 mg group, for 64 (97%) of 67 patients in the 500 mg group, and for 61 (97%) of 64 patients in the placebo group. Tolerability assessment by the patient was very good or good in 58 (87%) of 67 patients in the 1500 mg group, in 65 (99%) of 67 patients in the 500 mg group, and 59 (94%) of 64 patients in the placebo group. 15 patients prematurely discontinued the trial (eight patients in the 1500 mg group, six patients in the 500 mg group, three patients in the placebo group). The most common primary reason for premature withdrawal were intolerable adverse events (nine patients in total, five patients in the 1500 mg group, two patients in the 500 mg group, two patients in the placebo group). For three patients (two patients in the 500 mg group, one patient in the placebo group) the discontinuation was a result of the patient's request; other reasons applied in three patients (all in the 1500 mg group). There was striking agreement between investigators and patients in assessment of good or very good tolerability. The calculated treatment compliance was more than 90% for most patients (1500 mg: 62 [93%] of 67, 500 mg: 59 [88%] of 67, placebo: 60 [94%] of 64). In general, overall tolerability was assessed to be slightly better for placebo and the 500 mg group than the 1500 mg group. Tolerability was considered very good or good by 60 [90%] of 67 in the 1500 mg group, 65 [97%] of 67 in the 500 mg group, and 61 [95%] of 64 in the placebo group.

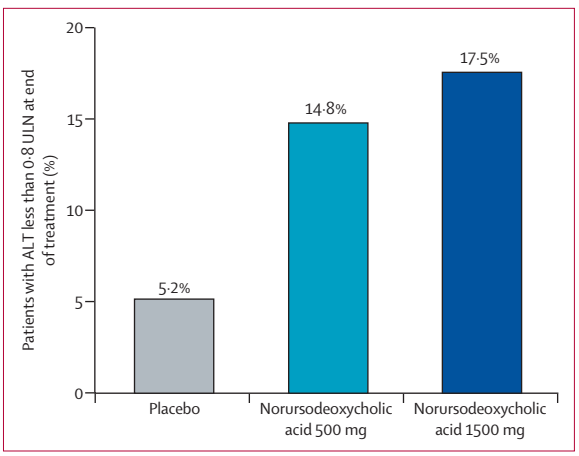

Figure 5: **Proportion of patients with ALT less than 0·8 times ULN at end of treatment in patients with ALT more than 0·8 times ULN at baseline**
ULN=upper limit of normal.

Discussion

In this double-blind, placebo-controlled, randomised, multicentre, comparative, exploratory, phase 2 dose

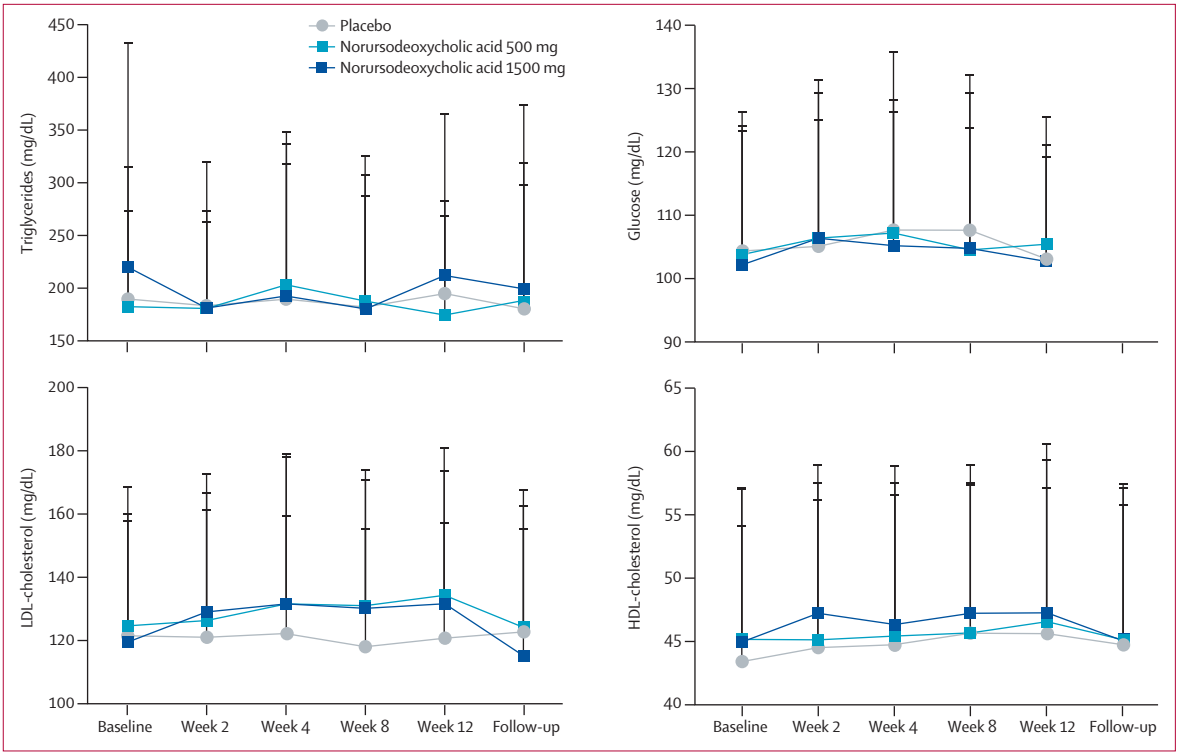

Figure 6: **Absolute changes in lipid and glucose concentrations from baseline to end of treatment and follow-up**
No follow-up data for glucose were available.

	1500 mg group (N=67)		500 mg group (N=67)		Placebo group (N=64)	
	Total	n (%)	Total	n (%)	Total	n (%)
Total	112	46 (69%)	99	44 (66%)	103	45 (70%)
Headache	11	10 (15%)	6	6 (9%)	3	3 (5%)
Nasopharyngitis	8	7 (10%)	7	6 (9%)	6	6 (9%)
Diarrhoea	7	7 (10%)	5	5 (8%)	7	6 (9%)
Abdominal pain upper	3	3 (5%)	7	6 (9%)	2	2 (3%)
Nausea	7	6 (9%)	2	1 (2%)	4	3 (5%)
Dizziness	4	3 (5%)	4	3 (5%)	3	3 (5%)
Fatigue	0	0	5	5 (8%)	4	4 (6%)
Abdominal pain	3	3 (5%)	1	1 (2%)	2	2 (3%)
Dry mouth	2	2 (3%)	2	2 (3%)	2	2 (3%)
Vomiting	3	3 (5%)	3	3 (5%)	0	0
Constipation	3	3 (5%)	0	0	2	2 (3%)
Abdominal distension	0	0	1	1 (2%)	3	3 (5%)
Dyspepsia	2	2 (3%)	1	1 (2%)	1	1 (2%)
Hypertension	1	1 (2%)	2	2 (3%)	1	1 (2%)
Rash	4	4 (6%)	0	0	0	0

Data are number of events or number (%) of patients.

Table 3: **Summary of treatment-emergent adverse events in patients with at least one treatment-emergent adverse event**

finding trial, 1500 mg per day of norursodeoxycholic acid significantly reduced ALT concentrations in patients with NAFLD compared with placebo. A 12-week treatment duration was chosen on the basis of previous studies in cholestatic diseases showing normalisation of liver enzymes within this timeframe.[19]

During the treatment phase, ALT concentrations decreased in a dose-dependent manner in the 1500 mg group and in the 500 mg group, but increased in the placebo group. The difference between the 1500 mg group and placebo group was significant, whereas the comparison between the 500 mg group and placebo was not. Also, mean absolute ALT concentrations at the end of the trial reflected efficacy of norursodeoxycholic acid—concentrations were lowest in the 1500 mg group, followed by the 500 mg and placebo groups. In the subsequent follow-up phase, ALT concentrations increased again in the two treatment groups, further supporting the causal relationship of ALT decrease with norursodeoxycholic acid treatment.

In a similar manner, dose-related decreases were observed in GGT concentrations and—to a lesser degree—in AST concentrations. The cholestatic parameters, bilirubin and alkaline phosphatase, in line with the expected effects of norursodeoxycholic acid in NAFLD, did not show any relevant changes. Notably, GGT has previously been shown as an indicator of oxidative stress and a strong predictor of cardiovascular and overall mortality,[20] possibly playing a distinct role in metabolic liver diseases.

Consistent with the observed decrease of liver enzymes, in ultrasound examinations, the proportion of patients

with severe steatosis decreased over the course of the trial in the two treatment groups but remained unchanged in the placebo group, which should be interpreted carefully as an ultrasound-based assessment of steatosis is rather subjective. The results from additional imaging diagnostics such as fibroscan or controlled attenuation parameter, acoustic radiation force impulse, MRI-chemical shift index, and MRS showed similar trends but were obtained from too few patients to allow firm conclusions. Stiffness stage and grade of hepatic steatosis, measured with these technologies, either stayed the same or improved moderately. Nevertheless, there are additional limitations with interpreting these data given that fibroscan and acoustic radiation force impulse results had to be pooled because of different methodological availability in the participating study centres. The mean value of hepatic fat fraction had a relative decrease from baseline to end of treatment of 23·5% (in the range predictive of histological improvement in NASH) in the 1500 mg group, whereas in the placebo and 500 mg groups the mean hepatic fat fraction increased. Given the short study period of this phase 2A trial, the observed trend for changes in liver stiffness might also reflect an improvement in inflammation rather than fibrosis. Overall, the observed trends might be at least in line with the observations of laboratory parameters and ultrasound findings.

We did subgroup analyses for the primary efficacy variable—ie, for the relative change in ALT during the trial. The results of these subgroup analyses suggest that men benefit in terms of reducing ALT concentrations from the norursodeoxycholic acid 1500 mg dose, whereas no differences between 500 mg dose and placebo were observed. In women, a dose-dependent effect, with pronounced differences between 500 mg and placebo, was observed. In both treatment groups, the ALT decrease was similar across different ages. Other subgroups, including those based on diagnosis with diabetes, the stage of liver fibrosis, or relevant weight change yielded small patient numbers. Thus, no clear conclusion can be drawn in terms of differences in treatment response.

Increased serum concentrations of FGF21 are known to be associated with development of NAFLD.[21,22] Fasting FGF19 concentrations have been shown to decrease in patients with NAFLD.[23] In the context of this study, both markers were evaluated to further investigate their usefulness in monitoring of patients with NAFLD. In line with the fact that norursodeoxycholic acid is not an agonist for FXR, the upstream regulator of FGF19, FGF19 remained unchanged in the 1500 mg group and even decreased in the 500 mg and the placebo groups. FGF21 decreased in both treatment groups, yet increased in the placebo group. The clinical relevance of these observations should be investigated further.

Total cholesterol concentrations remained fairly constant in all three groups. Triglyceride concentrations decreased in a dose-related manner in both treatment groups, whereas in the placebo group an increase was observed. HDL concentrations remained fairly stable in all three treatment groups. However, LDL concentrations increased in a dose-related manner in both treatment groups and decreased slightly in the placebo group, also seen in the LDL to HDL ratios. Overall, observed changes appeared to be low-grade, and high SDs do not allow for meaningful interpretation. Of patients included in this study who had an elevated mean body-mass index and increased triglyceride and LDL concentrations, only 10% were on lipid modifying agents. We speculate that more patients in this study would have benefited from statin therapy. Compared with epidemiological data from North America, mean body-mass index values were lower but reflect a European NAFLD population. Effects on lipid metabolism were also of interest given the ongoing discussion about the long-term safety of bile acid derivates or signalling agents with increasing concentrations of LDL and decreasing concentrations of HDL, as shown for obeticholic acid.[10] 2018 data indicate that all therapeutic strategies (ie, steroidal and non-steroidal FXR agonists and FGF19 mimetics) that inhibit bile acid synthesis and thereby increase hepatocellular cholesterol concentrations will result in downregulation of LDL receptors and subsequent increase in serum LDL.[24] Collectively, the clinical relevance of these low-grade effects on lipid metabolism, and their further course after treatment cessation, warrants further investigation.

This trial has several limitations, including the absence of histological outcomes. As this was a phase 2 dose-finding trial, a short treatment duration was chosen and liver biopsy did not seem to be an appropriate method to evaluate treatment response because of its invasive nature. In the subgroup analysis only few data were available regarding imaging diagnostics due to a limited and variable access to the different techniques at each individual centre. Furthermore, the number of people with type 2 diabetes was higher in the placebo group than the treatment groups, but was generally low.

Overall, oral treatment with norursodeoxycholic acid was well tolerated in most patients as assessed by both investigators and patients. More adverse drug reactions and treatment-emergent adverse events leading to study withdrawal were observed in the 1500 mg group than the 500 mg and placebo groups. During the trial, nine (5%) serious adverse events occurred, six (3%) of which emerged during the treatment phase. No serious adverse event was assessed as causally related to the study treatment. In the 1500 mg group, specifically, skin and subcutaneous tissue disorders and nervous system disorders occurred more frequently, both among all treatment-emergent adverse events and among adverse drug reactions. The relatively high number of dermatological events in the 1500 mg group was unexpected, as such events had not been observed in the

preceding human studies and warrant attention during future studies in this patient population. Importantly, and contrasting with the use of obeticholic acid, pruritus was not observed. In six (9%) patients in the 1500 mg group, however, tolerability was assessed as poor by investigators and patients, which demands further exploration.

In summary, during a 12-week treatment with norursodeoxycholic acid, a dose-related decrease of ALT concentrations was observed, with a significant difference in the 1500 mg group compared with the placebo group. Dose-related effects on the courses of AST and GGT were observed with a marked decrease in the 1500 mg group, a moderate decrease in the 500 mg group, and unchanged or increased concentrations in the placebo group. Investigators assessed more patients as experiencing a therapeutic benefit on a once a day dose of 1500 mg norursodeoxycholic acid than on 500 mg norursodeoxycholic acid or placebo, warranting a phase 2B trial to further evaluate the beneficial effects of norursodeoxycholic acid in patients with NAFLD or NASH at the histological level.

Contributors

All authors contributed to the acquisition of data, review, and critical revision of the manuscript and approved the final version of the manuscript. ST was responsible for study design and conception, interpretation of data, and manuscript writing. RG was responsible for study design and conception. MP was responsible for study design and conception. MPM was responsible for study design and conception and interpretation of data. MT was responsible for study design and conception, interpretation of data, and manuscript writing.

Declaration of interests

ST has received travel grants from Falk Foundation. JMS has been a speaker for Falk Foundation, Abbvie, and Takeda; participated in advisory boards for Intercept Pharmaceuticals, Genfit, Gilead Sciences, Bionorica, Medimmune, Novartis, and Pfizer; received travel grants from Janssen; and received research funding from Gilead Sciences and Yakult Europe. MD has been an advisor or speaker for AbbVie, BMS, Gilead, Janssen, MSD, and Bayer; and has received travel grants from AbbVie, Gilead, Janssen, BMS, Roche, MSD, and Bayer. JW has been a speaker for Falk Foundation, Abbvie, and Intercept Pharmaceuticals; participated in advisory boards for Intercept Pharmaceuticals and Abbvie; and has received research support from Siemens and Echosens. AG has been an advisor or speaker for AbbVie, Alexion, BMS, CSL Behring, Gilead, Intercept, Ipsen, MSD, Merz, Novartis, Pfizer, Falk Foundation, and Sequana; received travel grants from AbbVie, Gilead, Falk Foundation, and MSD; and has received an unrestricted research grant from Novartis and Intercept (for NAFLD clinical study group). GT has been a speaker for Abbvie, Gilead, and MSD; participated in advisory boards for Abbvie and Gilead; has received a research grant from Gilead; and a travel grant from Abbvie. WPH has been an advisor or speaker for AbbVie, Gilead, MSD, Falk Pharma, Intercept, and Norgine. AEK has been a speaker for Abbvie, BMS, Falk Foundation, Intercept, and MSD; participated in advisory boards for Beiersdorf, GSK, Intercept, Janssen, and MSD; and has received unrestricted research grants from Intercept. JK has participated in advisory boards for Novartis. TB has been a speaker for Falk Foundation, Gilead, Abbvie; participated in advisory boards for Abbvie, and received travel grants from Abbvie. EH has participated in advisory boards for Intercept and Novartis; and has received travel grants from Falk Foundation. MPM has been a speaker for Falk Foundation, participated in advisory boards for Dr Falk Pharma GmbH, Intercept, Aram Chol, Gilead, Genfit and Novartis; received research support from Falk Foundation and Gilead; and received travel grants from Gilead and Falk Foundation. PF has been a speaker for Falk Foundation, participated in advisory boards for Dr Falk Pharma GmBH and Intercept; and received travel grants and unrestricted research grants from Falk Foundation and an unrestricted research grant from Gilead. MT has been a speaker for BMS, Falk Foundation, Gilead, and MSD; participated in advisory boards for Albireo, Falk Pharma GmbH, Genfit, Gilead, Intercept, MSD, Novartis, Phenex, and Regulus; received travel grants from Abbvie, Falk, Gilead, and Intercept; and has received unrestricted research grants from Albireo, Cymabay, Falk, Gilead, Intercept, MSD, and Takeda. PF and MT are co-inventors of patents on the medical use of norursodeoxycholic acid filed by the Medical University of Graz. RG and MP are employees of Dr Falk Pharma GmbH. All other authors declare no competing interests.

Data sharing

Data collected for the study, including individual participant data and a data dictionary defining each field in the set, will not be made available to others, as per ethical committee agreements. With the investigators and sponsors' support, after approval of a proposal, with a signed data access agreement, de-identified participant data, other specified data, and additional, related documents (eg, study protocol, statistical analysis plan, informed consent form) will be made available once the Article is published upon request. For further information please contact markus.proels@drfalkpharma.de.

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