A review of the medical treatment of primary sclerosing cholangitis in the 21st century

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***Abstract*:** Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease that progresses to end-stage liver disease and cirrhosis. Recurrent biliary inflammation is thought to lead to dysplasia, and as such PSC confers a high risk of cholangiocarcinoma. PSC accounts for 10% of all UK liver transplants, although transplantation does not guarantee a cure with 20% recurrence in the graft. At present there are no effective medical treatment options for PSC, and trials of novel therapeutic agents are limited by the time taken to reach clinically significant endpoints with no well defined early surrogate markers for disease outcome. Moreover, PSC appears to be a heterogeneous disease with regards to disease distribution, associated inflammatory bowel disease and subsequent disease outcome, further compounding the issue. Thus existing trials have taken place in heterogeneous groups, are likely to be underpowered to detect any individual subgroups effect. The current mainstay of medical treatment is still with ursodeoxycholic acid, although there is no evidence that it alters long-term outcome. Small pilot studies of immunosuppressive agents have taken place, but despite evidence that may support studies in larger groups, these have not been conducted. Recent advances in our understanding of the disease pathogenesis may therefore pave the way for trials of novel therapeutic agents in PSC, even given the limitations described. This review explores the controversial evidence underlying current treatment strategies and discounted treatments, and explores prospective agents that may bring new hope to the treatment of PSC in the 21st century.

Keywords: cholestasis, primary sclerosing cholangitis, ursodeoxycholic acid

Introduction

Primary sclerosing cholangitis (PSC) is one of the archetypal autoimmune liver diseases alongside autoimmune hepatitis and primary biliary cirrhosis (PBC). Considered a rare disease, it characteristically affects young patients with a slight male predominance. At present a truly conceptual pathogenic framework is lacking and there is no recognized therapy that has been shown to alter the outcome of patients with this condition.

PSC is a chronic, cholestatic liver disease characterized by biliary inflammation and fibrosis of both small and large bile ducts, that can potentially lead to cholestasis and cirrhosis. This can result in end-stage liver failure and as such PSC is the fifth commonest indication for liver transplantation in the UK [Adam *et al.* 2003]. Patients with PSC carry a high lifetime risk of gastrointestinal malignancy; 44% of PSC deaths are cancer related, with 7–13% developing cholangiocarcinomas possibly due to inflammation-associated epithelial dysplasia [Bergquist *et al.* 2002; Fevery *et al.* 2007]. Inflammatory bowel disease (IBD) coexists in 60–80% of patients, with a 10-fold increased risk of colorectal carcinoma compared with the general population [Bergquist *et al.* 2002; Sano *et al.* 2011; Ye *et al.* 2011]. The IBD associated with PSC is unusual in that it is usually a pancolitis with activity worse on the right, backwash ileitis and rectal sparing [Loftus *et al.* 2005; Sano *et al.* 2011]. Furthermore, 25% of patients with PSC have concurrent autoimmune diseases [Saarinen *et al.* 2000]. Thus, despite being a rare disease with a

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prevalence of 0.2–16 per 100,000, PSC represents a significant burden on hepatobiliary and oncological services [Hurlburt *et al.* 2002; Bambha *et al.* 2003; Boonstra *et al.* 2012].

Existing evidence suggests that PSC is a heterogeneous condition, with different subgroups conferring different prognoses. For example, patients with concomitant ulcerative colitis (UC) and PSC have an increased risk of developing colorectal cancer compared with patients with UC or PSC alone [Broome *et al.* 1995]. Patients with PSC with small duct disease have an improved survival and lower risk of cholangiocarcinoma compared with patients with large-duct PSC [Bjornsson *et al.* 2002]. Patients with PSC and a raised immunoglobulin G4 (IgG4) have a more severe liver disease, and patients who demonstrate a significant reduction in their serum alkaline phosphatase (ALP) in a median time of 2 years following diagnosis have an improved transplant-free survival and reduced risk of cholangiocarcinoma [Mendes *et al.* 2006; Al Mamari *et al.* 2013].

Disease pathogenesis

An understanding of disease pathogenesis is fundamental to the selection of potential treatments. Although the exact pathogenesis remains unclear, PSC is thought to have both environmental and genetic causes, with 16 genetic loci currently identified and further genetic loci undergoing evaluation in international genome-wide-association studies (GWAS) meta-analysis [Liu *et al.* 2013]. These analyses and other experimental results are starting to shape a pathogenic model of PSC.

An autoimmune aetiology is strongly supported by several factors; the presence of concurrent autoimmune disease in up to 25% of patients, strong linkage of PSC to the human histocompatibility complex, tissue infiltration with immune cells, and GWAS/Immunochip (Illumina Infinium single-nucleotide polymorphism (SNP) microarray) genetic studies [Saarinen *et al.* 2000; Karlsen *et al.* 2010; Liu *et al.* 2013]. At the heart of the pathogenic model appears to be a dysregulated autoimmune network that results in the loss of peripheral tolerance to self cholangiocytes and colonocytes. There is strong evidence supporting the production of pathogenic T cells in the colon and small bowel, which subsequently attack the biliary tree and bowel. This is based upon the fact that pathogenic liver T cells are enriched in a subset that express the small intestinal homing C–C chemokine receptor type 9 (CCR9), and home to the liver endothelium by the expression of gut-enriched homing chemokines; mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1) and chemokine (C–C motif) ligand 25 (CCL25). In addition, infiltration of the liver by T-helper 17 (TH-17) cells further supports their proposed origin from the gut.

TH-17 are a subset of T-helper cells that produce interleukin 17 (IL-17), IL-22, tumour necrosis factor α (TNFα) and CCL20. They differentiate from naïve T cells in the presence of dendritic cells and cytokines IL-1β, IL-6, IL-23, transforming growth factor β (TGFβ) and IL-21. In order to maintain immunological tolerance, this production of TH-17 cells in the bowel and biliary tree in PSC should be kept in careful check. This is usually secured by the favoured production of T-regulatory cells (CD4+/CD25+/CTLA-4+/Foxp3 positive cell) by tolerogenic plasmacytoid dendritic cells. Therefore an alteration favouring the production of mucosal TH-17 axis (CD4+ and CD8+ T cells) may underpin PSC pathogenesis. This TH-17, T-regulatory imbalance would perhaps also suggest why single nucleotide polymorphisms of genome-wide significance are embedded in the IL-2, IL-2 receptor and CD28 axis, with macrophage stimulating 1 having a potential role in dendritic cell tolerance.

These autoreactive T cells are postulated to recognize cholangiocytes and colonocytes *via* class I and class II human leukocyte antigen, resulting in inflammation, apoptosis, necrosis and tissue fibrosis. Furthermore, increases in bile duct permeability result in leakage of bile into the surrounding peribiliary tissues, secondary vascular injury with endarteritis obliterans and biliary ischemia. The end result is activation of portal fibroblasts and stem cells that lay down elastin and collagen to form scar tissue, with associated biliary cell apoptosis/mesenchymal transition and the induction of cellular senescence. Furthermore damage and impaired drainage of the bile ducts promotes bacterial and fungal colonization, secondary invasion and tissue damage.

An additional role for involvement of the gut microbiota has been highlighted in PSC. *In vitro* studies of biliary epithelial cells (BECs) from patients with and without alcohol-related liver disease demonstrate inappropriate innate immune responses to intestinal endotoxins and subsequent endotoxin intolerance due to enhanced pattern recognition receptor signalling in BECs of

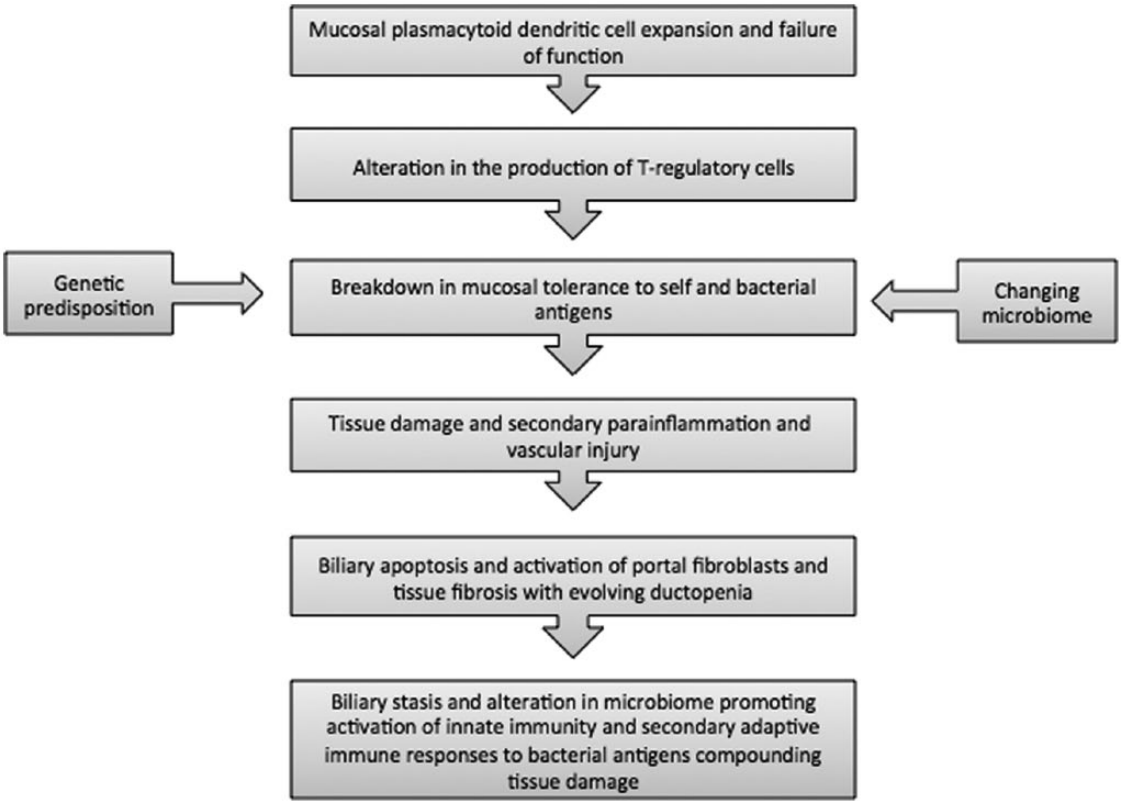

Figure 1. Pathogenesis of primary sclerosing cholangitis.

those with chronic liver disease [Mueller *et al.* 2011]. This is thought to contribute to chronic cholangitis. Rats with small bowel bacterial overgrowth induced by jejunual ligation develop liver lesions similar to those seen in PSC [Lichtman *et al.* 1990]. Subsequent antibiotic therapy with metronidazole and tetracycline leads to an improvement in these lesions, suggesting that modification of gut microbiota may be important in PSC [Lichtman *et al.* 1991]. Furthermore, a unique microbiome may be present in PSC [Sabino *et al.* 2015].

It is this growing understanding of the pathogenesis of PSC (see Figure 1) that brings new hope for emerging treatments in PSC. It is likely that by tackling the different aspects of pathogenesis, inflammation, ischaemia, fibrosis, vascular injury and alteration of the biome, a new successful treatment approach will be found either in isolation or with a combination of agents.

Factors hindering therapeutic trials in PSC

Despite some progress in our understanding of disease pathogenesis, medical therapy for PSC has been hindered by two major factors: disease characteristics and lack of clear endpoints for clinical trials.

As a disease of low prevalence, studies of PSC have, until recently, been limited by small cohort size, relying on meta-analyses to improve statistical power [Schrumpf and Boberg, 2001]. As previously discussed, PSC displays a multitude of clinical phenotypes that differ with respect to prognosis. These phenotypes encompass disease with and without ulcerative colitis, overlap with other autoimmune conditions, raised IgG4 and the location and site of the biliary injury [Trivedi and Hirschfield, 2012]. Thus patient heterogeneity with regards to level of baseline fibrosis and rate of disease progression means true randomization in clinical trials is difficult. A number of prognostic models have been developed to help identify at an early stage, patients who are more likely to progress to a poor outcome [Wiesner *et al.* 1989; Farrant *et al.* 1991; Dickson *et al.* 1992; Broome *et al.* 1996; Kim *et al.* 2000]. However, with the exception of the revised Mayo Clinic model, they all include a histological staging parameter, which necessitates invasive liver biopsy. In a disease diagnosed *via* cholangiogram, this limits the clinical utility of these prognostic models. Furthermore,

these models do not address the impact of cholangiocarcinoma in PSC, which is of paramount importance when considering prognosis and future treatments. Guidelines therefore suggest that the use of existing risk scores should be restricted to cohort studies rather than for individual patient outcomes [Wiesner *et al.* 1989; Farrant *et al.* 1991; Dickson *et al.* 1992; Broome *et al.* 1996; Kim *et al.* 1999; Chapman *et al.* 2010].

The lack of consensus over clinically relevant endpoints has further hindered interpretation of results from therapeutic trials. The most commonly used endpoints include liver biochemistry, symptoms, transplantation and disease-related death. However, the time taken for the latter to occur is not practical for most pharma-sponsored studies. Utility of histology is variable in study design, and importantly, whether histological progression directly reflects clinical progression is yet to be established given the heterogeneous distribution of fibrosis in PSC. Furthermore, use of fibroscan, magnetic resonance imaging (MRI) elastography, Enhanced Liver Fibrosis (ELF) and serum fibrosis panels is yet to be validated in PSC, although it is currently under study by the International PSC Study Group.

It is likely that future PSC trials will take two forms. The first is likely to be trials to establish if the drug of investigation has a biological signal (mediated *via* fibrosis, cholangiography or an immunological signal) without necessarily demonstrating that it alters clinically meaningful endpoints. The second is to stratify patients into groups that have either a low, intermediate or high risk of progression, and target the intermediate- or high-risk patients for recruitment into trials to establish if a clinically meaningful signal is detected. Whilst at present stratification according to risk of progression is not possible, large-scale phenotyping studies from the International PSC Study Group and UK PSC Consortium mean that this is likely to be possible in the near future.

This review will therefore explore the established and emerging medical therapies for PSC at a time that hopes to be a 21st century turning point in our understanding of the disease.

Therapies altering bile composition

Ursodeoxycholic acid

Ursodeoxycholic acid (UDCA) is the most commonly prescribed drug in PSC. Given its proven efficacy in the treatment of other cholestatic diseases such as PBC, UDCA has biological plausibility in the treatment of PSC. UDCA is postulated to have two mechanisms of action: reducing hydrophobicity of bile and a direct effect on adaptive immunity by inhibiting dendritic cell response. However, it is not an agonist for farnesoid X receptor (FXR) or pregnane X receptor (PXR), which is protective in cholestatic disorders [Paumgartner and Beuers, 2004; Beuers, 2006].

Low-dose UDCA. The first placebo-controlled pilot studies of low-dose UDCA (10–15 mg/kg/day) demonstrated efficacy in improving liver biochemistry, histology and symptoms [Chazouilleres *et al.* 1990; Beuers *et al.* 1992; Stiehl *et al.* 1994]. However, statistical power was limited by small sample sizes of 14–20 patients per trial. A larger placebo-controlled study of 105 patients confirmed improvement of liver biochemistry with low-dose UDCA (13–15 mg/kg), but did not demonstrate any effect on symptoms or time to transplantation [Lindor, 1997]. Yet unfortunately these early studies are notable for their short duration compared with the natural history of PSC (see Table 1).

The largest multicentre, randomized, double-blind, placebo-controlled study (RCT) to date recruited 219 patients, treated with UDCA (17–23 mg/kg/day) and followed up for 5 years [Olsson *et al.* 2005]. Despite significant efforts, researchers failed to recruit the 346 patients required to detect a statistically significant difference in primary endpoint, reflecting the difficulty of studying a rare disease. The study demonstrated only a non-significant trend towards improved survival and time to liver transplantation, which may reflect type II error resulting from an underpowered study. However, secondary outcome measures (change in symptoms, quality of life and change in liver biochemistry) were also not supportive of UDCA. Furthermore, a 15-year follow up to this trial supported no role for UDCA [Lindstrom *et al.* 2013].

High-dose UDCA. Pilot studies of UDCA up to 30 mg/kg/day demonstrated a significant improvement in Mayo risk score, which perhaps unadvisedly was used as a surrogate marker for improved survival [Harnois *et al.* 2001; Cullen *et al.* 2008]. However, the largest RCT of high-dose UDCA was terminated at interim analysis when, despite a statistically significant improvement in liver biochemistry, there was an unexpected, 2.3-fold

Table 1. Characteristics of trials of UDCA in PSC.

Study	Year	UDCA dose	Design	UDCA (*n*)	Placebo (*n*)	Trial duration (months)	Liver biochemistry improved	Symptomatic improvement	Mayo risk score	Liver histology improvement	Cholangiographic improvement	Progression to end-stage liver disease	Progression to transplant/ death
O'Brien *et al.*	1991	10 mg/kg/day	OL	12	N/A	30	Y	Y	Not done	Y	N	Not done	Not done
Beuers *et al.*	1992	13–15 mg/kg/day	DB, PC	6	8	12	Y	N	Not done	Y	N	Not done	Not done
Lo *et al.*	1992	10 mg/kg/day	DB, PC	8	10	24	Y	Not done	Not done	Y	N	Not done	Not done
Stiehl *et al.*	1994	750 mg/day	DB, PC	10	10	12–48	Y	N	Not done	Y	N	Not done	Not done
De Maria *et al.*	1996	600 mg/day	DB, PC	20	20	24	N	Not done	Not done	Not done	N	Not done	Not done
Lindor	1997	13–15 mg/kg/day	DB, PC	51	51	34	Y	N	Not done	N	Not done	N	N
Van Hoogstraten *et al.*	1998	10 mg/kg/day	DB, PC	24	24	24	Y	N	N	N	N	N	N
Mitchell *et al.*	2001	20 mg/kg/day	DB, PC	13	13	24	Y	N	Not done	Y	Y	Y	N
Harnois *et al.*	2001	25–30 mg/kg/day	OL	15	15	12	Y	Not done	Y	Not done	N	Not done	Y
Okolicsanyi *et al.*	2003	8–13 mg/kg/day	RA	69	17	N/R	Y	Y	N	N	N	Not done	Not done
Olsson *et al.*	2005	17–23 mg.kg.day	DB, PC	97	101	60	Y	N	N	Not done	N	Not done	Not done
Lindor *et al.*	2009	28–30 mg/kg/day	DB, PC	76	73	60	Y	Not done	Not done	Not done	N	N	N

DB, double blind; N/A, not applicable; N/R, not reported; OL, open label; PBC, primary biliary cirrhosis; PC, placebo controlled; RA, retrospective analysis; UDCA, ursodeoxycholic acid.

Table 2. Characteristics of trials of immunosuppressant agents in PSC.

Agent	Study	Year	Design	Treatment (Tx)	Tx *n*	Control (C)	C *n*	Trial duration (months)	Liver biochemistry improvement	Symptomatic improvement	Liver histology improvement	Cholangiographic improvement	Progression to end-stage liver disease	Transplant/ survival
Azathioprine	Schramm *et al*.	1999	Case series	Azathioprine 1–1.5 mg/kg/day Prednisolone 1 mg/kg/day UDCA 500–750 mg/day	15	N/A	N/A	41	N	Not done	Y	N	Not done	Not done
Cyclosporin	Wiesner *et al*.	1991	DB, PC	Cyclosporin	20	Placebo	10	N\R	N	N	Y	Not done	N	N
	Sandborn *et al*.	1993	DB, RCT	Cyclosporin 4.1 mg/kg/day	19	Placebo	11	12	N	N	N	N	N	N
Methotrexate	Knox and Kaplan	1991	OL	Methotrexate 0.2 mg/kg/week	10	N/A	N/A	12	Y	Not done	Y	Y	Not done	Not done
	Knox and Kaplan	1994	PC	Methotrexate	12	Placebo	12	24	N	Not done	N	N	Not done	Not done
Tacrolimus	Van Thiel *et al*.	1995	OL	Tacrolimus 3 mg twice daily	10	N/A	N/A	3	Y	Not done	Not done	Not done	Not done	Not done
	Talwalkar *et al*.	2007	OL	Tacrolimus 0.05 mg/kg twice daily	16	N/A	N/A	12	Y	Y	Not done	Not done	Not done	Not done

N/A, not applicable; DB, double blind; PC, placebo controlled; N/R, not reported; RCT, randomized controlled trial; OL, open label; C, control; Tx, treatment; *n*, number.

Table 3. Currently registered therapeutic trials in PSC.

Agent	ClinicalTrials.gov identifier	Principal investigators	Start date	Estimated completion date	Trial phase	Design	Treatment	Primary endpoint	Secondary endpoints
Human monoclonal anti-VAP-1 antibody (BTT1023)	NCT02239211	Hirschfield, GM, University of Birmingham, UK	March 2015	January 2017	II	Single arm, two stage, multicentre	BTT1023 8 mg/kg intravenous infusion, every 14 days (total of 7 infusions)	ALP	Liver fibrosis
Oral vancomycin	NCT01802073	Cox, KL, Stanford University	January 2012	December 2015	III	Single arm, open label	Adults and children >30 kg, vancomycin 500 mg 3 times per day. Dose increased to 750 mg 3 times per day for the second month and 1000 mg 3 times per day for the third month if bloods not normalized	ALT, ALP MRCI/MRCP Liver histology	
Obeticholic acid (OCA)	NCT02177136	Shapiro, D, Intercept Pharmaceuticals	November 2014	November 2018	II	Randomized, double blind, placebo controlled, dose finding	1.5 mg OCA, 5 mg OCA, or placebo for 12 weeks, followed by titration for further 12 weeks; 1.5 mg OCA treatment group then titrate to 3 mg, the 5 mg OCA treatment group then titrate to 10 mg OCA, placebo group remain on placebo	ALP	
Nor-ursodeoxycholic acid	NCT01755507	Trauna M, Med. Uni Wien Manns MP, Med. Hochschule Hannover, Boberg K, Dr Falk Pharma	December 2012	March 2014	II	Double blind, randomized, placebo controlled	500, 1000 or 1500 mg/day norursodeoxycholic acid capsules versus placebo	Change in serum ALP in 12 weeks	Proportion of patients with at least 50% reduction in serum ALP
LOXL-2 inhibitor simtuzumab (GS-6624)	NCT01672853	Myers R, Gilead Sciences	February 2013	July 2016	II	Dose ranging, randomized, double blind, placebo controlled	Subcutaneous injection weekly for 96 weeks	Change from baseline in morphometric quantitative collagen on liver biopsy	Safety of GS-6624 in subjects with PSC
All-trans retinoic acid (ATRA) and ursodeoxycholic acid (UDCA)	NCT01456468	Boyer JL, Yale University	October 2011	June 2014	I	Single-arm	ATRA 45 mg/m^2 divided into 2 doses, with UDCA 15 mg/kg/day	30% improvement in serum alkaline phosphatase	
LUM001 (apical sodium-dependent bile acid transporter inhibitor (ASBTi)	NCT02061540	Lumina Pharma	March 2014	December 2015	II	Open label, single arm	LUM001 administered orally once each day	Adverse events, changes in vital signs, laboratory and other safety parameters from baseline to week 14 Safety and tolerability	Changes in serum bile acids, pruritus, and other biochemical markers of cholestasis and liver disease from baseline to week 14

ALT, ALP, alkaline phosphatase; MRCI; MRCP, Magnetic Resonance Cholangiopancreatography.

increased risk of progression to liver transplantation and varices in the treatment group [Lindor *et al*. 2009]. Subgroup analysis revealed that the risk of adverse events, particularly oesophageal varices, were more apparent in patients with early histological stage disease and normal total bilirubin. Importantly, recent analysis has suggested that there is an increased serum concentration of lithocholic acid, a potent hydrophobic bile acid, in patients given high-dose UDCA, which may cause these adverse outcomes [Sinakos *et al*. 2010].

Due to conflicting evidence and the limited sample size in these major studies, we rely upon meta-analyses to improve statistical power. Recent meta-analysis of nine RCTs concluded that UDCA at any dose conferred no significant improvement in mortality, symptoms, cholangiocarcinoma and histological progression [Triantos *et al*. 2011]. Similarly, a Cochrane systematic review of eight RCTs found no significant reduction in the relative risk of death, treatment failure, liver transplant, varices, ascites or encephalopathy [Poropat *et al*. 2011]. Interestingly, a significant improvement in liver biochemistry was observed, the clinical significance of which is uncertain. Although meta-analyses are considered the highest class of evidence, the trials included were subject to high risk of publication bias. Variable dosage, treatment time course and follow up, and different primary endpoints in UDCA trials means that the role of UDCA is still uncertain; a point that is reflected in current international guidance, and the fact that many European countries no longer prescribe the drug [Chapman *et al*. 2010; Imam *et al*. 2011]. More recently, several studies have shown that patients with PSC, who normalize their serum ALP, whether this occurs spontaneously or more often with UDCA therapy, have a better prognosis. Despite this, recent guidelines from the American College of Gastroenterology recognize that many practitioners, particularly in the US and UK, are still prescribing UDCA at a dose of 15–20 mg/kg/day, but that data from well controlled trials are lacking [Lindor *et al*. 2015]. The only recommendation from these new guidelines is that UDCA of more than 28 mg/kg/day should not be used in the management of patients with PSC.

UDCA and chemoprotection

Current evidence demonstrates an increased incidence of right-sided colonic cancers in patients with PSC, perhaps caused by colonic exposure to secondary bile acids [Shetty *et al*. 1999]. *In vitro* and animal studies suggest that UDCA might act as a chemoprotective agent by modifying bile acid composition and reducing faecal levels of secondary bile acids [Rodrigues *et al*. 1995; Wali *et al*. 1995; Batta *et al*. 1998; Im and Martinez, 2004; Khare *et al*. 2008].

A phase III study of 1285 patients (without PSC) who had undergone removal of colorectal adenomas within the previous 6 months reported that low-dose UDCA (8–10 mg/kg/day) prevented adenoma recurrence [Alberts *et al*. 2005]. A cross-sectional study of 59 patients, and a retrospective analysis of 52 previously randomized patients with PSC IBD, reported a significantly decreased prevalence of colorectal dysplasia with UDCA: adjusted odds ratio (OR) 0.14 [95% confidence interval (CI) 0.03–0.64] ($p = 0.01$) [Tung *et al*. 2001]. However, a short follow-up period of 2 years, short 6-month exposure period, and low patient numbers limited the reliability of these conclusions.

Contrasting results were reported in a retrospective cohort study of patients with PSC IBD, comparing 28 cases treated with UDCA against 92 controls [Wolf *et al*. 2005]. Whilst UDCA appeared to confer a beneficial effect in decreasing mortality (adjusted relative risk for death 0.44; 95% CI 0.22–0.90), cumulative incidence of dysplasia or cancer was not significantly different between cases and controls ($p = 0.17$ by log-rank test). This was also suggested in a second long-term follow-up study of a previously randomized cohort of 98 patients with PSC IBD, treated with high-dose UDCA (17–23 mg/kg/day) or placebo. Frequency of colorectal dysplasia was similar in treatment and control groups, with no difference in cancer-free survival [Lindstrom *et al*. 2012]. Moreover, in a retrospective analysis of data from an RCT including 56 patients with PSC IBD treated with high-dose UDCA (28–30 mg/kg/day) and followed up for 235 patient-years, long-term intake of UDCA was surprisingly associated with an increased risk of colorectal neoplasia (hazard ratio 4.44; 95% CI 1.30–20.10; $p = 0.02$) [Eaton *et al*. 2011]. Conversely a recent meta-analysis of 763 patients with PSC IBD concluded that UDCA may reduce the risk of advanced colorectal neoplasia (OR 0.35; 95% CI 0.17–0.73), or all colorectal neoplasia at doses of 8–15 mg/kg/day (OR 0.19; 95% CI 0.08–0.49) [Singh *et al*. 2013]. However, the results of this meta-analysis should be treated

with caution, as several follow-up studies were included and no adjustment made for patients who switched between treatment groups. Furthermore, the observations with low-dose UDCA were based upon only two early studies of 70 cases and 41 controls.

There is even further limiting evidence for UDCA as a chemoprotectant for cholangiocarcinoma. A Scandinavian study of 255 patients with PSC awaiting liver transplantation over an 11-year period reported that lack of treatment with UDCA was an independent risk factor for development of hepatobiliary malignancy [Brandsaeter *et al.* 2004]. However, the two largest placebo-controlled RCTs observed no effect on rates of cholangiocarcinoma in patients with PSC, though they were not powered to detect a difference in rates of hepatobiliary malignancy [Olsson *et al.* 2005; Lindor *et al.* 2009].

Immunosuppressive-based therapies

Glucocorticoids

Glucocorticoids are the most commonly used drug in the treatment of immune-mediated conditions. Patients with PSC and autoimmune hepatitis overlap or high IgG4 levels benefit from corticosteroids [Gregorio *et al.* 2001; Boberg *et al.* 2003; Floreani *et al.* 2005; Mendes *et al.* 2006; Webster *et al.* 2009]. Surprisingly, there have been no published RCTs comparing oral corticosteroids with placebo in patients with PSC alone (See Table 2).

A cohort study of 21 patients treated with 9 mg of budesonide for 1 year showed an improvement in portal inflammation but no change in Mayo risk score [Angulo *et al.* 2000]. A significant decrease in serum ALP and Aspartate Transaminase (AST) compared with baseline was observed ($p = 0.001$), however this effect was lost 3 months post treatment cessation. Furthermore, significant loss of bone density prompted the authors to conclude that overall there was minimal benefit. A nonrandomized, placebo-controlled trial in 12 patients with PSC compared combined therapy with prednisolone 10 mg/day and colchicine 0.6 mg/twice daily with placebo, administered for 2 years [Lindor *et al.* 1991]. After 24 months, no significant difference in liver biochemistry or histology was detected, and only a nonsignificant trend towards less clinical deterioration. Furthermore bone density in the prednisolone group was significantly lower compared with the placebo group.

The only published meta-analysis of corticosteroids in PSC included just two trials [Giljaca *et al.* 2010]. The first, an unblinded trial of hydrocortisone administered *via* biliary lavage *versus* placebo in 11 randomized patients with PSC [Allison *et al.* 1986], and the second, an RCT comparing oral budesonide (3 and 9 mg/day) with oral prednisolone (10 mg/day) in 19 patients with PSC [Van Hoogstraten *et al.* 2000]. No effect on liver biochemistry, symptoms or mortality was observed. However, small sample sizes and the absence of power calculations or intention-to-treat analysis may have resulted in systematic error overestimating beneficial effects and underestimating harmful effects, such as increased rates of cholangitis and sepsis [Allison *et al.* 1986].

Azathioprine

Azathioprine is a steroid-sparing immunosuppressant and purine antimetabolite widely used for the maintenance of remission in IBD [Mowat *et al.* 2011]. However, studies of its efficacy in PSC have been limited. Azathioprine inhibits ribonucleotide synthesis and induces T-cell apoptosis by modulating Rac-1 cell signalling [Tiede *et al.* 2003]. Several cases of azathioprine use in PSC have been reported; two patients improved with treatment and one died of a liver abscess [Javett, 1971; Wagner, 1971]. A case series of 15 patients with PSC treated with combination azathioprine (1–1.5 mg/kg/day), prednisolone (1 mg/kg/day) and UDCA (500–750 mg/day) observed significant improvement in liver histology and biochemistry [Schramm *et al.* 1999]. Unfortunately the commonality of concomitant PSC IBD means that many patients with PSC are taking azathioprine at the time of PSC diagnosis and progression, which may explain the lack of enthusiasm for further evaluation of azathioprine. However, trials employing use of thiopurine metabolite measurements in PSC are also lacking.

Ciclosporin

Ciclosporin binds to cytosolic cyclophilin of T cells and inhibits calcineurin, subsequently inhibiting the transcription of IL-2, which whilst inhibiting T-cell response may also limit T-regulatory cell production. Following 24 months of treatment, ciclosporin prevented progression of liver histological change: 9 of 10 patients on placebo demonstrated histological progression compared with 11 of 20 patients on ciclosporin ($p < 0.05$) [Wiesner *et al.* 1991]. However, lack of effect

on symptoms, liver biochemistry or disease progression led to conclusions that cyclosporine was ineffective in the treatment of PSC. cyclosporine has also been further evaluated in a double-blind RCT of 35 patients with precirrhotic PSC with concomitant UC. Whilst patients with PSC UC experienced improvement in symptomatic bowel disease, the trial was primarily powered to establish an effect on UC and no difference in PSC-related endpoints were observed with this therapy [Sandborn *et al.* 1993].

Tacrolimus

A preliminary open-label trial of tacrolimus in 10 patients with PSC demonstrated a significant improvement of liver biochemistry [Van Thiel *et al.* 1995]. This effect was confirmed in an open-label, phase II study of 16 patients with PSC treated with tacrolimus (0.05 mg/kg/day). However, only 50% of patients completed 1 year of therapy and 31% withdrew from the trial due to drug-related adverse events. Moreover, inclusion of large numbers of patients with proctocolectomy may explain the greater frequency of gastrointestinal side effects. The study concluded that clinical benefit was limited and tacrolimus poorly tolerated in this patient group [Talwalkar *et al.* 2007]. Sirolimus and everolimus are inhibitors of mechanistic target of rapamycin (mTOR) and recent evidence shows these mTOR inhibitors improve liver fibrosis and reduce inflammation in bile duct ligated rats [Patsenker *et al.* 2011]. These could be potential therapeutic targets in cholangiocarcinoma and PSC [Herberger *et al.* 2007; Pignochino *et al.* 2010]. Further studies are therefore needed to evaluate these drugs.

Methotrexate

Methotrexate is a dihydrofolate reductase inhibitor that targets enzymes involved in purine metabolism, suppressing T-cell activation and adhesion molecule expression, thus conveying anti-inflammatory properties [Johnston *et al.* 2005]. A preliminary trial of 0.2 mg/kg/week oral methotrexate demonstrated a statistically significant improvement in liver biochemistry [Knox and Kaplan, 1991]. Six of none (66%) showed histological improvement at 1 year and three of six (50%) who underwent repeat cholangiograms showed improvement. In contrast, a prospective, placebo-controlled RCT of oral methotrexate in 24 patients with PSC demonstrated no change in liver histology, cholangiographic findings or liver biochemistry following 2 years of treatment [Knox and Kaplan, 1994]. However, it should be noted that 58% of patients in the treatment group had established cirrhosis compared with only 42% on placebo, which could explain the lack of observed efficacy with methotrexate in this trial [Lindor *et al.* 1996].

Mycophenolate

Mycophenolate mofetil (MMF) is a potent immunosuppressant that has largely replaced azathioprine as a second-line agent in solid-organ transplantation. MMF attenuates B- and T-lymphocyte proliferation by inhibiting *de novo* purine synthesis [Allison and Eugui, 1993; Eugui and Allison, 1993; Fulton and Markham, 1996]. A pilot study of 1–3 g MMF in 30 patients with PSC aimed to determine safety and efficacy; 77% completed 1 year of treatment, with 33% experiencing adverse reactions that resolved with dose reduction [Talwalkar *et al.* 2005]. A significant, but clinically marginal reduction in serum ALP was observed, and the pilot study did not support the sole use of MMF in PSC. These results were corroborated by a 2-year RCT of combined MMF (1 g/twice daily) and UDCA (13–15 mg/kg/day) (n = 12) *versus* UDCA alone (n = 13). Small sample size, open label and high dropout rate could have led to type II error, however results from this trial were not supportive of combination therapy with MMF in PSC [Sterling *et al.* 2004].

Antibiotics

The potential role of antibiotics in PSC therapy was initially derived from experimental evidence in rat models of small intestinal bacterial overgrowth, leading to biliary strictures and portal inflammation [Lichtman *et al.* 1990]. Importantly, patients with advanced PSC suffer repeated episodes of bacterial cholangitis, which may fuel disease progression [Broome *et al.* 1996]. Studies of bile fluid obtained at Endoscopic Retrograde Cholangiopancreatography (ERCP) and from explanted livers show a wide range of bacteria and fungi in both patients with multiple biliary interventions and 25% of patients who are ERCP naïve [Olsson *et al.* 1998; Bjornsson *et al.* 2000; Negm *et al.* 2010]. Current guidelines thus advocate the use of prophylactic antibiotics in patients with recurrent bacterial cholangitis and those undergoing biliary intervention [Chapman *et al.* 2010].

There have been a few small trials of antibiotics in PSC. A 1-year pilot study of minocycline, with

antiapoptotic and anti-inflammatory properties, in 16 patients with PSC observed a significant improvement in ALP ($p < 0.05$), however improvements in Mayo risk score were not statistically significant [Silveira *et al.* 2009]. The largest antibiotic trial including 80 patients with PSC evaluated the efficacy of metronidazole and UDCA *versus* UDCA alone in a 3-year RCT. A significant decrease in serum ALP and Mayo risk score was observed, however improvements in liver histology and cholangiographic findings were statistically insignificant [Farkkila *et al.* 2004]. Long-term treatment with oral vancomycin in 14 children with PSC IBD significantly improved liver biochemistry, inflammatory markers and symptoms, especially in the absence of cirrhosis [Cox and Cox, 1998; Davies *et al.* 2008]. More recently, a small RCT of 35 patients with PSC randomized to receive vancomycin 125 or 250 mg four times per day or metronidazole 250 or 500 mg three times per day for 12 weeks demonstrated some efficacy [Tabibian *et al.* 2013a]. The primary endpoint of ALP normalization was achieved in both the low- and high-dose vancomycin groups. Mayo risk score significantly decreased in both the low-dose vancomycin and metronidazole groups and pruritus significantly decreased in the high-dose metronidazole groups. This promising data have prompted a larger clinical trial to determine the efficacy of vancomycin in improving liver biochemistry in PSC, which is currently ongoing [ClinicalTrials.gov identifier: NCT01802073]. Therefore, antibiotic therapy appears promising, especially given mounting evidence for the role of the intestinal microbiome in PSC [Tabibian *et al.* 2013b], yet the concern of evolving resistance remains a real concern for clinicians. Future therapies involving faecal and bile transplantation which can alter the microbiome may be an important consideration in PSC.

Other treatments

Periductal fibrosis is a characteristic histopathological hallmark of PSC, however trials of antifibrotic agents including colchicine and pirfenidone have failed to demonstrate efficacy [Olsson *et al.* 1995; Angulo *et al.* 2002]. Fibrates are agonists at the nuclear peroxisome proliferator activated receptor α, thus decreasing IL-1 induced C-reactive protein expression. However, a case series of seven patients with PSC treated with bezafibrate for 6 months observed no effect on symptoms, progression or survival [Mizuno *et al.* 2010]. Other potential antifibrogenic agents include candesartan, an angiotensin II receptor blocker that attenuates liver fibrosis in rats [Ueki *et al.* 2006] and propranolol, a β-adrenoceptor antagonist [Strack *et al.* 2011]. No data are yet available for these agents.

Rats with small bowel overgrowth develop hepatobiliary injury from peptidoglycan polysaccharide mediated activation of Kupffer cells and release of proinflammatory cytokines such as TNFα, which is inhibited by xanthine-derived phosphodiesterase inhibitor, pentoxyfylline [Kucuktulu *et al.* 2007]. A pilot study of pentoxyfylline in 20 patients with variable stages of PSC showed no improvement in liver biochemistry, serum TNFα or TNF-receptor subtype following 1 year of treatment [Bharucha *et al.* 2000]. Cellular proliferation and liver-derived lymphocyte function are impaired in patients with PSC, perhaps a result of exposure to high levels of TNFα *in vivo* [Spengler *et al.* 1992; Bo *et al.* 2001]. A double-blind, placebo-controlled RCT of anti-TNFα antibody, infliximab, in 24 patients with PSC was halted following interim analysis demonstrating no difference in liver biochemistry or histology after 4–6 months [Hommes *et al.* 2008]. Furthermore, a pilot study of etanercept in 10 patients demonstrated no change in liver biochemistry or stricture formation, however two of five patients with symptomatic pruritus experienced resolution of pruritus during treatment, which returned on cessation of therapy, and resolved on reintroduction [Epstein and Kaplan, 2004].

Future treatments

A current priority in PSC research is the identification of short-term biomarkers for disease outcome. It is hoped that the identification of disease biomarkers will pave the way for the development and trial of new treatments in PSC (See Table 3).

CCR9 is a chemokine receptor, expressed on most lamina propria and intraepithelial T cells of the small intestine, with up to 25% of T cells on the large bowel positive for CCR9 [Zaballos *et al.* 1999; Norment *et al.* 2000]. CCR9 binds CCL25, causing activation of α4β7 T cells, thereby binding to MadCAM-1 in the bowel endothelium resulting in homing of T cells to the bowel in the healthy state. In IBD this process is enhanced and blocking of MAdCAM-1 and binding to α4β7 T cells by the VAP-1 blocker BTT1023 or vedolizumab alters the recruitment of pathogenic T cells. Furthermore vedolizumab

is now licensed for the treatment of IBD, particularly UC [Jin *et al.* 2015]. Aberrant expression of CCR9 and its ligand CCL25 in the liver of patients with PSC, but not healthy liver or liver disease controls, has been demonstrated [Eksteen *et al.* 2004]. Therefore, targeted blockade of this pathway using vedolizumab is a natural therapeutic development in PSC, a trial of which is currently ongoing [ClinicalTrials.gov identifier: NCT02239211]. CCX282-B is also a selective antagonist of CCR9 with some therapeutic efficacy in CD, which may inhibit B- and T-cell entry to the liver, and may also be therapeutically important [Keshav *et al.* 2013].

Matrix enzyme, lysyl-oxidase 2 (LOXL2), is implicated in nonorgan-specific pathological fibrogenesis by promoting cross-linking of type I collagen [Barry-Hamilton *et al.* 2010]. GS-6624 is a humanized monoclonal antibody with an immunoglobulin IgG4 isotype directed against human LOXL2. Pilot studies of its safety and tolerability in 10 patients with liver fibrosis of variable aetiology experienced no serious adverse effects, with a decline in serum Alanine Transaminase (ALT) [Talal *et al.* 2012]. Such agents may prove efficacious in attenuating the fibrosis of PSC and is the basis for a current phase II trial [ClinicalTrials.gov identifier: NCT01672853].

All-trans retinoic acid (ATRA) is a ligand for nuclear receptors involved in modulation of bile salt homeostasis [Cai *et al.* 2010]. ATRA possesses immunomodulatory effects through inhibition of proinflammatory cytokines [Montrone *et al.* 2009], and is currently used in the treatment of acute promyelocytic leukaemia, rheumatoid arthritis and psoriasis [Reichrath *et al.* 2007]. In some environments, ATRA can also induce T cells to become CCR9 positive [Eksteen *et al.* 2009]. In bile-duct ligated rats, treatment with UDCA and ATRA significantly reduces liver fibrosis, bile duct proliferation, liver necrosis and bile salt pool size compared with ATRA or UDCA alone [He *et al.* 2011]. However, this observation has not yet been verified in humans and phase II trials of ATRA and UDCA are now underway in PSC [ClinicalTrials.gov identifier: NCT01456468].

Manipulation of the UDCA molecule by shortening a side chain by one methylene group produces 24-norUDCA (norUDCA), a C_{23} homologue of UDCA. High-dose UDCA in patients with PSC leads to increased rates of adverse outcomes, especially in patients with normal serum bilirubin or early histological-stage disease [Imam *et al.* 2011]. *Post hoc* analysis of serum bile acid composition in 56 patients included in the aforementioned trial observed markedly increased levels of hepatotoxic bile acid: lithocholic acid (LCA) in patients on high-dose UDCA [Sinakos *et al.* 2010]. In rabbits, accumulation of toxic LCA following ingestion of UDCA causes inflammation of hepatic portal tracts and bile duct proliferation [Cohen *et al.* 1986]. In contrast to UDCA, norUDCA is secreted into bile in an unconjugated, glucoronidated form [Hofmann *et al.* 2005]. Its metabolite, nor-lithocholate, does not accumulate in hepatocytes or cause hepatotoxicity in animal models [Cohen *et al.* 1986]. Moreover, norUDCA administered to Mdr2–/– mice increases hydrophilicity of bile and stimulates canalicular flow [Fickert *et al.* 2006]. It remains unclear whether these promising results will translate to human studies, however phase II trials in humans are now also underway [ClinicalTrials.gov identifier: NCT01755507].

Activating protective pathways in hepatocyte cholestasis may limit hepatic damage in a cholestatic disorder. One of the master regulators of this is FXR. The natural ligand for FXR are bile salts and one of the key roles of FXR is down-regulating cytochrome P450 7A1, a rate-limiting enzyme in bile salt production. Obeticholic acid, an FXR agonist with encouraging results in PBC phase II studies, may also prove efficacious in PSC with a clinical trial ongoing [ClinicalTrials.gov identifier: NCT02177136]. Furthermore inhibition of the Apical Sodium Dependent Bile Acid Transporter (ASBT) in the terminal ileum may also reduce the enterohepatic circulation of bile salts, which could bring therapeutic benefit in PSC. Once again, a phase II study of an ASBT inhibitor, LUM001, is ongoing [ClinicalTrials.gov identifier: NCT02061540].

Conclusion

Over the past two decades many clinical trials of medical therapy in PSC have been conducted; however, none have demonstrated real improvements in hard clinical endpoints. This reflects our lack of understanding of disease heterogeneity, basic mechanisms of disease pathogenesis and perhaps a lack of robust biomarkers to act as early disease endpoints in clinical trial design. Several potential therapeutic agents have been widely accepted as ineffective despite inadequate trial data to support these

conclusions. A renewed approach to evaluation of such agents is thus justified. Future trials in PSC are speculated to focus on two areas: the search for a biologically plausible signal of drug efficacy (e.g. fibrosis markers, MRI changes, histological changes) and trials of therapeutic agents in high-risk individuals who have yet to reach end-stage disease, but whose disease is advanced enough to provide a true signal of drug efficacy. With GWAS highlighting new potential pathogenic mechanisms, the development of national collaborative disease consortia and new antifibrotic agents, the 21st century should hold new excitement for the treatment of PSC.

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