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Ustekinumab for Patients With Primary Biliary Cholangitis Who Have an Inadequate Response to Ursodeoxycholic Acid: A Proof-of-Concept Study

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The interleukin (IL)-12 signaling cascade has been associated with primary biliary cholangitis (PBC). This multicenter, open-label, proof-of-concept study evaluated the anti-IL12/23 monoclonal antibody, ustekinumab (90 mg subcutaneous at weeks 0 and 4, then every 8 weeks through week 20), in adults with PBC and an inadequate response to ursodeoxycholic acid therapy (i.e., alkaline phosphatase [ALP] >1.67× upper limit of normal [ULN] after ≥6 months). ALP response was defined as a >40% decrease from baseline and ALP remission as ALP normalization (if baseline ALP 1.67×-2.8× ULN) or <1.67× ULN (if baseline ALP >2.8× ULN). Changes in Enhanced Liver Fibrosis (ELF) scores and serum bile acids were also assessed. At baseline, patients had median disease duration of 3.2 years, median ELF score of 9.8, and highly elevated total bile acid concentration (median, 43.3 μmol/L); 13 of 20 (65%) patients had baseline ALP >3× ULN. Although steady-state serum ustekinumab concentrations were reached by week 12, no patient achieved ALP response or remission. Median percent ALP reduction from baseline to week 28 was 12.1%. ELF score decreased slightly from baseline to week 28 (median reduction: 0.173), and total serum bile acid concentrations decreased from baseline to week 28 (median reduction: 8.8 μmol/L). No serious infections or discontinuations resulting from adverse events were reported through week 28. One patient had a serious upper gastrointestinal hemorrhage considered unrelated to test agent by the investigator. *Conclusion:* Open-label ustekinumab therapy, though associated with a modest decrease in ALP after 28 weeks of therapy, did not otherwise appreciably change ALP and overt proof-of-concept was not established as per prespecified primary endpoint of proposed efficacy. No new ustekinumab safety signals were observed. (HEPATOLOGY 2016;64:189-199)

Primary biliary cholangitis (formally known as cirrhosis, PBC) is an autoimmune liver disease characterized by progressive lymphocytic cholangitis, ductopenia, cholestasis, and circulating antimitochondrial antibodies. Treatment-unresponsive disease is associated with development of biliary cirrhosis and end-stage liver disease (ESLD).[1,2] A combination of genetic and environmental influences are thought important to disease etiology and outcome, with significant genetic and immunological insights confirming an autoimmune component to disease, alongside the consequences of subsequent cholestasis.

Ursodeoxycholic acid (UDCA), which decreases plasma and biliary endogenous bile acid concentrations, inhibits hepatocyte apoptosis and stimulates hepatobiliary secretion, ultimately delaying the development of ESLD, is the only approved treatment for PBC. However, approximately 40% of UDCA-treated PBC patients have documented inadequate biochemical response and demonstrated progressive liver disease.[3]

Abbreviations: AIH, autoimmune hepatitis; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DSC, Dose Selection Committee; ELF, Enhanced Liver Fibrosis; ESLD, end-stage liver disease; FIS, Fatigue Impact Scale; HRQoL, health-related quality of life; IFN, interferon; Ig, immunoglobulin; IL, interleukin; IQR, interquartile range; LTE, long-term extension; PBC, primary biliary cirrhosis; PBC-40, 40-item assessment of PBC-related symptoms that impact HRQoL; SC, subcutaneous; STAT, signal transducer and activator of transcription; Th, T-helper cells; TLR, Toll-like receptor; TNF, tumor necrosis factor; UDCA, ursodeoxycholic acid; ULN, upper limit of normal.

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The cytokine profile in serum and liver specimens of PBC patients suggests activation and/or recruitment of T-helper (Th) 1 cells (e.g., interferon gamma [IFNγ]).[4,5] Additionally, plasma levels of chemokine ligand 9 (monokine induced by IFNγ) and induced protein 10 are increased in patients with PBC.[6,7] More recent data also suggest the involvement of Th17 cells in PBC pathology. For example, elevated interleukin (IL)-23p19 protein and IL-17$^+$ cells have been detected by immunohistochemistry around the damaged liver areas; elevated p19 messenger RNA levels and increased serum IL-23 levels have been detected in peripheral blood mononuclear cells in patients with PBC; and human biliary epithelium challenged with IL-17, Toll-like receptor (TLR) 4, or TLR2 ligand produced IL-23p19 and IL-12/23p40, demonstrating potential for IL-12/23 activity in relevant cell types.[8,9]

Consistent with these immunological findings, in patients with PBC evaluated by genome-wide case-control association analysis, significant genetic associations were detected for IL-12A (encoding IL-12p35), IL-12 receptor beta 2 subunit (IL-12Rß2), and signal transducer and activator of transcription (STAT) 4 variants.[10] These associations were confirmed in cohorts of patients with PBC and unaffected controls from Italy[11] and the United Kingdom.[12] The broader risk conferred by variants across a range of immunoregulatory loci was further confirmed by studies using single-nucleotide polymorphism arrays enriched for autoimmune disease risk loci. The role of IL-12 in PBC is also supported by experimental data demonstrated in an animal model of autoimmune cholangitis indicating that the IL-12p40 gene is crucial to disease development, given that deletion of IL-12p40 suppressed autoimmune cholangitis in dnTGFβRII mice.[13-15]

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Results from this study were presented, in part, at the International Liver Congress, 2014.

**Members of the Phase 2, Multi-center, Randomized, Double-blind, Placebo-controlled, Parallel-group Study Evaluating the Efficacy and Safety of Ustekinumab in Subjects with Primary biliary cholangitis Who had an Inadequate Response to Ursodeoxycholic Acid (UDCA) (PURIFI) Study Group are listed in the Supporting Appendix.*

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Cytokine analyses, genome-wide case-control association studies, and animal model data have provided evidence about the role of IL-12 and IL-23 in the pathogenesis of PBC, suggesting that IL-12/23p40 could be an important disease target in PBC.[16] Ustekinumab is a well-characterized human immunoglobulin (Ig) G1 kappa (IgG1κ) monoclonal antibody that specifically binds to the shared p40 protein subunit of human IL-12 and IL-23. Ustekinumab blocks IL-12 and IL-23 bioactivity by preventing their interaction with their cell-surface IL-12Rβ1 receptor protein. Therefore, we investigated the effects of ustekinumab on markers of disease activity in a multicenter, open-label, proof-of-concept clinical trial of patients with PBC unresponsive to UDCA.

Patients and Methods

PATIENTS

Eligible patients were ≥18 years of age, with PBC defined by at least two of the following three criteria at baseline: (1) a history of elevated alkaline phosphatase (ALP) for ≥6 months; (2) a positive antimitochondrial antibody titer or PBC-specific antibodies; and (3) a liver biopsy consistent with PBC. Eligible patients were to have an inadequate response to UDCA, as defined by a screening ALP level >1.67x upper limit of normal (ULN) despite treatment with a stable dose of UDCA for ≥6 months before baseline. Patients were to receive a stable dose of UDCA throughout the study. Patients were excluded if they had hepatic decompensation, a screening direct bilirubin value >1.0 mg/dL, previous liver histology with a diagnosis of steatohepatitis, a high risk of nonalcoholic steatohepatitis, chronic autoimmune hepatitis (AIH), or a high risk of AIH overlap syndrome. Written consent was obtained from all patients before screening. This study was approved by the institutional review board at each site and was conducted in compliance with the Declaration of Helsinki and Good Clinical Practice guidelines.

STUDY DESIGN

This phase 2 study (NCT01389973) was to comprise two parts. Part 1 of the study was conducted from September 2011 through June 2013.

Part 1 (Proof of Concept)

Part 1 was an open-label, proof-of-concept study (Supporting Fig. S1). All patients in Part 1 received open-label ustekinumab 90-mg subcutaneous (SC) injections at weeks 0 and 4, then every 8 weeks (q8w) through week 20. Part 1 of the study was planned to evaluate the efficacy of ustekinumab in reducing ALP, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin levels. Efficacy for Part 1 was primarily based on ALP response at week 12. Patients with ≥20% improvement in ALP and no worsening of other liver biochemistry tests at either week 20 or 28 were to be considered for entry into the long-term extension (LTE) of Part 1.

Part 2 (Double-Blind)

Part 2 was planned to be a multicenter, randomized, double-blind, placebo-controlled, parallel-group study evaluating the efficacy and safety of ustekinumab in patients with PBC who had an inadequate response to UDCA. The primary objective of Part 2 was to evaluate the efficacy of ustekinumab in achieving ALP response (a decrease from baseline of >40% in ALP). The primary endpoint was ALP response at week 28. However, based on the efficacy results of Part 1 (reported herein), the sponsor decided to not initiate Part 2.

STUDY ASSESSMENTS AND ANALYSES

Efficacy analyses were based on the modified intent-to-treat principle. All patients who received at least one administration of ustekinumab were included in the efficacy analysis. Efficacy assessments included changes in liver biochemistry tests (ALP, ALT, AST, and total bilirubin) and serum bile acid concentrations; changes in the Enhanced Liver Fibrosis (ELF) and Mayo risk scores; and changes in patient-reported outcomes assessing patient fatigue, pruritus, and quality of life.

ALP response was defined as >40% decrease from baseline. ALP remission was defined as either ALP normalization (for patients with baseline ALP between 1.67x and 2.8x ULN) or an ALP <1.67x ULN (for patients with baseline ALP >2.8x ULN). A cut-off of 1.67x ULN was chosen based upon previous findings that showed that ALP levels above this level are associated with an increased risk of disease progression.[17] For patients with baseline ALP between 1.67x ULN and 2.8x ULN, normalization of ALP ensured that

patients only met ALP remission criteria if they also met the ALP response criteria. Normalization of ALP is also associated with improved prognosis.[18] Bile acids were determined by liquid chromatography/mass spectrometry.[19]

The ELF assessment is used as a predictor of histological fibrosis in patients with chronic liver disease, including patients with PBC.[20] The ELF test measures three serum markers (serum hyaluronic acid, tissue inhibitor of metalloproteinase 1, and N-terminal propeptide of type III procollagen) that have been shown to correlate with extent of liver fibrosis based on biopsy. The ELF assessment was performed using week 0 and 28 serum samples (Siemens ELF test; iQur Ltd, Southhamptom, UK). The Mayo risk score is a six-item score that has been correlated with long-term risk of death or transplant in patients with PBC and includes age, bilirubin, albumin, prothrombin time, peripheral edema, and diuretic use.[21]

The Fatigue Impact Scale (FIS; also referred to as the Fisk Fatigue Severity Score) is a 40-item questionnaire that assesses the impact of fatigue on patient quality of life and employs a previous-month recall.[22] The three domains assessed by the FIS include psychosocial (20 items), cognitive (10 items), and physical activity (10 items). Each item of the FIS is rated on a scale from 0 to 4, with higher values indicating greater fatigue impact.

The 5D itch scale is a multidimensional patient-completed assessment of pruritus developed for use in a clinical setting.[23] The five dimensions of the 5D itch scale are Duration (one item), Degree (one item), Direction (one item), Disability (four items addressing impact on sleep, leisure, housework, and work/school), and Distribution (16-item checklist describing various regions of the body) and employs a past 2-week recall period.

Heath-related quality of life (HRQoL) was assessed using the PBC-40, a 40-item assessment of PBC-related symptoms that impact HRQoL, utilizing a past 4-week recall period.[24]

Excluding one patient who, after screening, was found not to have PBC, all patients who received at least one administration of ustekinumab and had at least one measurable serum ustekinumab concentration obtained after administration of a scheduled treatment were included in the pharmacokinetic and immunogenicity analyses. Blood samples were collected at weeks 0, 4, 8, 12, 20, and 28 for determination of serum ustekinumab concentrations using a validated enzyme-linked immunoassay. Presence of antibodies to ustekinumab was assessed using a validated immunoassay method.[25]

Blood samples collected at weeks 0 and 28 were also assessed for serum cytokine concentrations using a cytokine multiplex assay (Milliplex MAP 13-Plex Kit; EMD Millipore, Billerica, MA). Serum IL-17A and IL-17F levels were measured with Singulex (Alameda, CA) immunoassay kits, and IL-6 and IP-10 levels were measured with Mesoscale Discovery (Rockville, MD) kits.

Potential relationships between ustekinumab pharmacodynamics and clinical response were evaluated by using a modified definition of ALP responder, which included those who had >20% reduction in ALP from baseline to week 28. Because of small sample size, a threshold *P*-value cutoff <0.10 was used in these analyses.

All patients who received at least one administration of ustekinumab (full or partial) were included in the safety analysis in which adverse events were assessed. Descriptive statistics were employed to summarize all trial data. In the analysis of efficacy data, patients who had a missing value at a designated time point had their last available value carried forward. Change from baseline values were analyzed by the use of the matched-pairs *t* test.

Approximately 20 patients were planned for Part 1. The sample-size calculation for Part 1 was based on a one-sided exact binomial test for one population. Twelve patients were estimated to provide >90% power to detect an effect of ustekinumab treatment assuming that ALP response rate was 40% for ustekinumab treatment group and 5% for placebo population.

Results

PATIENT DISPOSITION AND BASELINE DEMOGRAPHIC AND DISEASE CHARACTERISTICS

Twenty patients were enrolled and treated at nine sites in Italy (1 patient), Canada (13 patients), and the United States (6 patients). One patient (5.0%) discontinued study agent before week 28 for "other" reasons. Four patients (20.0%) discontinued study agent before or at week 28 because of lack of efficacy. The remaining 15 patients (75.0%) discontinued study agent during the LTE of Part 1 for reasons of lack of efficacy (5, 25.0%), lost to follow-up (1, 5.0%), and study stopped by the sponsor (9, 45.0%).

The majority of patients were white (19; 95.0%) and female (19; 95.0%), and the median (interquartile range; IQR) age was 45.5 (42, 59) years. At study

TABLE 1. Demographics and Baseline Disease Characteristics; Treated Patients

Characteristic	Ustekinumab 90 mg SC (N = 20)
Age, years	
Mean (± SD)	48.8 (10.13)
Median (IQR)	45.5 (42.0; 59.0)
Range	(32.0; 69.0)
Sex, n (%)	
Male	1 (5.0)
Female	19 (95.0)
Race, n (%)	
White	19 (95.0)
Black or African American	1 (5.0)
Ethnicity (Hispanic/Latino), n (%)	
Yes	2 (10.0)
No	18 (90.0)
Weight, kg	
Mean (± SD)	67.8 (12.79)
Median (IQR)	63.0 (57.5; 76.5)
Range	(53.0; 95.0)
Height, cm	
Mean (± SD)	161.1 (8.31)
Median (IQR)	161.3 (154.5; 165.0)
Range	(146.0; 180.0)
Disease duration, years	
Mean (± SD)	5.19 (4.21)
Median (IQR)	3.2 (1.9; 8.4)
Range	(0.9; 13.2)
ALP, n (%)	
≤3× ULN	7 (35.0)
>3× ULN	13 (65.0)
Mayo Risk Score	
N	19
Mean (± SD)	3.7 (0.46)
Median (IQR)	3.7 (3.5; 3.9)
Range	(2.9; 4.6)

Abbreviation: SD, standard deviation.

outset, the median (IQR) duration of PBC was 3.2 years (1.9, 8.4), Mayo risk score was 3.7 (3.5; 3.9), and ALP concentration was >3x ULN for 65% (13 of 20) of patients (Table 1). Baseline ELF score indicated that 10 patients had moderate liver fibrosis (ELF ≥7.7 to <9.8) and 10 had severe liver fibrosis (ELF ≥9.8).

EFFICACY

Week 12

ALP levels and percent changes over time through week 76 are shown by individual patient in Fig. 1A and 1B, respectively. No patients achieved ALP response (0 of 20) or remission (0 of 20) at week 12. Median percent reduction from baseline in ALP concentration at week 12 was 8.4%. At week 12, 10 (50%), 4 (20%), and 0 patients had a ≥10%, ≥20%, and ≥40% decline from baseline in ALP, respectively.

There was little change in patient-reported outcomes of FIS, 5D itch scale, or PBC-40 from baseline to week 12 (Table 2).

Week 28

No patient achieved ALP response (0 of 20) or remission (0 of 20) at week 28. Median percent reduction from baseline in ALP concentration at week 28 was 12.1% (Table 3). ALP levels and percent changes over time through week 28 and beyond are shown by individual patient in Fig. 1A and 1B, respectively. At week 28, 10 (50%), 7 (35%), and 0 patients had a

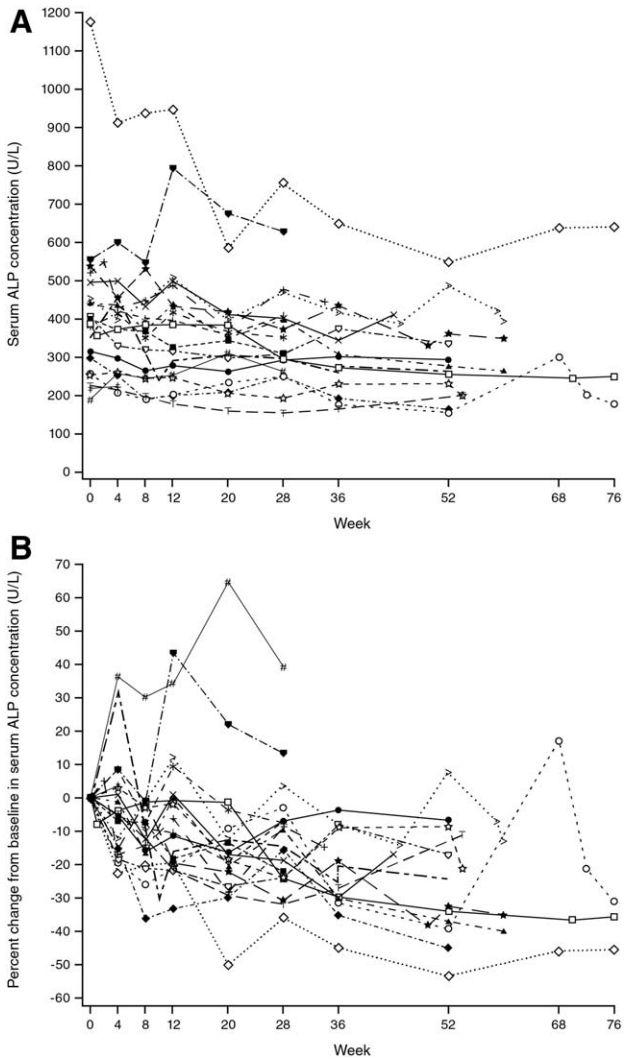

FIG. 1. Serum ALP levels (A) and percent change (B) over time through week 76 by patient among 20 treated patients. Key: U/L, units per liter.

TABLE 2. Change From Baseline at Week 12 and Week 28 in Patient-Reported Outcomes; Treated Patients

	Ustekinumab 90 mg SC		
Time Point	FIS* (N = 20)	5D Itch Scale* (N = 20)	PBC-40* (N = 20)
Baseline			
Mean (± SD)	40.3 (45.75)	11.1 (5.50)	86.8 (34.07)
Median (IQR)	20.0 (0.0; 69.0)	8.5 (8.0; 16.0)	83.0 (61.5; 109.5)
Change at week 12			
Mean (± SD)	−7.7 (36.55)	−0.6 (2.28)	−1.7 (25.11)
Median (IQR)	0.0 (−19.5; 5.5)	0.0 (−1.5; 0.0)	−2.0 (−15.0; 18.5)
P value	0.3610	0.2538	0.7653
Change at week 28			
Mean (± SD)	2.1 (36.51)	−0.7 (4.58)	2.7 (22.62)
Median (IQR)	0.0 (−12.5; 18.5)	0.0 (−3.5; 0.0)	2.0 (−15.0; 17.0)
P value†	0.8044	0.5023	0.5997

*Patients who had a missing value at the designated analysis time point had their last available value carried forward.
†*P* values from matched-pairs *t* test.
Abbreviation: SD, standard deviation.

≥10%, ≥20%, and ≥40% decline from baseline in ALP, respectively.

Eighteen of twenty patients had an abnormal baseline ALT or AST. At week 28, median percent reductions from baseline were 15.4% and 13.4% for patients with abnormal baseline ALT and AST values, respectively (Table 3). Three patients had an abnormal baseline bilirubin level, among which median percent reduction from baseline to week 28 in total bilirubin concentration was 3.7% (Table 3).

Median concentration of IgM was 3.51 g/L at baseline (normal range: 0.40-2.30) and reduced to 3.05 g/L at week 28. Median concentration of IgG was 12.15 g/L at baseline (normal range: 5.65-17.65) and was reduced to 11.00 g/L at week 28. Median concentration of gamma-glutamyl transpeptidase was 210.5 U/L at baseline (normal range: 4-50) and reduced to 197.0 U/L at week 28 (data on file).

Baseline total serum bile acid concentrations were highly elevated (median value: 43.3 μmol/L). At week 28, median reduction from baseline in total serum bile acid concentration was 8.8 μmol/L (Table 4). Overall, median changes from baseline at week 28 for individual serum bile acids were 0.1, 0.0, -1.3, 0.9, and -2.0 for lithocholic acid, deoxycholic acid, chenodeoxycholic acid, cholic acid, and UDCA, respectively (Table 4).

Only slight changes were observed from baseline through week 28 for the PBC-40, the FIS, and the 5D itch scale (Table 2). Small decreases in median ELF and Mayo risk scores were observed from baseline to week 28

TABLE 3. Percent Change From Baseline in Liver Biochemistry Tests at Week 28; Treated Patients With Abnormal Values at Baseline

	Ustekinumab 90 mg SC			
Time Point	ALP (U/L) (N = 20)	ALT (U/L) (N = 18)	AST (U/L) (N = 18)	Total Bilirubin (μmol/L) (N = 3)
Baseline				
Mean (± SD)	415.0 (210.34)	74.1 (31.10)	61.2 (19.21)	25.0 (2.65)
Median (IQR)	393.5 (278.0; 474.0)	62.0 (51.0; 100.0)	59.5 (45.0; 71.0)	26.0 (22.0; 27.0)
Week 28*				
Mean (± SD)	355.3 (144.79)	62.9 (23.09)	54.4 (15.91)	23.7 (2.52)
Median (IQR)	310.0 (257.0; 406.0)	61.0 (44.0; 79.0)	56.5 (39.0; 68.0)	24.0 (21.0; 26.0)
Percent change from baseline at week 28*				
Mean (± SD)	-11.3 (17.46)	-9.2 (32.36)	-7.2 (27.19)	-4.6 (14.18)
Median (IQR)	-12.1 (-23.8; -5.0)	-15.4 (-36.6; 16.0)	-13.4 (-30.1; 19.4)	-3.7 (-19.2; 9.1)
P value†	0.0096	0.2436	0.2753	0.6298

*Patients who had a missing value at week 28 had their last available value carried forward.
†*P* values from matched-pairs *t* test.
Abbreviation: SD, standard deviation.

TABLE 4. Change From Baseline in Serum Bile Acid Concentrations (μmol/L) at Week 28; Treated Patients

	Ustekinumab 90 mg SC					
Time point	Total serum bile acid (μmol/L) (N = 20)	Lithocholic acid (μmol/L) (N = 20)	Deoxycholic acid (μmol/L) (N = 20)	Chenodeoxy-cholic acid (μmol/L) (N = 20)	Cholic acid (μmol/L) (N = 20)	Ursodeoxy-cholic acid (μmol/L) (N = 20)
Baseline						
Mean (± SD)	52.8 (53.23)	0.6 (0.59)	3.6 (4.58)	10.1 (13.87)	9.3 (13.81)	29.3 (26.31)
Median (IQR)	43.3 (16.9; 56.2)	0.4 (0.2; 0.8)	2.4 (0.8; 3.8)	6.4 (1.7; 9.9)	3.9 (1.7; 9.4)	22.4 (9.8; 41.2)
Week 28*						
Mean (± SD)	50.0 (54.07)	0.6 (0.46)	3.4 (3.81)	8.2 (9.04)	7.7 (11.87)	30.1 (34.40)
Median (IQR)	26.7 (20.4; 49.5)	0.4 (0.2; 1.0)	2.1 (1.3; 3.5)	4.4 (1.4; 10.7)	3.9 (1.2; 8.0)	16.8 (9.4; 33.2)
Change from baseline at week 28*						
Mean (± SD)	-2.8 (61.44)	0.0 (0.54)	-0.2 (4.81)	-1.9 (13.58)	-1.5 (11.75)	0.8 (33.81)
Median (IQR)	-8.8 (-18.9; 4.2)	0.1 (-0.3; 0.3)	0.0 (-0.8; 0.4)	-1.3 (-4.8; 1.2)	0.9 (-2.8; 0.4)	-2.0 (-12.3; 3.6)
P value†	0.8415	0.9707	0.8671	0.5482	0.5650	0.9179

*Patients who had a missing value at week 28 had their last available value carried forward.
†*P* values from matched-pairs *t* test.
Abbreviation: SD, standard deviation.

(Supporting Table S1). Median ELF score decreased by 0.173 between weeks 0 and 28, with 6 (30%) patients having a decrease in ELF of ≥0.5 points and 2 (10%) demonstrating an increase of ELF score of ≥0.5 points.

PHARMACOKINETICS AND IMMUNOGENICITY

Mean serum ustekinumab concentrations at weeks 4 (4.26 μg/mL) and 8 (5.70 μg/mL) after administration of ustekinumab at weeks 0 and 4 (i.e., 4-weeks dosing interval) were higher than the mean trough serum ustekinumab concentrations at weeks 12, 20, and 28 (2.63, 2.54, and 2.71 μg/mL, respectively) during maintenance therapy with 8-weeks dosing interval. Mean trough serum ustekinumab concentrations at weeks 12, 20, and 28 were similar, suggesting that steady-state serum ustekinumab concentrations were reached by week 12. No clear relationship was observed between serum ustekinumab concentrations at steady state and percent change in ALP.

Through week 28, of the 19 patients with appropriate samples, 1 (5.3%) tested positive for antibodies to ustekinumab; this patient's antibodies to ustekinumab were neutralizing.

SERUM BIOMARKER CHANGES AFTER USTEKINUMAB TREATMENT

For serum analysis, patients were stratified based on a modified ALP response (>20% decrease in ALP from baseline to week 28). Nine serum proteins were significantly reduced at week 28 after ustekinumab treatment: IgM; granulocyte colony-stimulating factor; IFNα2; IL-17A; IL-6; tumor necrosis factor alpha (TNFα), granulocyte macrophage colony-stimulating factor, epidermal growth factor, and IFNγ (Supporting Table S2). Eight of these nine proteins were significantly decreased in ustekinumab responders, but not nonresponders. Only one of these serum proteins, IgM, was significantly modulated in both (Fig. 2A; Supporting Table S2). Reduction of serum IL-17A, IFNγ, and IFNα2 levels demonstrated a direct pharmacodynamic effect of IL-12p40 inhibition by the modified responder definition (Fig. 2B). These data suggest that despite the lack of efficacy observed in the overall clinical study, patients who reached >20% reduction in ALP showed modulation of biological pathways (i.e., Th17) related to ustekinumab response. No significant changes were noted for the other proteins measured (data not shown).

SAFETY

Eighteen of twenty patients reported one or more adverse events through the final safety visit, most commonly fatigue, headache, urinary tract infection, back pain, and nausea (Table 5 and Supporting Table S3). Eleven (55.0%) patients reported one or more infections (Table 5), and nine required antimicrobial therapy.

One patient experienced a serious adverse event of an upper gastrointestinal hemorrhage that was considered

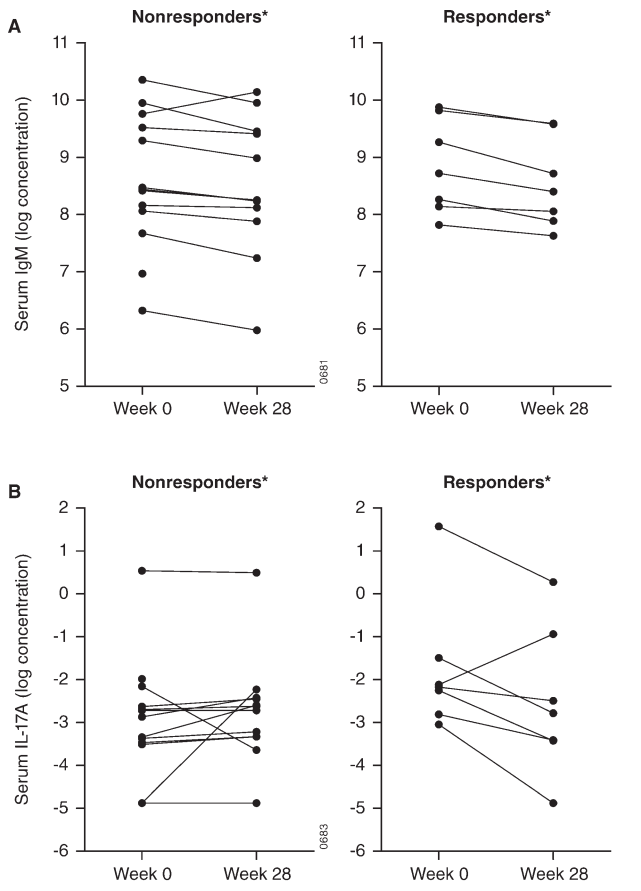

FIG. 2. Serum IgM (A) and IL-17A (B) concentrations before and after ustekinumab treatment.

not related to study drug secondary to possible portal hypertension 17 weeks after the week 20 dose. This patient had evidence of severe fibrosis or cirrhosis at baseline (ELF score = 12). No serious infections or discontinuations resulting from adverse events were reported through the final safety visit.

Discussion

PBC is an autoimmune biliary disease, wherein there are clear autoimmune disease features. However, equally it is a biliary disease, and the risk factors for disease development and progression need to be considered within the context of immune-mediated liver injury modulated by the complex consequences of cholestasis.

Multiple advances in understanding the pathophysiology of PBC strongly implicate the IL-12-signaling pathway in disease biology, but also provide contrasting insights as to why choosing a specific pathway inhibitor as a potential new therapy is challenging. Large-scale genetic studies in particular underscore the impact of adaptive regulatory immune pathways, highlighting IL-12 and downstream Janus kinase/STAT signaling.[10-12] IL-12 is a heterodimeric molecule made up of the two subunits, p35 and p40, encoded by the *IL12A* and *IL12B* genes, respectively. The latter protein also heterodimerizes with IL-23p19 to form IL-23, a key signaling component in the Th-17 pathway. The IL-12 receptor is also encoded by two genes, *IL12RB1*, which is constitutively expressed, and *IL12RB2*, which is up-regulated by IFNγ to act as a positive feedback loop in antigenic stimulation. The tyrosine kinase 2 protein is key to both IL-12 and IL-23 receptor signaling. IL-12 is central in generating effector Th1 cell responses directed toward clearance of intracellular pathogens, and IFNγ release suppresses IL-23-driven induction of IL-17-producing helper T lymphocytes (Th17). Impaired expression of the IL-12 receptor, IL-12Rβ2, has been shown to facilitate regulatory T-cell-suppressive functions in the context of a proinflammatory environment.

Herein, we tested the hypothesis that blockade of the IL-12/-23 pathway by ustekinumab could alleviate inflammation in cholestatic patients already unresponsive to standard therapy with UDCA. The prespecified primary endpoint was change in ALP, a biochemical surrogate of disease outcome, associated with efficacy of cholestatic-based therapies. Open-label ustekinumab therapy, though associated with a modest decrease in ALP after 28 weeks of therapy, did not otherwise appreciably change ALP values. However, despite the lack of efficacy observed based on the prespecified primary endpoint, patients who reached greater than 20% reduction in ALP showed modulation of biological pathways (i.e., Th17) related to ustekinumab response.

Our study population was consistent with a population of high-risk patients with PBC who had well-compensated liver disease with normal liver function.[26] The population median Mayo risk score reflected a population with compensated liver disease.[27-29] Nevertheless, the ELF score data suggest that most patients had moderate-to-severe liver fibrosis, of relevance given that speculatively ustekinumab might be more likely to have its greatest efficacy at a point in disease course when immune injury is most prominent.

The definition of ALP response (>40% decrease in ALP concentration of pretreatment or ALP

TABLE 5. Adverse Events Reported by >1 Treated Patient

Exposure/Adverse Event Data	Ustekinumab 90mg SC (N = 20)
Mean duration of follow-up, weeks	58.8
Mean exposure, no. of administrations	6.5
Patients with ≥1 adverse event, n (%)	18 (90.0)
General disorders and administration site conditions	11 (55.0)
Fatigue	8 (40.0)
Infections and infestations	11 (55.0)
Urinary tract infection	4 (20.0)
Influenza	2 (10.0)
Nasopharyngitis	2 (10.0)
Oral herpes	2 (10.0)
Sinusitis	2 (10.0)
Upper respiratory tract infection	2 (10.0)
Gastrointestinal disorders	8 (40.0)
Nausea	3 (15.0)
Abdominal pain	2 (10.0)
Abdominal pain upper	2 (10.0)
Nervous system disorders	8 (40.0)
Headache	5 (25.0)
Musculoskeletal and connective tissue disorders	7 (35.0)
Back pain	3 (15.0)
Arthralgia	2 (10.0)
Reproductive system and breast disorders	5 (25.0)
Menorrhagia	2 (10.0)
Skin and subcutaneous tissue disorders	5 (25.0)
Alopecia	2 (10.0)
Pruritus	2 (10.0)
Rash	2 (10.0)

normalization) in the present study was chosen based on the Barcelona Criteria established by Parés et al., accepting the limitations and differences in population and initial intent (stratification of risk vs. surrogate of new treatment efficacy), and the fact that ALP is a broad marker of cholestatic liver injury but not of specific immune active damage.[30]

Although serum ustekinumab concentrations achieved steady state by week 12, overall changes in ALP were modest and proof of concept was not established. No patients achieved ALP response or remission at week 12 or 28. Additionally, no improvement was noted in most other efficacy endpoints assessed. Overall, 20% of patients had a 20% decrease in ALP at week 12, and by week 28 of therapy, 35% of patients demonstrated a 20% drop in ALP. The ALP decrease at week 28 was overall modest (median ALP fell from 393.5 to 310.0 U/L), a change that was albeit statistically significant ($P = 0.0096$). Our proof-of-concept study lacked a placebo arm, but notably in a placebo-controlled study of obeticholic acid in a similar study population, the placebo-treated arm had a 3% ALP decrease after 12 weeks of therapy, whereas levels of ALP decreased 21%-25%, on average, from baseline in the treatment arms.[31]

In this trial, median ELF score decreased by 0.173 between weeks 0 and 28, whereas results of previous studies have shown an expected increase in ELF scores of 0.032 per year in patients with PBC.[20] Additionally, 30% of patients had a decrease in ELF of ≥0.5 points, whereas only 10% had an increase of ELF score of ≥0.5 points. Previous studies have shown that a 1-point increase in ELF score is associated with a doubling of the risk of liver-related events.[32]

Per protocol, if proof of concept was not established, the Dose Selection Committee (DSC) had the option to either discontinue the study or add a second open-label cohort in Part 1 with a maximum intravenous dose of 6 mg/kg instead of proceeding directly to Part 2. On the basis of the proof-of-concept efficacy results, the DSC elected to discontinue the study. The ustekinumab safety profile was acceptable in this group of patients with PBC; no new safety signals were observed.

Pathologically, PBC is characterized by a progressive lymphocytic cholangitis centered on small intrahepatic bile ducts.[1,4] With the PBC liver heavily infiltrated by $CD4^+$ and $CD8^+$ T lymphocytes (that can be demonstrated to recognize epitopes of PDC-E2), a predominant type I cytokine pattern with high levels of IFNγ, IL-5, IL-6, IL-10, IL-12, and IL-15 in the blood and liver is recognized. Whereas both T-cell subsets recognize similar sequences within the same epitope of the PDC-E2 lipoyl domain, supporting a common etiological trigger, it is believed that $CD8^+$ T cells play a role in the degeneration and death of cholangiocytes that aberrantly express PDC-E2. When liver tissue from patients with PBC is stained specifically for IL-12/IL-23 cell populations, localization is primarily around damaged interlobular bile ducts, whereas IL-12/Th1 and IL-23/Th17 staining is detected in all sections. Furthermore, Th17 skewing was prominent in advanced disease with intensive secretion of IL-23p19 by inflamed hepatocytes around IL-23R, IL-12RB2, and IFNγ expressing degenerated cholangiocytes. Thus, there appears to be a direct association of Th17 skewing and disease severity, and the IL-23/Th17 pathway may be relevant to the perpetuation of IL-12/Th1-mediated immunopathology in PBC.[33]

The significance of the genetic findings have been further illustrated in experimental cholangiopathy models, wherein mice that lack the p40 subunit of IL-12 ($IL12p40^{-/-}$) exhibit dramatic reductions in histological cholangitis and a significant decrease in intrahepatic, proinflammatory cytokine levels.[13] However, in an aggressive model of portal inflammation and colitis

(the IL-2Rα(−/−) knockout mice), investigators have studied groups of IL-2Rα(+/−), IL-2Rα(−/−), and p40(−/−)IL-2Rα(−/−) mice. In this setting, p40(−/−) IL-2Rα(−/−) mice manifest more severe portal inflammation and bile duct damage, including signs of portal hypertension and liver fibrosis, but a significant reduction in colitis. The p40(−/−)IL-2Rα(−/−) mice reveal a profound hepatic $CD8^+$ T-cell infiltrate, whose major component are effector memory cells as well as enhanced hepatic Th1, but reduced Th17, responses.[34]

It remains clear that PBC has fundamental autoimmune disease features, and ustekinumab and other immunomodulatory agents have clear potential to interfere with such dominant and relevant immunoregulatory pathways. Our observation that reduction of serum IL-17A, IFNγ, and IFNα2 levels demonstrated a direct pharmacodynamic effect of IL-12p40 inhibition, by the modified responder definition, suggest that despite the lack of efficacy observed in the overall study, patients who reached >20% reduction in ALP showed modulation of biological pathways (i.e., Th17) related to ustekinumab response. This raises a significant challenge for investigators given the parallel importance of the cholestasis observed in patients with PBC, and the possibility that, over time, the consequences of cholestasis become a more dominant pathological feature. Furthermore, whereas biochemical surrogates can be clinically important markers for individual risk stratification of future events, these same biochemical surrogates may not be optimal therapeutic trial endpoints for investigative therapeutics where modulation of immunological pathways is the mechanism of action. Equally, present risk stratification tools are unable to reflect the balance of immune-mediated and cholestatic injury. Beyond simple biochemical stratification, additional factors (e.g., age, ALT, IgG, and serology) and novel biomarkers of specific immune pathways may be needed as inclusion criteria for future biological-based treatment trials. Furthermore, investigators may need to consider patient selection based on liver biopsy evaluation, both classically and by applying tissue molecular diagnostics.

Collectively therefore, our study, aligned to that of tissue and murine models of disease, demonstrating the relevance and importance of the IL-12 pathway to PBC, and equally demonstrates the challenges of how, and when, to best intervene therapeutically. Future studies of immunomodulating therapy in patients with PBC thus remain important, but potentially need innovative, adaptive, and experimental medicine-based approaches, with initially fewer patients, but a higher level of individual phenotyping both pre- and post-treatment. Equally, an argument can be made to evaluate such therapies at the point of PBC diagnosis at the same time as, or perhaps before, initiation of UDCA. Ultimately, such approaches could translate into more personalized risk stratification and treatment, based on genomics and novel biomarkers.

In conclusion, in this proof-of-concept study of patients with PBC unresponsive to UDCA, open-label administration of ustekinumab was associated with a decrease in ALP values after 28 weeks of therapy, but this decrease was modest and no patient met the prespecified primary endpoint of a 40% decline in ALP at either 12 or 28 weeks of therapy. Translating basic immunological insights into new therapies for patients with PBC remains difficult, and particularly challenging given the lack of consensus as to optimal trial design and duration as well as appropriate endpoints of treatment efficacy.

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