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Therapeutic trials of biologics in primary biliary cholangitis: An open label study of abatacept and review of the literature

Christopher L. Bowlus[a,*], Guo-Xiang Yang[b], Chung H. Liu[a], Cole R. Johnson[a], Sandeep S. Dhaliwal[a], Darren Frank[a], Cynthia Levy[c], Marion G. Peters[d], John M. Vierling[e], M. Eric Gershwin[b]

[a] Division of Gastroenterology and Hepatology, University of California Davis, 4150 V Street, Sacramento, CA, 95817, United States
[b] Division of Rheumatology, Allergy, and Clinical Immunology, University of California Davis, 451 E. Health Sciences Dr., Davis, CA, 95616, United States
[c] Division of Hepatology, University of Miami, 1400 NW 12th Ave, Miami, FL, 33136, United States
[d] Division of Gastroenterology and Hepatology, University of California San Francisco, 350 Parnassus Ave, San Francisco, CA, 94117, United States
[e] Division of Gastroenterology and Hepatology, Baylor College of Medicine, 6620 Main St., Houston, TX 77030, United States

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ABSTRACT

Primary biliary cholangitis (PBC) is a classic autoimmune disease in which humoral, cytotoxic, and innate immune responses have been implicated with the specific targeting of a mitochondrial antigen. The mainstay of treatment remains the bile acid ursodeoxycholic acid (UDCA). Corticosteroids may have some benefits, but to date, clinical trials of biologics targeting B cells and IL-12/23 have not shown any efficacy. Because activated T cells target the intrahepatic bile ducts in PBC and pre-clinical models suggested that blocking CD80/CD86 with CTLA-4 Ig might have therapeutic benefit in PBC, we performed an open-label trial to determine if CTLA-4 Ig (abatacept) is safe and potentially efficacious in PBC patients with an incomplete response to UDCA. PBC patients with an alkaline phosphatase (ALP) > 1.67 × the upper limit of normal after 6 months on UDCA treatment or who were intolerant of UDCA received abatacept 125 mg s.q. weekly for 24 weeks. The co-primary endpoint was ALP normalization or a > 40% reduction from baseline. Among 16 subjects enrolled and who received at least 1 dose of abatacept, 1 (6.3%) met the co-primary endpoint. Absolute and percent changes in ALP [median (95% CI)] were +2.8 U/L (−90.9–96.6) and −0.28% (−21.1–15.5), respectively. No significant changes were observed in ALP, ALT, total bilirubin, albumin, immunoglobulins, or liver stiffness. Abatacept treatment decreased several non-terminally differentiated $CD4^+$ but not $CD8^+$ T cell populations, including decreases in $CD4^+$ CCR5+ ($p = 0.02$) and $CD4^+$ PD1+ ($p = 0.03$) lymphocytes. In contrast there were increases in $CD4^+$ CCR7+ lymphocytes ($p = 0.034$). Treatment emergent adverse events occurred in 4 subjects. Abatacept was well tolerated in this population of PBC patients but like other biologics in PBC was ineffective in achieving biochemical responses associated with improved clinical outcomes.

1. Introduction

Primary biliary cholangitis (PBC) is a progressive autoimmune disease in which biliary epithelial cells of the small to medium caliber intrahepatic bile ducts are targeted by autoreactive T cells resulting in destructive lymphocytic cholangitis, cholestasis, ductopenia, and biliary cirrhosis. The only approved medical therapies for PBC target bile acid homeostasis. Ursodeoxycholic acid (UDCA) is a hydrophilic bile acid that induces choleresis and alters the composition of the bile acid pool. Multiple studies suggest that UDCA delays progression of the disease, particularly in those that have a biochemical response which has been defined by multiple criteria [1–5]. More recently, the farnesoid X receptor agonist obeticholic acid (OCA) has been approved for use in PBC patients who are intolerant to UDCA or have had in inadequate response to UDCA [6]. However, 20–40% of PBC patients have an incomplete response to UDCA and while OCA has demonstrated efficacy in improving liver biochemistries associated with better clinical outcomes, only a minority of patients treated with OCA achieve

Abbreviations: PBC, primary biliary cholangitis; ALP, alkaline phosphatase; ULN, upper limit of normal; UDCA, ursodeoxycholic acid; OCA, obeticholic acid; AMA, antimitochondrial antibody; 2OA-BSA, 2-octynoic acid coupled to bovine serum albumin; APC, antigen presenting cell; CTLA-4, cytotoxic T lymphocyte-associated protein-4; PBMCs, Peripheral blood mononuclear cells; FITC, Fluorescein isothiocyanate-conjugated

* Corresponding author. Division of Gastroenterology and Hepatology 4150 V Street, PSSB 3500 Sacramento, CA, 95817, United States.
E-mail address: clbowlus@ucdavis.edu (C.L. Bowlus).

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complete biochemical normalization.

Although PBC is a classic autoimmune disease characterized by high titers of serum anti-mitochondrial autoantibodies (AMA) directed against the lipoic acid moiety on the pyruvate dehydrogenase complex E2 subunit and elevated plasma levels of immunoglobulin M (IgM), immunosuppressive therapies including corticosteroid [7], azathioprine [8], cyclosporine [9], methotrexate [10], and mycophenolate mofetil (MMF) [11] have been disappointing. Budesonide, a synthetic steroid with a high first-pass hepatic metabolism, is the exception that may have efficacy in combination with UDCA as second-line therapy [12–14] but this has not been universal and there remains concerns that budesonide could aggravate underlying osteopenic bone disease [12]. A recent phase III randomized placebo-controlled trial comparing budesonide (3 mg three times daily plus) and UDCA (12–16 mg/kg body weight) to placebo and UDCA reported significant reductions in serum ALP with budesonide compared to placebo after 12, 24, and 36 months of treatment [15]. These results suggest that despite the lack of success with immune-based therapies in PBC, the potential for this approach remains clear.

With the development of novel immune-based therapies with increased potency and better safety, the discovery of a biologic therapy with efficacy in PBC may be achieved. In addition, the recent success of small molecules inhibitors of Janus Kinase, TGF-β/Smad 7, and sphinosine-1-phosphate receptor offer additional avenues for possible treatments for PBC [16]. In this review, we summarize the previous experience of biologic therapies in PBC and present the results of an open-label clinical trial of abatacept in the treatment of PBC patients with an incomplete biochemical response to UDCA (Table 1).

2. Rituximab

In addition to AMA, the serological hallmark of PBC present in 90%–95% of sera of patients, PBC patients often have elevated serum levels of total immunoglobulin M (IgM) [17]. In addition, B cells from patients with PBC compared with healthy controls and patients with primary sclerosing cholangitis produce significantly greater amounts of IgM after stimulation with CpG-B [18]. Further, treatment of a mouse model of PBC which expresses a dominant-negative form of transforming growth factor-β (TGF-β) receptor II and develops autoimmune cholangitis and AMA, with anti-CD20 monoclonal antibody resulted in amelioration of liver inflammation, supporting a rationale for B-cell–-targeted therapies in PBC [19].

Rituximab is an anti-CD20 monoclonal antibody which depletes B cells and has demonstrated efficacy for B-cell lymphomas, rheumatoid arthritis [20], and other autoimmune diseases [21–25]. A total of 3 clinical trials of rituximab in patient with PBC have been reported. The first was an open label treatment trial which included 6 PBC patients with an incomplete response to ursodeoxycholic acid [26]. Patients were given two 1000 mg doses of rituximab separated by two weeks and followed to evaluate safety, liver biochemistries, and B-cell function from baseline to week 52. All patients completed 52 weeks of follow-up with no serious adverse event observed. During treatment total AMA titers decreased significantly from baseline to week 16 and 24. In addition, stimulation of B-cells with CpG-B after rituximab treatment resulted in significantly lower IgM secretion compared to pre-treatment B-cells. Importantly, significant decreases in serum ALP levels at weeks 2, 24, and 36 compared to baseline were observed.

A similar study by Myers et al. treated 14 PBC patients with an incomplete response to UDCA with the same dosing regimen [27]. Efficacy was measured as normalization and/or a 25% reduction in serum ALP levels at month 6. B-cell depletion was observed in all patients within a week of infusion. Although ALP was reduced significantly at month 6, it was not maintained. After month 12 and 18, ALP values returned to baseline levels. At month 6, only 3 of 14 patients were considered biochemical responders to rituximab treatment.

A phase 2, single center, randomized controlled, double blinded trial of rituximab was recently conducted for the treatment of fatigue, rather than improvement in liver biochemistries [28]. In this trial, patients were selected based upon the presence of moderate to severe fatigue. Rituximab was ineffective for fatigue in PBC patients in this study and ALP changed little over the course of the study. For the rituximab treated group mean ALP values were 157 ± 72 (baseline), 120 ± 65 (3 months), 128 ± 71 (6 months), 138 ± 72 (9 months), and 149 ± 86 (12 months) while values in the placebo treated group were 217 ± 167 (baseline), 224 ± 176 (3 months), 226 ± 170 (6 months), 244 ± 196 (9 months), and 204 ± 159 (12 months). Notably, these ALP values were relatively low at baseline compared to the prior studies of patients with an incomplete response to UDCA reflecting a less treatment-refractory population of patients.

3. Ustekinumab

Multiple genome-wide association studies identifying genes in the IL-12-JAK-STAT4 pathway (*IL12A, IL12RB2*, *STAT4*) contributing to PBC disease susceptibility led to an interest in targeting this pathway [29]. Several lines of evidence link IL-12 and IL-23-mediated Th1/Th17 signaling pathways with PBC and provide further evidence supporting the rationale to target IL-12/23. PBC patients demonstrate elevated IL-23p19 protein and IL-17 + cells on liver histology and increased serum IL-23 levels [30,31]. In addition, human biliary epithelium produced IL-23p19 and IL-12/23p40 after treatment with IL-17, or TLR 4, or TLR2 ligands [32]. Further, dissection of the roles of multiple cytokines using targeted gene-deleted mice immunized with 2-octynoic acid coupled to BSA (2OA-BSA) which induces an inflammatory cholangitis and AMA similar to PBC, demonstrated that in comparison to the $p19^{-/-}$ and $p35^{-/-}$ mice in which a portal mononuclear cell infiltration was readily detectable after immunization with 2OA-BSA, no detectable lymphocytic infiltration was noted in the $p40^{-/-}$ mice [33].

Based upon these studies, a phase 2 multicenter, open-label, proof of concept clinical trial of ustekinumab was planned. Ustekinumab is a human IgG1 kappa monoclonal antibody which binds to the shared p40 unit of IL-12 and IL-23 and also prevents IL-12 and IL-23 cell-surface interaction with IL12Rβ1 receptor [34]. In this clinical trial, 20 PBC patients with an incomplete response to UDCA were enrolled to receive 90-mg subcutaneous injections of ustekinumab at weeks 0 and 4 then every 8 weeks through week 20. No patients achieved a predetermined response or remission at weeks 12 or 28 which were defined as > 40% decrease in ALP from baseline and either normalization of ALP for patients with baseline ALP between 1.67 times and 2.8 times ULN or an ALP < 1.67 times ULN for patients with baseline ALP > 2.8 times ULN,

Table 1
Therapeutic trials of biologics in primary biliary cholangitis.

Drug	Target	Trial Design	Patient Population	Patients	Duration	Outcome
Rituximab [26]	CD20	Open-Label	Incomplete response to UDCA	6	52 weeks	14% median decrease in ALP at 36 weeks
Rituximab [27]	CD20	Open-Label	Incomplete response to UDCA	14	6 months	16% median decrease in ALP at 6 months
Rituximab [28]	CD20	Randomized, placebo-controlled	Moderate to severe fatigue	57	12 months	No improvement in fatigue.
Ustekinumab [34]	IL12/23	Open-Label	Incomplete response to UDCA	20	20 weeks	12% median decrease in ALP at 24 weeks
NI-0801 [41]	CXCL10	Open-Label	Incomplete response to UDCA	26	12 weeks	5.5% median increase in ALP 2 weeks after treatment
Abatacept	CD28	Open-Label	Incomplete response to UDCA	16	24 weeks	No significant change in ALP.

Table 2
Demographic and clinical characteristics of the participants at baseline[a].

Characteristic	*N = 16*
Age, *years*	54 (42, 59)
Female Sex, *n (%)*	15 (94%)
AMA Positive, *n (%)*	16 (100%)
UDCA	
Use at baseline, *n (%)*	15 (94%)
Daily Dose, *mg/kg*	15.3 (14.2, 16.5)
Alkaline phosphatase	
Median value, *U/L*	324 (216, 413)
> 1.67 × ULN, *n (%)*	16 (100%)
Alanine aminotransferase, *U/L*	57.5 (39.8, 93.3)
Total bilirubin, *mg/dL*	
Median value, *U/L*	0.9 (0.7,1.3)
> ULN, *n (%)*	4 (25%)
Platelets, *X 10^3/μL*	208 (172, 294)
Albumin, *g/dL*	3.7 (3.3, 3.9)
International normalized ratio	0.94 (0.90, 0.98)
Liver Stiffness, *kPa***	3.9 (2.8, 5.1)
> 4.13 kPa, *n (%)*	6 (37.5%)

[a] Continuous variables are displayed as median (IQR). AMA, anti-mitochondrial antibodies; UCDA, ursodeoxycholic acid; ULN, upper limit of normal reference range. **Liver stiffness was measured by magnetic resonance elastography in 15 patients. The cutoff of 4.13 kPa used for cirrhosis was based upon Venkatesh [59].

respectively. Median reduction in ALP from baseline was 8.4% and 12.1% at weeks 12 and 24, respectively, the latter being statistically significant. No improvements in patient-reported symptoms or bile acids were observed.

4. NI-0801

NI-0801 is a fully human IgG1 monoclonal antibody that binds human chemokine ligand 10 (CXCL10) and inhibits CXCL10-induced calcium flux, chemotaxis, and lymphocyte transendothelial migration across human sinusoidal endothelial cells [35]. CXCL10 is secreted in response to interferon-γ by several cell types, including monocytes, endothelial cells, fibroblasts, cholangiocytes and hepatocytes. CXCL10 is a ligand for chemokine receptor 3 (CXCR3), which is highly expressed on effector T cells and plays an important role in T-cell migration and function. In PBC patients, serum and liver levels of CXCL10 and CXCR3 levels are increased [36–38] and blocking CXCL10 in carbon tetrachloride treated animals, liver inflammation and fibrosis are ameliorated [39,40].

In an open-label, phase 2a study in patients with PBC and an incomplete response to UDCA, NI-0801 was administered every 2 weeks for a total of six doses in addition to UDCA and patients were followed up for 3 months after the last infusion [41]. The main outcome measurement was the percentage reduction in liver tests from baseline to 2 weeks after the final NI-0801 administration. A total of 29 patients were enrolled in the study and 26 patients completed. A small but statistically significant increase in serum ALP was observed after NI-0801 treatment while other liver tests remained unchanged. Interestingly, serum IgG also increased slightly but significantly. *Post hoc* analysis of NI-0801 exposure based upon the area under the curve found a significant difference in the percentage change in the enhanced liver fibrosis score, a serum marker of liver fibrosis, among those with the lowest, middle, and highest tertile of NI-0801 exposure (5.0%, 1.4%, and −1.8%, respectively). Although NI-0801 treatment increased patient serum CXCL10 to levels expected to induce chemotaxis, CXCL10-induced chemotaxis *in vitro* was not observed suggesting that the CXCL10 is neutralized by NI-0801. Importantly, NI-0801 increased expression of the CXCR3 on $CD8^+$ T cells and natural killer cells.

5. Abatacept

5.1. Rationale for abatacept

Evidence from both human and murine models support the role of $CD4^+$ and $CD8^+$ T-cells as key mediators of bile duct damage in PBC [42–46]. Activation of naive T cells requires an antigen-specific signal transmitted through the T cell receptor and a costimulatory signal delivered via CD28 on T cells binding to CD80 (B7-1) and CD86 (B7-2) on the antigen presenting cell (APC) [47–50]. Shortly after T cell activation, T cells express cytotoxic T lymphocyte-associated protein 4 (CTLA-4), which preferentially engages the CD80 and CD86 on the APC surface blocking co-stimulatory signaling of CD28 on the T cell surface required for activation and effector functions of T cells. Notably, PBC is associated with polymorphisms in the CTLA-4 gene [51–53].

Targeting CD80 and CD86 with CTLA-4 IgG (abatacept) has been successful in treating rheumatoid arthritis [54], juvenile idiopathic arthritis [55], and psoriatic arthritis [56]. Abatacept is a recombinant fusion protein consisting of the extracellular domain of human CTLA-4 and a fragment of the Fc domain of human IgG1 that has been modified to prevent complement fixation and antibody-dependent cellular cytotoxicity. Abatacept binds specifically to the CD80 and CD86 molecules and down-modulates the CD28-mediated co-stimulation of T cells.

Pre-clinical studies of abatacept in a murine model of PBC resulted in reduced intra-hepatic T cell infiltrates and biliary epithelial cell damage, although AMA levels were not affected [57]. Based upon these results, we performed an open-label study to determine the safety and potential efficacy of abatacept in PBC patients with an incomplete biochemical response to UDCA or whom could not tolerate UDCA.

5.2. Methods

5.2.1. Subjects

Patients between the ages of 18 and 80 years diagnosed with PBC based upon the presence of at least 2 out of 3 criteria including an AMA titer > 1:40, ALP > 1.5 times ULN, and/or liver histology consistent with PBC and who had an incomplete response to UDCA defined by an ALP > 1.67 times ULN after at least 6 months of UDCA treatment at a minimum dose of 13–15 mg/kg/d were eligible to participate. PBC patients were also eligible if they were intolerant of UDCA and otherwise met eligibility criteria. Patients were excluded if they had evidence of decompensated liver disease (Model of End Stage Liver Disease score of > 15, ascites, hepatic encephalopathy, or varices), other coexisting liver disease, treatment with immunosuppressive medications within 6 months of enrollment, or active infection.

5.2.2. Study design

This was an open-label study conducted at a single academic clinical research center (ClinicalTrials.gov Identifier: NCT02078882). After a screening visit, all subjects were treated with abatacept 125 mg by subcutaneous injection, self-injected weekly for 24 weeks. Safety assessments including a clinic visit and laboratory tests performed at treatment weeks 0, 2, 4, 12, and 24, and 12 weeks after treatment (week 36). Blood was also collected at the screening visit and weeks 12, 24, and 36 for flow cytometry. In addition, the PBC-40 questionnaire, a validated tool for the assessment of quality of life in PBC patients [58], was administered prior to treatment and at week 24.

The study was initially planned to enroll 20 patients but was closed after 19 patients were enrolled due to a lack of demonstrated efficacy. The study was approved by the Institutional Review Board and all subjects gave written informed consent prior to enrollment.

5.2.3. PBMC isolation

Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient using Histopaque-1077 (Sigma Chemical Co., St. Louis, MO), and the cells were washed and resuspended in phosphate-buffered

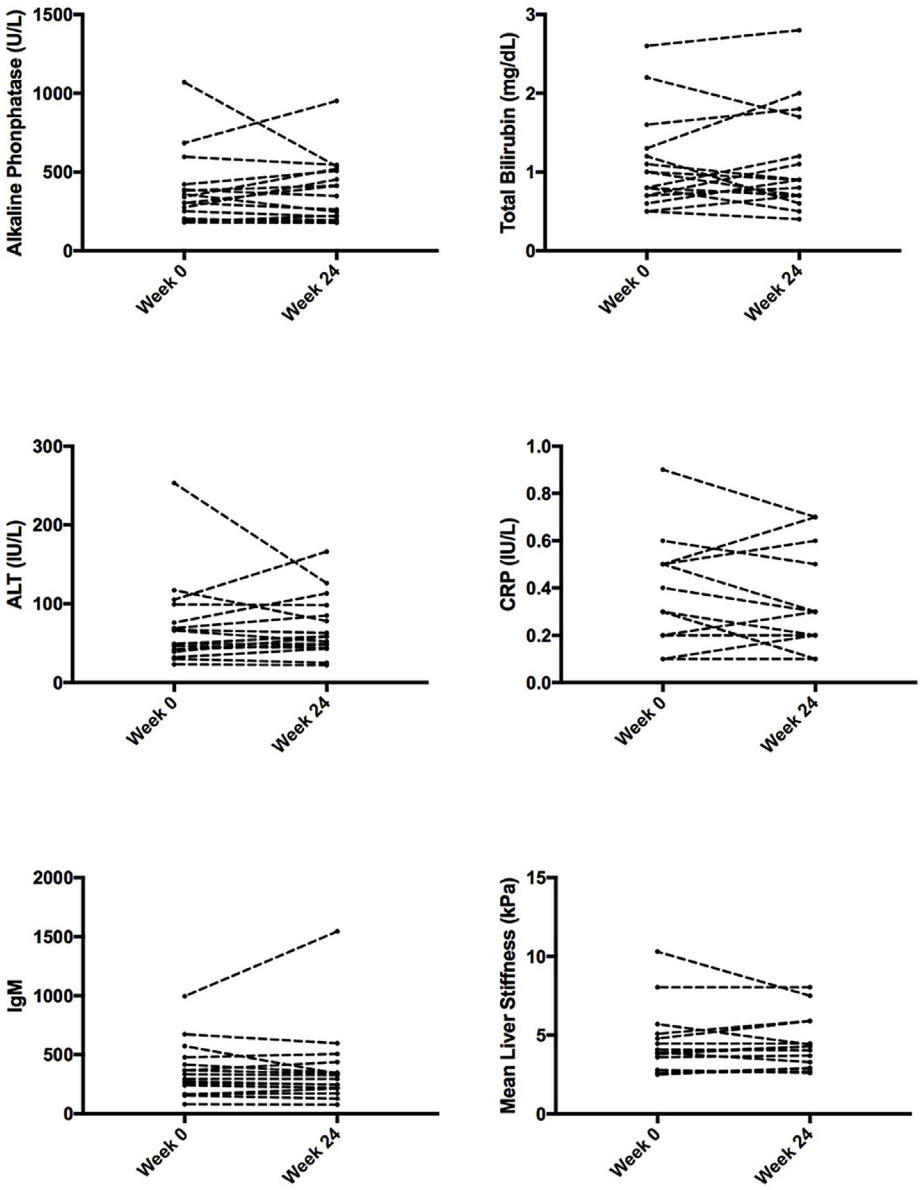

Fig. 1. Alkaline Phosphatase and Total Bilirubin Levels According to Study Visit. Shown are the geometric mean values of alkaline phosphatase (*top*) and total bilirubin (*bottom*) from baseline to week 36. Error bars indicate 95% confidence intervals. Comparisons with respect to the change from baseline were obtained with the use of a general linear model for repeated variables.

saline (PBS) (Mediatech Inc., Herndon, VA) containing 0.5% bovine serum albumin (BSA) (Fraction V, OmniPur; EMD Chemicals Inc., Gibbstown, NJ) and 0.05% EDTA (Sigma Chemical Co.). The viability of the cells was more than 98% by trypan blue dye exclusion.

5.2.4. Flow cytometry

PBMCs from PBC patients were resuspended in staining buffer (0.2% BSA, 0.04% EDTA, 0.05% sodium azide in PBS), divided into 25 µl aliquots, and incubated with anti-human FcR blocking reagent (eBioscience, San Diego, CA, USA) for 15 min at 4 °C. The cells were then washed and stained with the following antibodies for 30 min at 4 °C:

Fluorescein isothiocyanate-conjugated (FITC)-*anti*-CD4 (BD Pharmingen, San Diego, CA); CD8 (BD Pharmingen); FITC-*anti*-CD20 (eBioscience); Phycoerythrin-conjugated (PE)-*anti*-CD45RO (BD Pharmingen); PE-*anti*-CD38 (eBioscience); PE-Cy-Chrome (PE-Cy5)-*anti*-CD56 (BD Pharmingen); TRI-COLOR (TC)-*anti*-CD25 (Invitrogen/Caltag, Carlsbad, CA); Allophycocyanin-conjugated (APC)-antiCD19 (eBioscience); Alexa fluor 750 (AF750)-conjugated-*anti*-CD27 (eBioscience). IgG isotype controls were used for negative controls. The cells were then washed once with PBS containing 0.2% BSA. After staining, the cells were washed and fixed with 1% paraformaldehyde in PBS. For analysis, stained cells were counted on a FACScan flow cytometer (BD Immunocytometry Systems) that had been upgraded by Cytek Development (Fremont, CA) to allow for 5-color analysis. The acquired data were analyzed with Cellquest PRO software (BD Immunocytometry Systems).

5.2.5. Statistical analysis

The co-primary efficacy endpoint was ALP normalization or a > 40% reduction from baseline to week 24. Secondary endpoints included changes in liver tests and liver stiffness measured by magnetic resonance elastography from baseline to Week 24. Comparisons of laboratory tests, elastography, and lymphocytes were made using a Wilcoxon matched pairs test. Comparisons with respect to the change over time from baseline were made with the use of a general linear

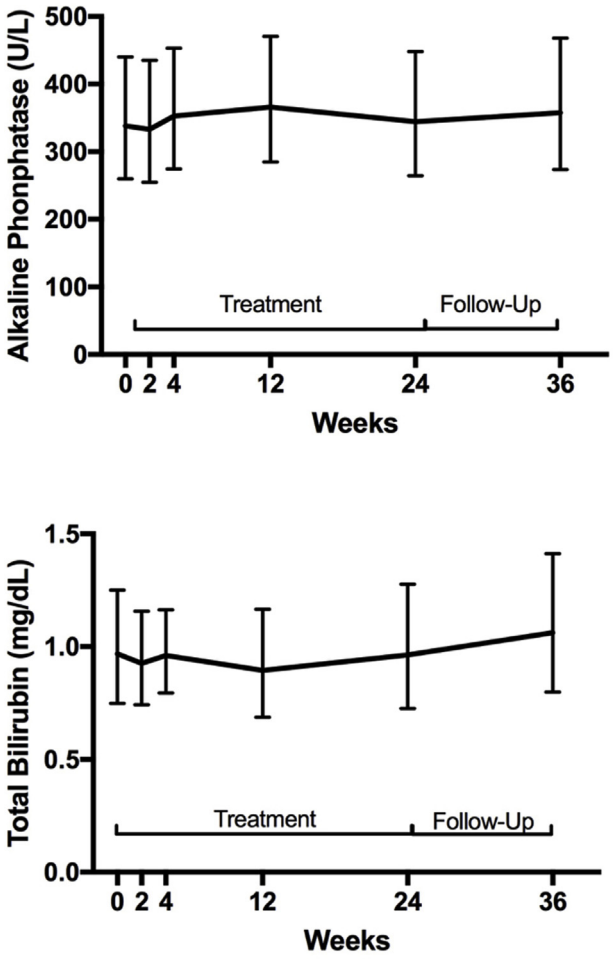

Fig. 2. Changes in Laboratory Values and Liver Stiffness from Baseline (Week 0) to End of Treatment (Week 24). Shown are subject level changes from week 0 to week 24 for alkaline phosphatase (*upper left*), total bilirubin (*upper right*), ALT (*middle left*), c-reactive protein (*middle right*), IgM (*bottom left*), and liver stiffness measured by MR elastography (*bottom right*). Comparisons were made using the Wilcoxon matched-pairs signed rank test.

model for repeated variables. All analyses were carried out at a two-sided 0.05 alpha level. Adverse events were summarized according to the *Medical Dictionary for Regulatory Activities* (MedDRA) System Organ Class, the MedDRA preferred term, severity, and causal relationship assessed by the investigators (CLB) and were reviewed by the Data Monitoring Board.

5.3. Results

5.3.1. Patient disposition and characteristics

A total of 22 patients were screened and 16 met all study criteria and received at least 1 dose of abatacept (Supplementary Figure 1). The subjects were predominantly female (15 female) with a mean age of 52 years (IQR:39-70) (Table 2). All participants were AMA positive and had an ALP ≥1.67 times ULN at baseline. A single participant was intolerant of UDCA and among the 15 other participants the median daily dose of UDCA was 15.3 mg/kg (IQR 14.2–16.5). Baseline total bilirubin was above the ULN in 4 (25%) participants and 6 (37.5%) of participants had cirrhosis based upon the mean liver stiffness measured by MR elastography [59].

5.3.2. Changes in liver biochemistries, inflammatory markers, liver stiffness, and PBC-40

Only 1 subject (6.3%) met the co-primary endpoint of ALP normalization or a > 40% reduction from baseline to week 24 (Fig. 1). No subject normalized ALP and there were no significant changes in ALP or total bilirubin over the course of the study (Fig. 2). Median absolute and percent changes in ALP from baseline to week 24 were +2.8 U/L (95% CI -90.9–96.6) and −0.28% (95% CI -21.1–15.5), respectively. No significant changes were observed between Week 0 and Week 24 in ALP, total bilirubin, ALT, C-reactive protein, IgM, or liver stiffness (Fig. 1). There was a marginally significant decrease in the fatigue domain of the PBC-40 from baseline to week 24 (median 21.5 (IQR 13.25–36.25) to 19.0 (IQR 13.0–33.0), p = 0.049). No significant difference from baseline to week 24 were observed for itch, cognitive, emotional, or social domains or the total PBC-40 score (Supplementary Table 1).

5.3.3. Immunologic assessments

To determine the pharmacologic effects of abatacept, a variety of cell surface markers were measured on PBMC. Following 24 weeks of abatacept treatment there was no significant change in the frequency of total $CD4^+$ or $CD8^+$ T cells (Supplementary Figure 2). However, among $CD4^+$ T cells, CCR5+ and PD1+ cells decreased (p = 0.02 and p = 0.02, respectively) and CCR7+ cells increased (p = 0.03) from Week 0–24 (Fig. 3). Restriction of these changes was to the KLRG1-cell populations. Similar changes were not observed in the $CD8^+$ T cell population and no changes in CD44, CD62L, CD45RO, CD69, CX3CR1, or CXCR5 expression were found on either $CD4^+$ or $CD8^+$ T cells (Supplementary Figure 2).

5.3.4. Safety and side effects

All 16 patients who received at least 1 dose of abatacept completed 24 weeks of treatment. Treatment emergent adverse events occurred in 4 subjects, including nausea, vomiting, right upper quadrant abdominal pain, upper respiratory and urinary tract infections, urticarial rash, chest pain, and hilar adenopathy (Table 3).

6. Discussion

Herein we review the literature on biologics for PBC and report the results of the first clinical trial in PBC patients involving the use of a therapeutic that targets T cell activation (Table 1). PBC patients who respond adequately to UDCA have a life expectancy that does not differ significantly from the general population [3,60], but response to treatment is markedly heterogeneous [5]. A significant minority of patients with PBC do not have an adequate biochemical response to UDCA and represent an unmet medical need due to their risk of disease progression. Although second-line agents targeting bile acid metabolism through FXR [6], FGF19 [61], and peroxisome proliferator-activated receptors [62] have demonstrated efficacy when added on to UDCA, some are limited by tolerability and few have resulted in a complete response in the majority of patients treated.

PBC is an archetypal autoimmune disease with a profound female predominance, nearly universal immune response to a specific autoepitope, and clear genetic and environmental predispositions [63]. However, unlike other autoimmune diseases, little efficacy has been reported with immunosuppressive medications other than prednisone [8] and budesonide [12,13,15] both of which have serious concerns related to long term safety. Because of this, targeted immunotherapies in PBC have been sought. B-cell depletion as a potential means to ameliorate PBC by decreasing autoantibody production and antigen presentation by B cells has been successful in antibody mediated autoimmune diseases [20,23,24,64,65] and showed potential efficacy in murine models of PBC [19,66]. Not surprisingly, the most extensively studied biologic in PBC has been rituximab which cumulatively has been given to 47 PBC patients in 3 clinical trials [26–28]. The 2 studies performed in patients with elevated ALP despite UDCA both demonstrated similar small but statistically significant reductions in ALP at 6–9 months after infusion. The third trial did not require an elevated

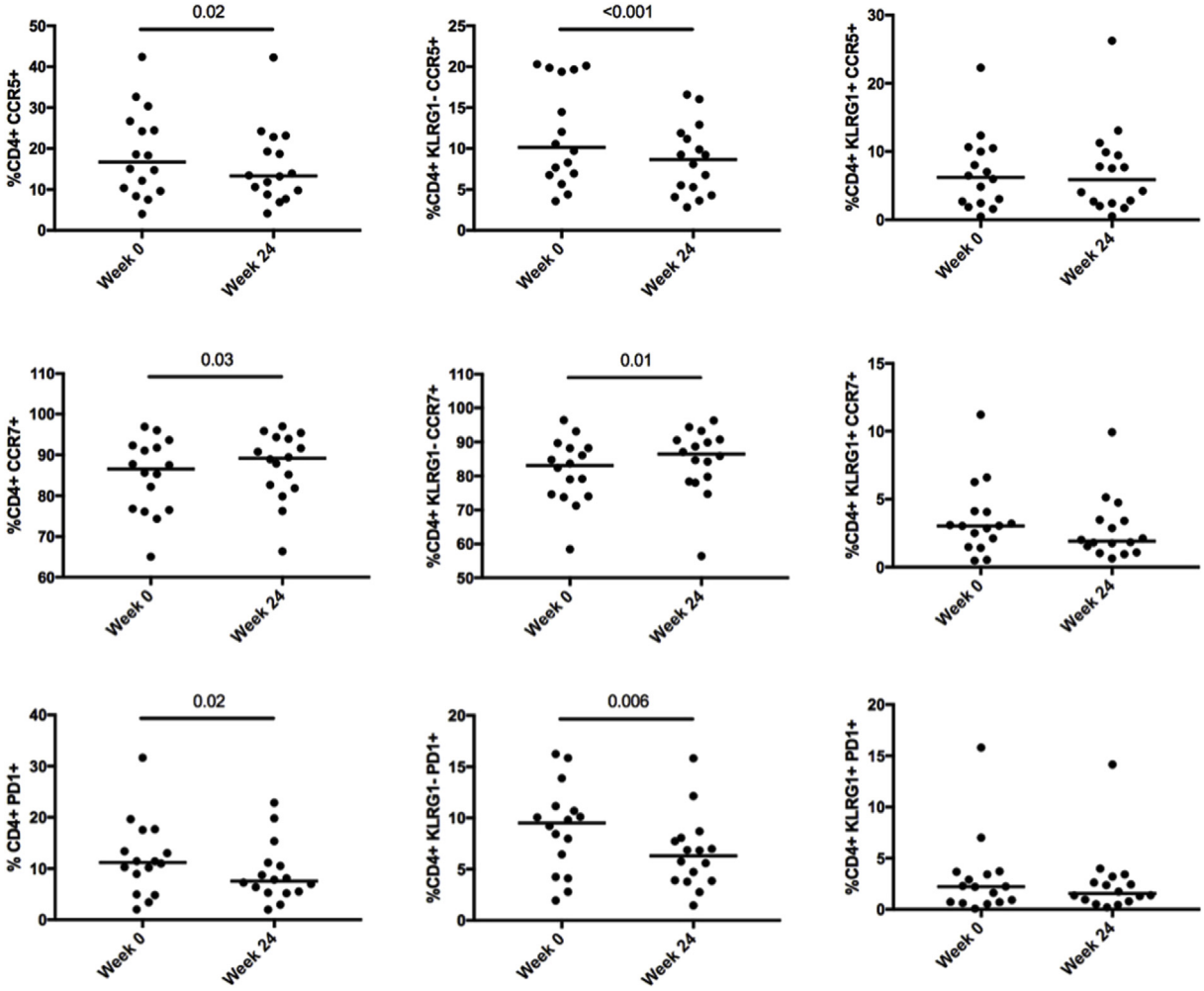

Fig. 3. Changes in CD4$^+$ T Cell Subpopulations in Peripheral Blood Mononuclear Cells from Baseline (Week 0) to End of Treatment (Week 24). Shown are subject level changes from week 0 to week 24 in CD4$^+$ T cells in peripheral blood mononuclear cells measured by flow cytometry. CCR5+ (*top row)* and PD1+ (*bottom row*) cells decreased and CCR7+ cells (*middle row*) increased among the KLRG1-cells after 24 weeks treatment with abatacept. Comparisons were made using the Wilcoxon matched-pairs signed rank test.

Table 3
Adverse events.

SYSTEM ORGAN CLASS	n (%)	No. of events
Any treatment emergent adverse event	4 (25%)	10
GASTROINTESTINAL		
Nausea	2 (12.5%)	2
Vomiting	1 (6.3%)	1
Elevated liver enzymes	1 (6.3%)	1
Right upper quadrant pain	1 (6.3%)	1
INFECTIONS		
Upper respiratory infection	1 (6.3%)	1
Urinary tract infection	1 (6.3%)	1
DERMATOLOGICAL		
Urticarial rash	1 (6.3%)	1
CARDIOVASCULAR		
Chest pain	1 (6.3%)	1
LYMPHATIC		
Hilar adenopathy	1 (6.3%)	1

ALP at enrollment and therefore it is not surprising that a biochemical effect of rituximab was not identified. Notably, this study determined that rituximab was not effective for the treatment of fatigue, a common and often debilitating symptom of PBC. Ustekinumab also has a small impact on ALP and based upon this criterium, is unlikely to be pursued for further development in PBC. Surprisingly, inhibition of CXCL10 with NI-0801 actually led to a small increase in ALP, but in a subset of patients receiving the highest drug exposure, a reduction in a serum liver fibrosis marker was found. Not only does this finding highlight the need for correct dosing, but also the need to consider non-traditional biomarkers which may be more sensitive to detect drug efficacy than the traditional ALP and other liver biochemistries.

In light of the lack of efficacy of these approaches, the rational next approach was to target T cells. This was further supported by evidence from PBC patients and murine PBC models that CD4$^+$ and CD8$^+$ T-cells play important roles in the bile duct injury that typifies PBC [42,45,46,63,67–69]. Using a murine model of PBC in which both AMA and biliary inflammation are induced by immunization with the 2-OA-BSA, we previously showed that CTLA-4 Ig treatment one day before 2-OA-BSA immunization, completely inhibited AMA production, intrahepatic T cell infiltrates, and bile duct damage [57]. More critically, treatment with CTLA-4 Ig initiated after the 2-OA-BSA immunization and development of disease, led to improved liver histology.

In the current study of a 24-week treatment period with abatacept, we assessed liver enzyme levels, antibody levels, and lymphocyte populations, with special emphasis on T-cell subsets in PBC patients with an inadequate response to first line therapy. Our results suggest that abatacept is safe, transiently reverses several of the immunologic abnormalities of PBC, but did not demonstrate a signal of therapeutic effect in this PBC population.

We chose to investigate abatacept because of its mechanism of action which blocks both $CD4^+$ and $CD8^+$ T cell activation through agonism of CTLA-4. The immunological effects of abatacept on PBMC in PBC patients was as expected with a decreased frequency of $CD4^+$ T cells expressing the inflammatory chemokine receptor CCR5 and an increased frequency expressing the homeostatic chemokine receptor CCR7, the latter being nearly absent among expanded T cell clones in PBC patients [70]. Similar effects have also been reported with juvenile idiopathic arthritis [71]. These effects, however, were limited to the KRLG1-population, whereas terminally differentiated KLRG1 + T cells which represent the antigen experienced T cells were not affected [72,73]. An alternative mechanism of action of abatacept through effects on regulatory T cells (Tregs) which are decreased in number but not function in PBC [74],has been considered in rheumatoid arthritis but the studies to date have reported inconsistent results [75].

Like rituximab and ustekinumab, abatacept was not associated with a biochemical response in PBC despite demonstrating the expected pharmacologic effects. The lack of clinical efficacy may have been due to a number of reasons. First, abatacept does not inhibit effector functions of terminally differentiated T cells, especially $CD8^+$ cytotoxic T cells implicated in the destruction of biliary epithelial cells [72,76]. Second, the effects of abatacept noted in peripheral immune cells may not reflect immune responses in the liver. To determine this would have required pre- and post-treatment liver biopsies. The use of biochemical endpoints in PBC is based on the established associations with biochemical responses to UDCA and clinical outcomes, however, immunologic therapies including abatacept may have beneficial effects not reflected in biochemistries. Future studies should consider direct measures of hepatic inflammatory activity. Third, our patient cohort had particularly advanced disease: over 30% had cirrhosis and 25% had an elevated total bilirubin at baseline. Treatment, particularly with an immunomodulatory therapy, may be of limited efficacy in late stage disease where bile acids may be the primary driver of injury due to established cholestasis. The identification of pretreatment predictors of non-response to UDCA including higher serum alkaline phosphatase and total bilirubin levels, younger age, and longer time from diagnosis to treatment may allow alternative risk stratification and earlier introduction of second liner therapies [77]. Finally, treatment duration may have been insufficient, though treatment responses have been seen with similar lengths of therapy in other diseases treated with abatacept [54–56].

In summary, there is growing experience with clinical trials of biologics for PBC and despite the lack of a highly efficacious biologic in PBC, the lessons learned should aid in future trial designs. Importantly, no safety concerns for the use of abatacept or other biologics in patients with PBC, including those with compensated cirrhosis, has been identified. Limitations specific to abatacept and rituximab may include the inability to inhibit terminally differentiated T or B cells. Alternatively, approaches that target chemokines such as CXCL10 may require higher doses to effectively inhibit the target or multiple targets may need to be targeted due to redundancy of pathways. In addition, current clinic study designs may not be appropriate for the investigation of biologic therapies. The ability of biologics to affect PBC disease progression may be at early disease stage and require limiting their use in the patient group. Further, their impact on disease progression may not be captured by serum ALP and future studies should be considered which incorporate more sensitive biomarkers not only of cholestasis, but also of fibrosis and inflammation. Regardless, further clinical studies targeting immune effector functions in PBC are warranted to achieve the unmet therapeutic needs of patients with PBC.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2019.04.005.

References

[1] A. Pares, L. Caballeria, J. Rodes, Excellent long-term survival in patients with primary biliary cirrhosis and biochemical response to ursodeoxycholic Acid, Gastroenterology 130 (2006) 715–720.
[2] E.M. Kuiper, B.E. Hansen, R.A. de Vries, J.W. den Ouden-Muller, T.J. van Ditzhuijsen, E.B. Haagsma, M.H. Houben, B.J. Witteman, K.J. van Erpecum, H.R. van Buuren, Improved prognosis of patients with primary biliary cirrhosis that have a biochemical response to ursodeoxycholic acid, Gastroenterology 136 (2009) 1281–1287.
[3] C. Corpechot, L. Abenavoli, N. Rabahi, Y. Chretien, T. Andreani, C. Johanet, O. Chazouilleres, R. Poupon, Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis, Hepatology 48 (2008) 871–877.
[4] T. Kumagi, M. Guindi, S.E. Fischer, T. Arenovich, R. Abdalian, C. Coltescu, E.J. Heathcote, G.M. Hirschfield, Baseline ductopenia and treatment response predict long-term histological progression in primary biliary cirrhosis, Am. J. Gastroenterol. 105 (2010) 2186–2194.
[5] M. Carbone, G.F. Mells, G. Pells, M.F. Dawwas, J.L. Newton, M.A. Heneghan, J.M. Neuberger, D.B. Day, S.J. Ducker, U.P. Consortium, R.N. Sandford, G.J. Alexander, D.E. Jones, Sex and age are determinants of the clinical phenotype of primary biliary cirrhosis and response to ursodeoxycholic acid, Gastroenterology 144 (2013) 560–569 e7; quiz e13-4.
[6] F. Nevens, P. Andreone, G. Mazzella, S.I. Strasser, C. Bowlus, P. Invernizzi, J.P. Drenth, P.J. Pockros, J. Regula, U. Beuers, M. Trauner, D.E. Jones, A. Floreani, S. Hohenester, V. Luketic, M. Shiffman, K.J. van Erpecum, V. Vargas, C. Vincent, G.M. Hirschfield, H. Shah, B. Hansen, K.D. Lindor, H.U. Marschall, K.V. Kowdley, R. Hooshmand-Rad, T. Marmon, S. Sheeron, R. Pencek, L. MacConell, M. Pruzanski, D. Shapiro, P.S. Group, A placebo-controlled trial of obeticholic acid in primary biliary cholangitis, N. Engl. J. Med. 375 (2016) 631–643.
[7] F.H. Wolfhagen, H.R. van Buuren, S.W. Schalm, Combined treatment with ursodeoxycholic acid and prednisone in primary biliary cirrhosis, Neth. J. Med. 44 (1994) 84–90.
[8] F.H. Wolfhagen, H.J. van Hoogstraten, H.R. van Buuren, G.P. van Berge-Henegouwen, F.J. ten Kate, W.C. Hop, E.W. van der Hoek, M.J. Kerbert, H.H. van Lijf, J.W. den Ouden, A.M. Smit, R.A. de Vries, R.A. van Zanten, S.W. Schalm, Triple therapy with ursodeoxycholic acid, prednisone and azathioprine in primary biliary cirrhosis: a 1-year randomized, placebo-controlled study, J. Hepatol. 29 (1998) 736–742.
[9] R.H. Wiesner, J. Ludwig, K.D. Lindor, R.A. Jorgensen, W.P. Baldus, H.A. Homburger, E.R. Dickson, A controlled trial of cyclosporine in the treatment of primary biliary cirrhosis, N. Engl. J. Med. 322 (1990) 1419–1424.
[10] B. Combes, S.S. Emerson, N.L. Flye, S.J. Munoz, V.A. Luketic, M.J. Mayo, T.M. McCashland, R.K. Zetterman, M.G. Peters, A.M. Di Bisceglie, K.G. Benner, K.V. Kowdley, R.L. Carithers Jr., L. Rosoff Jr., G. Garcia-Tsao, J.L. Boyer, T.D. Boyer, E.J. Martinez, N.M. Bass, J.R. Lake, D.S. Barnes, M. Bonacini, K.L. Lindsay, A.S. Mills, R.S. Markin, R. Rubin, A.B. West, D.E. Wheeler, M.J. Contos, A.F. Hofmann, Methotrexate (MTX) plus ursodeoxycholic acid (UDCA) in the treatment of primary biliary cirrhosis, Hepatology 42 (2005) 1184–1193.
[11] G. Treiber, P. Malfertheiner, Mycophenolate mofetil for the treatment of primary biliary cirrhosis in patients with an incomplete response to ursodeoxycholic acid, J. Clin. Gastroenterol. 39 (2005) 837–838 author reply 838.
[12] P. Angulo, R.A. Jorgensen, J.C. Keach, E.R. Dickson, C. Smith, K.D. Lindor, Oral budesonide in the treatment of patients with primary biliary cirrhosis with a suboptimal response to ursodeoxycholic acid, Hepatology 31 (2000) 318–323.
[13] H. Rautiainen, P. Karkkainen, A.L. Karvonen, H. Nurmi, P. Pikkarainen, H. Nuutinen, M. Farkkila, Budesonide combined with UDCA to improve liver histology in primary biliary cirrhosis: a three-year randomized trial, Hepatology 41 (2005) 747–752.
[14] M. Leuschner, K.P. Maier, J. Schlichting, S. Strahl, G. Herrmann, H.H. Dahm, H. Ackermann, J. Happ, U. Leuschner, Oral budesonide and ursodeoxycholic acid for treatment of primary biliary cirrhosis: results of a prospective double-blind trial, Gastroenterology 117 (1999) 918–925.

[15] G. Hirschfield, L. Kupcinskas, P. Ott, U. Beuers, A.M. Bergquist, M. Färkkilä, M.P. Manns, A. Pares, U. Spengler, R. Greinwald, M. Proels, R. Poupon, Results of a randomised controlled trial of budesonide add-on therapy in patients with primary biliary cholangitis and an incomplete response to ursodeoxycholic acid, J. Hepatol. 68 (2018) S38.
[16] B.S. Boland, S. Vermeire, Janus Kinase antagonists and other novel small molecules for the treatment of Crohn's disease, Gastroenterol. Clin. N. Am. 46 (2017) 627–644.
[17] C.L. Bowlus, M.E. Gershwin, The diagnosis of primary biliary cirrhosis, Autoimmun. Rev. 13 (2014) 441–444.
[18] K. Kikuchi, Z.X. Lian, G.X. Yang, A.A. Ansari, S. Ikehara, M. Kaplan, H. Miyakawa, R.L. Coppel, M.E. Gershwin, Bacterial CpG induces hyper-IgM production in CD27(+) memory B cells in primary biliary cirrhosis, Gastroenterology 128 (2005) 304–312.
[19] Y. Moritoki, Z.X. Lian, K. Lindor, J. Tuscano, K. Tsuneyama, W. Zhang, Y. Ueno, R. Dunn, M. Kehry, R.L. Coppel, I.R. Mackay, M.E. Gershwin, B-cell depletion with anti-CD20 ameliorates autoimmune cholangitis but exacerbates colitis in transforming growth factor-beta receptor II dominant negative mice, Hepatology 50 (2009) 1893–1903.
[20] S.B. Cohen, P. Emery, M.W. Greenwald, M. Dougados, R.A. Furie, M.C. Genovese, E.C. Keystone, J.E. Loveless, G.R. Burmester, M.W. Cravets, E.W. Hessey, T. Shaw, M.C. Totoritis, R.T. Group, Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks, Arthritis Rheum. 54 (2006) 2793–2806.
[21] I. Sanz, Connective tissue diseases: the conundrum of B cell depletion in SLE, Nat. Rev. Rheumatol. 5 (2009) 304–305.
[22] R. Stasi, G. Del Poeta, E. Stipa, M.L. Evangelista, M.M. Trawinska, N. Cooper, S. Amadori, Response to B-cell depleting therapy with rituximab reverts the abnormalities of T-cell subsets in patients with idiopathic thrombocytopenic purpura, Blood 110 (2007) 2924–2930.
[23] R. Stasi, A. Pagano, E. Stipa, S. Amadori, Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura, Blood 98 (2001) 952–957.
[24] P. Quartier, B. Brethon, P. Philippet, J. Landman-Parker, F. Le Deist, A. Fischer, Treatment of childhood autoimmune haemolytic anaemia with rituximab, Lancet 358 (2001) 1511–1513.
[25] R. Eming, A. Nagel, S. Wolff-Franke, E. Podstawa, D. Debus, M. Hertl, Rituximab exerts a dual effect in pemphigus vulgaris, J. Investig. Dermatol. 128 (2008) 2850–2858.
[26] M. Tsuda, Y. Moritoki, Z.X. Lian, W. Zhang, K. Yoshida, K. Wakabayashi, G.X. Yang, T. Nakatani, J. Vierling, K. Lindor, M.E. Gershwin, C.L. Bowlus, Biochemical and immunologic effects of rituximab in patients with primary biliary cirrhosis and an incomplete response to ursodeoxycholic acid, Hepatology 55 (2012) 512–521.
[27] R.P. Myers, M.G. Swain, S.S. Lee, A.A. Shaheen, K.W. Burak, B-cell depletion with rituximab in patients with primary biliary cirrhosis refractory to ursodeoxycholic acid, Am. J. Gastroenterol. 108 (2013) 933–941.
[28] A. Khanna, L. Jopson, D. Howel, A. Bryant, A. Blamire, J.L. Newton, D.E. Jones, Rituximab is ineffective for treatment of fatigue in primary biliary cholangitis: a phase 2 randomized controlled trial, Hepatology (2018) PMID: 29790196 (in press).
[29] A. Tanaka, P.S.C. Leung, M.E. Gershwin, The genetics and epigenetics of primary biliary cholangitis, Clin. Liver Dis. 22 (2018) 443–455.
[30] C.Y. Yang, X. Ma, K. Tsuneyama, S. Huang, T. Takahashi, N.P. Chalasani, C.L. Bowlus, G.X. Yang, P.S. Leung, A.A. Ansari, L. Wu, R.L. Coppel, M.E. Gershwin, IL-12/Th1 and IL-23/Th17 biliary microenvironment in primary biliary cirrhosis: implications for therapy, Hepatology 59 (2014) 1944–1953.
[31] G. Rong, Y. Zhou, Y. Xiong, L. Zhou, H. Geng, T. Jiang, Y. Zhu, H. Lu, S. Zhang, P. Wang, B. Zhang, R. Zhong, Imbalance between T helper type 17 and T regulatory cells in patients with primary biliary cirrhosis: the serum cytokine profile and peripheral cell population, Clin. Exp. Immunol. 156 (2009) 217–225.
[32] K. Harada, S. Shimoda, Y. Sato, K. Isse, H. Ikeda, Y. Nakanuma, Periductal interleukin-17 production in association with biliary innate immunity contributes to the pathogenesis of cholangiopathy in primary biliary cirrhosis, Clin. Exp. Immunol. 157 (2009) 261–270.
[33] K. Kawata, M. Tsuda, G.X. Yang, W. Zhang, H. Tanaka, K. Tsuneyama, P. Leung, X.S. He, S. Knechtle, A.A. Ansari, R.L. Coppel, M.E. Gershwin, Identification of potential cytokine pathways for therapeutic intervention in murine primary biliary cirrhosis, PLoS One 8 (2013) e74225.
[34] G.M. Hirschfield, M.E. Gershwin, R. Strauss, M.J. Mayo, C. Levy, B. Zou, J. Johanns, I.P. Nnane, B. Dasgupta, K. Li, C. Selmi, H.U. Marschall, D. Jones, K. Lindor, P.S. Group, Ustekinumab for patients with primary biliary cholangitis who have an inadequate response to ursodeoxycholic acid: a proof-of-concept study, Hepatology 64 (2016) 189–199.
[35] J.R. Groom, A.D. Luster, CXCR3 ligands: redundant, collaborative and antagonistic functions, Immunol. Cell Biol. 89 (2011) 207–215.
[36] Y.H. Chuang, Z.X. Lian, C.M. Cheng, R.Y. Lan, G.X. Yang, Y. Moritoki, B.L. Chiang, A.A. Ansari, K. Tsuneyama, R.L. Coppel, M.E. Gershwin, Increased levels of chemokine receptor CXCR3 and chemokines IP-10 and MIG in patients with primary biliary cirrhosis and their first degree relatives, J. Autoimmun. 25 (2005) 126–132.
[37] A. Lleo, W. Zhang, M. Zhao, Y. Tan, F. Bernuzzi, B. Zhu, Q. Liu, Q. Tan, F. Malinverno, L. Valenti, T. Jiang, L. Tan, W. Liao, R. Coppel, P. Invernizzi, Q. Lu, D.H. Adams, M.E. GershwinP.B.C.E.S. Group, DNA methylation profiling of the X chromosome reveals an aberrant demethylation on CXCR3 promoter in primary biliary cirrhosis, Clin. Epigenet. 7 (2015) 61.
[38] K. Nishioji, T. Okanoue, Y. Itoh, S. Narumi, M. Sakamoto, H. Nakamura, A. Morita, K. Kashima, Increase of chemokine interferon-inducible protein-10 (IP-10) in the serum of patients with autoimmune liver diseases and increase of its mRNA expression in hepatocytes, Clin. Exp. Immunol. 123 (2001) 271–279.
[39] H. Yoneyama, Y. Kai, J. Koyama, K. Suzuki, H. Kawachi, S. Narumi, T. Ichida, Neutralization of CXCL10 accelerates liver regeneration in carbon tetrachloride-induced acute liver injury, Med. Mol. Morphol. 40 (2007) 191–197.
[40] E. Hintermann, M. Bayer, J.M. Pfeilschifter, A.D. Luster, U. Christen, CXCL10 promotes liver fibrosis by prevention of NK cell mediated hepatic stellate cell inactivation, J. Autoimmun. 35 (2010) 424–435.
[41] K.L. de Graaf, G. Lapeyre, F. Guilhot, W. Ferlin, S.M. Curbishley, M. Carbone, P. Richardson, S. Moreea, C.A. McCune, S.D. Ryder, R.W. Chapman, A. Floreani, D.E. Jones, C. de Min, D.H. Adams, P. Invernizzi, NI-0801, an anti-chemokine (C-X-C motif) ligand 10 antibody, in patients with primary biliary cholangitis and an incomplete response to ursodeoxycholic acid, Hepatol Commun 2 (2018) 492–503.
[42] S. Shimoda, M. Nakamura, H. Ishibashi, A. Kawano, T. Kamihira, N. Sakamoto, S. Matsushita, A. Tanaka, H.J. Worman, M.E. Gershwin, M. Harada, Molecular mimicry of mitochondrial and nuclear autoantigens in primary biliary cirrhosis, Gastroenterology 124 (2003) 1915–1925.
[43] M.M. Kaplan, M.E. Gershwin, Primary biliary cirrhosis, N. Engl. J. Med. 353 (2005) 1261–1273.
[44] M.E. Gershwin, I.R. Mackay, A. Sturgess, R.L. Coppel, Identification and specificity of a cDNA encoding the 70 kd mitochondrial antigen recognized in primary biliary cirrhosis, J. Immunol. 138 (1987) 3525–3531.
[45] H. Kita, Z.X. Lian, J. Van de Water, X.S. He, S. Matsumura, M. Kaplan, V. Luketic, R.L. Coppel, A.A. Ansari, M.E. Gershwin, Identification of HLA-A2-restricted CD8(+) cytotoxic T cell responses in primary biliary cirrhosis: T cell activation is augmented by immune complexes cross-presented by dendritic cells, J. Exp. Med. 195 (2002) 113–123.
[46] H. Kita, S. Matsumura, X.S. He, A.A. Ansari, Z.X. Lian, J. Van de Water, R.L. Coppel, M.M. Kaplan, M.E. Gershwin, Quantitative and functional analysis of PDC-E2-specific autoreactive cytotoxic T lymphocytes in primary biliary cirrhosis, J. Clin. Investig. 109 (2002) 1231–1240.
[47] M. Azuma, D. Ito, H. Yagita, K. Okumura, J.H. Phillips, L.L. Lanier, C. Somoza, B70 antigen is a second ligand for CTLA-4 and CD28, Nature 366 (1993) 76–79.
[48] G.J. Freeman, J.G. Gribben, V.A. Boussiotis, J.W. Ng, V.A. Restivo Jr., L.A. Lombard, G.S. Gray, L.M. Nadler, Cloning of B7-2: a CTLA-4 counter-receptor that costimulates human T cell proliferation, Science 262 (1993) 909–911.
[49] R.J. Peach, J. Bajorath, W. Brady, G. Leytze, J. Greene, J. Naemura, P.S. Linsley, Complementarity determining region 1 (CDR1)- and CDR3-analogous regions in CTLA-4 and CD28 determine the binding to B7-1, J. Exp. Med. 180 (1994) 2049–2058.
[50] P.S. Linsley, J.L. Greene, W. Brady, J. Bajorath, J.A. Ledbetter, R. Peach, Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors, Immunity 1 (1994) 793–801.
[51] S. Oertelt, T.P. Kenny, C. Selmi, P. Invernizzi, M. Podda, M.E. Gershwin, SNP analysis of genes implicated in T cell proliferation in primary biliary cirrhosis, Clin. Dev. Immunol. 12 (2005) 259–263.
[52] B.D. Juran, E.J. Atkinson, J.J. Larson, E.M. Schlicht, X. Liu, E.J. Heathcote, G.M. Hirschfield, K.A. Siminovitch, K.N. Lazaridis, Carriage of a tumor necrosis factor polymorphism amplifies the cytotoxic T-lymphocyte antigen 4 attributed risk of primary biliary cirrhosis: evidence for a gene-gene interaction, Hepatology 52 (2010) 223–229.
[53] E.J. Walker, G.M. Hirschfield, C. Xu, Y. Lu, X. Liu, Y. Lu, C. Coltescu, K. Wang, W.G. Newman, V. Bykerk, E.C. Keystone, D. Mosher, C.I. Amos, E.J. Heathcote, K.A. Siminovitch, CTLA4/ICOS gene variants and haplotypes are associated with rheumatoid arthritis and primary biliary cirrhosis in the Canadian population, Arthritis Rheum. 60 (2009) 931–937.
[54] R. Westhovens, M. Robles, A.C. Ximenes, S. Nayiager, J. Wollenhaupt, P. Durez, J. Gomez-Reino, W. Grassi, B. Haraoui, W. Shergy, S.H. Park, H. Genant, C. Peterfy, J.C. Becker, A. Covucci, R. Helfrick, J. Bathon, Clinical efficacy and safety of abatacept in methotrexate-naive patients with early rheumatoid arthritis and poor prognostic factors, Ann. Rheum. Dis. 68 (2009) 1870–1877.
[55] D.J. Lovell, N. Ruperto, R. Mouy, E. Paz, N. Rubio-Perez, C.A. Silva, C. Abud-Mendoza, R. Burgos-Vargas, V. Gerloni, J.A. Melo-Gomes, C. Saad-Magalhaes, J. Chavez-Corrales, C. Huemer, A. Kivitz, F.J. Blanco, I. Foeldvari, M. Hofer, H.I. Huppertz, C. Job Deslandre, K. Minden, M. Punaro, A.J. Block, E.H. Giannini, A. Martini, G., Pediatric Rheumatology Collaborative Study and O. the Paediatric Rheumatology International Trials, Long-term safety, efficacy, and quality of life in patients with juvenile idiopathic arthritis treated with intravenous abatacept for up to seven years, Arthritis Rheum. 67 (2015) 2759–2770.
[56] P.J. Mease, A.B. Gottlieb, D. van der Heijde, O. FitzGerald, A. Johnsen, M. Nys, S. Banerjee, D.D. Gladman, Efficacy and safety of abatacept, a T-cell modulator, in a randomised, double-blind, placebo-controlled, phase III study in psoriatic arthritis, Ann. Rheum. Dis. 76 (2017) 1550–1558.
[57] A. Dhirapong, G.X. Yang, S. Nadler, W. Zhang, K. Tsuneyama, P. Leung, S. Knechtle, A.A. Ansari, R.L. Coppel, F.T. Liu, X.S. He, M.E. Gershwin, Therapeutic effect of cytotoxic T lymphocyte antigen 4/immunoglobulin on a murine model of primary biliary cirrhosis, Hepatology 57 (2013) 708–715.
[58] A. Jacoby, A. Rannard, D. Buck, N. Bhala, J.L. Newton, O.F. James, D.E. Jones, Development, validation, and evaluation of the PBC-40, a disease specific health related quality of life measure for primary biliary cirrhosis, Gut 54 (2005) 1622–1629.
[59] S.K. Venkatesh, M. Yin, R.L. Ehman, Magnetic resonance elastography of liver: clinical applications, J. Comput. Assist. Tomogr. 37 (2013) 887–896.
[60] W.J. Lammers, G.M. Hirschfield, C. Corpechot, F. Nevens, K.D. Lindor, H.L. Janssen, A. Floreani, C.Y. Ponsioen, M.J. Mayo, P. Invernizzi, P.M. Battezzati, A. Pares, A.K. Burroughs, A.L. Mason, K.V. Kowdley, T. Kumagi, M.H. Harms, P.J. Trivedi,

R. Poupon, A. Cheung, A. Lleo, L. Caballeria, B.E. Hansen, H.R. van Buuren, P.B.C.S.G. Global, Development and validation of a scoring System to predict outcomes of patients with primary biliary cirrhosis receiving ursodeoxycholic acid therapy, Gastroenterology 149 (2015) 1804–1812 e4.

[61] W.A. Mayo MJ, S.K. Roberts, et al., NGM282, a novel variant of FGF-19, demonstrates biologic activity in primary biliary cirrhosis patients with an incomplete response to ursodeoxycholic acid: results of a phase 2 multicenter, randomized, double blinded, placebo controlled trial, Hepatology 62 (2016) 263A–264A.

[62] D. Jones, P.F. Boudes, M.G. Swain, C.L. Bowlus, M.R. Galambos, B.R. Bacon, Y. Doerffel, N. Gitlin, S.C. Gordon, J.A. Odin, D. Sheridan, M.A. Worns, V. Clark, L. Corless, H. Hartmann, M.E. Jonas, A.E. Kremer, G.F. Mells, P. Buggisch, B.L. Freilich, C. Levy, J.M. Vierling, D.E. Bernstein, M. Hartleb, E. Janczewska, F. Rochling, H. Shah, M.L. Shiffman, J.H. Smith, Y.J. Choi, A. Steinberg, M. Varga, H. Chera, R. Martin, C.A. McWherter, G.M. Hirschfield, Seladelpar (MBX-8025), a selective PPAR-delta agonist, in patients with primary biliary cholangitis with an inadequate response to ursodeoxycholic acid: a double-blind, randomised, placebo-controlled, phase 2, proof-of-concept study, Lancet Gastroenterol Hepatol 2 (2017) 716–726.

[63] A. Lleo, S. Marzorati, J.M. Anaya, M.E. Gershwin, Primary biliary cholangitis: a comprehensive overview, Hepatol Int 11 (2017) 485–499.

[64] K.A. Keogh, M.E. Wylam, J.H. Stone, U. Specks, Induction of remission by B lymphocyte depletion in eleven patients with refractory antineutrophil cytoplasmic antibody-associated vasculitis, Arthritis Rheum. 52 (2005) 262–268.

[65] A. Pestronk, J. Florence, T. Miller, R. Choksi, M.T. Al-Lozi, T.D. Levine, Treatment of IgM antibody associated polyneuropathies using rituximab, J. Neurol. Neurosurg. Psychiatry 74 (2003) 485–489.

[66] Y. Moritoki, W. Zhang, K. Tsuneyama, K. Yoshida, K. Wakabayashi, G.X. Yang, C. Bowlus, W.M. Ridgway, Y. Ueno, A.A. Ansari, R.L. Coppel, I.R. Mackay, R.A. Flavell, M.E. Gershwin, Z.X. Lian, B cells suppress the inflammatory response in a mouse model of primary biliary cirrhosis, Gastroenterology 136 (2009) 1037–1047.

[67] L. Wang, Y. Sun, Z. Zhang, Y. Jia, Z. Zou, J. Ding, Y. Li, X. Xu, L. Jin, T. Yang, Z. Li, Y. Sun, J.Y. Zhang, S. Lv, L. Chen, B. Li, M.E. Gershwin, F.S. Wang, CXCR5+ CD4+ T follicular helper cells participate in the pathogenesis of primary biliary cirrhosis, Hepatology 61 (2015) 627–638.

[68] S. Shimoda, S. Hisamoto, K. Harada, S. Iwasaka, Y. Chong, M. Nakamura, Y. Bekki, T. Yoshizumi, K. Shirabe, T. Ikegami, Y. Maehara, X.S. He, M.E. Gershwin, K. Akashi, Natural killer cells regulate T cell immune responses in primary biliary cirrhosis, Hepatology 62 (2015) 1817–1827.

[69] H. Tanaka, W. Zhang, G.X. Yang, Y. Ando, T. Tomiyama, K. Tsuneyama, P. Leung, R.L. Coppel, A.A. Ansari, Z.X. Lian, W.M. Ridgway, T. Joh, M.E. Gershwin, Successful immunotherapy of autoimmune cholangitis by adoptive transfer of forkhead box protein 3(+) regulatory T cells, Clin. Exp. Immunol. 178 (2014) 253–261.

[70] W. Zhang, Y. Ono, Y. Miyamura, C.L. Bowlus, M.E. Gershwin, E. Maverakis, T cell clonal expansions detected in patients with primary biliary cirrhosis express CX3CR1, J. Autoimmun. 37 (2011) 71–78.

[71] M. Gattorno, I. Prigione, F. Morandi, A. Gregorio, S. Chiesa, F. Ferlito, A. Favre, A. Uccelli, C. Gambini, A. Martini, V. Pistoia, Phenotypic and functional characterisation of CCR7+ and CCR7- CD4+ memory T cells homing to the joints in juvenile idiopathic arthritis, Arthritis Res. Ther. 7 (2005) R256–R267.

[72] H.D. Ma, W.T. Ma, Q.Z. Liu, Z.B. Zhao, M.Z. Liu, K. Tsuneyama, J.M. Gao, W.M. Ridgway, A.A. Ansari, M.E. Gershwin, Y.Y. Fei, Z.X. Lian, Chemokine receptor CXCR3 deficiency exacerbates murine autoimmune cholangitis by promoting pathogenic CD8(+) T cell activation, J. Autoimmun. 78 (2017) 19–28.

[73] W. Huang, K. Kachapati, D. Adams, Y. Wu, P.S.C. Leung, G.-X. Yang, W. Zhang, A.A. Ansari, R.A. Flavell, M.E. Gershwin, W.M. Ridgway, Murine autoimmune cholangitis requires two hits: cytotoxic KLRG1(+) CD8 effector cells and defective T regulatory cells, J. Autoimmun. 50 (2014) 123–134.

[74] R.Y. Lan, C. Cheng, Z.X. Lian, K. Tsuneyama, G.X. Yang, Y. Moritoki, Y.H. Chuang, T. Nakamura, S. Saito, S. Shimoda, A. Tanaka, C.L. Bowlus, Y. Takano, A.A. Ansari, R.L. Coppel, M.E. Gershwin, Liver-targeted and peripheral blood alterations of regulatory T cells in primary biliary cirrhosis, Hepatology 43 (2006) 729–737.

[75] K. Langdon, N. Haleagrahara, Regulatory T-cell dynamics with abatacept treatment in rheumatoid arthritis, Int. Rev. Immunol. 37 (2018) 206–214.

[76] M. Tsuda, Y.M. Ambrosini, W. Zhang, G.X. Yang, Y. Ando, G. Rong, K. Tsuneyama, K. Sumida, S. Shimoda, C.L. Bowlus, P.S. Leung, X.S. He, R.L. Coppel, A.A. Ansari, Z.X. Lian, M.E. Gershwin, Fine phenotypic and functional characterization of effector cluster of differentiation 8 positive T cells in human patients with primary biliary cirrhosis, Hepatology 54 (2011) 1293–1302.

[77] M. Carbone, A. Nardi, S. Flack, G. Carpino, N. Varvaropoulou, C. Gavrila, A. Spicer, J. Badrock, F. Bernuzzi, V. Cardinale, H.F. Ainsworth, M.A. Heneghan, D. Thorburn, A. Bathgate, R. Jones, J.M. Neuberger, P.M. Battezzati, M. Zuin, S. Taylor-Robinson, M.F. Donato, J. Kirby, R. Mitchell-Thain, A. Floreani, F. Sampaziotis, L. Muratori, D. Alvaro, M. Marzioni, L. Miele, F. Marra, E. Giannini, E. Gaudio, V. Ronca, G. Bonato, L. Cristoferi, F. Malinverno, A. Gerussi, D.D. Stocken, H.J. Cordell, G.M. Hirschfield, G.J. Alexander, R.N. Sandford, D.E. Jones, P. Invernizzi, G.F. Mells, P.B.C.S.G. Italian and U.K.P.B.C.C. the, Pretreatment prediction of response to ursodeoxycholic acid in primary biliary cholangitis: development and validation of the UDCA Response Score, Lancet Gastroenterol Hepatol 3 (2018) 626–634.