norUrsodeoxycholic acid improves cholestasis in primary sclerosing cholangitis

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norUrsodeoxycholic acid improves cholestasis in primary sclerosing cholangitis

Graphical abstract

Highlights

- There is an urgent need for novel drugs for PSC.
- In this phase II clinical trial, norUDCA reduced serum ALP levels within 12 weeks.
- norUDCA's effects on liver enzymes were dose-dependent.
- The safety profile of norUDCA was excellent.

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Lay summary

Effective medical therapy for primary sclerosing cholangitis (PSC) is urgently needed. In this phase II clinical study in PSC patients, a side chain-shortened derivative of ursodeoxycholic acid, norursodeoxycholic acid (norUDCA), significantly reduced serum alkaline phosphatase levels in a dose-dependent manner during a 12-week treatment. Importantly, norUDCA showed a favorable safety profile, which was similar to placebo. The use of norUDCA in PSC patients is promising and will be further evaluated in a phase III clinical study.

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norUrsodeoxycholic acid improves cholestasis in primary sclerosing cholangitis

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Background & Aim: Primary sclerosing cholangitis (PSC) represents a devastating bile duct disease, currently lacking effective medical therapy. 24-norursodeoxycholic acid (norUDCA) is a side chain-shortened C23 homologue of UDCA and has shown potent anti-cholestatic, anti-inflammatory and anti-fibrotic properties in a preclinical PSC mouse model. A randomized controlled trial including 38 centers from 12 European countries, evaluated the safety and efficacy of three doses of oral norUDCA (500 mg/d, 1,000 mg/d or 1,500 mg/d) compared with placebo in patients with PSC.

Methods: One hundred sixty-one PSC patients without concomitant UDCA therapy and with elevated serum alkaline phosphatase (ALP) levels were randomized for a 12-week treatment followed by a 4-week follow-up. The primary efficacy endpoint was the mean relative change in ALP levels between baseline and end of treatment visit.

Results: norUDCA reduced ALP levels by −12.3%, −17.3%, and −26.0% in the 500, 1,000, and 1,500 mg/d groups (p = 0.029, p = 0.003, and p < 0.0001 when compared to placebo), respectively, while a +1.2% increase was observed in the placebo group.

Keywords: Alkaline phosphatase; Bile acid treatment; Cholestasis; Sclerosing cholangitis; Side chain-shortened bile acids; Cholehepatic shunting; Ursodeoxycholic acid.

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1 Full list study participants is listed at the end of the manuscript.

Similar dose-dependent results were found for secondary endpoints, such as ALT, AST, γ-GT, or the rate of patients achieving ALP levels <1.5× ULN. Serious adverse events occurred in seven patients in the 500 mg/d, five patients in the 1,000 mg/d, two patients in the 1500 mg/d group, and three in the placebo group. There was no difference in reported pruritus between treatment and placebo groups.

Conclusions: norUDCA significantly reduced ALP values dose-dependently in all treatment arms. The safety profile of norUDCA was excellent and comparable to placebo. Consequently, these results justify a phase III trial of norUDCA in PSC patients.

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Introduction

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease of unknown etiology characterized by a chronic inflammatory and fibro-obliterative destruction of extra-, and intrahepatic bile ducts.1 Currently there are no medical therapies with proven benefits on PSC patients’ survival. The list of drugs that have failed in PSC treatment is long, comprising of azathioprine, cyclosporine, methotrexate, tacrolimus, penicillamine, cholchicine, and infliximab.2 Consequently, liver transplantation is the only treatment option for PSC patients with end-stage liver disease. The use of Ursodeoxycholic acid (UDCA) in the treatment
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of PSC is highly controversial.3–5 Numerous studies showed biochemical or histological improvements, but failed to demonstrate significant differences in death rates, transplant-free survival, or development of cholangiocarcinoma.4–11 A randomized controlled trial testing doses of UDCA ranging from 17 to 23 mg/kg/day showed a favorable biochemical trend but lacked significant effects on hard clinical endpoints including transplant-free survival, probably due to being underpowered.10 Testing even higher doses of UDCA (28–30 mg/kg/day) in PSC patients revealed an overall improvement in serum liver tests but were associated with a higher risk of death, a need for liver transplantation, and increased rates of serious adverse events (SAEs).11 Interestingly, a study on the effects of UDCA withdrawal in PSC patients showed a significant deterioration of liver biochemistry and symptoms within 3 months.12 Taken together, effective medical therapy for PSC is still an unmet clinical need and novel drugs are urgently needed.2

24-norursodeoxycholic acid (norUDCA) is a side chain-shortened C24 homologue of UDCA and was previously shown to be highly effective in preclinical mouse models of cholestatic and fibrotic liver diseases.13–17 Comparing the therapeutic effects of UDCA and norUDCA in Abcb4 knockout mice (Abcb4−/−) as a model for sclerosing cholangitis and biliary fibrosis revealed specific and superior anti-inflammatory, anti-fibrotic, and anti-proliferative effects of norUDCA.14–16 The relative resistance of norUDCA to N-acetyl-amidation with taurine or glycine leads to cholehepatic shunting of norUDCA. This means that unconjugated norUDCA, as a weak acid, can be reabsorbed by cholangiocytes, returning to the sinusoids and hepatocytes via the periductular capillary plexus, and is re-secreted into bile.11,18–20 This process of cholehepatic shunting results in ductal targeting and leads to profound stimulation of cholangiocellular bicarbonate secretion and consequently induction of bile acid-independent bile flow and flushing of bile ducts. In addition, bile acid bicarbonate secretion may protect cholangiocytes as a bicarbonate umbrella2 against their rather unfriendly environment, where the apical surface membranes have to continuously face millimolar concentrations of potentially toxic bile acids.23 Moreover, norUDCA and its metabolites when secreted into human bile are present in mostly monomeric rather than micellar form, enhancing its osmotic effect thus making norUDCA a highly potent choleretic drug.18 These favorable mechanisms of norUDCA and excellent tolerability in phase I studies prompted us to initiate a multicenter placebo-controlled phase II clinical trial (NUC-3/PSC) in PSC patients. We aim to: (i) evaluate the efficacy of three doses of norUDCA vs. placebo; (ii) identify efficacious norUDCA dose(s) for further evaluation in a future phase III clinical trial; and (iii) to study safety and tolerability of norUDCA.

Patients and methods

This study was designed as a European multicenter, double-blind, randomized, placebo-controlled, comparative, and exploratory phase II dose finding trial with four treatment groups. The overall study design is summarized in Fig. 1. Patients were randomized to 500 mg/d, 1,000 mg/d or 1,500 mg/d norUDCA or placebo at 38 centers from 12 countries. Since the therapeutic efficacy of UDCA in PSC is unclear it seemed justifiable to introduce a placebo arm. UDCA was therefore discontinued at least 8 weeks prior to baseline visit in 113 of 161 patients enrolled. From 159 patients receiving the study medication 40 were documented as UDCA-naïve, 58 classified as UDCA responders, and 55 as UDCA non-responders. Response was defined as (partial) normalization of serum alkaline phosphate (ALP) at the investigator’s discretion (Table 2) and this study therefore lacks a strict definition of UDCA response. Importantly, all UDCA exposed patients discontinued UDCA at least for 8 weeks prior to study inclusion. The biochemical patterns following discontinuation of UDCA were in line with a previous report2 showing an increase in ALP (+27% in UDCA responder, +15% in UDCA non-responders). A 12-week treatment phase was followed by a 4-week follow-up period. The trial was performed between January 2013 and September 2015 following the principles of good clinical practice and in accordance with the ethical principles of the Declaration of Helsinki, was registered with EudraCT (No. 2011-002754-31), and approved by the independent local ethics committees responsible for the participating investigators. All patients provided written informed consent. Male or female PSC patients aged ≥18 and <80 years were included. PSC was verified by two of the three following criteria: (i) chronic cholestatic liver disease of at least 6 months duration; (ii) retrograde, operative, percutaneous, or magnetic resonance cholangiography demonstrating intrahepatic and/or extrahepatic biliary duct changes such as beading or narrowing consistent with PSC within 1 year prior to baseline; or (iii) liver biopsy available for review and compatible with the diagnosis of PSC. ALP had to be elevated ≥1.5× upper limit of normal (ULN) at baseline. Both PSC patients with or without irritable bowel disease (IBD) were included. Patients without a definite diagnostic exclusion of IBD required a colonoscopy with segmental biopsies prior to the baseline visit. Women of childbearing potential had to apply a highly effective method of birth control.

Key exclusion criteria included history or presence of other concomitant liver diseases such as secondary sclerosing cholangitis, primary biliary cholangitis, cholangiocarcinoma, hepatitis B or C infection, Wilson’s disease, haemochromatosis, autoimmune hepatitis, chronic alcoholic consumption (daily consumption ≥30 g/d), or biopsy proven NASH. Child-Pugh score B/C liver cirrhosis and patients with history or presence of hepatic decompensation were excluded. In addition, patients were excluded if receiving immunosuppressive drugs such as chlorambucil, pentoxifylline, penicillamine, pirfenidone, fibrates, biologicals (e.g. anti-tumor necrosis factor-α therapy), or rifampicin treatment 3 months prior to baseline recording. Endoscopic treatment for bile duct stenosis needed or planned within 5 months post randomization date and treatment for dominant bile duct stenosis within 6 months prior to baseline visit were also excluded. Moreover, patients with a total bilirubin ≥3.0 mg/dl (≥50 μmol/L) at screening or baseline or rise in total bilirubin of at least 50% within the last 6 months prior to baseline were excluded from this trial. Patients were to take 250 mg capsules of norUDCA for oral use. Groups were divided as follows: group A, 2 x 250 mg capsules norUDCA; group B, 4 x 250 mg capsules norUDCA; group C, 6 x 250 mg capsules norUDCA; group D, 0 (placebo). All patients were to take 6 capsules altogether (verum + placebo) in the morning to guarantee the blinding. The appearance, size, and taste of the placebo capsules for oral use were indistinguishable from the verum capsules.

Study assessments

After randomization, patients were monitored at baseline visit, interim visits at weeks 2, 4, 6, and 8, and end of treatment (EOT) at week 12, and follow-up visit at week 24 (Fig. 1). At all visits vital signs, weight, electrocardiography and body temperature were controlled and laboratory assessments included routine serum biochemistry (alanine [ALT] and aspartate aminotransferases [AST], gamma-
glutamyl transpeptidase (γ-GT), alkaline phosphatase (AP), total and conjugated bilirubin, albumin, c-reactive protein (CRP), total protein, serum creatinine, lipase, lact dehydrogenase [LDH]), hematology (blood count and differential blood count), and blood coagulation tests (international normalized ratio [INR], partial thromboplastin [PTT]). Abdominal ultrasound examination was done at the screening visit and EOT. This allowed for liver size and echogenicity, the bile duct system, gall bladder abnormalities, portosystemic collaterals, spleen size, and any other abnormalities to be evaluated. Patients suffering from ulcerative colitis were asked to start a diary one week before baseline until the EOT visit, to record the four sub-scores: number of stools, blood in or on stool, general wellbeing, and abdominal pain/cramps. This diary was used at every visit to calculate the clinical activity index (CAI) according to Rachmilewitz generated by the electronic Case Report Form. Pruritus was assessed by visual analogue scale (VAS) by making a vertical line from no itching (0) to unbearable itching (100). Fatigue was assessed by questionnaire adapted from the PBC-40 questionnaire. Each item was scored on a 5-point scale and the fatigue score was calculated as the sum of scores for the eleven items. To assess the physician’s general overall clinical impression of the therapeutic effect and changes in the patient’s condition the physician’s global assessment (PGA) scale was used. The short health scale (SHS) is a simplified four-item questionnaire and was used for testing the patients’ quality of life. Compliance was recorded by counting the returned daily boxes for study medication and occurring within the period of treatment. SAEs included death, any life-threatening event, requirement for in-patient hospitalization or prolongation of existing hospitalization, events with persistent or significant disablement/incapacity, and congenital anomaly/birth defects.

Study endpoints

The primary efficacy variable in this clinical trial was the relative change (%) in AP at the baseline visit and the EOT visit (LOCF), as serum AP is a relevant surrogate marker for prognosis in PSC patients. This was calculated for each patient as follows: \(100 \times \left(\frac{\text{ALP}_{\text{EOT}} - \text{ALP}_{\text{baseline}}}{\text{ALP}_{\text{baseline}}}\right)\).

A value of -25% calculated for a patient means a reduction by 25%, i.e., the ALP value at EOT was three-quarters the value that was measured at the baseline visit. Secondary efficacy endpoints included at least a 50% reduction in ALP between baseline and EOT; normalization (<ULN) or partial normalization (<1.5 × ULN) of ALP, absolute and relative changes of AP, γ-GT, AST, ALT, and serum bilirubin levels (total bilirubin and conjugated bilirubin) at each study visit (screening to follow-up), absolute and relative changes of pruritus course measured by VAS, fatigue course, and success and therapeutic benefit according to PGA.

Statistical methods

The primary efficacy variable was subject to a confirmatory statistical analysis (α = 0.025, one-sided) in the context of a two-stage group-sequential adaptive study design. The sample size has been chosen to ensure adequate power to detect absolute differences in the mean relative change (%) in serum AP of about 20%. Differences of this magnitude were regarded to be clinically meaningful in the proof of concept study. The sample size required was calculated using nQuery Advisor 6.01. This was done for an effect size of 0.20/0.25 = 0.8 and a power of 80%, with the result that 39 patients per treatment group were required. This calculation did not yet take the group-sequential adaptive study design into account. The study was conducted according to a 2-stage group-sequential test design with possible sample size adaptation at the interim analysis. To preserve the overall experiment with one-sided type I error rate of α = 0.025 in the analysis of the primary endpoint, boundaries calculated according to O’Brien and Fleming were applied with a critical value at the final analysis of 2.015. The inverse normal method of combining p values was used with prospectively planned information rates of 0.75 and 1.0 at the interim analysis and at the final analysis, respectively. This means that a sample size of 40 patients per treatment group was adequate (40/39 = 1.0256). The planned sample size was therefore 160 patients (1:1:1:1 randomization, i.e. about 40 patients per treatment group) in the full analysis set (FAS) if the study was completed without modification followed by the interim analysis, which was planned after and performed based on 120 FAS patients (about 30 per group). After the interim analysis, no sample size adaptation was required. The primary efficacy endpoint in this clinical trial was the mean relative change (%) in serum AP levels between the baseline visit and the EOT visit. The absolute change from baseline to a subsequent visit and to the EOT visit was calculated as the value at the visit minus the baseline value. Comparisons between treatment groups were performed in two steps: The first analysis step compared each norUDCA treatment group against placebo, the second analysis step was used for pairwise comparisons among the three norUDCA treatment groups. Only if all three hypotheses of the first step had been rejected, the second step was to be performed in a confirmatory sense. In order to adjust for multiplicity within each analysis step, a closed testing procedure using Simes intersection step tests was applied for each step. The inverse normal procedure was applied to Simes adjusted p values. Corresponding p values were combined, i.e., the p values for the global hypothesis based on patients included in the interim analysis was combined with the p value based on the remaining patients, the pairwise intersection p values were combined with their counterparts, as well as the p values for the elementary hypotheses. The global hypothesis (intersection of the 3 elementary hypotheses) could be rejected if the global test statistic (FAS) resulting from the inverse normal method exceeded the critical value. In this case, it was to be determined which of the elementary hypotheses could be rejected by the closed testing procedure. One-sided p values for the analysis of the primary endpoint were calculated using Wilcoxon rank sum tests. Calculations were performed using SAS® (version 9.3, SAS Institute, USA) and using ADDPLAN® (licensed by ADDPLAN, Inc., an ICON Clinical Research, LLC company). Descriptive statistical methods were used to analyze all variables. Continuous variables were summarized using the number of observations, arithmetic mean, and standard deviation. Categorical data are described using absolute and relative frequencies. There was no considerable number of centers contributing at least three patients per treatment group. Therefore, a possible center effect was not explored. A possible effect of geographical cluster was explored by presenting descriptive summary statistics for the primary efficacy endpoint stratified by geographical cluster. The following geographical clusters were used: Austria, Germany, Hungary, United Kingdom, Scandinavia (Denmark, Finland, Norway, Sweden), Benelux (Belgium, The Netherlands), and other countries (Lithuania, Spain).

For further details regarding the materials used, please refer to the CTAT table.

ClinicalTrials.gov number: NCT01755507.

Results

In total, 231 screenings were performed in 222 patients. Sixty-one out of 222 screened patients were not eligible for randomization. The main reason for screening failure was violation of inclusion or exclusion criteria in 51 patients. The study flow chart and additional reasons for screening failure are given in Fig. 2. Data of screening failures and data of two patients who did not take the
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study medication (one was withdrawn because of lack of cooperation, the other because of violation of an exclusion criterion after randomization) were not included in the statistical analysis. Consequently, 159 received the study medication comprising the intention to treat (ITT) analysis population. Out of the 159 patients who were treated with study medication, 21 patients discontinued the treatment phase prematurely (nor-UDCA 500 mg: 10, 25.6%; norUDCA 1,000 mg: 4, 9.8%; norUDCA 1,500 mg: 3, 7.7%, placebo: 4, 10.0%). The most frequent primary reasons were violation of inclusion or exclusion criteria (coming to light after randomization) or intake of prohibited concomitant medication. The number of patients terminating the treatment phase because of lack of efficacy was: norUDCA 500 mg: 1 patient, norUDCA 1,000 mg: 0 patient, norUDCA 1,500 mg: 1 patient, and two in the placebo group.

Patient characteristics

The main baseline characteristics of the 159 patients treated are shown in Table 1. Overall and for each treatment group, the majority of patients were male (109/159; 68.6%), reflecting the natural gender distribution in PSC patients. There was no significant difference between study groups with regard to age, weight, height, BMI, and ALP serum levels at baseline indicating that the four treatment groups were well balanced at enrollment. A range from 6 to 8 of newly diagnosed PSC patients was comparable between the different treatment groups, ranging from 15% and 20% (Table 1), with the highest percentage in the placebo group. The rate of concomitant IBD was highest within the norUDCA 500 mg group (77%) and lowest within the placebo group (50%), however this difference did not reach statistical differences. There was no correction for IBD presence with respect to allocation of treatment groups. In the active treatment groups, the median time since the first symptoms of IBD was about 13.5 years, and the median duration of IBD disease was about 11.5 years (Table 1). The median duration of PSC was 6.9 years in the placebo group and 6.9, 3.7, and 4.3 years in the 500, 1,000, and 1,500 mg norUDCA group respectively (variation not greater than expected by chance). Serum ALP values were profoundly elevated at baseline in all treatment groups ranging from 369 IU/L in the norUDCA 1,000 mg group to 495 IU/L in the norUDCA 500 mg group, indicating that all treatment groups had pronounced cholestasis. Out of 159 study patients, 113 (71.0%) were UDCA experienced, 58 (37.3%) of these were UDCA responders defined by partial normalization of ALP at investigator’s discretion and 55 (48.7%) UDCA non-responders. Forty out of 159 (25.2%) patients were UDCA naive, the response to previous UDCA treatment was unknown or not applicable for the remaining 6/159 (3.8%) randomized patients (Table 1). In the active treatment groups, the proportion of UDCA non-responders ranged between 51.8% in norUDCA 1,500 mg group and 54.8% in the norUDCA 1,000 mg group, whereas in the placebo group only 33.3% were UDCA non-responders, defined according to the investigators’ discretion (and therefore lacking a universal definition). The UDCA doses were in a similar range (between 12 and 15 mg/kg/d) in UDCA responders and non-responders (Table 1). There was no difference between the groups with regard to current pruritus or previous pruritus episodes reported by patients ranging from 40% in the placebo groups to 64% in the norUDCA 500 mg group. Fatigue at baseline was reported from 45% in the placebo group up to 70.7% in the norUDCA 1,000 mg group.

NorUDCA significantly reduces serum ALP levels in a dose-dependent fashion in PSC patients

The relative reduction of serum ALP values was chosen as the primary endpoint for this study, since ALP levels currently represent the best available surrogate parameter for PSC patients and therefore appeared most suitable for this short-term study.27–29 In each norUDCA group, the relative reduction of serum ALP was statistically significantly superior to placebo in the analysis of the primary endpoint showing dose-dependent reductions by...
NorUDCA significantly reduces γ-GT, ALT, and AST serum levels in PSC patients

NorUDCA significantly reduced serum γ-GT levels in a dose-dependent fashion, and was most pronounced with a mean reduction of 33.3% in the norUDCA 1,500 mg group (for absolute values see Fig. 4B). Notably, the dose-dependency of the biochemical norUDCA effects in the reduction of serum enzyme levels was most apparent for serum γ-GT compared to the other serum parameters (Fig. 4). In parallel to the reduction of the cholestatic liver enzymes, the median reduction of serum AST levels was 20.5% and 33.1% in ALT levels at EOT (for absolute values see Fig. 4C and D, respectively). As observed for ALP, following a significant reduction under norUDCA treatment, a pronounced rebound of γ-GT, AST, and ALT serum levels was evident from EOT to the follow-up visit.

NorUDCA response is not predicted by previous UDCA treatment, previous UDCA response, presence of IBD, or duration of PSC

Importantly, a dose-dependent reduction of ALP was observed in both sexes and independent of previous UDCA-pretreatment and response (Table 2). Similarly, presence of IBD, duration of PSC and
Table 2. Relative changes (%) in ALP serum levels from baseline to EOT according to UDCA pretreatment as well as UDCA treatment response and other clinical parameters.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (N = 40)</th>
<th>norUDCA 500 mg (N = 39)</th>
<th>norUDCA 1,000 mg (N = 41)</th>
<th>norUDCA 1,500 mg (N = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, mean % (N)</td>
<td>4.7% (25)</td>
<td>-14.1% (27)</td>
<td>-13.9% (28)</td>
<td>-27.8% (29)</td>
</tr>
<tr>
<td>Female, mean % (N)</td>
<td>-6.6% (15)</td>
<td>-8.3% (12)</td>
<td>-24.7% (13)</td>
<td>-20.5% (10)</td>
</tr>
<tr>
<td>UDCA responder, mean % (N)</td>
<td>-6.2% (18)</td>
<td>-9.9% (13)</td>
<td>-16.4% (14)</td>
<td>-19.5% (13)</td>
</tr>
<tr>
<td>UDCA non-responder, mean % (N)</td>
<td>-3.5% (9)</td>
<td>-10.1% (15)</td>
<td>-14.9% (17)</td>
<td>-27.3% (14)</td>
</tr>
<tr>
<td>UDCA naïve, mean % (N)</td>
<td>+12.3% (12)</td>
<td>-14.9% (9)</td>
<td>-24.8% (9)</td>
<td>-32.0% (10)</td>
</tr>
<tr>
<td>ALP &gt; 3 × ULN, mean % (N)</td>
<td>-0.9% (25)</td>
<td>-14.4% (23)</td>
<td>-19.7% (17)</td>
<td>-26.2% (22)</td>
</tr>
<tr>
<td>ALP ≤ 3 × ULN, mean % (N)</td>
<td>4.8% (15)</td>
<td>-9.2% (16)</td>
<td>-15.6% (24)</td>
<td>-25.7% (17)</td>
</tr>
<tr>
<td>PSC with IBD, mean % (N)</td>
<td>5.8% (20)</td>
<td>-10.4% (29)</td>
<td>-14.5% (27)</td>
<td>-24.0% (27)</td>
</tr>
<tr>
<td>PSC without IBD, mean % (N)</td>
<td>-4.8% (18)</td>
<td>-21.8% (8)</td>
<td>-22.8% (14)</td>
<td>-29.9% (11)</td>
</tr>
<tr>
<td>PSC duration ≤ 5 yr, mean % (N)</td>
<td>8.3% (15)</td>
<td>-10.5% (17)</td>
<td>-20.3% (22)</td>
<td>-28.2% (20)</td>
</tr>
<tr>
<td>PSC duration &gt; 5 yr, mean % (N)</td>
<td>-3.0% (24)</td>
<td>-13.7% (22)</td>
<td>-13.4% (18)</td>
<td>-23.7% (19)</td>
</tr>
</tbody>
</table>

* In N = 5 no classification to IBD or non-IBD was possible.
** In N = 2 data from the first PSC diagnosis was not documented.

Baseline ALP levels had no impact on the dose-dependent response to norUDCA (Table 2). Notably, the percentage of patients without therapeutic response to norUDCA (defined as an ALP at EOT above screening values when most patients were still on UDCA) declined sharply with increasing norUDCA doses, again independently of previous UDCA pretreatment and response (Fig. S1).

Patient related outcomes

Body weight, body temperature, and vital signs did not show any relevant changes from baseline to EOT in all four treatment groups. It is interesting to note, that 7 (17.9%) patients showed enlarged spleen at baseline in the norUDCA 500 mg group, 3 (7.3%) in the 1,000 mg group, and 3 in the 1,500 mg group. At EOT this number was reduced to 4 (10.3%), 0, and 0 patients in each verum-treated group respectively, while the number of patients with enlarged spleen was 2 (5%) in the placebo group at baseline and increased to 3 (7.5%) at EOT. The mean values of CAI sum were low in all treatment groups at baseline and increased to 3 (7.5%) at EOT. The sub-scores with the highest mean values at baseline were: score for number of stools, score for general wellbeing, score for bloody stools, score for abdominal pain/cramps and laboratory findings. Sub-scores showed some variability but no trend among treatment groups. In addition, there was no statistical significant difference in SHS scores between treatment groups but indicated a slight improvement from baseline to EOT for the norUDCA 1,500 mg group for each individual dimension. Using the sum of eleven questions for the calculation of the fatigue score there was no significant difference from baseline (placebo: 23.0, norUDCA 500 mg: 21.0, 1,000 mg: 19.7, 1,500 mg: 23.3) to EOT (placebo 21.3, norUDCA 500 mg: 20.7, 1,000 mg: 19.8, 1,500 mg: 20.2) and between treatment groups. However, a slight improvement of symptoms according to PGA of efficacy could be achieved at EOT for 43.6% of patients in the 1,500 mg norUDCA group, 29.3% in the 1,000 mg, 23.1% in the 500 mg, and 30% in the placebo group. These differences did not reach statistical significance.

NorUDCA showed a favorable safety profile

Tolerability of the study medication was described as very good in the vast majority of patients and investigators. A poor tolerability was described only by two patients (4.9%) in the norUDCA 1,000 mg group and by one patient (2.5%) in the placebo group. Tolerability was described as equally good in all treatment arms. No patient died during the course of this study. The percentage of treatment emergent AEs was 80%, 59%, 73%, and 67% in the placebo group, for the norUDCA 500 mg, the norUDCA 1,000 mg group, and norUDCA 1,500 mg groups respectively. In total eight treatment emergent SAEs were noted in 8 patients, 3 in the norUDCA 500 mg group, 1 in the norUDCA 1,000 mg group, and 1 in the norUDCA 1,500 mg group. The number of treatment emergent SAEs was therefore similar between treatment groups. Only one SAE in the placebo group was described by an investigator as possibly being related to investigational medicinal product (IMP) (ocular icterus/lack of clinical efficacy). An additional five SAEs, not related to treatment, were also categorized as “disease progression/lack of clinical efficacy”. The remaining two SAEs were a removal of meniscus and a diagnosis of colon cancer 4 days after start of treatment. Treatment emergent AEs reported in more than 10 patients were abdominal pain (11), fatigue (13), nasopharyngitis (30), headache (13), and pruritus (17). There were no differences in the incidence of AEs between treatment groups related to dose-dependent effects, and none compared to placebo. In addition, the percentage of adverse drug reactions was comparable in all groups with 28%, 23%, 32%, and 28% in the placebo group, for the norUDCA 500 mg, the norUDCA 1,000 mg, and norUDCA 1,500 mg groups respectively. The most common AEs with the most prominent being nasopharyngitis in all treatment groups are listed in Table 3. Notably, pruritus occurred similarly across treatment arms and generally at low frequencies: 7 events in the 500 mg/d group, 7 in the 1,000 mg/d group, 12 in the 1,500 mg/d group, and 10 in the placebo group. Importantly pruritus was mild and no patient discontinued treatment. There were no significant differences of scores for pruritus between baseline visit (placebo 15.4, 500 mg 9.9, 1,000 mg 14.8, 1,500 mg 16.5; scale 0–100 mm) and EOT (placebo 15.3, 500 mg 10.8, 1,000 mg 20.9, 1,500 mg 15.2) and between all treatment groups. There was no effect on IBD...
activity. Taken together the safety profile of norUDCA seems to be favorable and no warning signs emerged from the data of this study.

**Discussion**

For decades there has been no significant progress in the medical therapy of PSC. This double-blind, randomized, placebo-controlled, dose finding study is a proof of concept study and the first human study with norUDCA to assess its efficacy, safety and tolerability in PSC patients. The main findings of the study are: (i) norUDCA resulted in a significant reduction of ALP values in PSC patients within 12 weeks of treatment compared to placebo, (ii) the positive effects occurred in a dose-dependent manner, and (iii) the safety profile of norUDCA was comparable to placebo in our study.

All three norUDCA doses tested in this study resulted in a significant reduction in ALP, which was most pronounced in the 1,500 mg arm and consequently all norUDCA treatment arms reached the primary efficacy endpoint at ITT analysis as defined for this study. In addition, the decline in ALP levels was paralleled by significant reductions in ALT, AST, and γ-GT levels. In particular, the time course of γ-GT levels uncovered the most pronounced relationship between norUDCA doses and its biochemical effect. In addition to serving as a biochemical parameter of cholestasis in PSC patients, serum γ-GT levels may also reflect inflammation-triggered hepatic oxidative stress, which may be significantly reduced upon norUDCA treatment as observed previously in preclinical models. Since spleen size may also reflect PSC activity it is worth noting that 13 verum-treated patients showed increased spleen size at baseline but only 4 patients at EOT. Potential anti-inflammatory effects of norUDCA need detailed future mechanistic studies. Interestingly, patients in the placebo arm showed a decrease in all serum parameters tested in the follow-up period of the study comparable to the effect of norUDCA in the verum group. This maybe primarily related to the more frequent reintroduction of UDCA treatment in this study group of patients who continued to show profoundly elevated cholestasis parameters throughout the treatment period, after discontinuation of UDCA in the pre-study washout phase. It is also important to note that the most pronounced relative decrease in serum ALP levels was observed in the 1,500 mg norUDCA group with a 26% reduction indicating a dose-dependent effect of norUDCA. This observation, as well as the fact the 1,500 mg norUDCA dose was well tolerated, is also relevant for the dose selection of future phase III clinical trial in PSC patients. Importantly 12.8% of patients achieved a reduction of serum ALP levels ≤1.5 × the ULN in the norUDCA 500 mg group, 41.5% in the 1,000 mg norUDCA group, 30.8% in the 1,500 mg norUDCA, and 12.5% in the placebo group, which was previously shown to be of potential prognostic importance in PSC patients.

Although a direct comparison of the current study with previous UDCA trials in PSC is not possible due to substantial differences in study design, UDCA has also demonstrated pronounced ALP reduction in the range observed for norUDCA herein or even higher. Along these lines, it is also important to note that in the post-hoc analyses of the very high dose UDCA trials as reported by Stanich et al. and of the Scandinavian UDCA trial by Lindström et al. patients with normalization of ALP had a better prognosis, although this was not necessarily related to UDCA use. This may underline the prognostic relevance of ALP normalization in PSC patients, but also points towards a potential dissociation from active drug (UDCA) treatment. Importantly, based on our preclinical findings norUDCA can be expected to have profoundly different/additional mechanisms than UDCA, which may not be fully reflected by biochemical improvement in short-term phase II studies. For our current study, it is essential to note that the observed response to norUDCA treatment was independent of whether patients were UDCA-naïve or -experienced and whether the physicians categorized them as UDCA non-responders or responders. There may however still be PSC patients who may benefit from UDCA treatment, potentially those with a significant reduction in ALP serum levels. Interestingly, the norUDCA-induced fall in serum ALP levels was most pronounced within the first two weeks of treatment. Consequently, it appears plausible that norUDCA may reduce the viscosity of bile immediately after starting the treatment, since it represents a potent pharmacological inducer of biliary bicarbonate secretion and therefore protects the liver.

The safety profile of norUDCA as observed in our study was very encouraging. Most importantly there was no statistical difference with relation to treatment emergent AEs between norUDCA and placebo arms. The most frequently reported complaints were related to nasopharyngitis, which was also comparable between all treatment arms. The number of reported SAEs was generally low and equally in all treatment arms. Consequently, the reported AEs in this study do not raise alarm for norUDCA treatment in PSC patients. This is particularly important for pruritus, which was not more frequently observed in the norUDCA arms, as pruritus may represent a serious problem associated with bile acid treatment in cholestatic patients. We did not observe any significant differences concerning fatigue score, CAI, and PGA between baseline and EOT in all treatment arms. However, it may be overambitious to expect significant differences within 12 weeks of norUDCA treatment; notably we also observed no worsening in these scores, which indicates at least that norUDCA was well tolerated in all active treatment arms. Importantly, this norUDCA study, the first conducted in humans, excluded patients with main bile duct strictures or recent need for endoscopic therapy, as we had potential concerns regarding the potent choleretic actions of norUDCA observed preclinically. Predicting the relative risk for potential aggravation of portal hypertension or the development of bile infarcts in norUDCA-treated PSC patients with more advanced disease or main bile duct strictures will be very challenging. This may become even more complex when combination therapy of norUDCA with...
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UDCA is considered in future studies, since the compounds may have additive beneficial effects.\textsuperscript{23} Therefore closely spaced, clinical, and biochemical controls will be necessary for the best safety of our patients in a future phase III clinical trial.

This norUDCA phase II dose finding study in PSC has several limitations including the lack of C4 bile acids and FGF19 serum levels. The primary efficacy variable of this phase II clinical trial was defined as the relative change \( \% \) in serum ALP between the baseline visit and the EOT, since ALP, transient elastography, and histology are currently seen as the most promising candidate surrogate endpoints for clinical trials in PSC patients.\textsuperscript{23,36,39,44} However, pronounced changes in liver stiffness or liver histology may be rather ambitious to expect within a short treatment period of 12 weeks in PSC patients and therefore have not been chosen as primary endpoints for this study. Moreover, the observed significant reduction in ALP values in all norUDCA treatment arms in our study should not be overestimated at this stage. A randomized controlled trial testing high doses of UDCA (17 to 23 mg/kg/day) showed a favorable biochemical trend but lacked significant effects on hard clinical endpoints such as transplant-free survival and was in addition probably underpowered.\textsuperscript{10} Testing even higher doses (28–30 mg/kg/day) in PSC patients again revealed an improvement in serum liver tests including ALP but were associated with adverse clinical outcomes.\textsuperscript{11} In the current study, PSC patients with more advanced disease with Child-Pugh class B and C and those with serum bilirubin levels higher than 3 mg/dl have been excluded due to potential safety reasons. Consequently, it is currently unclear whether norUDCA will be safe to use in these patients.

In summary, the herein presented findings justify a phase III clinical trial for norUDCA treatment in PSC patients. The design of such a trial poses several key questions: (i) a future study should consider combined endpoints with serum ALP levels, transient elastography, proper assessment of spleen size, and liver histology. Hard clinical endpoints such as need for liver transplantation or death will be unrealistic study endpoints for such a rare disease with a highly variable clinical course. (ii) The different therapeutic mechanisms and pharmacodynamics of norUDCA and its mother compound UDCA may provide a potential rationale for combining norUDCA and UDCA in the treatment of PSC, which has to be demonstrated in a combination therapy arm. (iii) Based on our current data the daily norUDCA dose of 1,500 mg was proven to be efficacious and safe, and therefore should be further tested in a phase III clinical trial.

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Conflict of interest

The Medical University of Graz has filed two patents for the use of norUDCA in the treatment of liver diseases and arteriosclerosis, and P.F. and M.T. are listed as co-inventors (publication numbers WO2006119803 and WO20099013334).

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Authors’ contributions

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Supplementary data

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