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Research Article
DILI, Autoimmune, Cholestatic and Genetic Diseases

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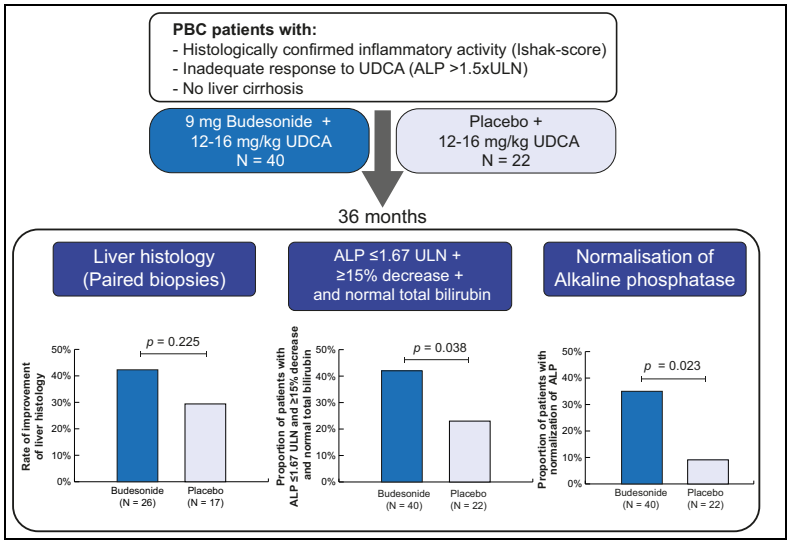
Graphical abstract



Highlights

- In patients with PBC at high-risk of disease progression, budesonide in addition to UDCA did not improve liver histology.
- Addition of budesonide was associated with improved biochemical markers of PBC disease activity.
- Future trials of new therapies in PBC will benefit from the use of a variety of clinical endpoints.

Authors

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Lay summary

Around one-third of patients with primary biliary cholangitis (PBC) needs additional medical therapy alongside ursodeoxycholic acid (UDCA) treatment. In this clinical trial, the addition of the corticosteroid budesonide did not improve liver histology; there were however relevant improvements in liver blood tests.

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A placebo-controlled randomised trial of budesonide for PBC following an insufficient response to UDCA

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Background & Aims: In patients with primary biliary cholangitis (PBC), the efficacy of budesonide, a synthetic corticosteroid displaying high first-pass metabolism, is unresolved. In a placebo-controlled, double-blind trial, we evaluated the added-value of budesonide in those with PBC and ongoing risk of progressive disease despite ursodeoxycholic acid (UDCA) treatment.

Methods: We evaluated 62 patients with PBC who had histologically confirmed hepatic inflammatory activity, according to the Ishak score, and an alkaline phosphatase (ALP) >1.5× upper limit of normal (ULN), after at least 6 months of UDCA therapy. Participants were randomly assigned 2:1 to receive budesonide (9 mg/day) or placebo once daily, for 36 months, with UDCA treatment (12–16 mg/kg body weight/day) maintained. Primary efficacy was defined as improvement of liver histology with respect to inflammation and no progression of fibrosis. Secondary outcomes included changes in biochemical markers of liver injury.

Results: Recruitment challenges resulted in a study that was underpowered for the primary efficacy analysis. Comparing patients with paired biopsies only (n = 43), the primary histologic endpoint was not met (p >0.05). The proportion of patients with ALP <1.67×ULN, a ≥15% decrease in ALP and normal bilirubin was higher in the budesonide group than in the placebo group at 12, 24, and 36 months (p <0.05, each). In contrast to placebo, budesonide reduced mean ALP and 35% of budesonide-treated patients achieved normalisation of ALP (placebo 9%; p = 0.023). Serious adverse events occurred in 10 patients receiving budesonide and 7 patients receiving placebo.

Conclusion: Budesonide add-on therapy was not associated with improved liver histology in patients with PBC and insufficient response to UDCA; however, improvements in biochemical markers of disease activity were demonstrated in secondary analyses.

Lay summary: Around one-third of patients with primary biliary cholangitis (PBC) needs additional medical therapy alongside ursodeoxycholic acid (UDCA) treatment. In this clinical trial, the addition of the corticosteroid budesonide did not improve liver histology; there were however relevant improvements in liver blood tests.

ClinicalTrials.gov number: NCT00746486.

Introduction

Primary biliary cholangitis (PBC) is a chronic and usually progressive cholestatic liver disease with autoimmune features.[1] End-stage biliary cirrhosis arises when patients have chronic cholestatic hepatitis driven by a lymphocytic and destructive cholangitis, as well as persistent interface hepatitis.[2] Although an autoimmune disease with regards to its underpinning risk factors, attempts at immunosuppressive treatment with agents such as predniso(lo)ne, azathioprine, methotrexate, or cyclosporine have largely failed due to a lack of efficacy and/or concern over long-term side effects.[3,4] Standard therapy in PBC remains with ursodeoxycholic acid (UDCA) and second line treatment with obeticholic acid (licenced) and fibric acid derivatives (off-label); these therapies derive benefit by modulating bile acid signalling at the level of hepatic and bile duct epithelial cells and thereby, improve bile secretory capacity.[5–7] In large cohort studies, it has become robustly evident that a failure

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to control disease as indicated by worsening of serum liver tests such as alkaline phosphatase (ALP), aspartate aminotransferase (AST), bilirubin or albumin is associated with progressive liver fibrosis and development of cirrhosis.[8,9] Current clinical trial design for new therapies in PBC is thus based on biochemical surrogate markers of disease activity which are reasonably likely to predict patient outcome.

Patients with PBC frequently have histopathologic evidence of an interface hepatitis, the features of which can be indistinguishable from corticosteroid responsive autoimmune hepatitis; furthermore, the histologic appearances can be accompanied by other features of autoimmune hepatocellular injury such as increased serum aminotransferase activity as well as co-existent autoantibodies.[10] Budesonide is a potent synthetic corticosteroid displaying high first-pass metabolism within the liver, resulting in fewer systemic side effects compared to predniso(lo)ne. In patients with PBC exhibiting 'florid' interface hepatitis on biopsy, the efficacy of budesonide in improving liver histology and biochemistry when used in combination with UDCA has been reported in 2 studies conducted in non-cirrhotic, early stage patients.[11,12] Administration to patients with more advanced disease provided only minimal benefits but enhanced the risk of osteoporosis[13]; additionally budesonide is avoided in cirrhosis because of the risk of portal vein thrombosis and uncontrolled systemic shunting of the drug.[14] The intent of therapy in part reflects the association between serum aminotransferase activity and interface hepatitis, and the association with disease progression in PBC. Equally relevant are *in vitro* data showing that corticosteroids and UDCA are synergistic in increasing expression of the biliary chloride/bicarbonate anion exchanger 2 (AE2)[15] and, thereby, biliary secretion of bicarbonate. This process may stabilise the biliary bicarbonate umbrella, a protective mechanism of biliary epithelia against uncontrolled invasion of toxic bile acids.[16]

Herein, we report the final results of a 3-year phase-III double-blind randomised placebo-controlled trial evaluating UDCA + budesonide *vs.* UDCA + placebo. The aim of the present study was to evaluate the impact of budesonide in patients incompletely responding to standard UDCA treatment.

Patients and methods

Study design

This was a randomized, double-blind, placebo-controlled, multicentre, comparative phase III clinical study in patients with non-cirrhotic PBC (Fig. 1); EudraCT number 2007-004040-70; trial protocol available on request from Dr Falk Pharma GmBH. The study took place from January 2009 until July 2015 in 23 study sites across 13 countries, with 6 active sites in Germany, 3 active sites in Denmark and 2 active sites in Austria. The study of patients with PBC was conducted with 2 treatment arms in the form of a parallel group comparison and served to compare the efficacy, tolerability and safety of a combination therapy of UDCA plus budesonide, against UDCA plus placebo.

The target population was non-cirrhotic patients with PBC already treated with UDCA, who were at risk of disease progression based on ≥1 of the following criteria: a) serum ALP ≥3 ×ULN at any time since diagnosis of PBC and alanine aminotransferase (ALT) ≥2×ULN; b) total bilirubin ≥1 mg/dl; c) moderate to severe periportal or periseptal lymphocytic interface hepatitis or; d) periportal and portal fibrosis with numerous septa (Ludwig stage III) without cirrhosis. A delay in patient recruitment due to these strict criteria led to a protocol modification 33 months after launching the study to allow for additional inclusion of patients with incomplete response to UDCA as defined by the failure to achieve serum ALP values <1.5×ULN after at least 6 months of treatment with UDCA and with inflammatory activity according to the modified hepatic activity index (mHAI) sum score[17] in the histological assessment of the liver. Histological assessment had to be conducted no more than 6 months before baseline. In total, 48/62 (77%) treated patients fulfilled the original inclusion criterion, 34/40 (85%) in the budesonide group and 14/22 (64%) in the placebo group.

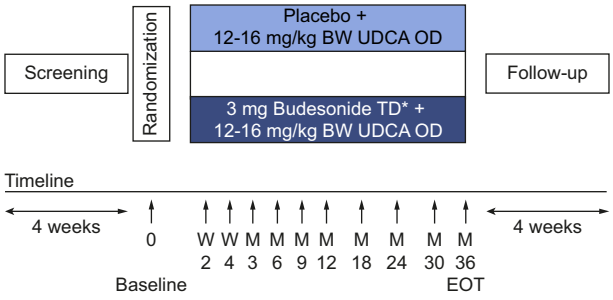

Fig. 1. Trial design. Patients were screened for eligibility and eligible patients were randomly assigned 2:1 to receive either 9 mg/d budesonide or placebo on top of 12–16 mg/kg bodyweight/day of UDCA. 6 mg/d budesonide was allowed if aspartate aminotransferase (AST) values were normalized, i.e. dose adjustment according to disease activity. After randomization, control visits were performed at week 0, 2, and every 3 months. A follow-up visit was performed 4 weeks after end of study. AST, aspartate aminotransferase; BD, twice daily; EOT, end of treatment; OD, once daily; TD, three times daily; UDCA, ursodeoxycholic acid. *Dose adjustment down to 3 mg Budesonide BD allowed if AST normal.

Patients were randomized centrally with an allocation ratio of 2:1 to 9 mg/d budesonide (1 × 3 mg capsule 3× daily) or placebo (1 × capsule 3× daily). All patients concomitantly received 12–16 mg UDCA acid/kg bodyweight/day. The individual dose of budesonide/placebo could be reduced depending on the AST values (*i.e.* dose adjustment according to disease activity). If AST values normalised at any time from the start of trial treatment, 2 capsules of budesonide/placebo were allowed (1 capsule in the morning and 1 capsule in the evening). Randomisation was performed using randomly permuted blocks and patients were allocated to the next available randomisation number according to the centrally prepared randomisation list. Participance for an individual patient consisted of a 36-month treatment period with 11 scheduled trial visits (V1–V11).

After the interim analysis in September 2017, the Independent Data Monitoring Committee recommended terminating the study, treating the remaining patients until they had completed at least 1 year of treatment and then performing a termination visit. The study was conducted in accordance with good clinical practice, the Declaration of Helsinki, all applicable national laws, and was approved by independent local ethics committees. Dr. Falk Pharma GmbH were the study sponsor; all authors had access to the results.

Population

This study included female and male patients aged ≥18 years with a biopsy-proven diagnosis of PBC, who were anti-mitochondrial antibody (AMA) positive on immunofluorescence >1:40, had received UDCA treatment for at least 6 months

prior to baseline and had an incomplete response to UDCA treatment.

Patients with histologically proven cirrhosis, HIV infection and other clinically dominant chronic liver diseases including hepatitis B or C infection, primary sclerosing cholangitis, Wilson's disease, α1-anti-trypsin-deficiency, haemochromatosis and auto-immune hepatitis (AIH) were excluded. Patients with PBC and features of AIH treated insufficiently with UDCA monotherapy, could be enrolled. In addition, patients with densitometry proven osteoporosis, hypertension (persistently raised blood pressure >140/90 mmHg), diabetes mellitus and cataract(s) were excluded. Further exclusion criteria can be found in the supplementary material.

Procedures

Efficacy and safety assessments were conducted at baseline, at the 9 interim visits (week 2, 4 and every 3 months thereafter), and at end of treatment (EOT) at month 36 or withdrawal visit. The baseline examinations were performed within 4 weeks. At all visits vital signs were controlled and routine laboratory parameters were assessed including routine serum biochemistry (ALT, AST, gamma-glutamyltransferase, ALP, total bilirubin, albumin, total protein, serum creatinine, cholinesterase, glutamate dehydrogenase), haematology (blood count and differential blood count), and blood coagulation tests (international normalised ratio [INR], partial thromboplastin time).

A liver biopsy was performed, according to standard medical procedures, at baseline, if no liver biopsy had previously been performed or if the last biopsy dated back more than 6 months. A second liver biopsy was performed at the EOT/withdrawal visit. Biopsies were analysed by 2 central pathologists (U. Drebber, D. Wendum). The samples were processed according to standard pathological methods. The severity of fibrosis/cirrhosis was staged according to Ludwig.[18] Inflammatory activity was rated according to Desmet *et al.*[19] and Ishak *et al.*[17] Additional histological parameters were classified according to Pares *et al.*[20] Further procedures are described in the supplementary material.

Study endpoints

Study design was based on regulatory feedback and discussion. The primary efficacy variable was the response rate defined as rate of patients with improvement of liver histology with respect to inflammation (≥3 points in the mHAI sum score or no inflammatory activity according to Ishak *et al.*[17]) and no progression of fibrosis (staging according to Ludwig[18]) at the individual last patient visit within the study compared to baseline.

A number of clinically meaningful secondary efficacy variables were evaluated. This included the rate of patients presenting with cirrhosis or oesophageal varices and/or ascites at the end of treatment; the rate of patients registered on the liver transplant waiting list; or patients with liver-related death during up to 3 years of treatment; the rate of patients with improvement of liver histology with respect to stage (according to Ludwig[18]); the rate of patients with improvement of liver histology with respect to grade (according to Desmet *et al.*[19]) and stage (according to Ludwig[18]); the rate of patients with improvement of liver histology with respect to grade (according to Desmet *et al.*[19]). Secondary efficacy variables included normalisation of serum levels of ALP, or reduction of baseline ALP levels by at least 40%; absolute (and % change in) ALP, bilirubin, ALT and AST; rate of patients with ALP ≤1.67×ULN AND ≥15% decrease in ALP AND total bilirubin within the normal limits at 12 months (last observation carried forward [LOCF]), 24 months (LOCF), 36 months (LOCF); course of pruritus (measured by visual analogue scale [VAS]), course of fatigue and quality of life (measured by PBC-40), course of the adapted Mayo Risk Score[21] and the modified globe score, assessment of inflammatory activity (mHAI score[17]), quality of life by PBC-40 and global assessment of efficacy by patient and investigator.

Sample size and statistical analysis

The primary objective of the study was to demonstrate the superiority of a combination therapy with UDCA plus budesonide compared to UDCA plus placebo in terms of the primary efficacy variable. The primary efficacy variable was subjected to a confirmatory statistical analysis ($\alpha = 0.025$, one-sided). Analysis was based on the methodology of Cochran-Mantel-Haenzel-Test method with "patients at risk of disease progression" as a stratification factor. Evaluations of secondary efficacy and safety variables were performed for exploratory purposes.

After the pre-defined interim analysis, the sponsor decided to stop the study due to insufficient recruitment but followed the independent data monitoring committee recommendation to treat patients already recruited for at least 1 year. Early termination of this study resulted in a lack of power. For details see the supplementary methods section.

Results

Patients

A total of 90 patients were enrolled with all individuals providing informed consent prior to study participation; 62 patients (69%) fulfilled inclusion and exclusion criteria and were randomised. All received at least 1 dose of study medication, and, were thus evaluated in the safety and intention-to-treat (ITT) population. Forty patients (65%) were allocated to the 9 mg/d budesonide group, while 22 patients (36%) received placebo in addition to UDCA (Fig. 2).

Among 62 patients treated with study medication, 33 (53%) patients withdrew from the trial prematurely (budesonide: 23/40 patients [58%]; placebo: 10/22 patients [46%]). The only differences between the treatment groups regarding the percentages of patients withdrawn from the study concerned AEs (budesonide: 8/40 patients [20%]; placebo: 0/22 patients [0%]). A total of 42 patients were excluded from the ITT population to form the per protocol (PP) analysis set (n = 20). The most frequent reasons for exclusion from the ITT analysis set were premature termination (25/62 patients [40%]), efficacy data after baseline not available (19/62 patients [31%]), and adherence (17/62 patients [27%]). Three patients in the budesonide group and 1 patient in the placebo group stopped the study at 1 year. Of these, 1 patient in the budesonide group and the placebo patient had a final biopsy.

The percentages of patients excluded from the PP analysis set, by reason for exclusion, were similar in both treatment groups. Table 1 shows baseline demographics; no clinically relevant differences were present between treatment groups, neither for the ITT nor the PP population. There were minor imbalances in the proportion of patients at risk of disease progression and the intensity of pruritus was higher in the placebo group. Histological evaluation showed a qualitative difference for less fibrosis, portal inflammation and lower mHAI score in the placebo group (Table 2). Median treatment duration was 32.3 months and 29

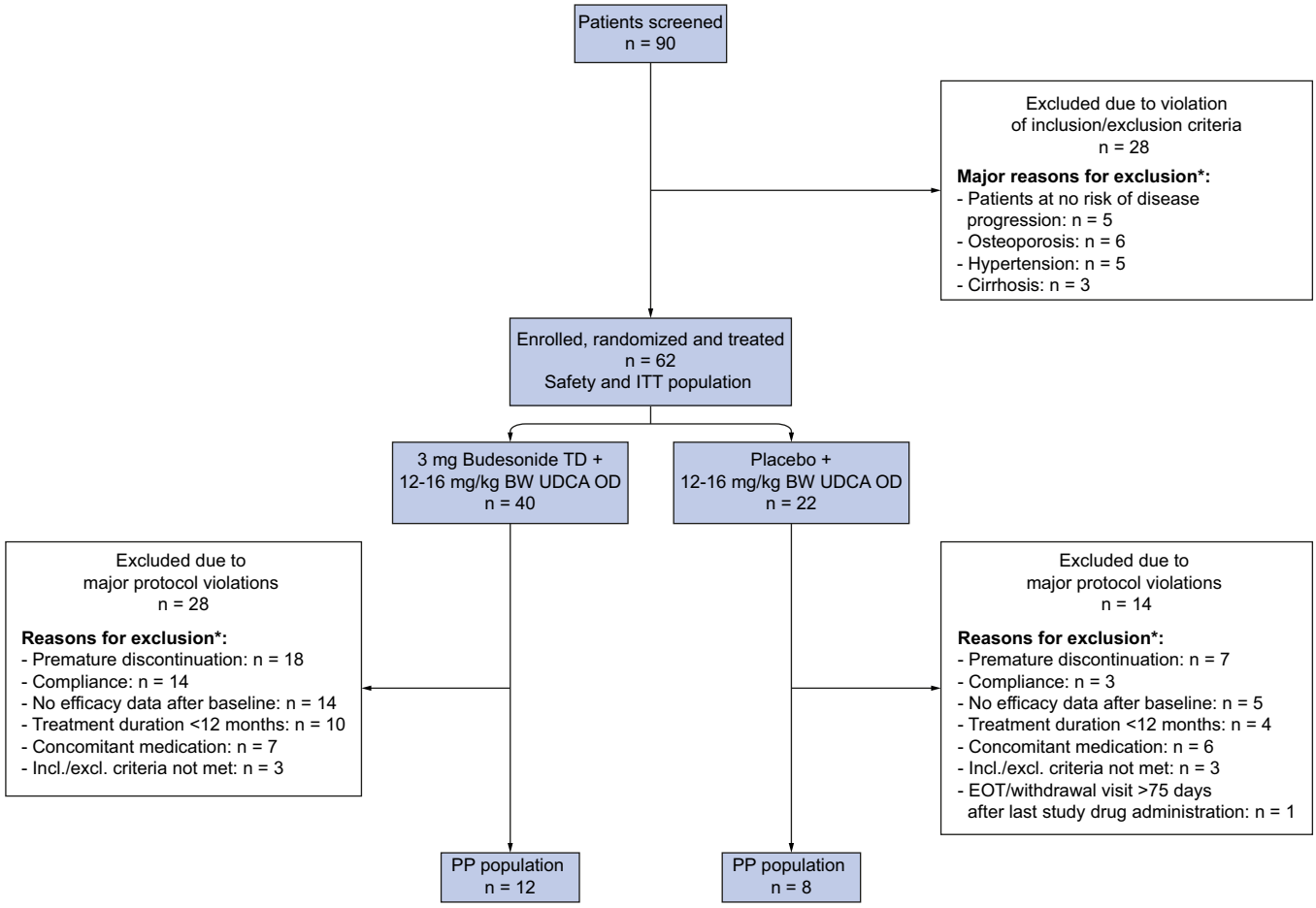

Fig. 2. Patient characteristics. Flow diagram of the progress through the phases of the parallel randomised trial (enrolment, intervention allocation, follow-up, and data analysis). EOT, end of treatment; ITT, intention to treat; OD, once daily; PP, per-protocol; TD, three times daily; UDCA, ursodeoxycholic acid. *Multiple reasons for exclusion were applicable for some patients.

patients completed 3 years of treatment (17 patients in the budesonide group and 12 patients in the placebo group). All patients included during the first 33 months before the modification of the trial population showed incomplete response to UDCA as defined by ALP <1.5×ULN.

Primary efficacy

In the ITT analysis of the primary endpoint, 11/40 (28%) patients in the budesonide and 5/22 (23%) patients in the placebo group showed a response at 36 months (LOCF). Thus, the observed difference (4%; $p = 0.36$) could not confirm superiority of budesonide compared to placebo regarding the primary endpoint. The results of the subgroup analyses by country, by duration of study participation and by being at risk of disease progression did not support any differences between the treatment groups within each subgroup.

With regard to the primary efficacy variable among the 43 patients with paired biopsies (baseline and EOT), the response rate in the budesonide group (11/26 patients [42%]) was numerically higher than in the placebo group (5/17 (29%); Fig. 3A). Further histological evaluations were inconclusive (Fig. 3B). However, portal inflammation improved more in the budesonide group (mean [SD] change in mHAI score −1.3 [2.1]) than the placebo group (−0.3 [3.6]).

Secondary efficacy analyses

In the ITT population (budesonide n = 40, placebo n = 22) starting from Month 12 (LOCF), the proportion of patients with ALP ≤1.67 ×ULN and ≥15% decrease in ALP AND total bilirubin in the normal limits was higher in the budesonide group than the placebo group ($p = 0.029$; 12 months, and $p = 0.021$; 24 months) (Fig. 4A). At Month 36 (LOCF), these criteria were met in 17/40 patients (43%) in the budesonide group and 5/22 patients (23%) in the placebo group ($p = 0.038$). ALP improved in the budesonide group compared to the placebo group ($p < 0.05$; Fig. 4B and Table 3) and a reduction of at least 40% in ALP values was observed in 19/40 (48%) and 4/22 (18%) patients in the budesonide and placebo group, respectively. Consistently, normalisation of ALP occurred more often after budesonide treatment (35%) than after placebo treatment (9%; $p = 0.023$; Fig. 4C and Table 3). Serum bilirubin increased in the placebo group (0.6 mg/dl), but not in the budesonide group (−0.02 mg/dl; Fig. 4D and Table 3). Serum ALT and AST improved in the budesonide group compared to the placebo group ($p < 0.05$ each; Fig. 4E,F and Table 3).

The adapted Mayo Risk Score did not change in the budesonide group (LOCF, mean change from baseline [SD] 0.006 [0.3]), but slightly increased in the placebo group (0.2 [0.8]). Changes in modified GLOBE score were small, showing a small reduction in the budesonide group (LOCF, mean change from baseline [SD] 0.3 [0.5]) and essentially no change in the placebo group (0.06 [0.8]).

Table 1. Baseline demographics and disease characteristics (intention-to-treat population).

	Budesonide (n = 40)	Placebo (n = 22)
Female, n (%)	38 (95)	22 (100)
Age, years	54 [10]	51 [12]
BMI, kg/m^2	24.2 [8.8]	25.7 [5.0]
Duration of disease, year	7.4 [9.6]	5.0 [10.8]
Patients at risk of disease progression*	34 (85)	14 (64)
Alkaline phosphatase, U/L	262 [286]	256 [210]
Alkaline phosphatase >1.67×ULN	30 (75)	17 (77)
Total bilirubin, mg/dl	0.55 [0.63]	0.61 [0.23]
Total bilirubin >ULN at baseline	9 (23)	1 (5)
ALT, U/L	70 [56]	76 [62]
AST, U/L	44 [37]	54 [33]
GGT, U/L	183 [307]	197 [351]
Albumin, U/L	4.4 [0.3]	4.2 [0.5]
Prothrombin time, INR	0.9 [0.1]	0.9 [0.1]
Pruritus VAS, cm	1.2 [4.0]	2.2 [7.0]

Categorical variables are presented as n (%) and continuous variables are presented as median [IQR].
ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyltransferase; INR, international normalised ratio; PBC, primary biliary cholangitis; ULN, upper limit of normal; VAS, visual analogue scale.
*Risk of disease progression was defined by fulfilling one or more of the following criteria: serum alkaline phosphatase ≥3× the upper limit of normal (corresponding to 312 U/L for women and 387 U/L for men) at any time since diagnosis of PBC OR ALT or AST ≥2× upper limit of normal (corresponding to 70 U/L for women and 100 U/L for men) at inclusion OR moderate to severe periportal or periseptal lymphocytic interface hepatitis OR periportal and portal fibrosis with numerous septa (Ludwig stage III) without cirrhosis.

Table 2. Histological characterization at baseline (intention-to-treat population).

	Budesonide (n = 40)	Placebo (n = 22)
Fibrosis (Ludwig score), n (%)*		
Stage 1	1 (3)	3 (14)
Stage 2	17 (43)	12 (55)
Stage 3	22 (55)	7 (32)
Inflammatory activity (Desmet score)*		
Grade 1	2 (5)	4 (18)
Grade 2	30 (75)	15 (68)
Grade 3	8 (20)	3 (13)
Periportal or periseptal interface hepatitis (piecemeal necrosis), n (%)*		
Absent	1 (3)	1 (5)
Mild (focal, few portal areas)	15 (38)	10 (46)
Mild/moderate (focal, most portal areas)	12 (30)	6 (27)
Moderate (continuous around <50% of tracts or septa)	10 (25)	4 (18)
Severe (continuous >50% of tracts or septa)	2 (5)	1 (5)
Confluent necrosis, n (%)		
Absent	29 (73)	17 (77)
Focal confluent necrosis	11 (28)	5 (23)
Portal inflammation, n (%)*		
Mild, some or all portal areas	3 (8)	4 (18)
Moderate, some or all portal areas	15 (38)	10 (46)
Moderate/marked, all portal areas	16 (40)	8 (36)
Marked, all portal areas	6 (15)	0 (0)
mHAI sum score*, median (IQR)	7 (3)	6 (2)

mHAI, modified hepatic activity index.
*Derived by using the 'consent' value. In case of mHAI scores equally assessed by the 2 pathologists the values of the first central pathologist were used for the single items of mHAI score at baseline.

During up to 3 years of treatment, the rate of clinical events (cirrhosis or oesophageal varices and/or ascites at EOT, or patients registered on the liver transplant waiting list, or patients with liver-related death) was low and did not differ between the budesonide group (1/40 [3%]) and the placebo group (2/22 [9%]; p = 0.93, Cochran-Mantel-Haenzel-Test with "patients at risk of disease progression" as a stratification factor).

Symptoms

Pruritus as measured by VAS remained unchanged in the budesonide group (LOCF, mean change from baseline [SD]: −0.3 [2.3]) compared to a slight increase in the placebo group (0.6 [3.1]). Quality of Life remained unchanged (Table 3).

Safety

In total, 60/62 patients (97%) experienced AEs (Table 4; 98% and 96% in the budesonide group and placebo group, respectively). Arthralgia, osteopenia, cataract(s), muscle spasms, hypertension, dyspepsia, weight increase, abdominal pain, peripheral oedema and blood cortisol decrease were noticeably more frequent in the budesonide group than in the placebo group, some of these are commonly observed effects after long-term therapy with glucocorticoids. On the other hand, pruritus, diarrhoea and nausea were more common in the placebo group (Table 4).

No deaths were reported. Serious adverse events (SAEs; defined as per international practice) were reported in 25/40 patients (63%) in the budesonide group and in 14/22 patients (64%) in the placebo group. Most of the SAEs were related to the planned investigational liver biopsy (22/40 patients [55%] in the budesonide group and 11/22 patients [50%] in the placebo group). Next to SAEs of liver biopsy, 17 SAEs occurred in 12 patients during the study and were distributed across diverse preferred terms without relevant differences between the 2 treatment groups: 8/40 patients (20%) with 10 SAEs in the budesonide group and 6/22 patients (27%) with 7 SAEs in the placebo group.

Frequencies of AEs leading to discontinuation of study drug were higher under budesonide than under placebo treatment, i.e. 9/40 patients (23%) in the budesonide group and 2/22 patients (9%) in the placebo group. Adverse drug reactions were reported in 24/40 patients (60%) in the budesonide group and in 8/22 patients (36%) in the placebo group. The proportion of drug-related AEs (budesonide/placebo) was higher in the budesonide group, especially regarding the preferred terms blood cortisol decrease (6 patients [15%]), cataract (5 patients [13%]), hypertension (5 patients [13%]) and osteopenia (4 patients [10%]), for which no cases were reported in the placebo group.

In the budesonide group, 8/40 (20%) patients reduced their budesonide dose at various time points; in the placebo group, 2/22 (9%) patients reduced their daily medication. Of the drop-outs, 3/23 (13%) in the budesonide group reduced their dose before the premature termination. In 2 cases, the drop-out was after 28 months budesonide therapy and the dose reduction was close to the study termination in only 1 case (3 months). In contrast, 5/8 (63%), of the patients with dose reduction completed the study.

Laboratory assessments showed slight suppression of adrenal function and effects on bone metabolism in the budesonide group (Table S1). Serum cortisol was slightly reduced in the budesonide group compared to baseline and placebo. Furthermore, there was a slight reduction in bone-specific ALP, N-MID Osteocalcin and the T-Scores of femoral neck and lumbar spine in the budesonide group (Table S1).

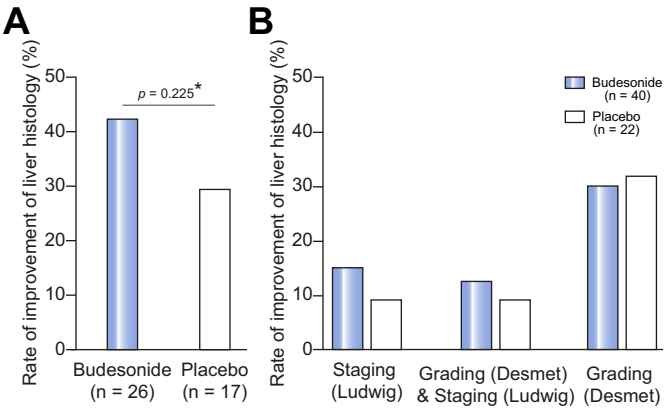

Fig. 3. Histological efficacy variables. (A) Improvement of liver histology with respect to inflammation (an improvement by at least 3 points in the Ishak sum score or no inflammatory activity) and no progression of fibrosis (Ludwig staging), compared to baseline at the individual last patient visit within the study, paired biopsies only. (B) Improvement of liver histology with respect to stage (Ludwig) and grade (Desmet). *Cochran-Mantel-Hentzel Test stratified according to stratification factor "patients at risk of disease progression".

Discussion

Herein, we report on a prospective, randomised, placebo-controlled trial assessing the addition of oral budesonide for patients with PBC and an incomplete response to UDCA alone. We describe the therapeutic impact of budesonide, as measured by change over time in histology and serum liver tests. We found no additional improvement in predefined histologic outcomes compared to placebo, even though the mHAI-score decreased more from baseline (−1.3 [2.1]) in the budesonide group than in the placebo group (−0.3 [3.7]). However, improvements in relevant biochemical surrogates of disease activity were found. Therapy was well tolerated but systemic effects of long-term corticosteroid use were evident. We also demonstrate the significant real-world challenges of completing an adequately powered clinical trial in PBC if serial liver biopsies are mandatory.

Although there are 2 licenced therapies for PBC,[5] as well as off-label data on fibric acid derivatives,[7] there remains an unmet need for treatment options that address the clinically evident spectrum of inflammatory liver disease. Budesonide is a non-halogenated corticosteroid with potent dual agonism for the glucocorticoid and xenobiotic pregnane X receptor, which exhibits a high (90%) first-pass effect through the liver when administered orally, thus mitigating systemic bioavailability and related side effects.[14,22] Prior clinical studies have suggested a benefit when budesonide is added to treatment approaches in PBC.[11,12]

Significant challenges in trial recruitment, possibly related to the regulatory need for paired liver biopsies, finally resulted in the termination of this study prior to the recruitment of sufficient patient numbers. However, we now report the findings of this double-blind, placebo-controlled, but underpowered trial in patients with non-cirrhotic PBC and an incomplete biochemical response to UDCA (ALP >1.5×ULN) as well as a liver biopsy showing inflammatory activity. In our study, patients were randomly assigned 2:1 to oral budesonide 9 mg/day or placebo, alongside UDCA (12–16 mg/kg/d), for 36 months. The primary endpoint agreed with regulators during the design of the trial – an improvement of ≥3 points in the mHAI sum score or no inflammatory activity according to Ishak and no progression of fibrosis stage – did not differ between groups. However, the proportion of patients with serum ALP values <1.67×ULN and a ≥15% drop in ALP from baseline and normal bilirubin – more recently acknowledged by regulatory authorities in Europe and North America as a valid surrogate criterion reflecting favourable long-term prognosis in PBC – was significantly higher in the budesonide group than in the placebo group at 12, 24, and 36 months. Budesonide was associated with significantly reduced serum cortisol concentration and lumbar spine bone density; adverse event monitoring was also reported more frequently in patients treated with budesonide.

Several notable themes arise when evaluating these data. Firstly, the study was designed with a histologic endpoint. PBC is a rare and heterogeneous inflammatory liver disease, wherein features of disease are classic but not archetypal.[2] In addition to the challenges of consistent biopsy interpretation and the slow nature of histologic changes in a disease, liver biopsy is invasive and increasingly not used clinically in the diagnosis and management of PBC.[5] Therefore, the trial design and the trial activities differed from clinical practice in the treatment of patients with PBC. In this setting, recruitment of patients was ultimately a factor in study deliverability, even accepting that liver histology provides important information on safety and efficacy in the development of new therapies for patients with liver disease. Furthermore, data on histology are inconclusive for the following limitations: first, only a much smaller number of patients than initially planned in the sample size calculation had biopsies at baseline and at 3 years. Thus, the study is underpowered for histology. The secondary analyses were not formally powered; such power calculations allow only a statement about whether an actually existing effect may not be detected. In the analyses, we report a statistically significant difference between budesonide and placebo for the secondary endpoint. Thus, the degree of confidence in the result itself is already covered by the level of significance. Second, a subset of patients had biopsies at baseline and at 1 year only due to early termination caused by the substantial recruitment problems. One year is most likely too short to reflect potential treatment effects on histology. Furthermore, it is notable that even for the standard therapy, UDCA, very well powered and longer duration clinical trials were needed to meet a histologic end-point.[23] At the time of study design and biopsy interpretation, more contemporary PBC histologic grading and staging approaches (*e.g.* Nakanuma) were not in widespread use, and as a result we were unable to evaluate treatment efficacy with methods beyond those described. In addition, a recent trial assessing the efficacy of bezafibrate as add-on therapy for patients with PBC could not detect changes in liver histology despite a strong reduction of biochemical surrogate markers of disease activity.[7] Equally whilst non-invasive tests such as elastography are not designed to replace careful histologic assessment, they do provide valuable adjunctive data on liver stiffness related to inflammation and fibrosis. However, at the time of study conduct, elastography was not widely available. Overall, this study points to the need to carefully consider the role of liver biopsy in PBC trials.

Within PBC, there is also the ongoing challenge of correctly defining overlap syndromes, particularly AIH. In the context of a study such as this, ultimately some discretion rests with investigators and our approach was to focus on the patient history in this regard: based on a review of case histories, there was 1 patient with 'AIH' and 1 patient with 'suspicion of AIH' in the

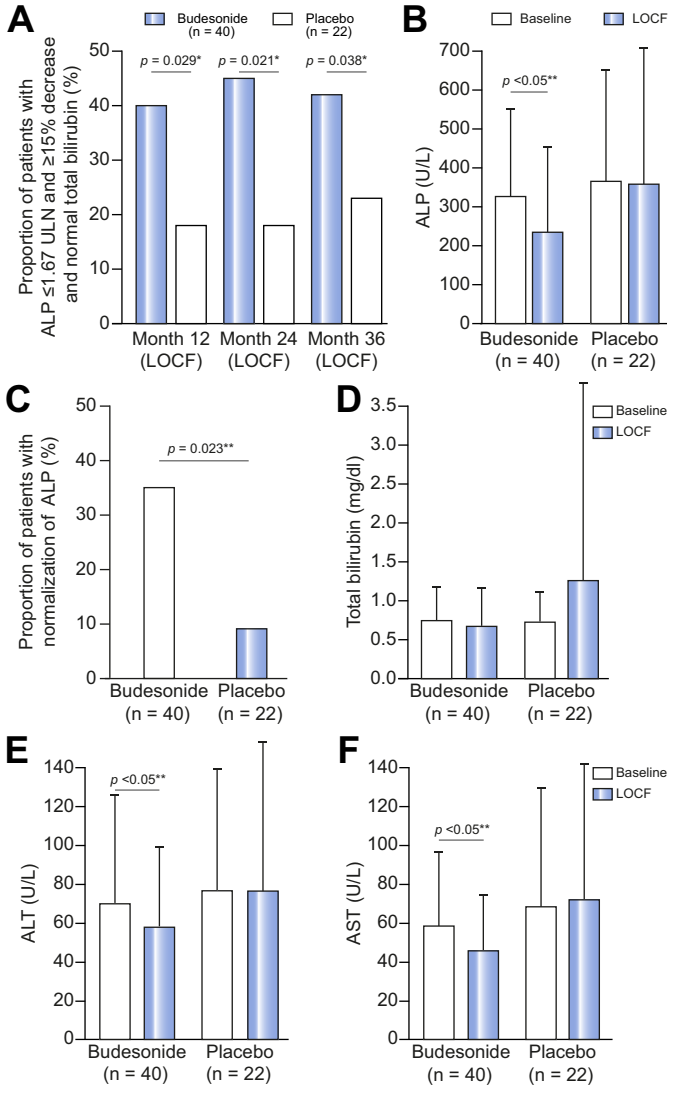

Fig. 4. Biochemical efficacy variables. (A) Proportion of patients with serum levels of ALP ≤1.67 ULN and ≥15% decrease and normal total bilirubin at different months after baseline (LOCF). (B) Serum levels of ALP at baseline and LOCF. (C) Proportion of patients with normalized ALP at month 36 (LOCF). (D) Serum levels of total bilirubin at baseline and LOCF. (E) Serum levels of ALT at baseline and LOCF. (F) Serum levels of AST at baseline and LOCF. *Cochran-Mantel-Hentzel Test stratified according to stratification factor "patients at risk of disease progression"; **Fisher's Exact Test; ALP, alkaline phosphatase; ALT, alanine aminotransferase, AST, aspartate aminotransferase; LOCF, last observation carried forward.

budesonide group (2/40; 5%); in the placebo group, there was only 1 patient with 'AIH' (1/22; 5%). A final limitation we acknowledge but believe not to be material is a late biochemical effect of UDCA therapy: 8/40 individuals (20%) in the treatment group had <12 months between diagnosis and first use of study medication; 5/22 (27%) individuals in the placebo arm had <12 months between diagnosis and first use of study medication.

Current clinical trial activity, in part because of many of the issues we report herein, now has a focus on robust surrogates of disease activity.[24] In this respect, the secondary analyses provide more consistent evidence that add-on budesonide may be beneficial. However, this must be interpreted in the context of secondary analyses, for which there were distinctions in patient

Table 3. Secondary efficacy variables.

	Budesonide (n = 40)	Placebo (n = 22)
ALT [U/L]		
Baseline	70 (56)	76 (50)
Change from baseline (LOCF)	−12 (30)	0 (47)
AST [U/L]		
Baseline	58 (39)	68 (62)
Change from baseline (LOCF)	−13 (19)	4 (47)
ALP [U/L]		
Baseline	327 (227)	367 (289)
Change from baseline (LOCF)	−95 (166)	−9 (177)
Normalization (LOCF), n (%)	14 (35)	2 (9)
≥40% reduction (LOCF), n (%)	19 (47)	4 (18)
Total bilirubin [mg/dl]		
Baseline	0.75 (0.46)	0.67 (0.41)
Change from baseline (LOCF)	−0.02 (0.44)	0.59 (2.22)
mHAI-Score		
Baseline	6.9 (2.1)	6.1 (2.2)
Change from baseline (LOCF)	−1.3 (2.1)	-0.3 (3.7)
Pruritus VAS [cm]		
Baseline	2.4 (2.9)	3.3 (3.4)
Change from baseline (LOCF)	−0.3 (2.3)	0.6 (3.1)
PBC-40 - Quality of Life		
Domain: Symptoms		
Baseline	17.0 (5.2)	18. 5 (4.7)
Change from baseline (LOCF)	0.1 (3.0)	1.7 (2.8)
Domain: Itch		
Baseline	5.7 (3.4)	6.5 (4.1)
Change from baseline (LOCF)	−0.5 (2.8)	0.5 (1.2)
Domain: Fatigue		
Baseline	26.8 (11.8)	32.8 (13.3)
Change from baseline (LOCF)	−1.6 (7.1)	−0.4 (9.3)
Domain: Cognition		
Baseline	11.7 (5.4)	14.0 (4.8)
Change from baseline (LOCF)	0.4 (4.0)	0.4 (3.0)
Domain: Social		
Baseline	18.4 (8.6)	19.7 (7.4)
Change from baseline (LOCF)	−0.1 (5.4)	2.5 (8.6)
Domain: Emotional		
Baseline	7.6 (3.4)	8.8 (3.7)
Change from baseline (LOCF)	0.1 (2.6)	0.2 (3.5)

Data presented as mean (SD).
ALP, serum alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LOCF, last observation carried forward; mHAI, modified hepatic activity index; VAS, visual analogue scale.

ascertainment and inclusion criteria compared to other recent PBC clinical trials. Nevertheless, if compared to an end-point that has been applied to a conditionally licenced therapy (obeticholic acid),[6] we report that the proportion of patients with ALP ≤1.67 ×ULN and a ≥15% decrease in ALP AND normal total bilirubin was significantly higher in the budesonide group than the placebo group (Fig. 4A). Consistently, normalisation of ALP occurred more often after budesonide treatment than after placebo treatment and a ≥40% reduction in ALP was also more often observed in the budesonide group.[6]

Tolerability of budesonide was in line with prior experience, and as expected there were treatment-related dropouts. Nevertheless, recommending budesonide use for a chronic disease such as PBC remains a challenge if duration of therapy is considered. The induction dose of budesonide used in the present study was higher (9 mg/day) than in a previous study.[12] In that regard, whilst no new safety signals emerged from our study, long-term clinical use of budesonide, even dose adjusted, will need to accommodate the potential for harmful effects on bone health for example.[25] Further evaluation of the impact of

Table 4. Overview of treatment-emergent AEs (safety population).

	Budesonide (N = 40) n (%)	Placebo (N = 22) n (%)
AEs	39 (98)	21 (96)
TEAEs	39 (98)	21 (96)
Follow-up AEs	3 (8)	3 (14)
Serious TEAEs	25 (63)	14 (64)
AEs leading to premature discontinuation of the study medication	9 (23)	2 (9)
ADRs[a]	24 (60)	8 (36)
Most common AEs[b]		
Headache	18 (45)	9 (41)
Nasopharyngitis	10 (25)	5 (23)
Pruritus	6 (15)	7 (32)
Back pain	7 (18)	4 (18)
Hypertension	8 (20)	2 (9)
Bronchitis	6 (15)	3 (14)
Influenza-like illness	6 (15)	3 (14)
Abdominal pain upper	5 (13)	4 (18)
Pain in extremity	5 (13)	3 (14)
Nausea	4 (10)	4 (18)
Cataract	6 (15)	2 (9)
Muscle spasms	6 (15)	1 (5)
Blood cortisol decreased	6 (15)	0 (0)
Arthralgia	6 (15)	0 (0)
Osteopenia	6 (15)	0 (0)
Diarrhoea	2 (5)	4 (18)
Osteoporosis	5 (13)	1 (5)
Oropharyngeal pain	4 (10)	2 (9)
Cough	3 (8)	3 (14)
Dyspepsia	5 (13)	1 (5)
Weight increased	5 (13)	0 (0)
Abdominal pain	5 (13)	0 (0)
Oedema peripheral	5 (13)	0 (0)
Fatigue	4 (10)	1 (5)

ADRs, adverse drug reactions; AE, adverse event; TEAE, treatment emergent adverse event; PT, preferred term.
[a]TEAE or post-treatment AE with causal relationship assessed at least as possible to Budesonide and/or Placebo. AEs due to the planned liver biopsies within the study are not listed.
[b]Occurred in at least 5 patients of the safety population.

budesonide on symptoms is required, but of note, pruritus did not deteriorate on treatment.

In conclusion, add-on budesonide did not confer a histologic benefit (defined by a ≥3-point reduction in mHAI sum score or no inflammatory activity according to Ishak) in UDCA-treated patients with PBC and a high risk of disease progression. Addition of budesonide was associated with improvements in biochemical surrogate markers of liver injury; these surrogate markers of disease activity have been associated with patient outcomes in other studies. Delivery of clinical trials in PBC that are designed with histologic endpoints, in a manner that recruits and retains appropriate cohort sizes, remains challenging. The use of alternative surrogates of disease severity, such as elastography, alongside serum liver tests requires ongoing consideration.

Abbreviations

AE, adverse event; AE2, biliary chloride/bicarbonate anion exchanger 2; AIH, auto-immune hepatitis; ALP, alkaline phosphatase; ALT, alanine aminotransferase, AST, aspartate aminotransferase; EOT, end of treatment; mHAI, modified hepatic activity index; INR, international normalized ratio; ITT, intention to treat; LOCF, Last observation carried forward; PBC, primary biliary cholangitis; PP, per-protocol; SAE, serious adverse event; UDCA, ursodeoxycholic acid; ULN, upper limit of normal; VAS, visual analogue scale.

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Conflict of interest

G Hirschfield: personal fees and advisory board fees from Dr Falk Pharma GmbH, Cymabay, Gilead, Intercept, GSK, Roche, Pliant and Genfit. U Beuers: support for investigator-initiated studies by Dr.Falk and Intercept. No lecture fees in 2019 and 2020 (current member EASL Clinical Practice Guideline Committees). A Pares: received grant funding, personal fees and advisory board fees from Intercept Pharmaceuticals; advisory board fees and personal fees from Novartis; and personal fees from CymaBay Therapeutics and Inova Diagnostics. M Manns: grants, personal fees and non-financial support from Gilead; grants, personal fees and non-financial support from Intercept; grants, personal fees and non-financial support from Falk; grants, personal fees and non-financial support from Novartis, outside the submitted work. R. Greinwald, M. Pröls and M. Stiess: employees of Dr Falk Pharma GmbH. All other authors had no relevant conflicts of interest to declare.

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

Authors' contributions

All authors have had access to the trial report and approved the final submission, contributing to study conduct and reporting. GMH and RP were chief investigators; GMH, UB, MS and MP wrote the paper and finalised for submission; they vouch for the integrity of the data analyses.

Data availability statement

Available on request to study sponsor, Dr Falk Pharma GMBH.

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Supplementary data

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