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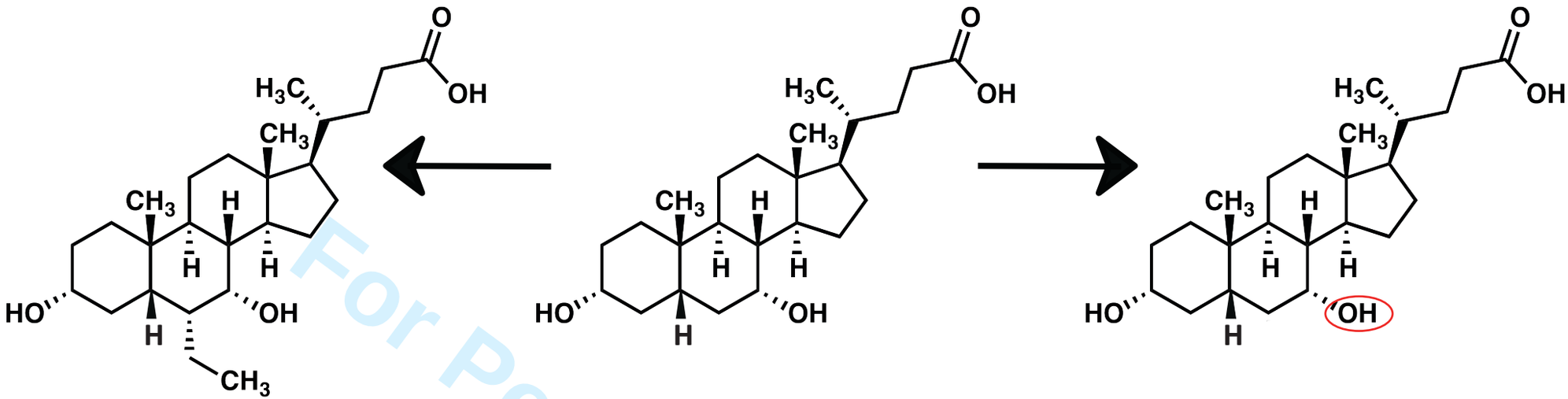
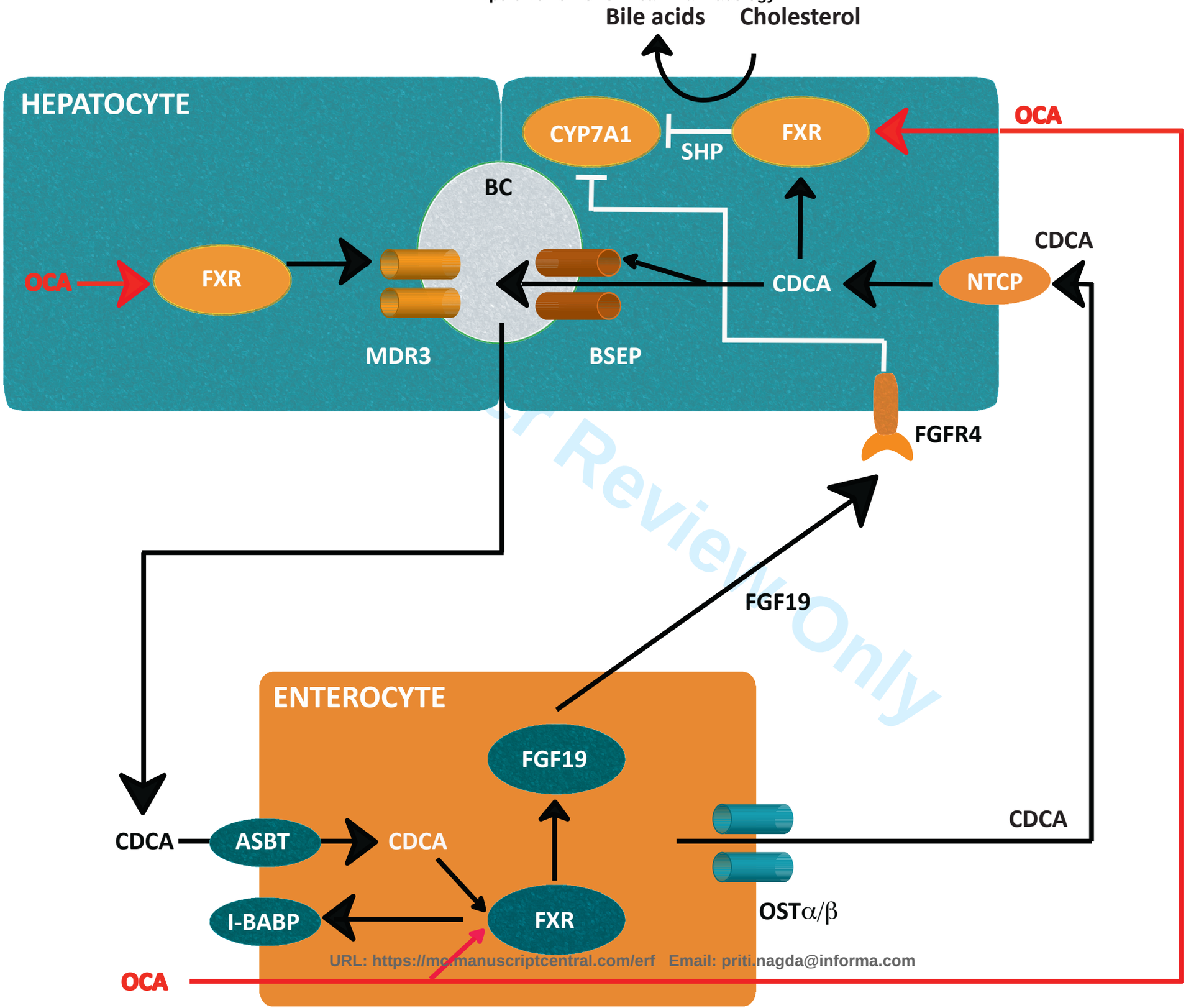
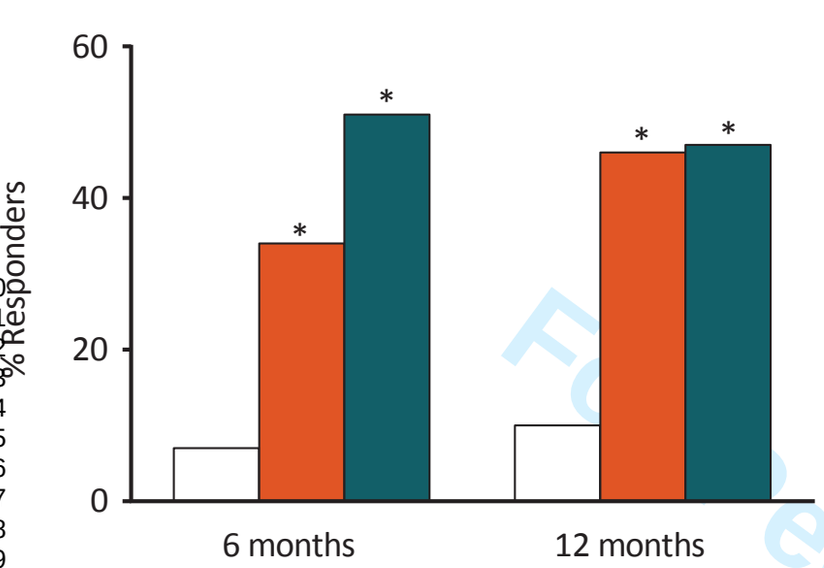
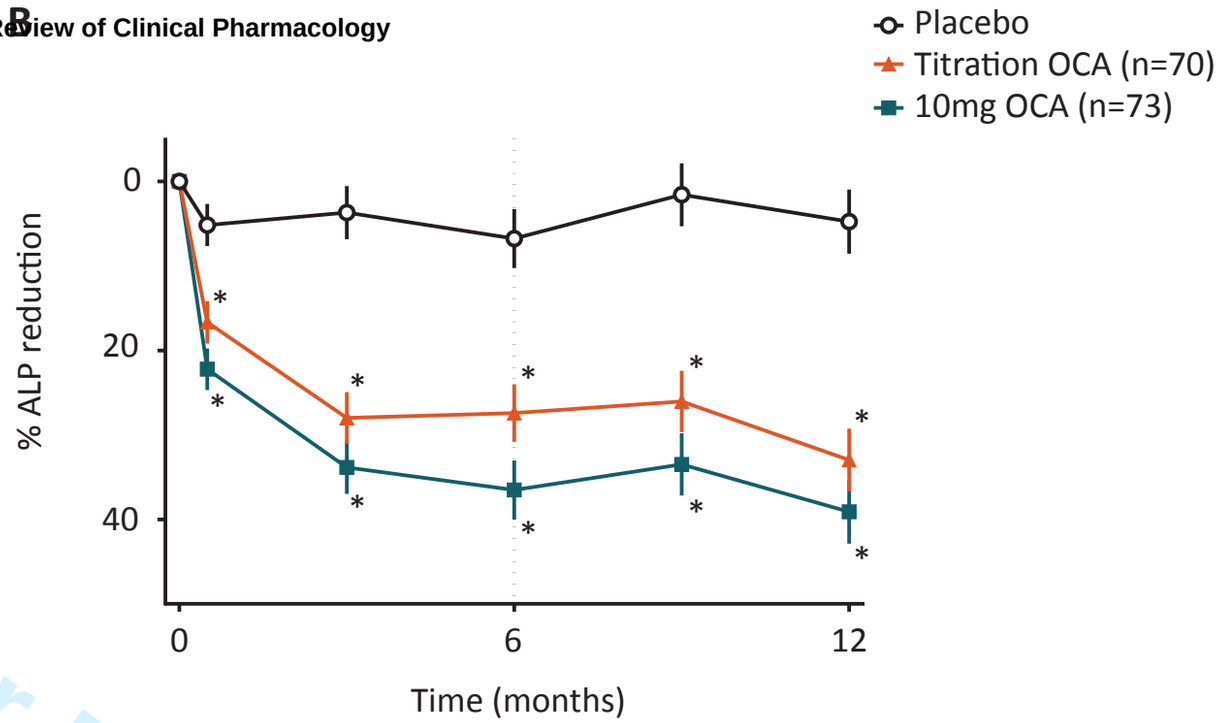
Stereoisomers

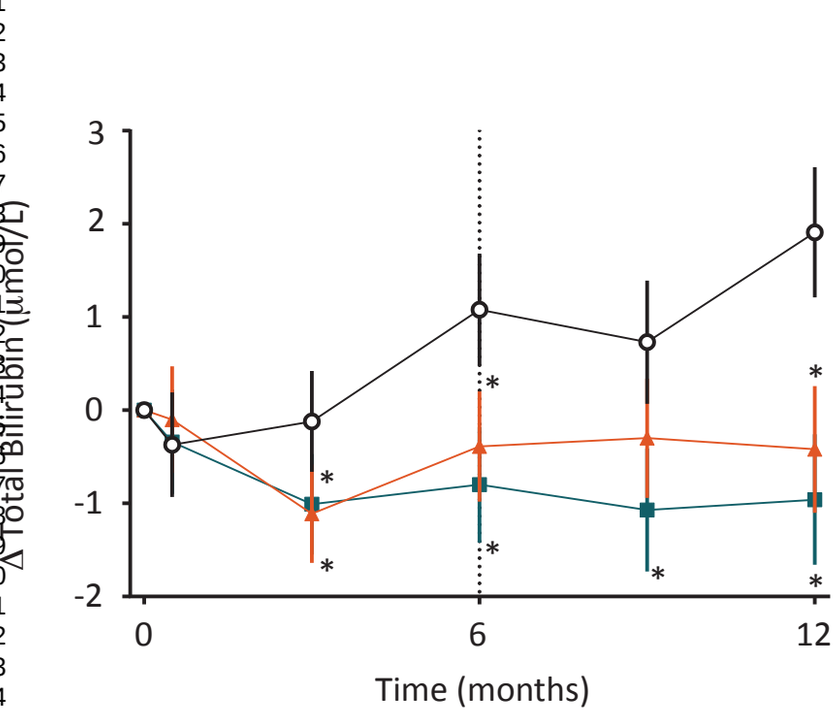


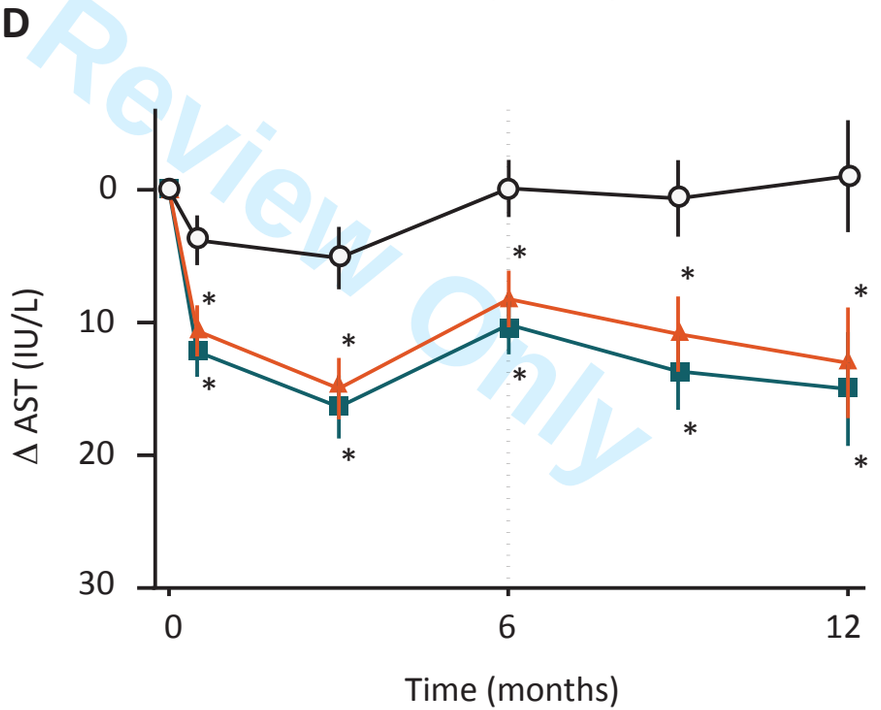
Agent	Obeticholic acid	Chenodeoxycholic acid	Ursodeoxycholic acid
Potency (FXR EC_{50})	0.09μM	8.6μM	No activity

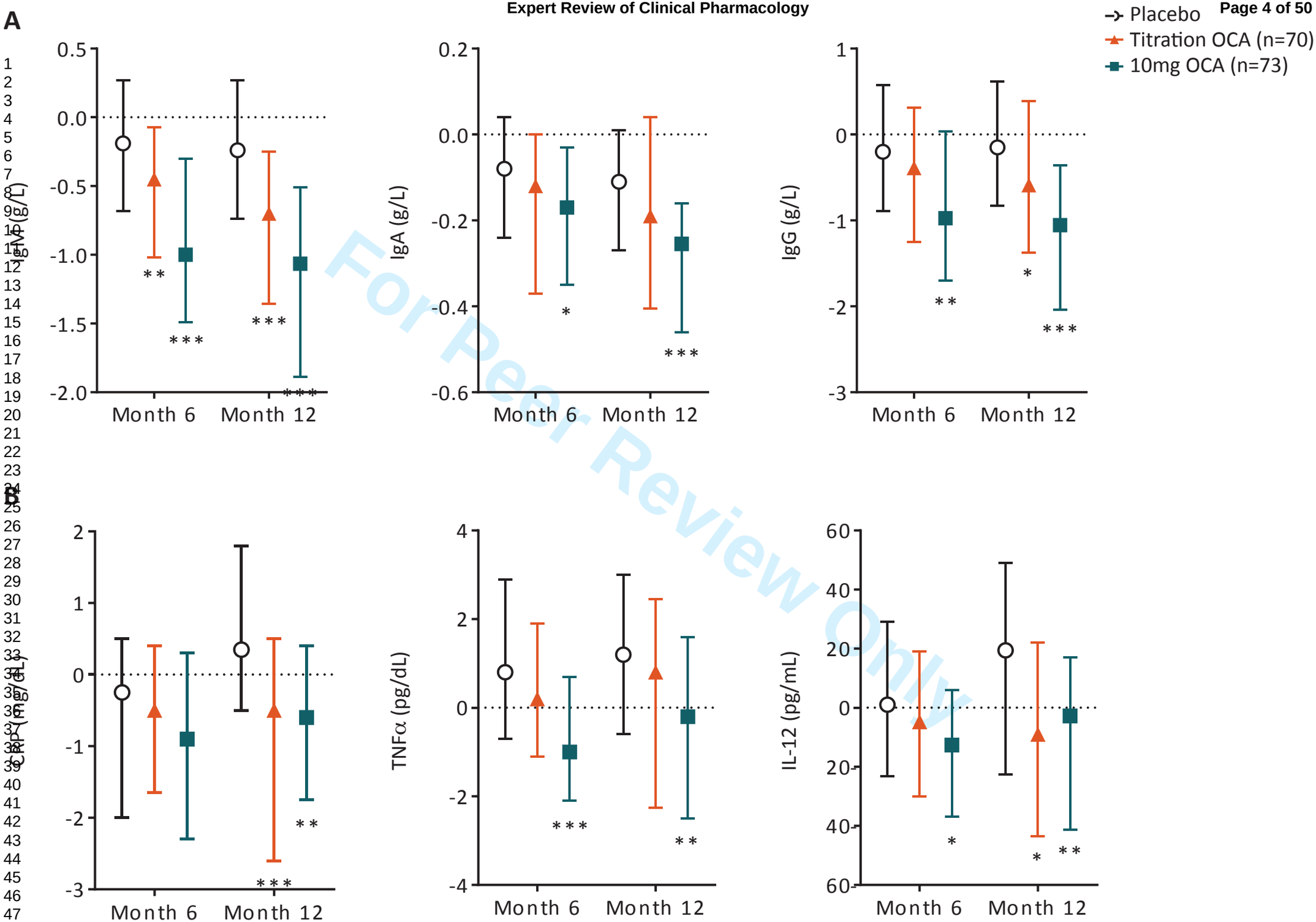


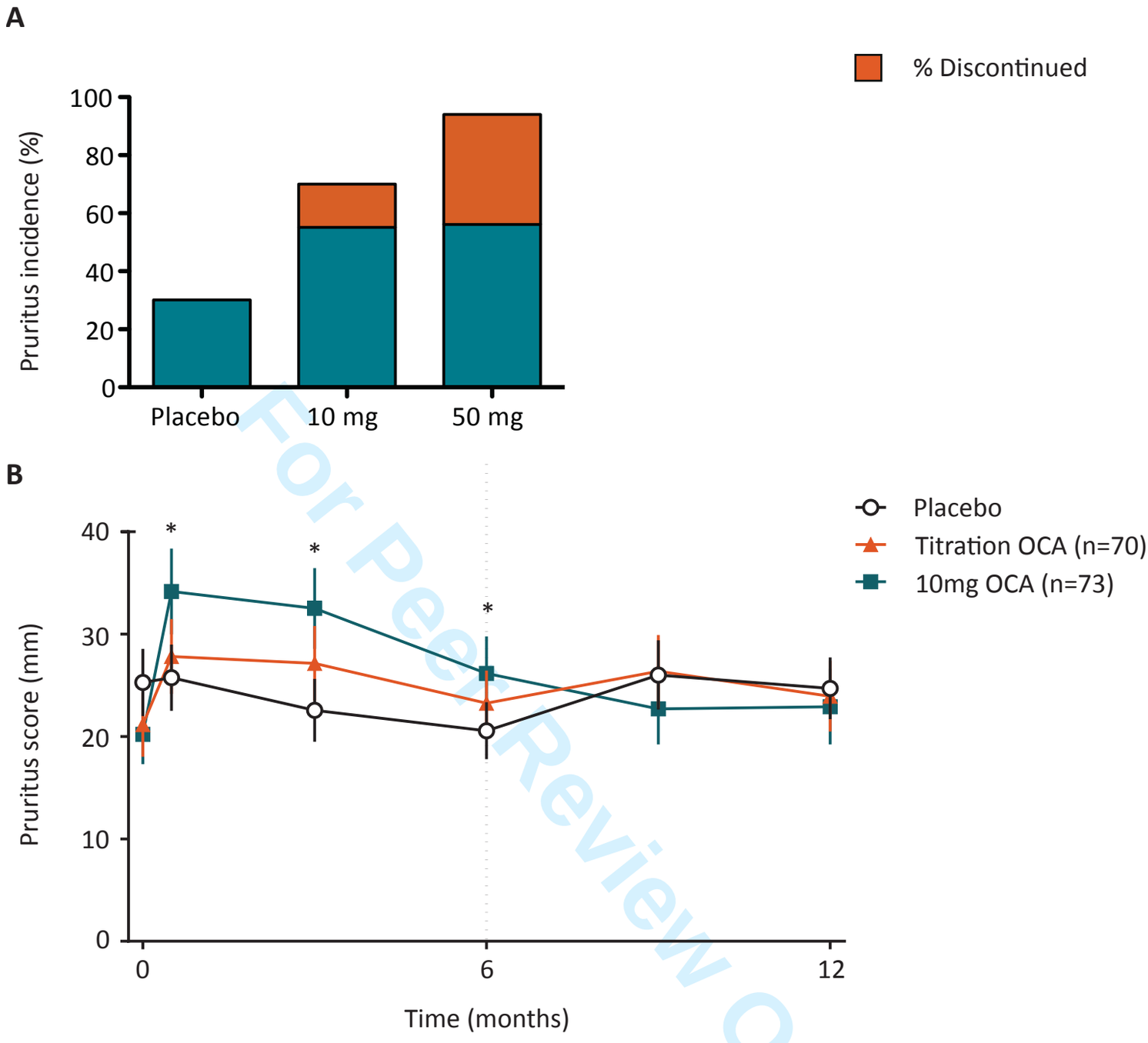












Expert Review of Clinical Pharmacology

Drug profile

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Obeticholic Acid for the Treatment of Primary Biliary Cirrhosis

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ABSTRACT

Primary biliary cirrhosis (PBC) is characterized by progressive non-suppurative destruction of small bile ducts resulting in intrahepatic cholestasis, fibrosis and ultimately end-stage liver disease. Timely intervention with ursodeoxycholic acid is associated with excellent survival; although approximately one-third of all patients fail to achieve biochemical response, signifying a critical need for additional therapeutic strategies. Obeticholic acid (OCA) is a potent ligand of the nuclear hormone receptor farnesoid X receptor (FXR). Activation of FXR inhibits bile acid synthesis and protects against toxic accumulation in models of cholestasis and facilitates hepatic regeneration in pre-clinical studies. Data from recent phase II and phase III controlled trials suggests a therapeutic impact of OCA in PBC biochemical non-responders, as evidenced by change in proven laboratory surrogates of long-term outcome. Dose-dependent pruritus is a common adverse effect, but may be overcome through dose-titration. Longer-term studies are needed with focus on safety and long-term clinical efficacy.

Introduction

Primary biliary cirrhosis (PBC) is an immune-mediated hepatobiliary disorder characterised by ductopenia, chronic cholestasis and progressive liver fibrosis [1–4]. Although there are multiple animal models proposed, often with similar immunological abnormalities as patients with PBC, therapeutic efforts have thus far been based entirely on human clinical trials [5–9]. Presently, the mainstay of treatment is ursodeoxycholic acid (UDCA), which in addition to stimulating hepatobiliary secretions is a potent intracellular signaling molecule, inducing choleresis through mitogen-activated protein kinase (MAPK) and integrin-dependent mechanisms [10]; as well as protecting epithelia from 'toxic' effects of low pH bile acids [11]. Survival advantage is demonstrated best for UDCA-treated patients with early-stage PBC receiving timely and appropriately dosed therapy (13 – 15 mg/kg/d) [12]; specifically those attaining well-defined biochemical response criteria in whom survival parallels that of an age and sex-matched control population [13–20]. However, therapeutic failure is evident in up to 40% of PBC patients [13,14], with biochemical non-response independently associated with disease progression, liver transplantation (LT) and death, highlighting a critical need for alternative / adjuvant pharmaco-intervention.

Farnesoid X receptor (FXR) is a nuclear 'ligand-activated' receptor abundantly expressed in tissues that engage in the enterohepatic circulation of bile acids. However, unlike UDCA which operates at a post-translational level, FXR-signalling directly regulates genes involved in bile acid synthesis, secretion, transportation, absorption and detoxification [21]. Chenodeoxycholic acid (CDCA) and its semi-synthetic analogue obeticholic acid (OCA) are selective ligands for FXR, with the latter illustrating exponential activation potency relative to its endogenous counterpart (Figure 1) [22]. OCA also induces expression of fibroblast growth factor (FGF)-19,

which could plausibly explain the puissant anti-inflammatory and portal hypotensive effects observed in several experimental models [23–25]. Moreover, the clinical impact of FXR-agonism has recently been explored through phase II and phase III trials of OCA in PBC, which demonstrate significant improvement in validated biochemical surrogates of disease outcome [26]. Herein, we present a synthesis of the available experimental and clinical evidence that have driven emergence of OCA as a viable therapeutic option for PBC patients.

Natural history of PBC

Primary biliary cirrhosis is the most common of all autoimmune hepatobiliary diseases, with an estimated prevalence of 1 per-1000 in women over the age of 40 [27,28]. Diagnosis is reliant upon features of chronic biochemical cholestasis, circulating disease-specific anti-mitochondrial antibodies (AMA; present in >90% of patients), and/or or characteristic biopsy findings of destructive non-suppurative granulomatous/lymphocytic cholangitis [29]. Pruritus and fatigue represent the archetypal symptoms in chronic cholestasis and need not necessarily relate to liver disease severity, nor abate with UDCA therapy [30]. Although non-specific, both significantly impact quality of life and represent a significant unmet need [31].

The classical ‘textbook’ presentation of PBC implies disease restriction to middle-aged, often post-menopausal women with a median survival of approximately 9 – 10 years from presentation [32,33]. Advanced histological stage is a clear determinant of poor clinical outcome and in an era where liver biopsy was the norm and effective therapy lacking, the median time to develop extensive fibrosis was ~2 years and probability of remaining in early stage disease <30% over a 4-year period [34,35]. However, the advent of high-accuracy AMA testing largely obviates

routine, diagnostic histological assessment [27,36], and PBC is increasingly identified at an earlier, pre-cirrhotic stage with progressive recognition of male patients and young women of child-bearing age [14,37–39]. Approximately 60% of patients with PBC are asymptomatic at time of diagnosis, however as little as 5% remain symptom-free over time [33]. Furthermore, it is now apparent that presenting age and gender influence the presenting phenotype, with young women – a group who fail UDCA therapy more commonly – having the greatest symptom burden.

Ursodeoxycholic acid is currently the only approved drug for treating PBC, demonstrating consistent evidence in improving liver biochemistry [40,41] and a lower progression rate from early stage disease to extensive fibrosis/cirrhosis compared with placebo in clinical trials (7% vs. 35% per-year; $p<0.01$) [42]. Observed 10-year transplant-free survival indices are significantly better in UDCA-treated patients (78% vs. 59%; $p<0.001$ [38]) however remain lower than age- and sex-matched control populations in the current era [13,14,43].

On-treatment liver biochemical changes are variable in PBC, with some patients experiencing complete normalisation and others only minor improvements. Serum bilirubin is well-established as an independent predictor of prognosis, regardless of treatment, although is limited to prediction of short-term survival (<2 years) during relatively late-stage disease [44]. A relationship between ALP and risk of adverse outcomes has been extensively documented across several studies; however, it was only recently that a systematic, international multi-centre study validated worth as a biochemical surrogate by demonstrating a near log-linear relationship between serum ALP and risk of transplantation/death across several time points [38]. Serum

ALP bestows incremental prognostic information to bilirubin alone; an association independent of patient follow-up time that holds true irrespective of presenting age, sex, disease stage and treatment status. To this effect, several studies illustrate a strong association between normalisation, percentage reductions or absolute decreases in serum ALP (either in isolation, or in association with other biochemical covariates) and improved transplant-free survival on UDCA-therapy [13–15,17]. Conversely, biochemical 'non-responders' exhibiting persistent elevations in serum ALP represent the current target population for evaluation of new therapies in PBC.

PBC as a cholestatic disease

Understandings of PBC disease pathophysiology are incomplete; with initiation of immune-mediated small bile duct cholangitis likely reflecting strong genetic risk coupled with environmental induction [2,45–47]. Ductopenia is propagated by a multi-lineage humoral and cellular adaptive response, but may also include innate immune reactions against cholangiocytes. The autoimmune nature of PBC is characterised by a distinct loss of tolerance to a series of mitochondrial autoantigens and integrally related to bile duct loss, which progresses through an iterative inflammatory process from lymphocytic cholangitis to progressive ductopenia, associated cholestasis and eventually biliary fibrosis [2]. OCA is an agent that predominantly has an impact on cholestasis; hence immune aspects of PBC are not discussed further.

An overview of bile secretion

Conjugated bile acids are the major component of bile, and transported across the basolateral hepatocyte membrane by the sodium taurocholate co-transporting polypeptide (NTCP; or solute carrier family 10 member 1, SLC10A1); whereas unconjugated bile acids and a large variety of other organic anions (including bilirubin) are taken up from the circulation via a series of organic anion transporters (OATP) [48]. The canalicular secretion of bile acids is facilitated by a number of adenosine triphosphate binding cassette (ABC)-transporters, with passage specifically dependent upon the bile salt export pump (BSEP; or ABC11). A further ABC transporter, the multi-drug resistance 3 P-glycoprotein (MDR3; or ABCB4) is also required for biliary secretion of phosphatidylcholine which facilitates packaging of bile as mixed micelles, therein protecting biliary epithelium from oxidative injury [48]. In addition, cholangiocytes secrete an alkaline-rich fluid, with the cystic fibrosis transmembrane conductance regulator (CFTR; or ABCC7) responsible for the efflux of chloride that is subsequently exchanged against bicarbonate via the anion exchanger 2 (AE2; SLC4A2). The resultant 'bicarbonate umbrella' serves to maintain an alkaline pH near the apical surface of hepatocytes and cholangiocytes to prevent uncontrolled membrane permeation by protonated, glycine-conjugated bile acids [49]. This protective mechanism is also reliant on an intact biliary glycocalyx on human cholangiocytes, and dysregulation or functional impairment of the biliary bicarbonate umbrella may lead to enhanced vulnerability of cholangiocytes and peri-portal hepatocytes towards hydrophobic bile acids.

The basolateral hepatocyte membrane possesses a number of additional transporters that are normally expressed at very low levels, but compensatory up-regulated in cholestasis. These include multidrug resistance-associated protein 4 (MRP4; or ABCC4) which transports bile acids

together with glutathione, MRP3 (or ABCC3) which transports conjugated bilirubin and other organic anions, and the organic solute transporter (OSTα/OSTβ) that transports bile acids and sterols [50]. Reduced immunostaining of the chloride-bicarbonate anion exchanger AE2 and other ion-exchange pumps have been demonstrated in the PBC liver [51], which fail to respond to adenosine triphosphate stimulation with secretin; and their ability to dilute and alkalinize canalicular bile upon stimulation lost in untreated disease, but restored in patients receiving UDCA [52]. Conjugates of UDCA have also been shown to activate vesicular exocytosis and carrier insertion into the apical membrane of hepatocytes and cholangiocytes, resulting in additional choleresis via MAPK- and α5β1 integrin-dependent mechanisms. Anti-cholestatic effects of UDCA are predominantly dependent on Ca^{2+}-dependent signalling (inositol-triphosphate receptor and protein kinase), with well-known anti-apoptotic functions conferring additional hepatoprotective and cholangioprotective benefit [53,54].

The expression of certain other hepatocellular transporter genes may also vary depending on disease stage [55]. Early reports indicate activity of hepatic cholesterol 7 alpha-hydroxylase (CYP7A)-1 – the rate-limiting enzyme involved in bile acid synthesis – as significantly increased in early PBC, but reduced to levels 10 – 20 % of that found in normal controls as the condition progresses [55,56]. Down-regulation of OATP2 and increased mRNA expression of NTCP and BSEP have also been reported in late-stage disease and likely represent protective mechanisms that have evolved to prevent accumulation of toxic bile acids. Nuclear hormone receptors regulate such adaptive responses, and include the pregnane X receptor (PXR), the constitutive androstane receptor (CAR) and the peroxisome-proliferator-activator receptor α (PPARα) [21], with FXR representing the key sensor involved in bile acid feedback regulation [57]. In contrast

to hydrophobic bile acids such as CDCA or lithocholic acid (LCA), UDCA does not markedly affect transport protein expression at the transcriptional level, and has limited effect on post-transcriptional carrier modification [58].

The farnesoid X receptor

FXR is predominantly expressed in the liver and small intestine, with highest levels observed at the terminal ileum. Upon ligand binding within the liver, FXR suppresses transcription of CYP7A1 via the target gene short heterodimer protein (SHP); as well as promoting canalicular bile salt secretion through up-regulation of BSEP and MDR3 and alternative routes of bile acid elimination such as OSTα/OSTβ [59,60]. In addition, ileal FXR ligation up-regulates expression of FGF19 that upon entry into the portal circulation binds to the receptor FGFR4 on hepatocytes. This in turn activates a series of MAP-kinases which further suppress CYP7A1 expression synergistically with SHP [61], as well as exerting potent pro-glycogenic and anti-lipogeneic effects (Table 1) (Figure 2). These findings have been successfully translated into clinical trials for non-alcoholic fatty liver disease (NAFLD) [62,63].

Although FXR response elements exist in the proximal region of the human *MDR3* promoter (*MDR3* mRNA levels increasing following FXR ligation [64]), *Fxr* knockout mice still respond to a cholic acid rich diet with an increase in transcription of *Mdr2* (the murine orthologue of MDR3 [65]). Indeed, activation of the nuclear hormone receptor PPARα by its synthetic ligand Fenofibrate enhances MDR3 mRNA and protein expression on primary human hepatocytes to a greater degree than CDCA, although a synergistic effect is not observed [66]. Moreover, ligand activated PPAR can attenuate bile acid synthesis (through direct inhibition of CYP7A1 and

CYP27A1), regulate bile acid detoxification (increased hepatic glucoronidation activity) and stimulate biliary excretion of phosphatidylcholine (enhanced MDR3 expression) [67]; suggesting that FXR-mediated effects may also be exerted indirectly via PPARα activation.

Pre-clinical models targeting FXR

The critical role of FXR in curbing BA synthesis can be illustrated in *Fxr* knockout mice, which exhibit an increased bile acid pool size and phenotypic features akin to the human condition progressive familial intrahepatic cholestasis [65,68]. These models illustrate a prominent role for intestinal FGF15 signalling (murine orthologue of FGF19) over SHP in the repression of CYP7A1 activity [69]. By contrast, constitutive FXR activation in the intestine has been shown to protect against chemical and obstructive cholestasis in at least two murine models [70]. Further anti-cholestatic effects are evident following systemic pharmacologic activation of FXR in rats, which exhibit decreased expression of bile acid biosynthetic genes and up-regulation of those involved in bile acid transport [71]. More incipient studies have suggested that effective bile acid signalling plays a critical role in liver regeneration [72]; and in an elegant series of experiments using humanised mouse livers, Naugler et al. illustrated that FGF19-signalling was able to increase hepatocyte proliferative responses in a murine model of PBC [24]. Of further interest was the observation that FGF19 transcription although virtually absent from normal healthy liver in man, was readily detected in sorted cholangiocytes from PBC liver, suggesting the existence of more local regulatory networks in chronic cholestasis.

Liver natural killer T-cells may also express functional FXR, and regulatory functions are suggested by mouse models in which congenital *Fxr* deletion confers enhanced susceptibility to

T-cell-mediated hepatitis (an effect likely mediated through NFκB-signalling), increased expression of pro-inflammatory cytokines and oncogenes, and spontaneous development of hepatic tumours [73–76].

Pre-clinical data also indicates that FXR is expressed by rat hepatic stellate cells (HSC) which are the precursors of hepatic myofibroblasts (MFB) – key drivers of hepatic fibrogenesis [77–79]. Indeed, FXR activation was associated with reduced secretion of extra-cellular matrix (ECM) as well as prevention and resolution of liver fibrosis in carbon-tetrachloride (CCl_4) and bile-duct ligated (BDL) rats [77]. These findings have not, however, been recapitulated in all murine studies; and Fickert *et al*. report cell-type specific expression of FXR as relatively low in mouse as well as human HSC and hepatic MFB compared to that observed in whole liver [80]. Nevertheless, *Fxr* loss significantly attenuated liver fibrosis specifically in models of biliary (BDL, or dietary 3,5-diethoxycarbonyl-1,4-dihydrocollidine application), but not parenchymal liver disease (*Schistosoma mansoni* infection, or CCl_4).

FXR agonism has also been shown to reduce portal hypertension *in vivo* through a direct action on endothelial nitric oxide synthase (eNOS) activation, inhibition of endothelin-1 mediated HSC contraction, and consequently a reduction in intra-hepatic vascular resistance [25,81]. This observation is of particular relevance in the risk reduction profile of PBC, given the negative prognostic implications of portal hypertensive disease on clinical outcomes [18,82,83].

Obeticholic acid – development for PBC

CDCA and cholic acid (CA) are the natural ligands for FXR. Shortly following identification of the latter, OCA was generated as the 'first-in-class' synthetic agonist, which incorporates a 6α ethyl substitution to CDCA (synonymously known as 6-ECDCA). This modification endows OCA with >100-fold activating potency as evident in cell-free ligand sensing as well as reporter gene assays, relative to all previously identified bile acids [22]. Moreover, in a rat model of lithocholic acid induced cholestasis, OCA (5–30mg/kg) significantly improved bile flow and hepatocellular injury; however, the *in vivo* effects were short-lived. In keeping with FXR agonistic effects, significant increases in FGF19 were observed following OCA provision as part of assessment in a recent Phase-II clinical trial [26]. These changes were paralleled by reductions in 7α-hydroxy-4-cholesten-3-one (C4) as a precursor of bile acid synthesis, in addition to lower total circulating bile acid concentrations.

OCA is rapidly absorbed and extensively conjugated to the amino acids glycine and taurine. The conjugates are near equipotent on the FXR receptor and undergo enterohepatic recirculation akin to CDCA, making estimation of drug half-life somewhat difficult (no data available at time of writing). Pharmacokinetic characteristics are also broadly similar, with the exception of the 7-dehydroxylation step being restricted to CDCA [84].

Phase II / III clinical trials

Clinical outcomes in PBC are largely dictated by development of cirrhosis and portal hypertensive complications; however, the slowly progressive nature effectively precludes evaluation of classical endpoints such as hepatic decompensation or transplant-free survival in clinical trials. Histological assessment although a robust indicator of disease progression in liver disease, is not ideal given its invasiveness and well-known sampling variability in chronic cholestasis. These barriers have fostered the development of several plausible surrogates, of which serum ALP is validated as most secure in predicting long-term outcome [38].

Results from the first randomised, double-blind controlled trial in PBC were recently published, wherein the therapeutic efficacy of UDCA and three doses of OCA (10, 25, and 50 mg/d) compared against UDCA/placebo [26]. Entry into this internationally representative trial was restricted to patients exhibiting persistent elevations in serum ALP >1.5 times the upper limit of normal (ULN) whilst on a stable dose of UDCA for at least 6 months. The primary endpoint herein was a significant reduction in serum ALP from baseline, and met across all three doses of OCA versus placebo (Table 2). Moreover, 87%, 69% and 7% of all OCA-treated patients completing therapy achieved a decline in serum ALP of at least 10%, 20% or complete normalization (vs. 14%, 8% and 0% with placebo). Statistical differences in absolute ALP reductions were also evident in all OCA-treated patients, with attainment rates of established PBC biochemical response criteria being significantly greater in the 25 mg/d group at end of trial [26]. Another phase II study adopted similar inclusion criteria but evaluated OCA monotherapy (10 and 50 mg) versus placebo in the absence of concomitant UDCA provision [85]. Statistically significant relative and absolute reductions in ALP were reported for both doses of OCA versus

placebo (from baseline: –233.5 IU/L and –161 IU/L vs. +12 IU/L; $p<0.0001$), as well as improvements in serum bilirubin. Results from this trial await formal reporting and currently available data is restricted to abstract form.

Preliminary data from the only phase III study of OCA (PBC OCA International Study of Efficacy; POISE) have also recently been presented [86] [submitted; under review]. Akin to its predecessors the POISE study recruited PBC patients with persistent elevation in serum ALP (prior biochemical non-response according to Toronto criterion) but also those intolerant to UDCA. The primary endpoint during the 12-month double-blind period was attainment of both an ALP level <1.67 × ULN (with a ≥15% reduction from baseline) and a normal serum bilirubin. Patients were randomised to receive either placebo, 10 mg OCA, or 5 mg OCA for 6 months titrated to 10 mg OCA based on clinical response; and pre-existing UDCA therapy was continued at a stable dose (7% UDCA intolerant). All test groups were well-matched and in an intention-to-treat analysis response was met in 10% of the placebo group relative to 47% and 46% in the 10 mg and dose-titrated OCA groups, respectively ($p<0.0001$ for both; Figure 3). The mean decrease in ALP from baseline was 39 % and 33 % in the 10 mg and titrated OCA-groups, respectively, versus 5% for patients in receipt of placebo ($p<0.0001$ for both). In addition, both OCA groups met pre-defined secondary endpoints including reduction in serum AST and total serum bilirubin (both OCA groups $p<0.001$ vs. placebo).

The encouraging results from phase II and phase III studies represent a significant milestone in PBC; however, longer-term efficacy of obeticholic acid (OCA) and generalizability to the patient population as a whole need confirmation in prospective follow-up studies. This is of particular

importance given the limited number of patients enrolled in aforementioned clinical trials – a reflection of the relative infrequency of PBC globally [87]. Up till now enrolment into clinical trials has been restricted to individuals demonstrating persistent elevations in serum ALP, with therapeutic efficacy gauged through percentage change or absolute decline. It is plausible therefore, that the beneficial effect of OCA (and other novel bile acid therapies) will be restricted to patients failing to achieve biochemical response based on ALP criteria. However, there is no currently available data regarding therapeutic efficacy stratified according to the magnitude of serum ALP elevations at point of trial inclusion. Assessment of further surrogates of clinical outcome (including for instance AST/platelet ratio, liver stiffness measurements derived via transient elastography) would be of additional clinical benefit in this regard [18,88].

Safety and tolerability

The favourable changes observed in serum liver biochemistry were mirrored by significant reduction in circulating immunoglobulin levels and serum inflammatory markers (**Figure 4**); which although not directly linked with prognosis, suggests mediation of underlying immunological and inflammatory processes driving disease pathogenesis [26]. However, treatment with OCA is not without concern. Pruritus was a major side effect in the clinical trials, leading to treatment discontinuation in >10% of subjects in phase II studies (**Figure 5a**) [26]. Moreover, in the open-label long-term safety extension phase (n=78, 12 months – 61 patients completed) ALP reductions were sustained in all of the completer population, yet 87% reported pruritus at some point with ~10% overall needing to discontinue therapy. OCA-induced pruritus appears to be a dose-dependent effect and in the phase III clinical study was addressed (in part) through the dose-titration arm (Figure 5b) and provision of colestyramine (proportion of patients

receiving colestyramine: 11%, 19% and 26%; in the placebo, dose-titration and 10mg arms, respectively). However, it is unclear whether attenuation in itch severity over time (10mg group) was specifically due to symptom-specific intervention or due to development of OCA-tolerance, and data pertaining to colestyramine initiation date/duration is currently unavailable [63]. In addition to being a selective ligand for FXR, OCA may act as a partial agonist for transmembrane G-protein-coupled receptor (TGR)-5 – a receptor widely distributed in brown adipose tissue, skeletal myocytes, Kupffer cells, cholangiocytes, and enteroendocrine cells within the intestine [21,89]. More recently, TGR5 transcriptive signals were identified in cutaneous afferent neurons wherein direct bile acid stimulation was able to induce pruritus in mice [90,91], with receptor overexpression associated with enhanced basal scratch activity. In support of the TGR-5 hypothesis of cholestatic pruritus, intradermal injection of the bile-salt deoxycholate (a TGR5 agonist) has been shown to induce scratch activity in wild type mice; however, this was only partially attenuated in animals harboring a congenital *Tgr5* deletion suggesting additional pathogenic mechanisms. Bile salts may also lead to activation and degranulation of mast cells [92], although a wealth of research effort has failed to illustrate causal relationships between pruritus and either serum bile salt concentration or histaminergic activity [93]. Moreover, antihistamines have historically proven ineffective in the treatment of cholestatic pruritus.

PBC patients with early disease have elevated total lipid levels although risks of cardiovascular morbidity do not appear disparate relative to the general population [94]. Changes in serum lipids were observed in trials of OCA; specifically, a decrease in high-density lipoprotein (HDL) as early as 2 weeks of treatment coupled with an increase in total and LDL cholesterol (levels returned to baseline within 2 weeks of treatment cessation). One possible explanation is that by

attenuating hepatic bile acid synthesis cholesterol elimination is consequently reduced. Lipid effects in animals relate to up-regulation of reverse cholesterol transport, wherein *Fxr* knockout mice exhibit hypercholesterolemia. However, in this instance lipid changes reflect marked elevation in HDL levels secondary to down-regulation of scavenger receptor class B member 1 (SCARB)-1 – a receptor for hepatic clearance of cholesterol from HDL [65]. Indeed, OCA has been shown in animal models to lower atherogenic plaque formation via selective reduction of HDL2c or ApoAI [95,96]. Of interest, lipophilic bile acids which bind to FXR have been shown to promote insulin sensitivity and decrease hepatic gluconeogenesis and circulating triglyceride levels, ostensibly mediated by decreased lipid synthesis within the liver and enhanced peripheral clearance of VLDL, in addition to SCARB-1–mediated actions [97–100]. Based on these metabolic effects, OCA has also been proposed as a target for the treatment of NASH [62,63]; however, dedicated studies in PBC are needed to specifically delineate the vascular implications stemming from current findings.

Expert Commentary and 5 Year View

The FXR axis is the most dedicated of all signalling pathways that mediate bile acid homeostasis. Proven beneficial effects of receptor ligation and/or functional overexpression encompass choleresis, anti-inflammatory and anti-fibrotic properties, as well as tissue regeneration in pre-clinical models. Presently, OCA represents the most powerful of all (clinically-trialed) FXR agonists, and results from randomized trials are supportive of therapeutic merit in PBC, at least gauged by non-invasive yet highly validated and robust clinical outcome predictors. Biochemical efficacy is apparent across all currently tested dosages (5 mg – 50 mg) which given the dose-dependent effect on pruritus explains the use of lower-dose regimens in the

Phase III trial. Longer-term safety and durability monitoring is of importance in view of the persistently elevated FGF19 titres in OCA-treated patients. The role of FXR/FGF19 in cancer development is complex, with both protective and deleterious concerns. For instance, HCC develops spontaneously in aged *Fxr* null mice, but can be prevented by re-expressing a constitutively active form of FXR in the intestine [101]. Conversely FGF19 transgenic mice are seen to develop spontaneous hepatocellular carcinoma (HCC) [102], and FGF19 amplifications have been implicated in driving hepatic carcinogenesis in man [103,104]. Given the positive effects on hepatocyte proliferation, such oncogenic properties are likely a consequence of supraphysiological circulating FGF19 levels and support the need for appropriate safety monitoring of drugs modulating the FXR axis, particularly as biochemical non-responders in PBC represent a group in which HCC-risk is greatest [105]. Of interest, the ostensible mitogenic effects of FGF19 can be abrogated through substitution of a 7-amino acid region that comprises an FGFR4-interaction domain [106], and is of particular relevance for emerging FGF19 agonists.

The role of FXR/FGF19 in cancer development is complex, with both protective and deleterious concerns. For instance, development of HCC in aged *Fxr* null mice can be prevented by re-expressing a constitutive active form of FXR in the intestine [101]; conversely FGF19 transgenic mice are seen to develop spontaneous hepatocellular carcinoma (HCC) [102], and FGF19 amplifications have been implicated in driving hepatic carcinogenesis in man [104]. These discrepant findings support the need for appropriate safety monitoring of drugs that modulate the FXR axis; particularly given that biochemical non-responders in PBC represent a group in which HCC-risk is greatest [105].

The requirement for additional medical treatment in PBC is clearly recognized; and in addition to OCA, synthetic agonists of PPAR are perhaps furthest in terms of clinical study. To this effect, several non-controlled studies report an improvement in serum ALP following administration of Fenofibrate in addition to UDCA (mean decrease from a recent meta-analysis of 6 case series: 114 IU/L [107]). Similar results have also been garnered with regard to Bezafibrate (PPARα/δ/γ agonist), which has the added benefit of alleviating pruritus of cholestasis [108,109]. Unfortunately, many studies of fibrates are limited in terms of patient number follow-up (some <8 weeks in duration), lack of appropriate controls, application of sub-optimal UDCA dosages and ill-defined patient populations (biochemical response not consistently defined). Moreover, fibrates have been associated with development of renal dysfunction; and long-term efficacy and safety data eagerly awaited. A phase III randomized control trial of Bezafibrate in PBC is currently underway.

Further avenues for therapeutic development in PBC include targeting additional nuclear bile acid receptors (e.g. TGR5, PXR, CAR), immunomodulatory therapies (e.g. targeting pathways such as CD40, CTLA-4, CD80, JAK-STAT signaling), and cell-based therapies (mesenchymal stromal cells) are in development, all alongside drugs that modulate FXR function (INT-767; a dual FXR/TGR5 agonist with greater FXR-activating potency than OCA), or change bile acid re-circulation (ASBT-inhibitors)/biliary function (norUDCA). A detailed discussion pertaining to each of these approaches is beyond the scope of this review, and covered elsewhere [7,110–116].

To apply OCA and other therapies there remains a need to better understand the underlying pathogenesis of disease so that drugs target the predominant immunologic, metabolic and fibrotic

components to achieve best prevention and treatment of progressive disease. With an increasing understanding of the effector mechanisms that lead to biliary destruction, a major focus should take advantage of basic science observations so that they can be transferred to viable therapeutic tools, much like that which has occurred in rheumatoid arthritis and inflammatory bowel disease [117–120]. We would emphasize that such efforts will need to take advantage of newer technologies involving pathway analysis, microRNA, high throughput assays, gene replacement and dissection of the microbiome [7,121].

In conclusion there is clearly a significant unmet need for new therapies in patients with cholestatic liver diseases [110–113]. Thus, as well as its development programme in non-alcoholic steatohepatitis; OCA, a selective FXR agonist, represents one new potential treatment on the horizon for patients with PBC.

Key Issues:

- Primary biliary cirrhosis (PBC) is a chronic autoimmune liver disease in which clinical course is characterized by cholestasis, and outcome dictated by development of cirrhosis and portal hypertension.
- Presently, ursodeoxycholic acid (UDCA) is the only licensed medical treatment; and for those who meet specific biochemical response criteria (60 – 70% of all patients), clinical outcome is excellent.

- However, therapeutic shortfall of UDCA is evident in approximately one-third of the PBC population, particularly those failing to attain biochemical response, who experience progressive liver disease despite adequate UDCA provision.
- Obeticholic acid (OCA) is a semi-synthetic analogue of chenodeoxycholic acid that demonstrates potent anti-cholestatic, anti-inflammatory, anti-fibrotic and cell-proliferative effects in pre-clinical models of hepatobiliary injury, mediated through activation of the nuclear farnesoid X receptor (FXR).
- In phase II and phase III PBC clinical trials, OCA (monotherapy, or in addition to UDCA) resulted in significantly greater improvement to proven biochemical surrogates of clinical outcome, relative to placebo (± UDCA).
- Pruritus is the most significant side effect of OCA treatment, although this can be overcome through appropriate dosage titration and provision of bile acid sequestrants.
- Longer-term studies are needed to explore safety concerns and validate clinical efficacy.

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Table 1: Pleiotropic effects of FXR signaling [21]

Natural ligands and potency	Actions	Target tissues
CDCA > DCA > LCA > CA	*Up-regulated pathways* - Bile acid export - Tumor suppression - Hepatic regeneration - Phase I/II drug metabolism - Intestinal barrier function *Down-regulated pathways* - Bile acid synthesis - Hepatic inflammation - Lipogenesis - Gluconeogenesis - ECM production and tissue fibrosis	Liver Intestine Kidney

CDCA: Chenodeoxycholic acid, DCA: deoxycholic acid, LCA: lithocholic acid, CA: cholic acid, ECM: extracellular matrix

Table 2: Randomized, Double-Blind Placebo Controlled Trials of Obeticholic Acid in Primary Biliary Cirrhosis

Study phase *	OCA dose	No. Participants	1° Endpoint Met
II [26] *1° endpoint:* % reduction in serum ALP *Duration:* 12 weeks *Inclusion:* ALP >1.5 x ULN + <10 x ULN		*165; 136 completed*	*Mean % ALP decrease* **
	Placebo	38	3%
	10 mg	38	34%
	25 mg	48	25%
	50 mg	41	21%
II [85] – OCA monotherapy vs. placebo *1° endpoint:* % reduction in serum ALP *Duration:* 12 weeks *Inclusion:* ALP >1.5 x ULN and no UDCA >6 mo.		*59* ***	*Mean % ALP decrease* **
	Placebo	23	N/A (+0.4%)
	10 mg	20	45%
	50 mg	16	38%
III [86] *1° endpoint:* serum ALP <1.67 × ULN (with ≥15 % reduction from baseline) and normalization of bilirubin *Duration*: 12 months *Inclusion:* ALP ≥1.67 x ULN and/or total bilirubin >ULN to <2 x ULN		*216; 198 completed*	*Attainment rate* **
	Placebo	73	10%
	10 mg	70	47%
	10 mg to 50 mg; - dose titration at 6 months	73	46%

* All studies included patients on a stable dosage of UDCA for > 6 months except that by Kowdley et al.
** Indicate statistically significant differences
*** Completer population figures not available at time of writing

Figure Legends

Figure 1: Obeticholic acid structural characteristics

Obeticholic acid is a synthetic analogue to the endogenous farnesoid-X-receptor (FXR) ligand chenodeoxycholic acid (CDCA), albeit with >2 log potency as regards to receptor activation, as illustrated by the half maximal effective concentration (EC_{50}). By contrast, the CDCA epimer ursodeoxycholic acid (UDCA) does not harbour any FXR agonistic effects.

Figure 2: FXR agonism and bile acid homeostasis

Bile acids (BA) are synthesis by hepatocytes via cholesterol 7 alpha-hydroxylase (CYP7A1)-mediated conversion of cholesterol. Bile salts are secreted via the bile salt export pump (BSEP) into canaliculi for subsequent transport to the gut. Reclamation of bile salts occurs via the apical sodium bile acid transporter (ASBT) on ileal enterocytes, and facilitates activation of FXR and transcription of fibroblast growth factor (FGF)-19. The latter is subsequently transported to the liver via the portal circulation where ligation of its cognate receptor (FGFR4) initiates a cascade of intracellular signals that suppress CYP7A1 expression and consequently BA synthesis. Bile acids can also be shuttled from enterocytes to the liver via organic solute transporters (OST), and taken up by the sodium-taurocholate transporter (NTCP). In the liver, bile acids bind hepatic FXR, which in turn up-regulates small heterodimer (SHP) as another repressive mechanism of CYP7A1 transcription. FXR agonists such as obeticholic acid (OCA) exert their functional effects in the liver and gut, to a greater degree than natural ligands in a fervent effort to down-regulate CYP7A1 expression. FXR agonists may also mediate bile salt excretion via up-regulation of BSEP, and provide additional protective effects through up-regulation of the flippase multidrug resistance protein 3 (MDR3).

Figure 3: Attainment of primary and secondary endpoints in the POISE study.

The primary endpoint in the obeticholic acid (OCA) phase III trial in PBC was biochemical response at the end of treatment (12 months; response defined as: ALP < 1.67 x ULN *and* ≥15 decline from baseline; *and* normal serum bilirubin) phase (A). Percentage reductions in serum ALP (B) and fluctuations in bilirubin (C) as well as AST (D) are also presented for comparison. Statistically significant differences are indication by asterisks (*p<0.001 vs. placebo) [86].

Figure 4: Immunoglobulin and pro-inflammatory cytokine profiles

Provision of obeticholic acid (OCA) led to reduction in circulating immunoglobulin levels (A), as well as notable fluctuations in the serum concentration of pro-inflammatory cytokines (B). Data is presented for the phase III study only. Statistically significant differences are indication by asterisks (*p<0.05, **p<0.01, **p<0.001 vs. placebo) [86].

Figure 5: Pruritus and obeticholic acid in primary biliary cirrhosis

The most common side effect of obeticholic acid (OCA) therapy is (dose-dependent) pruritus. Summary data is shown for the phase II trial of OCA-monotherapy (A), wherein bar height indicates overall incidence of symptoms, and orange box the proportional discontinuation rate [85]. Similar rates were manifest in the phase II trial of OCA + ursodeoxycholic acid (UDCA) and have been presented elsewhere [26]. The phase III study incorporated a dose titration step in an effort to minimise severity of pruritus (B), with 8 patients overall discontinuing therapy due to refractory symptoms (none in the placebo group, n=7 in the 10mg group and n=1 in the titration

group; data not shown). Statistically significant differences are indication by asterisks (*p<0.0001 vs. placebo) [86].

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