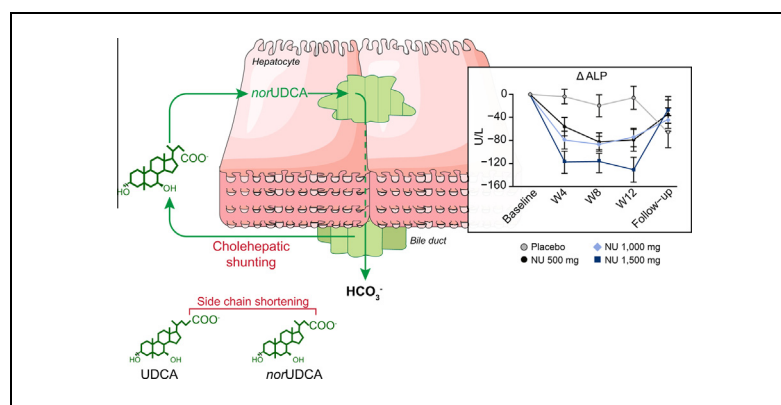


# *nor*Ursodeoxycholic acid improves cholestasis in primary sclerosing cholangitis

## Graphical abstract



## Highlights

- There is an urgent need for novel drugs for PSC.
- In this phase II clinical trial, *nor*UDCA reduced serum ALP levels within 12 weeks.
- *nor*UDCA's effects on liver enzymes were dose-dependent.
- The safety profile of *nor*UDCA was excellent.

## Authors

Peter Fickert, Gideon M. Hirschfield, Gerald Denk, ..., Markus Pröls, Michael P. Manns, Michael Trauner

## Correspondence

michael.trauner@meduniwien.ac.at  
(M. Trauner)

## 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, *nor*ursodeoxycholic acid (*nor*UDCA), significantly reduced serum alkaline phosphatase levels in a dose-dependent manner during a 12-week treatment. Importantly, *nor*UDCA showed a favorable safety profile, which was similar to placebo. The use of *nor*UDCA in PSC patients is promising and will be further evaluated in a phase III clinical study.

## ***nor*Ursodeoxycholic acid improves cholestasis in primary sclerosing cholangitis**

Peter Fickert<sup>1</sup>, Gideon M. Hirschfield<sup>2</sup>, Gerald Denk<sup>3</sup>, Hanns-Ulrich Marschall<sup>4</sup>, Istvan Altorjay<sup>5</sup>, Martti Färkkilä<sup>6</sup>, Christoph Schramm<sup>7</sup>, Ulrich Spengler<sup>8</sup>, Roger Chapman<sup>9</sup>, Annika Bergquist<sup>10</sup>, Erik Schrumpf<sup>11</sup>, Frederik Nevens<sup>12</sup>, Palak Trivedi<sup>2</sup>, Florian P. Reiter<sup>3</sup>, Istvan Tornai<sup>5</sup>, Emina Halilbasic<sup>13</sup>, Roland Greinwald<sup>14</sup>, Markus Pröls<sup>14</sup>, Michael P. Manns<sup>15</sup>, Michael Trauner<sup>13,\*</sup>,  
for the European PSC norUDCA Study Group<sup>†</sup>

<sup>1</sup>Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University of Graz, Graz, Austria; <sup>2</sup>Centre for Liver Research and NIHR Biomedical Research Unit, University of Birmingham, United Kingdom; <sup>3</sup>Department of Medicine II, Liver Center Munich, Ludwig Maximilians University (LMU), Munich, Germany; <sup>4</sup>Department of Molecular and Clinical Medicine, Sahlgrenska Academy, Institute of Medicine, University of Gothenburg, Gothenburg, Sweden; <sup>5</sup>Department of Gastroenterology, School of Medicine, Debrecen University, Debrecen, Hungary; <sup>6</sup>University of Helsinki and Clinic of Gastroenterology, Helsinki University Hospital, Helsinki, Finland; <sup>7</sup>1st Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; <sup>8</sup>Department of Internal Medicine 1, Rheinische Friedrich-Wilhelm's University Bonn, Bonn, Germany; <sup>9</sup>Translational Gastroenterology Unit, John Radcliffe Hospital, Oxford, United Kingdom; <sup>10</sup>Department of Gastroenterology and Hepatology, Karolinska University Hospital, Karolinska Institute, Huddinge, Stockholm, Sweden; <sup>11</sup>Section of Gastroenterology, Department of Transplantation Medicine, Division of Cancer Medicine, Surgery and Transplantation, Oslo University Hospital, Rikshospitalet, Oslo, Norway; <sup>12</sup>Hepatology, University Hospital Gasthuisberg, KU Leuven, Leuven, Belgium; <sup>13</sup>Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria; <sup>14</sup>Dr. Falk Pharma GmbH, Freiburg, Germany; <sup>15</sup>Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany

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**Background & Aim:** Primary sclerosing cholangitis (PSC) represents a devastating bile duct disease, currently lacking effective medical therapy. 24-*nor*ursodeoxycholic acid (*nor*UDCA) is a side chain-shortened C<sub>23</sub> 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 *nor*UDCA (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:** *nor*UDCA 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. Similar dose-dependent results were found for secondary endpoints, such as ALT, AST,  $\gamma$ -GT, or the rate of patients achieving ALP levels  $< 1.5 \times$  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 1,500 mg/d group, and three in the placebo group. There was no difference in reported pruritus between treatment and placebo groups.

**Conclusions:** *nor*UDCA significantly reduced ALP values dose-dependently in all treatment arms. The safety profile of *nor*UDCA was excellent and comparable to placebo. Consequently, these results justify a phase III trial of *nor*UDCA in PSC patients.

**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, *nor*ursodeoxycholic acid (*nor*UDCA), significantly reduced serum alkaline phosphatase levels in a dose-dependent manner during a 12-week treatment. Importantly, *nor*UDCA showed a favorable safety profile, which was similar to placebo. The use of *nor*UDCA in PSC patients is promising and will be further evaluated in a phase III clinical study.

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

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\* Corresponding author. Address: Department of Internal Medicine III, Medical University of Vienna, Waehringer Guertel 18–20, A-1090 Vienna, Austria. Tel.: +43 1 40 40047410.

E-mail address: [michael.trauner@meduniwien.ac.at](mailto:michael.trauner@meduniwien.ac.at) (M. Trauner).

<sup>†</sup> Full list study participants is listed at the end of the manuscript.

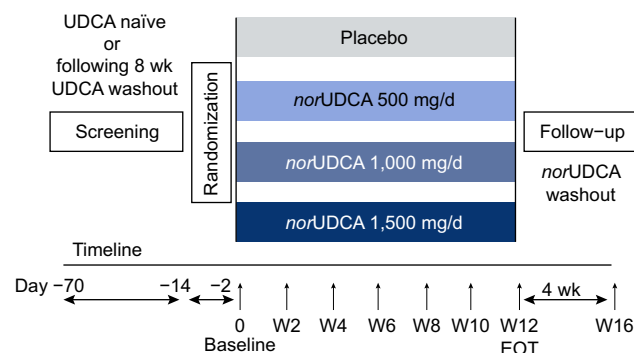


# Research Article

## 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.<sup>1</sup> 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.<sup>2</sup> 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 of PSC is highly controversial.<sup>3–5</sup> Numerous studies showed biochemical or histological improvements, but failed to demonstrate significant differences in death rates, transplant-free survival, or development of cholangiocarcinoma.<sup>4–11</sup> 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.<sup>10</sup> 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).<sup>11</sup> Interestingly, a study on the effects of UDCA withdrawal in PSC patients showed a significant deterioration of liver biochemistry and symptoms within 3 months.<sup>12</sup> Taken together, effective medical therapy for PSC is still an unmet clinical need and novel drugs are urgently needed.<sup>2</sup>

24-*nor*ursodeoxycholic acid (*nor*UDCA) is a side chain-shortened C<sub>23</sub> homologue of UDCA and was previously shown to be highly effective in preclinical mouse models of cholestatic and fibrotic liver diseases.<sup>13–17</sup> Comparing the therapeutic effects of UDCA and *nor*UDCA in *Abcb4* knockout mice (*Abcb4*<sup>−/−</sup>) as a model for sclerosing cholangitis and biliary fibrosis revealed specific and superior anti-inflammatory, anti-fibrotic, and anti-proliferative effects of *nor*UDCA.<sup>14–16</sup> The relative resistance of *nor*UDCA to *N*-acyl-amidation with taurine or glycine leads to cholehepatic shunting of *nor*UDCA. This means that unconjugated *nor*UDCA, 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.<sup>13,18–21</sup> This process of cholehepatic shunting results in ductal targeting and leads to profound stimulation of cholangiocyte bicarbonate secretion and consequently induction of bile acid-independent bile flow and flushing of bile ducts. In addition, biliary bicarbonate secretion may protect cholangiocytes as a bicarbonate umbrella<sup>22</sup> against their rather unfriendly environment, where the apical surface membranes have to continuously face millimolar concentrations of potentially toxic bile acids.<sup>23</sup> Moreover, *nor*UDCA and its metabolites when secreted into human bile are present in mostly monomeric rather than micellar form, enhancing its osmotic effect thus making *nor*UDCA a highly potent choleretic drug.<sup>18</sup> These favorable mechanisms of *nor*UDCA 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 *nor*UDCA vs. placebo; (ii) identify efficacious *nor*UDCA dose(s) for further evaluation in a future phase III clinical trial; and (iii) to study safety and tolerability of *nor*UDCA.



**Fig. 1. Trial design of the NUC-3 PSC study.** Patients were screened for eligibility and eligible patients were randomized in an equal ratio to either 500 mg/d, 1,000 mg/d, 1,500 mg/d *nor*UDCA, or placebo. After randomization, control visits were performed at week 0, 2, 4, 6, 8, 10, and 12. A follow-up visit was performed 4 weeks after end of study (week 16). UDCA, ursodeoxycholic acid; *nor*UDCA, *nor*ursodeoxycholic acid; wk or w, weeks; EOT, end of treatment.

## 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 *nor*UDCA 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 phosphatase (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 report<sup>12</sup> 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  $\geq 18$  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  $\geq 1.5 \times$  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, cholangiocellular carcinoma, 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- $\alpha$  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 ( $>50$   $\mu$ mol/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, end of treatment (EOT) at week 12, and follow-up visit at week 16 (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 [ $\gamma$ -GT], alkaline phosphatase [AP], total and conjugated bilirubin, albumin, c-reactive protein [CRP], total protein, serum creatinine, lipase, lactic 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.<sup>24</sup> 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.<sup>25</sup> The short health scale (SHS) is a simplified four-item questionnaire and was used for testing the patients' quality of life.<sup>26</sup> Compliance was recorded by counting the returned daily boxes for study medications. Adverse events (AEs) representing any unfavorable and unintended sign and symptom including abnormal laboratory findings were recorded at each control visit. Treatment emergent AEs were defined as any event from the start of 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 disability/incapacity, and congenital anomaly/birth defects.

#### Study endpoints

The primary efficacy variable in this clinical trial was the relative change (%) in ALP between the baseline visit and the EOT visit (LOCF), as serum ALP is a relevant surrogate marker for prognosis in PSC patients.<sup>27–29</sup> This was calculated for each patient as follows:

$$100 \times (\text{ALP}_{\text{LOCF}} - \text{ALP}_{\text{baseline}}) / \text{ALP}_{\text{baseline}}$$

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 x ULN) of ALP, absolute and relative changes of ALP,  $\gamma$ -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.

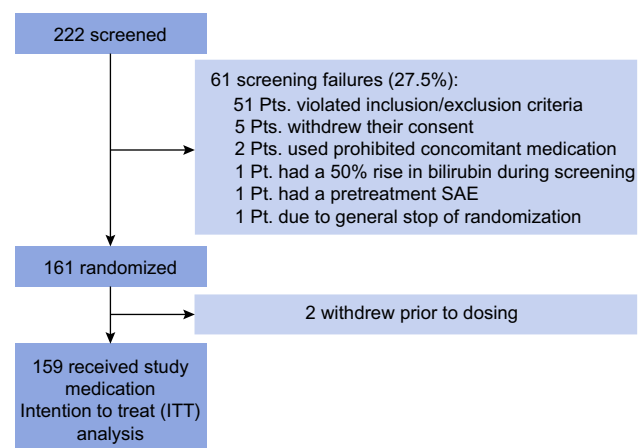
#### Statistical methods

The primary efficacy variable was subject to a confirmatory statistical analysis ( $p = 0.025$ , 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 this 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-wise) one-sided type I error rate of  $\alpha = 0.025$  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<sup>30</sup> 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 following 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 ALP 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 tests<sup>31</sup> 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 are 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.



**Fig. 2. Patient recruitment.** Of 222 patients assessed for eligibility, 161 were randomized and 159 received the study medication. 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. ITT, intention to treat; SAE, serious adverse event; Pts, patients; Pat, patient.

# Research Article

## 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 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 (*norUDCA* 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.

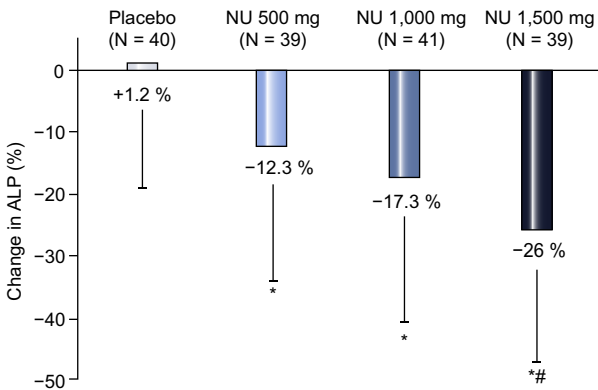
## Patient characteristics

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 (51.3%) 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 naïve, 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 40.0% in the placebo groups to 64.1% in the *norUDCA* 500 mg group. Fatigue at baseline was reported from 45.0% in the placebo group up to 70.7% in the *norUDCA* 1,000 mg group.

**Table 1. Demographic and baseline characteristics of randomized and treated patients.**

	Placebo (N = 40)	<i>norUDCA</i> 500 mg (N = 39)	<i>norUDCA</i> 1,000 mg (N = 41)	<i>norUDCA</i> 1,500 mg (N = 39)
Male, N (%)	25 (62.5%)	27 (69.2%)	28 (68.3%)	29 (74.4%)
Female, N (%)	15 (37.5%)	12 (30.8%)	13 (31.7%)	10 (25.6%)
Age (yr), (mean SD)	44.1 (14.89)	40.9 (12.24)	42.3 (13.08)	41.0 (13.11)
Weight (kg), (mean SD)	78.7 (13.63)	75.5 (13.51)	79.1 (15.37)	77.7 (15.65)
Height (cm), (mean SD)	176.0 (12.14)	176.0 (9.01)	175.7 (8.06)	176.9 (11.30)
BMI (kg/m <sup>2</sup> ), (mean SD)	25.5 (3.93)	24.3 (3.87)	25.6 (4.94)	24.8 (3.78)
Duration of PSC (Months), (median range)	83 (2–343)	83 (1–345)	45 (2–242)	52 (2–311)
New diagnosis of PSC, N (%)	8 (20.0%)	6 (15.4%)	8 (19.5%)	7 (17.9%)
IBD at screening	50%	77%	65%	64%
Ulcerative colitis, N (%)	15 (37.5%)	27 (69.2%)	22 (53.7%)	24 (61.5%)
Crohn's disease, N (%)	5 (12.5%)	3 (7.7%)	5 (12.2%)	1 (2.6%)
Time since first IBD symptoms (Months), (median range)	162 (36–451)	183 (10–416)	151 (4–526)	140 (11–476)
ALP at baseline (U/L), (mean SD)	456 (234.4)	495 (282.4)	369 (200.6)	464 (241.6)
UDCA naïve, N (%)	12 (30.0%)	9 (23.1%)	9 (22.0%)	10 (25.6%)
UDCA pre-treatment, N (%)	27 (67.5%)	28 (71.8%)	31 (75.6%)	27 (69.2%)
Responder, N (%)	18 (66.7%)	13 (46.4%)	14 (45.2%)	13 (48.2%)
Mean previous dose, mg/kg/d	14.5	13.7	12.2	13.3
Non-responder, N (%)	9 (33.3%)	15 (53.6%)	17 (54.8%)	14 (51.8%)
Mean previous dose, mg/kg/d	14.3	13.0	14.4	15.3
Unknown UDCA exposure, N (%)	1 (2.5%)	2 (5.1%)	1 (2.4%)	2 (5.1%)



**Fig. 3. Percentage change in serum levels of ALP after 12 weeks compared to baseline values.** Serum ALP levels decreased in a dose-dependent manner in response to *norUDCA* treatment with the most pronounced reduction in the 1,500 mg *norUDCA* arm. NU, *norUrsodeoxycholic acid*. \*Indicates  $p < 0.01$  compared to placebo; #Indicates  $p < 0.025$  comparison of NU 1,500 mg vs. NU 500 mg. A closed testing procedure using Simes intersection tests<sup>31</sup> was applied for each group comparison (comparison of each *norUDCA* treatment group against placebo and pairwise comparisons among the three *norUDCA* treatment groups). The inverse normal procedure was applied to Simes adjusted  $p$  values. One-sided  $p$  values for the analysis of the primary endpoint were calculated using Wilcoxon rank sum tests.

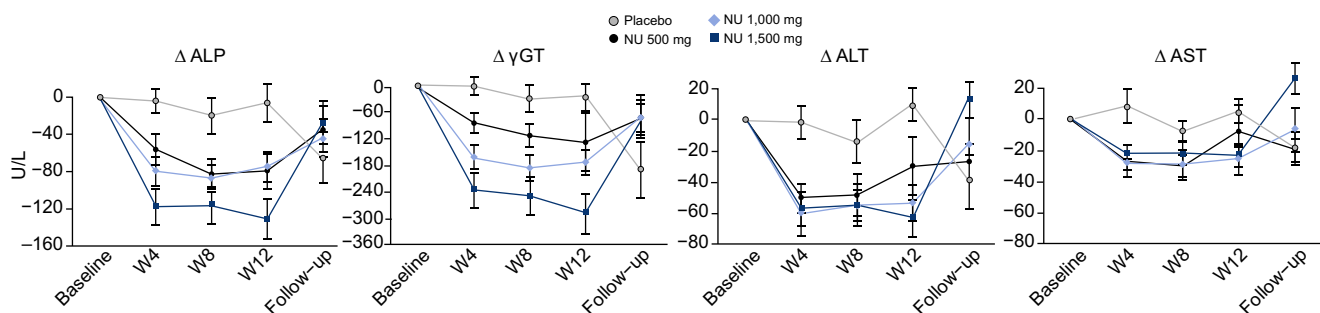
*NorUDCA significantly reduces serum ALP levels in a dose-dependent fashion in PSC patients*

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.<sup>27–29</sup> 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 –12.3%, –17.3%, and –26.0% in the 500, 1,000, and 1,500 mg/d *norUDCA* groups respectively (Fig. 3). Thus, the study was positive on ITT for all *norUDCA* treatment groups. Statistically significant differences compared to placebo were also seen for all three *norUDCA* groups in the per protocol set (data for per protocol analysis not shown). Possible confounding effects of geographical clusters were not detected by stratified analysis of the relative reduction of serum ALP (data not shown). The percentage of treated patients reaching prognostically meaningful reductions of ALP defined as serum levels  $\leq 1.5$ -fold the ULN<sup>27,28</sup> was: 12.5% (5/40) in the placebo group, 12.8% (5/39) in the *norUDCA* 500

mg group, 41.5% (17/41) in the 1,000 mg group (the group starting with the lowest ALP levels), and 30.8% (12/39) in the 1,500 mg group. ALP levels within ULN were found in 2/40 (5.0%) of the placebo group, 1/39 (2.6%) of the *norUDCA* 500 mg group, 5/41 (12.2%) in the 1,000 mg group, and 4/39 (10.3%) of the 1,500 mg group. Moreover, all three *norUDCA* doses induced a rapid decrease in serum ALP levels within 4 weeks, which was again most pronounced in the highest *norUDCA* treatment group (Fig. 4A). A post-hoc analysis with respect to the daily *norUDCA* dose/kg body weight confirmed the main result of this study, as well as a dose selection for the phase III program (data not shown). Serum ALP levels remained relatively stable throughout the treatment period in the *norUDCA* treatment arms, but returned nearly to baseline levels at the end of the follow-up period, indicating a pronounced rebound of serum ALP levels following discontinuation of *norUDCA* treatment. In contrast, in the placebo group ALP levels increased 1.2% during the study period until EOT (Fig. 3). Interestingly, ALP serum levels decreased in the placebo group from EOT to the follow-up visit. This could at least in part be related to more frequent reintroduction of UDCA treatment in the placebo arm (9/40 = 22.5% patients; vs. 4/39 = 10.3% *norUDCA* 500 mg; 2/41 = 4.9% *norUDCA* 1,000 mg; 5/39 = 12.8% *norUDCA* 1,500 mg) following the active treatment period upon physician's discretion. It may also be of interest to note that 8 out of these 9 patients were classified as UDCA responders before study entry and that the proportion of patients receiving UDCA after placebo in the follow-up period was about double the number in the active treatment groups (Table S1). Serum bilirubin levels did not significantly differ between treatment groups at the different time points (Table S2).

*NorUDCA significantly reduces  $\gamma$ -GT, ALT, and AST serum levels in PSC patients*

*NorUDCA* significantly reduced serum  $\gamma$ -GT levels in a dose-dependent fashion, and was most pronounced with a mean reduction of 33.9% 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  $\gamma$ -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



**Fig. 4. Absolute changes in serum ALP,  $\gamma$ GT, ALT, and AST levels throughout the study period.** Y axis indicates absolute changes (U/L) compared to baseline levels. NU, *norUrsodeoxycholic acid*. Placebo group, gray circles; 500 mg NU group, black circles; 1,000 mg NU group light diamonds; 1,500 mg NU group, dark squares. Descriptive statistical methods were used to analyze all variables. Continuous variables are summarized using the number of observations, arithmetic mean, and standard deviation.

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**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.**

	Placebo (N = 40)	norUDCA 500 mg (N = 39)	norUDCA 1,000 mg (N = 41)	norUDCA 1,500 mg (N = 39)
Male, mean % (N)	+4.7% (25)	−14.1% (27)	−13.9% (28)	−27.8% (29)
Female, mean % (N)	−4.6% (15)	−8.3% (12)	−24.7% (13)	−20.5% (10)
UDCA responder, mean % (N)	−6.2% (18)	−9.9% (13)	−16.4% (14)	−19.5% (13)
UDCA non-responder, mean % (N)	−3.5% (9)	−10.1 (15)	−14.9% (17)	−27.3% (14)
UDCA naïve, mean % (N)	+12.3% (12)	−14.9% (9)	−24.8% (9)	−32.0% (10)
ALP > 3 × ULN, mean % (N)	−0.9% (25)	−14.4% (23)	−19.7% (17)	−26.2% (22)
ALP ≤ 3 × ULN, mean % (N)	4.8% (15)	−9.2% (16)	−15.6% (24)	−25.7% (17)
PSC with IBD, mean % (N)*	5.8% (20)	−10.4% (29)	−14.5% (27)	−24.0% (27)
PSC without IBD, mean % (N)*	−4.8% (18)	−21.8% (8)	−22.8% (14)	−29.9% (11)
PSC duration ≤ 5 yr, mean % (N)**	8.3% (15)	−10.5% (17)	−20.3% (22)	−28.2% (20)
PSC duration > 5 yr, mean % (N)**	−3.0% (24)	−13.7% (22)	−13.4% (18)	−23.7% (19)

\* In N = 5 no classification to IBD or non-IBD was possible.

\*\* In N = 2 data from the first PSC diagnosis was not documented.

rebound of  $\gamma$ -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 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 EOT (1.3/1.7 norUDCA 500 mg group, 0.9/1.0 1,000 mg group, 2.0/1.3 1,500 mg group, and 2.1/1.5 in the placebo group). 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 1000 mg, 1 in the 1500 norUDCA group, 3 in the placebo 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

Table 3. Most common adverse events.

	Placebo (N = 40)	norUDCA 500 mg (N = 39)	norUDCA 1,000 mg (N = 41)	norUDCA 1,500 mg (N = 39)
Abdominal pain	5 (12.5%)	1 (2.6%)	4 (9.8%)	1 (2.6%)
Diarrhea	4 (10.0%)	0 (0.0%)	1 (2.4%)	3 (7.7%)
Fatigue	4 (10.0%)	2 (5.1%)	2 (4.9%)	5 (12.8%)
Nasopharyngitis	7 (17.5%)	6 (15.4%)	9 (22.0%)	8 (20.5%)
Arthralgia	4 (10.0%)	1 (2.6%)	1 (2.4%)	0 (0.0%)
Back pain	4 (10.0%)	1 (2.6%)	0 (0.0%)	3 (7.7%)
Headache	3 (7.5%)	2 (5.1%)	1 (2.4%)	7 (17.9%)
Pruritus	4 (10.0%)	3 (7.7%)	4 (9.8%)	6 (15.4%)

Number of patients (%) with treatment emergent adverse events.

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  $\gamma$ -GT levels. In particular, the time course of  $\gamma$ -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  $\gamma$ -GT levels may also reflect inflammation-triggered hepatic oxidative stress,<sup>32–34</sup> which may be significantly reduced upon *norUDCA* treatment as observed previously in preclinical models.<sup>14–17</sup> Since spleen size may also reflect PSC activity<sup>35</sup> 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 also 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  $\leq 1.5 \times$  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* group, and 12.5% in the placebo group, which was previously shown to be of potential prognostic importance in PSC patients.<sup>28,36,37</sup>

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.<sup>6–8</sup> 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.*<sup>27</sup> and of the Scandinavian UDCA trial by Lindström *et al.*,<sup>38</sup> patients with normalization of ALP had a better prognosis, although this was not necessarily related to UDCA use. This may underscore 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-responder 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.<sup>39</sup>

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.<sup>40–42</sup> 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.<sup>23,14,15</sup> Predicting the relative risk for potential aggravation of portal hypertension or the development of bile infarcts in

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*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 UDCA is considered in future studies, since the compounds may have additive beneficial effects.<sup>23</sup> 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.<sup>29,36,43,44</sup> 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.<sup>10</sup> 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.<sup>11</sup> 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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**I.A.:** Travel grants from Falk Foundation.

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**M.P.:** Employee of Dr. Falk Pharma GmbH

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Please refer to the accompanying ICMJE disclosure forms for further details.

### Authors' contributions

All authors contributed to acquisition of data, review and critical revision of the manuscript and approved the final version of the manuscript. **P.F.:** study design/conception, interpretation of data, manuscript writing; **R.G.:** study design/conception; **M.P.:** study

design/conception; **M.P.M.:** study design/conception, interpretation of data; **M.T.:** study design/conception, interpretation of data, manuscript writing.

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#### \*Complete list of participating countries and study centers

**AUSTRIA: Vienna:** Principle Investigator (PI) M. Trauner; Sub-investigators (SIs): H. Hofer, S. Traussnigg, E. Halilbasic; **Graz:** PI P. Fickert; SIs: E. Krones, F. Durchschein; **Innsbruck:** PI W. Vogel; SIs: H. Zoller, H. Steinle, H. Schwaighofer, A. Viveiros; **BELGIUM: Leuven:** PI F. Nevens; SIs: W. van Steenberghe, S. Van der Merwe; **DENMARK: Odense:** PI T. Havelund; SI: A. A. Krag; **FINLAND: Helsinki:** PI: M. Faerkkilae; SI: K. Jokelainen; **GERMANY: Hannover:** PI M. Manns; SIs: M. Cornberg, K. Deterding, H.-W. Wedemeyer; K. Port; **Mainz:** PI P. Galle; SIs: J. Schattenberg, A. Grambihler; **Essen:** PI G. Gerken; SIs: A.E. Canbay, A. Kahraman, J. Best, C. Jochum; **Heidelberg:** PI D. Gotthardt; SIs: K.-H. Weiss, A. Wannhoff, C. Rupp; **Kiel:** PI R. Guenther; SIs: T. Bielefeld, P. Wietzke-Braun, H. Vollbrecht, A. Koepke; **Homburg (Saar):** PI F. Lammert; SIs: B. Appenrodt, C. Juengst; **Munich:** PI G. Denk; SIs: S. Hohenester, F. Reiter; **Hamburg:** PI C. Schramm; SIs: C. Weiler-Normann, S. Lueth, M. Lang, M. Wehmeyer; **Bonn:** PI U. Spengler; SIs: R. Hueneburg, Z. Balta, C. Bless-Paar; **Aachen:** PI C. Trautwein; SIs: J. Tischendorf, H. Lutz, S. Voigt, F. Tacke; **Frankfurt (Main):** PI S. Zeuzem; SIs: T. Welzel, S. Klein, S. Luhne, J. Schrewe; **Wuerzburg:** PI A. Geier; SIs: T. Kudlich, O. Goetze, J. Weiss, C. Hoell; **Erlangen:** PI A. Kremer; SIs: S. Zopf, J. Siebler, M. Waldner, R. Goertz; **Tuebingen:** PI C. Berg; SIs: D. Egetemeyr, C. Werner, J. Schwarz, V. Scheble; **Duesseldorf:** PI D. Haeussinger; SIs: H. Bock, V. Keitel, U. Heinzel-Pleines; **Berlin:** PI E. Schott; SIs: D. Beha, T. Mueller, C. Plehm, M. Schulz; **Mannheim:** PI C. Antoni; SIs: T. Zimmerer, W. Reindl; **HUNGARY: Budapest:** PI Z. Tulassay; SI: G. Lengyel; **Debrecen:** PI I. Altorjay; SIs: K. Palatka, I. Tornai; **LITHUANIA: Kaunas:** PI L. Kupcinskis; SIs: V. Petrenkiene, J. Kondrackiene; **NETHERLANDS: Amsterdam:** PI U. Beuers; SIs: R. Van Dijk, L. Ailette de Buy Wenninger, S. Aronson; **Rotterdam:** PI H. van Buuren; SI: W. Lammers; **Nijmegen:** PI J. Drenth; SIs: F. Berden, M. Broekman, S. Pape; **Leiden:** PI B. van Hoek; SIs: M. Coenraad, A. Anderson; **NORWAY: Oslo:** PI K. M. Boberg; SI: E. Schrumpf; **Bergen:** PI G.G. Dimcevski; SI: M. Vesterhus; **SPAIN: Barcelona:** PI A. Pares; SI: L. Caballería; **SWEDEEN: Gothenburg:** PI H.-U. Marschall; SIs: I. Friis-Liby, A. Schult; **Stockholm:** PI A. Bergquist; SIs: K. Said, S. Haas; **UNITED KINGDOM: Oxford:** PI R. Chapman; SIs: K. Williamson, J. Ryan; **Norwich:** PI S. Rushbrook; SI: E. Goode; **Birmingham:** PI G. Hirschfield; SIs: P. Trivedi, P. Eddowes.

#### Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jhep.2017.05.009>.

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## Research Article

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