EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

24 September 2015
EMA/689761/2015
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Kolbam

International non-proprietary name: cholic acid

Procedure No. EMEA/H/C/002081/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

3β-HSD	3β-hydroxy-Δ5-C27-steroid oxidoreductase
Δ^4-3-oxo-R	Δ4-3-oxosteroid 5β-reductase
AE	Adverse Event
ALT	Alanine Aminotransferase
AKR1D1	Δ4-3-oxosteroid 5β-reductase
AMACR	2- (or α-) methylacyl-CoA racemase
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Classification
AUC	Area Under the Concentration Curve
CA	cholic acid
CCHMC	Cincinnati Children's Hospital Medical Center
CDCA	Chenodeoxycholic acid
CI	Confidence Interval
Cmax	Maximum Plasma Concentration
CNS	Central Nervous System
CSR	Clinical Study Report
CTX	Cerebrotendinous Xanthomatosis
CYP7A1	Cholesterol 7α-Hydroxylase
CYP7B1	Oxysterol 7α-Hydroxylase
GGT	Gamma Glutamyl Transferase
DHCA	Dihydroxycholestanoic acid
EMA	European Medicines Agency
EU	European Union
FAB-MS	Fast Atomic Bombardment-Mass Spectroscopy
FXR	Faresoid X Receptor
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GCP	Good Clinical Practice
HPLC	High Pressure Liquid Chromatography
HSD3ß7	3β-hydroxy-Δ5-C27-steroid oxidoreductase
ICH	International Conference on Harmonization
IRB	Institutional Review Board
ITT	Intent To Treat

LC-MS	Liquid Chromatography-Mass Spectrometry
LFT	Liver Function Test
mITT	Modified Intent To Treat
MS	Mass Spectroscopy
NALD	Neonatal Adrenoleukodystrophy
PK	Pharmacokinetic
SAE	Serious Adverse Event
SmPC	Summary of Product Characteristics
SOC	System Organ Class
TBM	To Be Marketed
THCA	Trihydroxycholestanoic acid
THCA-CoA	Trihydroxycholestanoic acid Coenzyme A
ULN	Upper Limit Normal
URSO	Ursodeoxycholic acid

1. Background information on the procedure

1.1. Submission of the dossier

The applicant FGK Representative Service GmbH submitted on 29 February 2012 an application for Marketing Authorisation to the European Medicines Agency (EMA) for cholic acid FGK, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 29 May 2009.

Cholic acid FGK, was designated as an orphan medicinal product EU/3/09/683 on 9 December 2011 in the following indication: Treatment of inborn errors of primary bile acid synthesis responsive to treatment with cholic acid. At the time of designation, inborn errors in primary bile acid synthesis responsive to treatment with cholic acid affected approximately 0.07 in 10,000 people in the European Union (EU).

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Kolbam as an orphan medicinal product in the approved indication. The outcome of the COMP review can be found on the Agency's website: ema.europa.eu/Find medicine/Rare disease designations.

The applicant applied for the following indications:

Cholic acid FGK is indicated for the treatment of inborn errors of primary bile acid synthesis, responsive to treatment with cholic acid, in infants from one month of age for continuous lifelong treatment through adulthood.

Inborn errors of primary bile acid synthesis involve congenital defects in the primary enzymes responsible for catalysing key reactions in the synthesis of cholic and chenodeoxycholic acids. The primary enzyme defects include but are not limited to:

- *3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase (also known as 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase/isomerase or 3β-HSD or HSD3β7) deficiency*
- *Δ^4-3-oxosteroid 5β-reductase (Δ^4-3-oxo-R or AKR1D1) deficiency*
- *Sterol 27-hydroxylase (presenting as cerebrotendinous xanthomatosis, CTX) deficiency*
- *Defective bile acid amidation due to failure to conjugate with glycine and/or taurine*
- *2- (or α-) methylacyl-CoA racemase (AMACR) deficiency*
- *Oxysterol 7α-hydroxylase (CYP7B1) deficiency*
- *Cholesterol 7α-hydroxylase (CYP7A1) deficiency*
- *Trihydroxycholestanoic acid (THCA) CoA oxidase deficiency*
- *Side-chain oxidation defect in the sterol 25-hydroxylation pathway*

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application. The applicant indicated that cholic acid was considered to be a new active substance.

The application submitted is composed of administrative information, complete quality data, non-clinical based on bibliographic literature substituting all non-clinical tests and clinical data based on bibliographic literature and the own applicant's clinical data.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/206/2011 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/206/2011 was not yet completed as some measures were deferred.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

Derogation of market exclusivity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant submitted a claim addressing the following derogation laid down in Article 8.3 of the same Regulation; the applicant can establish in the application that the medicinal product, although similar to the orphan medicinal product already authorised, is safer, more effective or otherwise clinically superior.

Applicant's request for consideration

Marketing Authorisation under exceptional circumstances

The applicant requested consideration of its application for a Marketing Authorisation under exceptional circumstances in accordance with Article 14(8) of Regulation (EC) No 726/2004 based on the following claims: the applicant justified that he is unable to provide comprehensive data on the efficacy and safety under normal conditions of use, because: the indications for which the product in question is intended are encountered so rarely that the applicant cannot reasonably be expected to provide comprehensive evidence, and that it would be contrary to generally accepted principles of medical ethics to collect such information. The applicant proposed as specific obligation to expand the data available on the clinical safety and efficacy of cholic acid in the treatment of inborn errors of bile acid synthesis by establishing a patient registry.

New active Substance status

The applicant requested the active substance (cholic acid) contained in the above medicinal product to be considered as a new active substance in itself, as the applicant claims that it is not a constituent of a product previously authorised within the Union.

Protocol Assistance

The applicant did not seek Protocol Assistance at the CHMP.

Licensing status

The product was not licensed in any country at the time of submission of the application. At present, several products containing combinations of cholic acid with other active substances are marketed in a

few EU countries. On 13 September 2013 Orphacol (cholic acid) was granted a marketing authorisation in the EU for the treatment of treatment of inborn errors in primary bile acid synthesis due to 3β-Hydroxy-Δ^5-C_{27}-steroid oxidoreductase deficiency or Δ^4-3-Oxosteroid-5β-reductase deficiency in infants, children and adolescents aged 1 month to 18 years and adults.

It should be noted that cholic acid is an endogenous substance and is used in foods as a food additive (E 1000) in low concentrations. Historically medicinal products containing cholic acid have been used for other indications, such as laxatives and choleretics since at least the early part of the 20th century.

1.2. Manufacturers

Manufacturer responsible for batch release

Lucane Pharma
Immeuble Le Dorian
Bâtiment B1
172 rue de Charonne
FR – 75011 Paris

1.3. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Robert Hemmings (UK)	Co-Rapporteur: Patrick Salmon (Ireland)

- The application was received by the EMA on 29 February 2012.
- The procedure started on 21 March 2013.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 08 June 2012. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 11 June 2012.
- During the meeting on 19 July 2012, the CHMP agreed on the consolidated List of Questions to be sent to the applicant.
- A GCP inspection triggered by the CHMP was conducted at the site of the sponsor (Cincinnati Children's Hospital Medical Centre, USA) between 22 and 25 October 2012. The inspection report including the responses from the sponsor dated 15 March 2013 was circulated to the CHMP on 15 March 2013.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 25 April 2013.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 30 May 2013.
- During the CHMP meeting on 27 June 2013, the CHMP agreed on a list of outstanding issues to be addressed in writing and/or in an oral explanation by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 19 August 2013.

- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 29 August 2013.
- During the CHMP meeting on 19 September, the CHMP agreed on a list of outstanding issues to be addressed in writing and/or in an oral explanation by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on October 2013.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 30 October 2013.
- During the November 2013 CHMP meeting, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- The CHMP adopted on 21 November 2013 a report on similarity of cholic acid FGK with orphan medicinal product(s) authorised in the same orphan condition.
- The CHMP adopted a report on the claim for clinical superiority derogation applicable to similar orphan medicinal products on 21 November 2013.
- Following the oral explanation the applicant withdrew from the application the two indications that the CHMP considered similar to Orphacol, i.e. 3β-HSD and Δ^4-3-oxoR deficiencies. As a result of the withdrawal, the report on the clinical superiority derogation is not relevant for the purpose of this opinion as cholic acid FGK is not anymore similar to Orphacol in the claimed indications.
- During the meeting on 21 November 2013, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, adopted a positive opinion for granting a Marketing Authorisation under exceptional circumstances to cholic acid FGK for the following therapeutic indications: Sterol 27-hydroxylase (presenting as cerebrotendinous xanthomatosis, CTX) deficiency; 2- (or α-) methylacyl-CoA racemase (AMACR) deficiency; Cholesterol 7α-hydroxylase (CYP7A1) deficiency.
- The EMA received letters dated 28, 29 November 2013 and 09 January 2014 from third parties regarding the positive opinion for cholic acid FGK dated 21 November 2013.
- The EMA received letters from the applicant dated 29 December 2013 and 17 January 2014 in response to the letters received from third parties.
- During the December 2013 meeting, the CHMP discussed these interventions and concluded on the need to revise the CHMP assessment report in order to further motivate its positive opinion.
- During the meeting on 23 January 2014, the CHMP adopted a revised positive opinion for granting a Marketing Authorisation under exceptional circumstances to cholic acid FGK for the following therapeutic indications: Sterol 27-hydroxylase (presenting as cerebrotendinous xanthomatosis, CTX) deficiency; 2- (or α-) methylacyl-CoA racemase (AMACR) deficiency; Cholesterol 7α-hydroxylase (CYP7A1) deficiency.
- On 4 April 2014, the Commission adopted a Decision granting a marketing authorisation under exceptional circumstances for Cholic Acid FGK.
- On 06 August 2014, the name of the medicinal product was changed to Kolbam.
- On 12 August 2014, the marketing authorisation for Kolbam was transferred to ASK Pharmaceuticals GmbH.

- On 05 June 2015, the marketing authorisation for Kolbam was transferred to Retrophin Europe Limited.
- On 11 June 2015, the General Court annulled the Commission Decision of 4 April 2014 granting, in exceptional circumstances, a marketing authorisation for Kolbam.
- On 7 September 2015, the European Commission requested the CHMP to review its opinion dated 23 January 2014 in light of the above-mentioned court case.
- During the meeting on 24 September 2015, the CHMP, in light of the scientific discussion within the Committee, adopted a revised positive opinion for granting a Marketing Authorisation under exceptional circumstances to Kolbam for the following therapeutic indications: Sterol 27-hydroxylase (presenting as cerebrotendinous xanthomatosis, CTX) deficiency; 2- (or α-) methylacyl-CoA racemase (AMACR) deficiency; Cholesterol 7α-hydroxylase *(*CYP7A1) deficiency.

2. Scientific discussion

2.1. Introduction

Problem statement

The inborn errors of bile acid synthesis are a category of metabolic liver disease (Setchell & Heubi, 2006). These conditions are extremely rare genetic disorders and cholic acid has been granted orphan medicinal product status pursuant to Regulation (EC) No 141/2000 for the treatment of inborn errors in primary bile acid synthesis (EU/3/09/683) with a calculated prevalence of 0.07 per 10,000 people in the European Union (EU). Individuals with inborn errors of bile acid synthesis lack the enzymes needed to synthesize the primary bile acids, cholic acid (CA) and chenodeoxycholic acids (CDCA). This deficiency results in diminished production of primary bile that are essential for promoting bile flow and the concomitant production of high concentrations of atypical bile acids and bile acid intermediates (Heubi *et al.* 2007). The enzyme deficiency allows these bile acid intermediates that are the substrates for a particular enzyme, to accumulate and these can be metabolised to an array of unusual bile acids, several of which have been shown to be hepatotoxic. The absence of primary bile acids causes hepatocytes to continuously metabolize cholesterol in an attempt to established normal bile acid pool. The result is the continued production of high concentrations of these hepatotoxic metabolites, which cause a progressive cholestasis. The liver disease associated with these inborn errors in bile acid synthesis is progressive and, if untreated, may lead to death from cirrhosis and liver failure (Heubi *et al.* 2007).

Disorders in bile acid synthesis and metabolism can be broadly classified as primary or secondary. Primary enzyme defects involve congenital deficiencies in enzymes responsible for catalysing key reactions in the synthesis of cholic and CDCA. The primary enzyme defects include:

- 3β-hydroxy-Δ^5-C27-steroid oxidoreductase (also known as 3β-hydroxy- Δ5-C27-steroid dehydrogenase/isomerase or 3β-HSD or *HSD3*β*7*)deficiency
- Δ^4-3-oxosteroid 5β-reductase (Δ^4-3-oxo-R or *AKR1D1*) deficiency
- Sterol 27-hydroxylase (presenting as cerebrotendinous xanthomatosis,CTX) deficiency
- Defective bile acid amidation due to failure to conjugate with glycine and/or taurine
- 2- (or α-) methylacyl-CoA racemase (*AMACR*) deficiency

- Oxysterol 7α-hydroxylase *(CYP7B1)* deficiency
- Cholesterol 7α-hydroxylase *(CYP7A1)* deficiency
- Trihydroxycholestanoic acid (THCA) CoA oxidase deficiency
- Side-chain oxidation defect in the sterol 25-hydroxylation pathway

The most common enzyme deficiency appears to be 3β-hydroxy-Δ5-C27-steroid oxidoreductase deficiency, followed by Δ4-3-oxosteroid 5β-reductase (*AKR1D1*) deficiency, sterol 27-hydroxylase (CTX) deficiency and amidation defects (Heubi *et al.* 2007).

At the time of the submission of the application by the applicant no treatment had a marketing authorisation in the EU. The only therapeutic option in severely affected cases was liver transplant. On 13 September 2013 Orphacol (cholic acid) was granted a marketing authorisation in the EU for the treatment of treatment of inborn errors in primary bile acid synthesis due to 3β-Hydroxy-Δ^5-C_{27}-steroid oxidoreductase deficiency or Δ^4-3-Oxosteroid-5β-reductase deficiency in infants, children and adolescents aged 1 month to 18 years and adults. Deficiencies in 3β-HSD and Δ^4-3-oxoR are two of the nine indications initially claimed by the applicant.

Oral administration of cholic acid, the substance missing in affected patients, inhibits the production of the hepatotoxic bile acid precursors by down-regulating cholesterol 7α-hydroxylase, the rate limiting enzyme in bile acid synthesis (Setchell and O'Connell 2007). In addition, it provides a stimulus for bile flow facilitating the hepatic clearance of toxic bile acid precursors and toxic substances, including bilirubin. It also improves growth by facilitating the absorption of fat-soluble vitamins and fat (Setchell & Heubi, 2006).

About the product

The active substance of Kolbam is cholic acid. Cholic acid is a primary bile acid and is classified as a bile and liver therapy, bile acid preparations. Primary bile acids are biosynthesised in the liver of healthy men and are key constituents of normal bile. The two primary bile acids synthesised by the human liver, cholic acid and chenodeoxycholic acid, serve several important physiological functions (Setchell and O'Connell 2007). Cholic acid represents between approximately half and two thirds of the primary bile acids produced in adult humans. In addition to facilitating fat absorption from the gastrointestinal tract and to participating in the regulation of cholesterol homeostasis, the primary bile acids provide the major driving force for the promotion and secretion of bile. They are essential to the development of the enterohepatic circulation, which is necessary for the elimination of toxic endogenous or exogenous substances. The applicant applied for 2 strengths: 50 and 250 mg both as hard capsules.

Type of application and aspect on development

The application submitted is composed of a complete dossier including quality data; non-clinical data are based on bibliographic literature substituting all non-clinical tests and clinical data are based on the own applicant's clinical data supported by bibliographic literature. The applicant has submitted one pivotal clinical study involving a total of 85 patients and a second study which was actually a subset of the pivotal study. The findings of these studies are supplemented by clinical studies reported in the literature. The applicant has complied with the requirement of providing a paediatric investigation plan (PIP). As part of the PIP, an oral suspension of the product is being developed. The applicant did not seek scientific advice at the CHMP regarding this application.

In accordance with Article 14(8) of Regulation (EC) No 726/2004 and Annex I, part II of the Directive 2001/83/EC the applicant applied for a marketing authorisation under exceptional circumstances. The applicant argued that he was unable to provide comprehensive data on the efficacy and safety under normal conditions of use because the indications for which cholic acid are intended are encountered so rarely that he cannot reasonably be expected to provide comprehensive evidence. In addition, it would also be contrary to generally accepted principles of medical ethics to collect such information. The applicant proposed as specific obligation to monitor the clinical safety and efficacy of cholic acid in the treatment of the claimed bile acid deficiencies by establishing a patient registry.

2.2. Quality aspects

2.2.1. Introduction

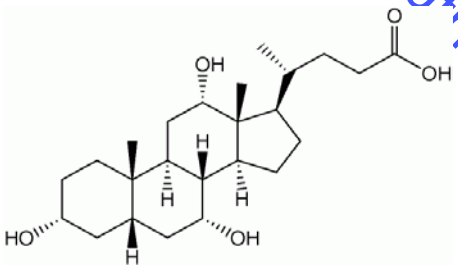
Kolbam is presented as hard gelatine capsules containing 50 mg or 250 mg of cholic acid as the active substance. The 50 mg strength is presented as size 2 capsules with Swedish orange caps (black imprint “ASK001”) and bodies (black imprint “50mg”). The 250 mg strength is presented as size 0 capsules with white caps (black imprint “ASK002”) and bodies (black imprint “250 mg”).

Other ingredients are: silicified microcrystalline cellulose and magnesium stearate (components of the capsule contents), gelatin, titanium dioxide and red iron oxide (components of the capsule shells for the 50 mg strength) or gelatin and titanium dioxide (components of the capsule shells for the 250 mg strength). For the printing ink the excipients are shellac (E904), propylene Glycol (E1520), strong Ammonia Solution (E527), potassium Hydroxide (E525), black Iron Oxide (E172).

The capsules are packed in white high density polyethylene (HDPE) bottles with a white, child-resistant closure (screw caps).

2.2.2. Active Substance

Cholic acid is chemically designated as cholan-24-oic acid or (3α, 5, 7α, 12α)-3α, 7α, 12α-trihydroxy-5β-cholan-24-oic acid and has the following structure:



In nature, cholic acid is produced in the liver from cholesterol. The liver converts cholesterol into the conjugated salts of glycocholic and taurocholic acid which are secreted into the bile. Bile is released into the intestine where the bile salts emulsify fats and promote digestion. Cholic acid is produced from bovine and ovine bile which is a by-product of the meat processing industry.

It is a white powder comprised of aggregated polycrystalline particles. It is practically insoluble in water and in 0.1M HCl and is sparingly soluble in 0.1M NaOH. It is reported to be soluble in glacial acetic acid and some organic solvents (e.g. alcohols, acetone). The saturated solution in water at 20°C has a pH of 4.4. The substance is not hygroscopic unlike its sodium salt (sodium cholate). Cholic acid has a melting point of approximately 200°C and is very heat stable.

Sufficient evidence was provided to demonstrate that only one crystal form is obtained by the utilised manufacturing process.

The applicant claimed that cholic acid should be classified as New Active Substance (NAS) and provided justification for such claim. However the NAS status could not be confirmed because medicinal products containing cholic acid have historically been used as laxatives and choleretics since at least the early part of the 20th century. Many of these products are no longer authorised but nevertheless as previously authorised products they were relevant in determining the NAS status. Furthermore, during evaluation of this application, another product containing cholic acid as the active substance has been authorised. Therefore, it was concluded that as cholic acid was previously been authorised as a medicinal product, it can not be considered a NAS.

Manufacture

Information about manufacturing process of cholic acid has been provided using Active Substance Master File (ASMF) procedure.

Cholic acid is manufactured using bovine and ovine bile as a raw material. The safety of the bile with regard to TSE is assured by the EDQM Certificate of Suitability. The applicant's documentation included details on slaughter, collection processes, geographical sourcing, and traceability. Bile is supplied as either liquid bile or concentrated bile. Satisfactory specifications for these starting materials have been provided.

Bile acids sourced from ox and sheep are rich in cholic acid conjugated to amino acids taurine and glycine. The amino acids are removed from the cholic acid by hydrolysis under alkaline conditions. Purification by precipitation and solvent extraction then occurs, before crystallisation and milling. The manufacturing process has been well described. Critical parameters and accompanying in-process controls to ensure quality of the final compound have been defined.

Confirmation of the chemical structure of cholic acid was provided by elemental analysis (confirmation of the determined elementary composition), UV, FTIR, ^{1}H-NMR, ^{13}C-NMR, MS, X-ray powder diffraction (XRD) and differential scanning calorimetry (DSC). X-ray diffraction and DSC studies confirmed the morphology of cholic acid and absence of polymorphic forms.

Potential impurities have been well discussed in relation to their origin (raw materials, manufacturing process and degradation products) and potential carry-over into the final substance. The possibility of genotoxic impurities was also addressed. Most of the impurities (chenodeoxycholic acid, deoxycholic acid, degraded bile salts, glycine, taurine, and other naturally occurring impurities) are naturally occurring *in vivo*. As the active substance is being administered to correct bile acid deficiencies the potential risk from these impurities would be no greater than present in healthy humans.

Particle size of the active substance is considered a critical attribute for the manufacture of the finished product, as cholic acid is not dissolved in the dosage form. Therefore an appropriate test on particle size determination was included in the active substance specification.

Specification

The active substance specification includes tests for physical appearance, identification (FTIR and HPLC), specific rotation, assay (HPLC), melting point, loss on drying, acidity, impurities (HPLC), residue on ignition, colour index, heavy metals, particle size distribution, residual solvents (GC) and microbiological purity (total aerobic microbiological count, yeasts and moulds, *Escherichia coli*).

A detailed description for all analytical methods was provided. Some of the proposed methods are in accordance with the Ph. Eur. Full method validation data was provided for the non compendial (*in-house*) analytical methods.

Limits proposed for known and unknown impurities are acceptable and in line with the ICH guideline Q3A (R2). All impurities typically present in cholic acid at a level greater than 0.05% are reported and those typically present at a level greater than 0.10% have been identified.

In general analytical methods proposed are suitable to control the quality of the active substance.

Data on 3 consecutive commercial scale batches of cholic acid were provided by the ASMF Holder and by the manufacturer of the finished product. All batches complied with the requirements in the active substance specification.

In general, sufficient information regarding the manufacturing process, raw materials, critical steps and intermediates and manufacturing process have been provided.

Stability

Stability data was provided from studies performed with different grade of the active substance (grades 01 and 03). The grade 01, which is subject of this application, is obtained from the grade 03. The 01 grade is subject to an additional re-crystallization and therefore is purer than the 03 grade.

For grade 01 stability data on three commercial scale batches performed in accordance to ICH conditions were provided. Samples of cholic acid were placed under long term (25°C/60% RH), intermediate (30°C/75% RH) and accelerated (40°C/75% RH) conditions. The batches were produced by the proposed manufacturer and stored in the proposed container closure system. Results were provided from up to 18 months of storage at long term and intermediate conditions and up to 9 months of storage at accelerated conditions.

For the 03 grade stability programme included full testing in accordance with ICH conditions. Results were provided from up to 60 months of storage at 25°C/60% RH and up to 6 months of storage at 40°C/75% RH.

In both cases (grades 01 and 03) the batches were monitored for appearance, identification by HPLC, loss on drying, assay, related impurities, microbial enumeration and colour index. All results reported were within proposed specification limits. No trends were seen.

Although only 18 months stability was presented for the 01 grade it is considered that data from the 03 grade supports longer re-test period. This was considered acceptable because cholic acid is extremely stable, there is no reason why the purer 01 grade would be less stable than the 03 grade and the packaging for the 01 grade has been upgraded with the use of moisture resistant foil.

In addition stability data from forced degradation studies were provided. The following conditions were studies: acid degradation (0.1 M and 1.0 M HCl) for up to 48 hours, alkaline degradation (0.1M and 1.0M NaOH) for up to 48 hours, oxidative degradation (5% H_2O_2) for up to 72 hours and thermal degradation (40°C) up to 93 hours. During stress studies cholic acid was shown to be stable in acidic, alkaline and oxidative environment and also resistant to elevated temperatures.

The stability studies demonstrated that the active substance is stable and confirmed the proposed re-test period. Furthermore, one batch of cholic acid per year will be added to the stability program, stored at 25°C/60% RH and tested annually for at least up to and including the re-test period. This reduced (annual) testing protocol was accepted based on the integrity of the available data for cholic

acid. In accordance with EU GMP guidelines, any confirmed out of specification result or significant negative trend will be reported.

2.2.3. Finished Medicinal Product

Pharmaceutical Development

Only limited information was provided on pharmaceutical development of the finished product. Development of the product began as a hospital special preparation.

The finished product, powder-filled capsules, is rather simple dosage form and no formal development studies were performed. Initially powder filled capsules with the active substance and lactose mix were made by the hospital pharmacy for the clinical investigations. This formulation was replaced by a to-be-marketed formulation, which is subject of this application. As there were no particular formulation issues with the active substance, a simple capsule formulation could be employed. Standard well known compendial excipients, commonly used in capsule formulations, were chosen for the formulation and stability studies have been performed confirming there were no problems of compatibility of the active substance with the excipients. In the to-be-marketed formulation lactose was replaced by silicified microcrystalline cellulose as a filler and magnesium stearate added as a lubricant. The critical component characteristics that can influence batch reproducibility, product performance, and finished product quality are primarily rheological properties affecting blending and powder flow during filling which were confirmed during process validation.

A relative bioavailability study was conducted to demonstrate similarity of the to-be-marketed formulation to the clinical trial formulation. The two oral capsule formulations had similar PK profiles although the standard criteria for bioequivalence were not met. Clinical trial/therapeutic equivalence study was conducted to demonstrate similarity between formulations. Patients treated with the clinical trial formulation were switched to the to-be-marketed formulation. The study was not specific to a specific formulation in the same way that a bioequivalence study would be. Both strengths of capsule and the sprinkle were considered in the therapeutic equivalence study. As the same powder mix is used in both strengths of capsule it is considered that there is no difference in bioavailability between the different strengths. The to-be-marketed formulation has been shown to be therapeutically equivalent to a formulation shown to be effective clinically. The design of the study might not be as clear cut as a standard bioequivalence study.

A suitable method for dissolution was developed and validated for routine control of the finished product at release and shelf life. Specifications were defined and presented. A summary of the method and method validation have been provided.

In addition, discussion about the palatibility and compatibility of the capsule contents mixed with some types of foods or drinks was investigated. Mixing of the capsule contents is designed to mask any unpleasant taste which results from the capsules being opened and to make the formulation age-appropriate in case of children who are not able to swallow oral solid dosage forms. This method of administration has been used during the 20 years of product development as a hospital special. The method of administration appears qualified by experience. The applicant has justified the size of capsules in relation to the paediatric population and gave more guidance on how to administer the product as a sprinkle. For infants, from one month of age, the contents of the capsules should be mixed with a small amount of infant formula, expressed breast milk or fruit puree and given from a spoon. In weaned infants and children under 6 years, who may be unable to swallow capsules, the capsule contents should be mixed with a small amount of soft food such as mashed potatoes or apple puree. This has been refelected in the Product inormation.

It can be concluded that the formulation development of the product was satisfactorily described.

Adventitious agents

Cholic acid is manufactured using bovine and ovine bile as a source material. The safety of the bile with regard to TSE is assured by the Ph. Eur. Certificate of Suitability.

Among excipients used in the finished product only gelatin (component of the capsule shell) is of animal origin. For gelatin TSE Certificates of Suitability were provided from suppliers.

Magnesium stearate used in the formulation is of vegetal origin.

Manufacture of the product

The manufacturing process is standard and relatively simple and includes sieving (the active substance and the filler) followed by pre-lubrication and blending of the pre-lubrication blend with the lubricant and filling of capsules.

The in-process controls were considered to be adequate as the mixing parameters are well defined in the manufacturing process and the active substance constitutes substantial amount of the blend. Blend uniformity and content uniformity were identified as critical process parameters which need to be controlled as in-process controls. Satisfactory process validation data were provided.

Product specification

The finished product specifications at release and shelf-life include tests for appearance, identification (FTIR, melting point and HPLC), content uniformity (Ph. Eur.), assay (HPLC), related substances and degradation products (HPLC), dissolution, water content (KF) and microbiological purity (total aerobic viable count, total yeasts and moulds, *Escherichia Coli*, *Salmonella*).

A detailed description for all analytical methods was provided. Complete method validation data was provided for the non compendial (*in-house*) analytical methods.

Batch analysis results on batches of each strength of the finished product demonstrated compliance with the proposed specification and confirmed consistency and uniformity of the product. The batches were manufactured by the proposed manufacturer and packaged in the packaging intended for commercial use.The results were consistent from batch to batch and proved that the product can be manufactured reproducibly according to the agreed specifications.

Stability of the product

Stability has been studied on 3 industrial scale batches of each strength of the finished product. Stability data were generated using the storage conditions listed in the ICH Guidance and contained results up to 24 months from long term storage at 25°C/60% RH and from storage under intermediate conditions at 30°C/75% RH, and 6 months from accelerated conditions at 40°C/75% RH. The selection of 30°C/75% RH as a long term condition was made to support the use of the product in hot and humid climatic zones. As this storage condition is considered to be more challenging than 30°C/65% RH it was considered to be suitable as a long term storage conditions.

The following parameters were tested appearance, assay, water content, degradation products, dissolution and microbiological attributes. No trends were observed in the data and all results complied with the specification. No stability trends were observed for any of the attributes studied.

In addition stability data from photostability studies were provided. Studies were performed in accordance with ICH Q1B. Formation of a degradation product in amounts above the qualification limit was observed. This degradation product was not observed during long term or accelerated storage of the finished product, and this indicates that the product should be protected from light. The storage precaution “Store in original package in order to protect from light” has been added to the product information.

It is foreseen that the capsule content could be mixed with a small amount of food or drinks to facilitate the administration of the product to children who are not able to swallow capsules. Normally stability studies would be required to support such claim in the SmPC. However in case of this product stability studies were not required because stress testing has shown that cholic acid was resistant to degradation by acid, base or oxidising agents. Furthermore cholic acid is a food additive (E1000, an emulsifying agent) so presumably spends long periods in contact with food and it is an endogenous substance secreted by the bile duct to digest food. It is not degraded by food but is subject to repeated entero-hepatic circulation. If there were any degradants produced by contact with food they would be qualified, as the degradation products would be endogenous substances.

The overall stability data showed that Kolbam is chemically, physically and microbiologically stable. The results generated during the stability studies and statistical analyses support the proposed shelf life and storage conditions as defined in the SmPC.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

The active substance, cholic acid, is a well-known and well-characterized substance. Information about manufacturing process of cholic acid has been provided using Active Substance Master File (ASMF) procedure. Cholic acid is manufactured using bovine and ovine bile as a source material and the safety of the bile with regard to TSE is assured by the EDQM Certificate of Suitability. ASMF Holder has a long experience in manufacture of cholic acid and has manufactured more than 10,000 batches of this substance.

In general analytical methods proposed are suitable to control the quality of the active substance. Cholic acid was shown to be stable, even when stored at elevated temperatures.

The finished product is formulated as hard capsules. It is a simple formulation and only limited information was provided on the formulation development. This was however acceptable in view of the long record of use of the product and the bridging data to the applied formulation.

The method of manufacture is considered standard and has been satisfactorily described, including in-process tests. The data shows consistent manufacture and is considered sufficient for this manufacturing process. The proposed specifications were justified based on the batch and stability results, and are in general adequate for assuring the product quality and therefore were accepted.

The stability program is considered satisfactory. The batches placed on stability are considered representative of the product to be marketed. The results generated during the stability studies support the proposed shelf life and storage conditions as defined in the SmPC.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The active substance and the finished product have been appropriately characterised and overall satisfactory documentation has been provided. The results indicate that the drug substance and the drug product can be reproducibly manufactured and therefore the product should have a satisfactory and uniform performance in the clinic.

2.3. Non-clinical aspects

2.3.1. Introduction

No new non-clinical studies have been submitted in the application. The non-clinical data consists of literature references published up to 2011. Due to the bibliographic nature of the data and the date of origin of some of the studies the GLP statuses are not fully covered according to the present regulatory standards. This is however acceptable as CA is an endogenous substance and has been investigated in animals and humans over a considerable number of years without showing overt major toxicity.

2.3.2. Pharmacology

Primary pharmacodynamic studies

Cholic acid is synthesised in the liver from cholesterol. It undergoes enterohepatic circulation, in which its principal functions include induction of bile flow; feedback inhibition of bile acid synthesis; modulation of cholesterol synthesis; elimination of cholesterol; and the facilitation of dispersion and absorption of lipids and fat-soluble vitamins through the formation of micelles (Setchell and O'Connell 2007). The effects of CA on the metabolism and secretion of bile acids are well known. Metabolism of bile acids is tightly controlled via a negative feedback regulation of bile acid synthesis. The cytochrome P450 enzymes CYP7A1 (cholesterol 7α-hydroxylase) and CYP8B1 (sterol 12α-hydroxylase) that are involved in bile acid synthesis are regulated by negative feedback from bile acids such as CA (Li-Hawkins *et al*. 2002; Murphy *et al*. 2005; Shea *et al*. 2007).

Several of the murine knock-outs models reviewed in the literature, targeting specific enzymes that in humans result in inborn errors of bile acid synthesis, have not proven suitable as predictive animal models. However, two murine models have been used to demonstrate that when bile acid synthesis has been depressed, or the composition of the bile acid pool has been altered so that no CA is available, dietary supplementation with CA can positively influence resultant pathologies. Mice deficient in HSD3β7 (the homolog of the human gene coding for 3β-HSD), saw survival rates increase after feeding with CA and vitamin supplements (Shea *et al*. 2007). The 3-hydroxyl group conferred by HSD3β7 is required to maintain the functional and regulatory properties of bile acids in mice, and this is similarly required in humans. In mice deficient with 12α-hydroxylase gene (CYP8B1) a similar finding was seen (Murphy *et al*. 2005).

Taken together, the data from both studies demonstrated that in the HSD3B7 knock-out mouse (a representative model for one of the documented errors of human bile acid biosynthesis) and in the CYP8B1 knockout mouse (a surrogate model for inborn errors, in which CA is absent), CA, supplemented in the diet, has the capacity to both prolong and maintain survival in addition to preventing characteristic features of the phenotype in the HSD3B7 knockout mouse and to normalize the absorption of cholesterol in a CYP8B1 knockout mouse that does not generate CA. The reported actions of exogenous CA in both of these models support the use of CA in the treatment of inborn errors of bile acid biosynthesis in patients.

Secondary pharmacodynamic studies

No published studies have been identified by the applicant describing the secondary pharmacological effects of CA. Cholic acid being an endogenous substance, no secondary pharmacological effects is

expected. The dose of bile acid administered is normally titrated against the extent of disappearance of atypical bile acid intermediates and metabolites in urine, which correlate with the degree of improvement in liver function tests. To date, no serious or persisting adverse effects following administration of CA in therapeutic doses of up to 15 mg/kg body weight/day have been reported in man. Data from animal toxicology studies suggest that there is no pharmacologic or toxic action of CA outside the organs of the enterohepatic circulation (see toxicology section).

Safety pharmacology programme

CA has been used clinically to treat patients with inborn errors of bile acid synthesis since 1994. In these patients, the dose of bile acid administered was titrated against the extent of disappearance of atypical bile acid intermediates and metabolites in urine, which correlate with the degree of improvement in liver function tests. To date, no serious or persisting adverse effects following administration of CA in therapeutic doses of up to 13 mg/kg body weight/day for up to 15 years have been reported in man (Gonzales *et al.* 2009). Consequently, formal safety studies examining the effects of CA on the cardiovascular, respiratory and central nervous system in animals defined in ICH Topic S7A Safety Pharmacology Studies for Human Pharmaceuticals (CPMP/ICH/539/00) have not been undertaken. Upon request the applicant provided the following review of the literature analysing several pharmacology studies that have previously reported the effects of CA on potential target systems.

Administration of CA (10 - 40 mg/kg i.v.) in the rat induced a negative chronotropic effect that was dose dependent *in vitro* and reduced by atropine or vagotomy suggesting both a vagally mediated and a direct effect on heart rate. The response was further diminished by ganglion blockade or decerebration (Joubert 1978). In feline isolated right ventricular papillary muscles low concentrations (3x10-9-3x10-7M) of CA induced a mild positive inotropic effect as well as an endocardial endothelium-dependent and β-receptor mediated positive inotropic response. Higher concentrations of CA (>3x10-7M) or prolonged exposure to a single low concentration (3x10-8M) caused extensive morphological damage to the endothelium (Colpaert *et al*. 1992).

Two separate studies examined the influence of bile acids, including CA, on the actions of isoprenaline, adrenaline, noradrenaline and acetylcholine on the rat autonomic system on the heart *in vivo* and *in vitro*, using an isolated blood vessel preparation (Kadlubowski *et al*. 1984) and isolated rat intestine (Szkudlinski, 1984). Kadlubowski *et al*. reported that bile acids reduced the stimulating effect of isoprenaline, adrenaline and noradrenaline on the rat heart. The spasmolytic effect of isoprenaline on blood vessels was reduced but, the vasoconstrictor effects of adrenaline and noradrenaline were enhanced. Furthermore, action of acetylcholine on the heart and blood vessels was reversed (Kadlubowski *et al*. 1984). In a separate study, using isolated rat intestine, bile acids attenuated the relaxant effect of isoprenaline and reversed the actions of adrenaline and noradrenaline. These changes were suggested due to bile acids stimulating α-adrenoceptors whilst reducing stimulation at β-receptors. The actions of acetylcholine on the intestine were enhanced (Szkudlinski, 1984).

Furthermore, a study investigating the effects of CA on systolic blood pressure (SBP) in rats, that received CA (80 mg/kg/day p.o.) for 30 days, reported an increased SBP from 95±9 to 123±10 mmHg (n=7) (Wu *et al*.1999) and increased mesenteric pressor responses to norepinephrine *in vitro*. It is not clear whether this effect was dose-related. The clinical relevance of these observations is unclear since increases in blood pressure following administration of lower doses of CA have not been reported in man.

Under normal conditions, the heart is exposed to concentrations of CA in the micromolar range, when CA is confined to the enterohepatic circulation. However, this is 10 to 1000-fold below the

concentrations used in the experiments described above. Only under conditions of cholestasis or continued CA acid administration does its concentration in peripheral blood approach these concentrations. Such situations are considered unlikely to persist due to the self-limiting toxicity of CA as evidenced by pruritus and diarrhoea as well as elevated serum bile acids, transaminases, and particularly GGT (Güldütuna *et al*. 1993; Gonzales *et al*. 2009). Precautions against chronic overdose have been specified in the SmPC (see section 4.8) and include regular monitoring of serum and urine bile acid levels as well as liver parameters.

Pharmacodynamic drug interactions

No studies were performed or identified in the literature detailing possible pharmacodynamic drug interactions of CA. Studies which describe the pharmacokinetic drug interactions with CA are discussed in the section below.

2.3.3. Pharmacokinetics

Absorption

The absorption of CA is well recognised and established. Following ingestion, absorption of CA will first be by the small intestine, and is then transported to the liver by the blood for further processing. Orally administered CA is absorbed by passive diffusion along the length of the gastrointestinal tract by virtue of its hydrophobicity (Hofmann & Hagey, 2008; Keating *et al*. 2009). Following oral administration of ^{14}C-cholic acid to rats, 85% of radioactivity was recovered in bile by 6 hours post-dose (Norman & Sjovall, 1958). More than 50% of administered radioactivity was recovered in bile within 32 hours of a dose injected into the caecum. Once absorbed, exogenous CA enters into the body's bile acid pool and is likely to undergo multiple cycles of enterohepatic circulation. Following intravenous administration of ^{14}C-cholic acid to healthy dogs (n=7), plasma concentrations of radioactivity declined rapidly in a biphasic manner, with median half-lives of 1.5 and 7.6 minutes for the first and second phase respectively (Bosje *et al*. 2005).

Distribution

In portal venous blood conjugated and unconjugated CA are 60-80% bound to protein (Hofmann & Hagey, 2008). Cholic acid is mostly bound to albumin in human (Rudman & Kendall, 1956) and rat serum (Fukuda & Iritani, 1981). Despite being highly albumin bound, bile acids, including conjugated and unconjugated CA, are extracted from portal blood (Hofmann, 2007). Hepatocellular uptake of conjugated CA is mainly mediated by sodium co-transporting polypeptide (NTCP) [>75%], while uptake of unconjugated CA is mainly mediated through Oatp1 and passive diffusion (Dawson *et al*. 2009; Hofmann & Hagey, 2008). Once inside the hepatocyte, unconjugated bile acids are conjugated with taurine or glycine in a species dependent manner. Conjugated bile acids are actively transported across the canalicular membrane via the bile salt excretory pump (BSEP) (Gerloff *et al*. 1998). Following a single intravenous administration of CA (29 mg) to a pregnant ewe, a significant increase was observed in the total bile acid concentration in foetal serum, indicating that despite a high hepatic extraction and a low volume of distribution, placental transfer of CA is achieved (Perez *et al*. 1994).

Metabolism

Unconjugated bile acids entering the liver are almost entirely conjugated with taurine and/or glycine via bile acid CoA:amino acid N-acyltransferase, forming glycocholic acid and taurocholic acid as well as glycochenodeoxycholic acid and taurochenodeoxycholic acid , before being secreted into bile. There are large species differences in the ratio of bile acids conjugated with either taurine or glycine, ranging

from exclusively with glycine in the rabbit to exclusively with taurine in the cat and dog (Zhang *et al*. 1992). Normal bile in the rat has a ratio of approximately 8:1 in favour of taurine conjugation, whereas in human bile the ratio is in favour of glycine conjugation.

Upon secretion into the intestine, bile acids are largely reabsorbed in the ileum to return to the liver via the portal blood. Primary bile acids that escape reabsorption into the colon are deconjugated and converted to secondary bile acids through the action of intestinal bacteria. The most important reaction in this context is the 7α-dehydroxylation where CA is converted to deoxycholic acid.

Excretion

Excretion studies in rat showed that CA is almost exclusively excreted in the faeces in the form of metabolite. Only minor amounts of CA were found in the unconjugated form in rat faeces. Urinary excretion of bile acids is minimal and in mice fed a 1% CA diet, the excretion of bile acids was 2000-fold higher in faeces than in urine (Soroka *et al*. 2011).

No data were presented on potential excretion of CA to milk in animals, however feeding pregnant and nursing mice carrying a targeted inactivation of the HSD3B7 gene 0.1% or 0.5% CA during late gestation and through postnatal day 28 resulted in an approximate doubling or normalisation, respectively, of the survival frequency of homozygous mutant offspring mice (Shea *et al*. 2007). This indicates that CA may be secreted in milk in mice.

Pharmacokinetic drug interaction

Oral administration of bile acids has been advocated as a mechanism for increasing the oral bioavailability of poorly absorbed drugs (Mikov *et al*. 2006). In most cases this will be achieved by enhanced micelle formation in the small intestine and hence the bile acids have been administered in conjunction with other typical components of mixed micelles in order to achieve optimal effect.

The membrane permeability of clofazimine in a rat gut perfusion model was shown to be enhanced by high concentrations of CA (80 mM), but not significantly altered at lower concentrations (O'Reilly *et al*. 1994). In rabbits, the systemic exposure to cefpirom following intra-duodenal administration (100 mg/kg) was increased approximately 5-fold by coadministration with CA (100 mg/kg) (Mrestani *et al*. 2003).

The enterohepatic circulation of bile acids involves several transporters and at least some of these also transport a variety of drugs (Hofmann, 2007). Inhibition of bile salt excretory pump (BSEP) has been implicated as a mechanism of drug-induced cholestasis, with several cholestatic drugs being shown to be competitive inhibitors of BSEP in vitro (Pauli-Magnus *et al*. 2005). Using drug-induced inhibition of fluorescent probe substrate transport across confluent monolayers of cells expressing BSEP, it has been shown that several drugs inhibit BSEP, with cyclosporin A being the most potent tested (IC50 = 8 μM) (Wang *et al*. 2003). Cyclosporin A, along with other BSEP inhibitors such as troglitazone and pravastatin, have been known to be responsible for clinical cases of drug-induced cholestasis, however, in the case of pravastatin it has been questioned whether the relatively modest BSEP inhibition could be responsible for clinical effect (Hirano *et al*. 2005).

2.3.4. Toxicology

Nonclinical toxicology of CA was reviewed in a series of publications. The GLP status of the studies reviewed cannot be verified and in some cases pre-date implementation of this requirement.

Single dose toxicity

No studies on the effects of CA after a single administration in animals have been performed or identified in the literature. A single *in vitro* study investigating cytotoxicity of CA to hepatocytes has been provided. In this study (Delzenne *et al*. 1992), isolated rat hepatocytes, at a density of $0.5x10^6$ hepatocytes/mL, were incubated with either CA, deoxycholic acid (DCA) or lithocholic acid at concentrations of 1 to 5 mM for 45 minutes. The percentage of lactate dehydrogenase (LDH) leakage into the medium was measured as an index of membrane integrity. Cholic acid caused a progressive and time-dependant lytic effect at increasing concentrations between 1 and 5 mM, but was significantly less toxic than DCA.

Repeat dose toxicity

No new repeated-dose toxicity studies with CA were conducted. A review of the published literature (seven studies) has been supplied examining effects in rats, hamsters, rabbits and primates.

Table 1 Repeated dose studies with cholic acid

Species	Route	Duration of Dosing	Doses	Gender and No. per Group	Reference
Rat	Oral (diet)	10 days	1.25 or 5mmol/kg (ca 500 or 2000 mg/kg/day)	M/No. not specified	Saiful Islam *et al.* 2011
Rat	Oral (diet)	2 weeks	0.5 or 1% (ca 500 or 1000 mg/kg/day)[1]	M/10	Delzenne *et al*. 1992
Rat and Hamster	Oral (diet)	3 weeks	0.1% (ca 100 mg/kg/day)[1]	F & M No. not specified	Spady *et al.* 1986
Rat	Oral (diet)	30 days	80 mg/kg/day[1]	M/14	Wu *et al.* 1999
Rat and Rabbit	Oral (diet)	8 weeks	1% (ca 1000 mg/kg/day)[2]	M/8-11 M/5-9	Rosenman *et al.* 1953
Rat	Oral (diet)	26 weeks	1% (ca 250 mg/kg/day)	M/ No. not specified	Deschner *et al.* 1981
Primate (rhesus monkeys)	Oral (diet)	15 weeks	20 mg/kg/day	Gender not specified/3	Webster *et al.* 1975

Saiful *et al*. 2011 showed significant reductions on epididymal adipose tissue and two other adipose tissue was observed in rats fed 5 mmol/kg CA (equivalent to 2000 mg/kg/day). This appeared to be associated with increased energy expenditure due to thyroid hormone activation by taurocholic acid which was found at a high concentration in the faeces of rats. Serum adiponectin was also increase at both 1.25 mmol/kg (equivalent to 500 mg/kg/day) but did not manifest in an inflammatory reaction on the large intestinal mucosa. No clinical or histological (caecum & colon) effects were observed in this study. No effect on body weight was noted.

In the Delzenne *et al*. 1992 publication, DCA was the most hepatotoxic and cholestatic compound while CA represented the least hepatotoxic and cholestatic compound of the three examined. Significant elevations of transaminases and total serum bile acids were only seen in rats fed with a diet supplemented with 1% CA.

Spady *et al*. 1986 examined the effects of different bile salts on receptor dependent and independent LDL uptake in the liver and intestines. The authors concluded that while CA has no acute, direct effect on rates of receptor-dependent LDL transport or cholesterol synthesis, it can alter these processes indirectly by inducing changes in cholesterol balance across the liver. The applicant argued that while elevated plasma LDL-cholesterol levels caused by reduced hepatic receptor-dependent LDL uptake after CA administration in human might be considered a long term risk to health, no changes in LDL-cholesterol levels were observed in 2 week clinical study in human volunteers, nor were there any safety issues reported in long term treatment of patients with CA.

Wu *et al*. 1999 showed that systolic blood pressures were increased in Wistar rats treated with CA for 1 month. Pressor responses in isolated mesenteric arteries were significantly higher in the arteries treated with CA while the production of aldosterone in mesenteric artery perfusates treated with CA was decreased, but that of corticosterone was increased compared to those of controls. The author concluded that the altered autocrine system in vessels may potentiate the development of hypertension induced by CA.

In the Rosenman *et al*. 1953 publication hypercholesteremia and increased plasma phospholid levels were evident in rats treated with both CA and cholesterol together – CA by itself did not induce such increases. No atherosclerosis was seen in any of the rat aortas examined. In rabbits however, moderate hypercholesteremia was seen in animals receiving CA alone and was associated with scattered atherosclerotic plaques in the aortas examined.

In Webster *et al*. 1975, rhesus monkeys gained weight during the treatment period but with the lack of contemporaneous controls, no effect of CA treatment could be discerned. Biliary bile acid composition was significantly altered compared to control level, DCA was found to be 10-fold higher than untreated controls, while the level of CA was found to be around 50% of control levels. No increase in serum triglycerides or serum cholesterol was evident and liver morphology was unaffected by treatment.

Overall, no evidence of toxicity was observed in repeat dose oral dietary studies in rats (up to approximately 1000 mg/kg/day, approximately 50 times the therapeutic dose in humans), approximately 100 mg/kg/day in hamsters (approximately 5 times the therapeutic dose), approximately 500 mg/kg/day in rabbits (approximately 25 times the therapeutic dose) and at approximately the therapeutic dose (20 mg/kg/day) in primates. Limited parameters were investigated in these studies and histology was often not performed. However, these data support the administration of CA orally for up to 26 weeks during which time CA was well tolerated with no mortalities, no apparent effects on bodyweight or food consumption and no evidence of significant macroscopic or microscopic findings in the liver.

Genotoxicity

The applicant has performed no new genotoxicity studies for CA and has provided a review of available literature for CA.

In vitro

In Watabe and Bernstein *et al*. 1985 the authors concluded that CA, deoxycholic acid and chendeoxycholic acid were mutagenic for both TA98 and TA100, however, from the data presented,

the number of revertant colonies did not increase in a dose-dependent manner, and often the highest dose level tested was actually comparable with control. Vernit *et al*. 1987 showed that at the concentrations tested, there were no dose-related, statistically significant increases in mutagenicity compared to controls for both TA98 and TA100. These results did not support the claim of Watabe and Bernstein, 1985, that these bile acids are mutagenic. Mori *et al.* 1991 tested CA in the fluctuation test at concentrations up to 75 µg/mL using 50/50 ethanol/water, ethanol and DMSO as solvents and showed no evidence for mutagenicity with TA98 and TA100. Overall, the weight of published evidence would point to CA not being mutagenic. It is acknowledged that genotoxicity studies were not completed according to OECD 471 or ICH S2 (R1) guidelines. Irrespective of whether the genotoxicity testing was conducted fully in accordance with these guidelines, the dose of CA to be administered to the patients is intended to restore a concentration that is equivalent to that physiologically present in healthy humans. Therefore any perceived genotoxic risk from CA and deoxycholic acid to the patients would be equivalent to that of a normal healthy adult that produces these bile acids intrinsically. Overall, CA showed non significant mutagenic activity in a battery of genotoxicity tests performed *in vitro*.

In vivo

No *in vivo* data has been identified in the literature. Cholic acid being an endogenous substance it cannot be considered to be genotoxic at physiological concentrations and the lack of *in vivo* genotoxicity data is justified. According to the "Note for guidance on genotoxicity (ICH S2(R1) or EMEA/CHMP/ICH/126642/2008) there are compounds for which standard *in vivo* tests do not provide additional useful information. Cholic acid can be considered such a compound, in addition CA has been shown to not interact with DNA via adduct formation (Scates *et al*. 1995).

Carcinogenicity

No long-term oral carcinogenicity studies of CA or other bile acids have been identified in the literature. A study was presented in a carcinogenicity model where a known carcinogen was given to initiate the formation of tumours. In McSherry *et al*. 1989, ten groups of male Fischer 344 rats were fed a basal diet supplemented with CA (0.2%) and/or calcium (1.6%) throughout the 28 week study period. On study Days 1 and 4, all animals were administered intrarectally with either N-Methyl-N-nitrosourea (MNU) at 2mg/dose or saline (control). At the end of the 28 week period, no tumours were observed in the groups receiving only saline, which included a group supplemented with CA alone. CA did however increase the incidence of tumour formation from 55% to 80% in the MNU treated animals.

Overall, the results showed a carcinogenic promotion potential that is carcinogen dependent. No carcinogenic effects were seen under CA treatment without the addition of the carcinogen. Despite this, it is not possible to eliminate bile acids from human metabolism as they play a vital role. Elevated gastrointestinal bile acid concentrations are probably best regarded as a potential risk factor for gastrointestinal cancer. In the case for treatment to patients with inborn errors in bile acid synthesis the restoration of normal, functional enterohepatic circulation, absorption of fat and fat-soluble vitamins together with the long-term survival and avoidance of liver disease (risk of carcinogenicity in itself) largely outweighs the potential risk of carcinogenicity. Long-term monitoring of the risk for carcinogenicity is included as an element in the Risk Management Plan.

Reproduction Toxicity

No studies examining the effects on pre-natal, early embryonic and post natal development have been identified in the literature. Cholic acid has been showed to be present in foetal blood and to pass the placenta sheep (Perez *et al*. 1994; Campos *et al*. 1986). Administration of a single dose of CA

intravenously to pregnant ewes in late gestation demonstrated systemic exposure of CA in the foetus with no effect on the mortality and general wellbeing (cardiovascular parameters) of the foetus or the mother, and following birth the offspring appeared normal (Perez *et al*. 1994). When given after at the end of gestation, uterine contractions were observed coinciding with the increase in maternal and foetal plasma bile acid levels with labour occurring within 24 hours of administration. However, the foetuses delivered appeared normal. A similar study, in pregnant ewes (Campos *et al*. 1986), also demonstrated an increase in early deliveries (19-26 days early) following intravenous CA administration on Day 124 of gestation (normal gestation period 150 days). No nonclinical studies were identified investigating the effects of the use of CA during lactation and paediatric development. From a mouse study examining deficiency of the HSD3B7 gene, there is evidence that CA is secreted in murine milk and has beneficial effect in inborn error of primary bile acid synthesis (Shea *et al*. 2007).

Toxicokinetic data

No toxicokinetic data has been provided or identified in the literature. Due to the amount of clinical data available on CA, the absence of animal data is acceptable.

Local Tolerance

No studies were identified investigating local tolerance. As the administration roure is oral, dedicated studies are not considered relevant.

Other toxicity studies

Metabolites

Deoxycholic acid is the main metabolite of CA. Deoxycholic acid has for a number of years been recognized as highly hepatotoxic *in vivo* in rats and cytotoxic *in vitro* in cultured rat hepatocytes (Delzenne *et al.* 1992), probably by its potential to induce lipid peroxidation. Mori *et al.* 1991 confirmed that deoxycholic acid does not induce mutagenicity *in vitro* in 2 bacterial strains, but more recently, Bernstein *et al.* 2011a revealed that high physiologic concentrations of deoxycholic acid induce the formation of reactive oxygen species with increased DNA damage, mitotic aberrations and development of resistance to apoptosis *in vitro*. *In vivo* supplementation of 0.2% deoxycholic acid to the diet of mice for up to 10 months (resulting in levels of deoxycholic acid in the faeces similar to those of humans eating a high fat diet) induced colonic tumours indicating that deoxycholic acid can act as a carcinogen under certain conditions. Therapeutic administration of CA is intended to restore bile acid concentration equivalent to those physiologically present in healthy humans. Therefore any perceived toxicity risk from deoxycholic acid would be equivalent to that in healthy adults producing normal levels of bile acids intrinsically. Delzenne *et al* used dose levels in vivo of 0.5 and 1% of the daily diet which, taking average daily consumption for a rat to be 20 g, is equivalent to 0.1-0.2g/day, or 400-800 mg/kg/day; far in excess of the therapeutic CA dose of 10 15 mg/kg/day. The *in vitro* cytotoxicity reported by Delzenne *et al* was observed over a deoxycholic acid concentration range of 1-5 mM (1000-5000 µmol/L), again far in excess of the expected human plasma concentration of 1 µmol/L. These findings are therefore not considered relevant to CA supplementation for the treatment of inborn errors of bile acid metabolism. Furthermore, Gonzales *et al*. 2009 demonstrated that treatment with CA (13.4 mg/kg tapering to 6 mg/kg) in children with inborn errors of bile acid metabolism for an average of 12.4 years was well tolerated, safe and effective, with no evidence of tumour formation in liver biopsies.

Impurities

The limit for impurity methyl cholate has been tightened. Methyl cholate did not cause significant numbers of revertants of Salmonella strains TA1535, TA1537, TA1438, TA98 and TA100 in an Ames assay, indicating no potential for mutagenicity (McDonald *et al.* 1978). This is in excess of methyl cholate concentrations found at maximal daily dose of Kolbam. Kolbam is available in 50 mg and 250 mg capsules and will be administered at doses no higher than 15 mg/kg/day. To a 70 kg human this represents a maximum CA dose of 1050 mg and hence total daily intake of methyl cholate no higher than 2.1 mg. This is well below the ICH Q3 thresholds for toxicological evaluation and it is accepted that cholesterol synthesis of patients would not be adversely affected at this concentration.

At least 15 patients reported by Gonzales *et al.* 2009, have been treated, most of them for more than 10 years, with CA preparations that have contained between 0.4 to 0.5% of methyl cholate. No adverse events have been reported in this population at therapeutic doses of CA.

2.3.5. Ecotoxicity / environmental risk assessment

Given that CA is an endogenous bile acid and a physiological substance in mammals, it would occur naturally in the environment as faeces. Therefore, it is exempt from the requirement of an ERA in line with the "Guideline on the Environmental Risk Assessment of the medicinal products for human use" (EMEA/CHMP/SWP/4447/00).

2.3.6. Discussion on non-clinical aspects

The effects of CA on the metabolism and secretion of bile acids are well known. Metabolism of bile acids is tightly controlled via a negative feedback regulation of bile acid synthesis. The cytochrome P450 enzymes CYP7A1 (cholesterol 7α-hydroxylase) and CYP8B1 (sterol 12α-hydroxylase) that are involved in bile acid synthesis are regulated by negative feedback from bile acids such as CA. Mice deficient in HSD3β7 (the homolog of the human gene coding for 3β-HSD), saw survival rates increase after feeding with CA and vitamin supplements. The 3-hydroxyl group conferred by HSD3β7 is required to maintain the functional and regulatory properties of bile acids in mice, and this is similarly required in humans. In mice deficient with 12α-hydroxylase gene (CYP8B1) a similar finding was seen. Only one primary enzyme defect has been addressed, HSD3β7 deficiency, while the use of CYP8B1 is used as a surrogate model for CYP7B1 (Oxysterol 7α-hydroxylase) deficiency. Indeed polymorphisms of CYP8B1 have not been associated with any disease in man. Although, the CHMP considered that the pharmacology data to support the claimed indications for treatment of inborn errors of primary bile acid synthesis was very limited, it was acknowledged that patients with inborn errors of bile acid synthesis have received successful CA treatment for over 20 years.

No studies have been presented describing the secondary pharmacological effects of CA. Cholic acid being an endogenous substance, no secondary pharmacological effects is expected. No formal safety pharmacology studies have been performed with CA. The applicant has presented initially minimal study data to address safety pharmacology of CA, focussing predominantly on the cardiovascular findings from a rat study performed in 1999. Wu *et al*. 1999 have reported increased SBP and mesenteric pressor responses following administration of CA (80 mg/kg p.o.) for 30 days in the rat. However, the clinical relevance of these observations is uncertain since increases in blood pressure following administration of lower doses of CA have not been reported in man. During the procedure the applicant provided a comprehensive and acceptable review of the non-clinical findings for safety pharmacology. No other findings were identified. In addition, no concerns were raised from a safety pharmacology standpoint. In addition clinical experience for CA has been gained in patients and to date, no serious or persisting adverse effects following administration of CA in therapeutic doses of up to 15 mg/kg body weight/day have been reported in man.

The pharmacokinetics of CA is well recognised and established. The applicant provided satisfactory literature review concerning the absorption, distribution, metabolism and elimination of CA. No data has been presented on potential excretion of CA to milk in animals, however indications following feeding pregnant and nursing mice carrying a targeted inactivation of the HSD3B7 gene 0.1% or 0.5% CA during late gestation and through postnatal day 28 resulted in an approximate doubling or normalisation, respectively, of the survival frequency of homozygous mutant offspring mice (Shea *et al*. 2007). This indicates that CA is excreted in milk in mice. This was reflected in section 4.6 of the SmPC.

Cholic acid and drugs that strongly interact with BSEP (cyclosporine A, troglitazone and pravastatin), together both substances interfere with the transport of CA. Inhibition of BSEP has been implicated as a mechanism of drug-induced cholestasis. Actions of clofazimine (membrane permeability) and cefpirom appear to be enhanced with co-administration with CA. The SmPC contains appropriate warnings over drug-drug interactions and further non-clinical data are not needed.

Toxicities studies from published literature have been summarised and reviewed by the applicant in rats, hamsters, rabbits and primates. The studies reviewed are of small size and systematic dosing data is not available. No toxicokinetic data has been provided and the toxicity findings in the studies were not particularly detailed. The results presented showed that CA administered to animals was generally well tolerated with no evidence of mortalities, no major effects on bodyweight or food consumption and no evidence of significant macroscopic or microscopic findings in the liver. This was at doses up to 50-fold the therapeutic dose in humans for up to 26 weeks in duration. In general the target organs for toxicity for CA are in the enterohepatic circulation, the liver and the administration caused diarrhoea. Although not discussed by the applicant, in an eight month feeding study (Beasancon *et al.* 1970), in combination with a high-cholesterol containing diet, CA administration leads to increased liver weight and the formation of gallstones in mice. The basis for this toxic effect appears to be related to a particular combination of metabolically related substances. The formation of gallstones has been linked to the cholesterol super-saturation of bile provoked by this diet. Overall, the CHMP concluded that due to the amount of clinical data available on CA, the absence of robust animal data was considered acceptable.

A number of studies have shown conflicting evidence as to whether CA is genotoxic and whether it has mutagenic potential. The *Salmonella tyrphimurium* and Ames test has been used to evaluate the mutagenic potential of CA. The positive results seen in the fluctuation assay by Watabe and Bernstein have been challenged by Venitt *et al.* 1987 and Mori *et al.* 1991. It is acknowledged that genotoxicity studies were not completed according to OECD 471 or ICH S2 (R1) guidelines and this has been adequately justified by the applicant. Overall, CA showed non significant mutagenic activity in a battery of genotoxicity tests performed *in vitro*.

Animal models indicated that administered bile acids show carcinogenic promotion potential and that this is carcinogen dependent. No carcinogenic effects were seen under CA treatment without the addition of carcinogens. Despite this, it is not possible to eliminate bile acids from human metabolism as they play a vital role. Elevated gastrointestinal bile acid concentrations are probably best regarded as a potential risk factor for gastrointestinal cancer. In the case for treatment to patients with inborn errors in bile acid synthesis the restoration of normal, functional enterohepatic circulation, absorption of fat and fat-soluble vitamins and long-term survival largely outweighs the potential risk of carcinogenicity. As conservative approach, given the limited data provided the risk of carcinogenicity is added as potential risk in the RMP and the applicant will monitor the risk on the long term a part of the patients' registry.

Studies with high dose CA have been reviewed in sheep. There was no evidence of systemic exposure of CA in the foetus, and no effect of teratogenicity was observed. There was increased incidence of

early deliveries, however no further fertility, embryo-foetal of post natal developmental effects were observed. Further evidence, from hamsters (Siviero *et al*. 2008) and chickens (Verrett *et al*. 1980) showed no adverse or teratogenic effects to foetus following administration of CA. Overall, animal studies showed that CA did not induce any teratogenic effect or foetal toxicity. No data on the effects of CA on fertility are available. Clinical data from Gonzales *et al* (2009) included two women treated with CA therapy having four normal pregnancies.

2.3.7. Conclusion on the non-clinical aspects

The applicant submitted no new non-clinical study in support of this application. The nonclinical data have been compiled from published literature as CA is an endogenous product and has been investigated in animals and humans over a considerable number of years. Since 1992 CA has been administered to infants with inborn errors of bile acid synthesis and been shown to be an effective treatment. The extensive literature review of the pharmacology, pharmacokinetics and toxicology of CA was considered appropriate and acceptable to support the non-clinical aspect of Kolbam. For the majority of cited literature the GLP status of the studies cannot be verified as either they pre-date the implementation of GLP requirements or no information on GLP status was available. In view of the available data from use of CA in humans, both for the treatment of various medical conditions as well as non-medical use, and taken into account the endogenous nature of the substance, the CHMP considers the lack of confirmation of the GLP status acceptable.

The analysed non-clinical data from the literature reveal no special hazard for humans based on studies of safety pharmacology, repeated dose toxicity, genotoxicity, carcinogenic potential, toxicity to reproduction. The review of the studies has taken into account that CA is a physiological substance in animals and humans.

CA showed non significant mutagenic activity in a battery of genotoxicity tests performed *in vitro*. Data reviewed suggest that CA does not act as a carcinogen *per se* but as a promoter of carcinogenicity only following treatment with an alkylating agent. Evidence for a tumour-promoting potential of some bile acids, particularly deoxycholic acid, have been shown. Animal studies showed that CA did not induce any teratogenic effect or foetal toxicity. No data on the effects of CA on fertility are available. At therapeutic doses, no effect on fertility is anticipated. Animal studies did not indicate reproductive toxicity; however, one study showed that when administered intravenously to pregnant ewes in late stage gestation, CA appeared to induce premature labour. Any similar risk in pregnant women receiving the recommended oral dose of CA is unlikely. Slightly increased blood pressure was evident in rats after 30 days of CA at approximately 4 fold therapeutic dose with increased vasoconstrictor responses to norepinephrine, together with decreased levels of aldosterone and increased corticosterone, but no adverse clinical signs were observed. As conservative approach, given the limited data provided, the long-term monitoring for the potential risks of carcinogenicity, reproductive toxicity and elevated systolic blood pressure are included as an element in the Risk Management Plan.

Available data in animals have shown excretion of CA in milk. At therapeutic doses, no effects on the breast-fed newborn infant are anticipated. Cholic acid can be used during breast-feeding.

2.4. Clinical aspects

2.4.1. Introduction

GCP

The academic, investigator-initiated study CAC-91-10-10 and its sub-study CAC-92-8-19 were started in 1992, prior to the initiation of global GCP requirements. Although the program was subject to the institution's internal IRB, the studies were not conducted according to currently applied GCP standards. While the original protocols were reviewed and approved by the IRB, the amendments to the studies were only reported to the IRB via letters or changes to the informed consent documentation. No formal protocol amendment was provided until June 2010, after the data cut-off point for the final CAC-91-10-10 study report. Thus in order to verify the robustness of the data supporting this application the CHMP required a GCP inspection to be conducted. The inspection (INS/GCP/2012/016) identified 4 critical findings relating to data integrity, clinical study report, investigational medicinal products and reliability of laboratory data. There were 8 major findings relating to trial documents, sponsor oversight, protocol compliance, oversight of the follow up of trial patients, data management, efficacy source data, pharmacovigilance and monitoring. There were 2 minor findings relating to trial documents and data management. The principal outcome of the inspection was that the data and corresponding clinic study report (CSR) were not reliable, not complete, did not reflect the actual conduct of trial in sufficient details and were regarded as unsuitable for assessment.

During the procedure the applicant addressed the inspection findings and provided a revised CSR for which an extensive re-monitoring of the study data was performed resulting in an increased reliability of the data integrity. As a result of the re-monitoring, more patients could be included in the analysis. In addition, sensitivity analyses comprising additional data analysis sets and subpopulation analyses were performed to further investigate the robustness of the results. The results of the revised CSR are presented in this report. The limitations of this non-compliant academic trial, and start date has to be balanced against the orphan designation status of CA. Despite a non-optimal documentation of study data due to the early stage academic nature of the study and the long-term study period, the results presented consistently show improvements in all efficacy parameters analyzed and across the majority of subpopulations and analysis sets. Even given the sparseness of data documentation, GCP lapses and remaining uncertainties in data integration, the CHMP considered that this is the consistency and maintenance of improvement in patients' symptoms that provides evidence of the therapeutic efficacy of CA in the treatment of patients with inborn errors of bile acid synthesis (see efficacy section).

The applicant has provided a statement to the effect that clinical trials CAC-001-01 conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Study ID	Study Centres Location	Study Start Enrolment status, date Enrolment/ goal	Design	Study & Ctrl Drugs Dose, Route &Regimen	Study Objective	# subj by arm entered/ compl.	Duration	Gender M/F Median age (Range)	Primary Endpoint(s)
CAC-91-10-10 (inc. sub-study 92-8-19)	1 CCHMC Cincinnati, Ohio, USA	Jan, 1992 Completed Dec, 2009 85/open	Open-label, single arm, non-compar-ative	cholic acid, no ctrl about15 mg/kg/day oral	Efficacy & Safety	85 ITT 79 Safety 70 mITT	Up to 17 years	31/50 unknown Age at diagnosis 2+/- 4 years	Urinary bile acids, Liver function, Liver histology Height/weight

CAC-001-01	1 CCHMC Cincinnati, Ohio, USA	April, 2010 Completed Aug, 2010 16/open	Open-label, single arm, Cross-over	cholic acid, no ctrl 10-15 mg/kg/day oral	Compara-tive Efficacy & Safety	16/16	30 days	11/5 7.15 yr (0.6 – 20 yrs)	Urine & serum bile acids, Liver function

2.4.2. Pharmacokinetics

The applicant has provided a literature review of studies on bile acid kinetics and conducted a bioavailability study in order to show similarity between the formulations used in the pivotal trial CAC-91-10-10 and the formulation of Kolbam intended for marketing.

The kinetics and dynamics of CA have generally been studied as an endogenous molecule and to a much lesser degree as an exogenously administered pharmacological agent. Its pharmacokinetic characteristics and metabolism are different from that of a conventional synthetic small molecule in that once administered, any exogenous CA will behave like an endogenous molecule. This is particularly true since exogenous oral CA will simply enter the normal enterohepatic circulation of endogenous bile acids. Data on the kinetics and dynamics of endogenous CA thus provides relevant information on the clinical pharmacology of exogenously administered CA.

Cholic acid is subject to first-pass hepatic extraction and enterohepatic recirculation. To provide an accurate determination of the absorption kinetics, blood samples would be required from the portal vein, which is not feasible or ethical except during surgical interventions (Angelin *et al.* 1982). Peripheral plasma sampling is useful primarily for studying relative bioavailability and bioequivalence of bile acids that have a low endogenous concentration (Setchell, 2004).

The bile acid pool is largely confined within the enterohepatic circulation and there is poor systemic distribution, resulting in low total serum bile acid concentrations of about 1-12 µmol/L. The total bile acid concentration in human peripheral serum fluctuates in relation to meal intake between 2-5 µM during night time (fasting) lows to 10-16 µM during daytime highs (Everson, 1987). In the hepatic venous portal plasma (i.e. within the enterohepatic circulation), fasting CA concentration averaged 6.13 ± 2.57 µmol/L while maximum postprandial concentrations averaged 18.42 ± 4.17 µmol/L (Angelin *et al.* 1982). Cholic acid is present in serum and plasma of normal humans, including children and pregnant women (Barth *et al.* 2005), in a range beween 0.05-6.7 µmol/L. Values for normal children are broadly in the same range as for healthy adults. As noted above, serum bile acid concentrations vary considerably over the day and in relation to meal intake, so this broad normal range may be expected. Cholic acid concentration (as its glycocholic acid and taurocholic acid conjugates) in gallbladder bile is in the range of 4-74 mmol/L (Perwaiz *et al.* 2001) and it constitutes about 35% of gallbladder and hepatic bile (Ahlberg *et al.* 1981).

Absorption

Because it is hydrophobic, CA is absorbed rapidly by passive non ionic diffusion. Once absorbed, exogenously administered CA becomes part of the bile acid pool. The CA pool size has been measured in healthy adult volunteers using a stable isotope dilution/GLC/MS technique (Stellard *et al.* 1984; Everson, 1987; Koopman *et al.* 1988) and a microscale stable isotope dilution/GLC/electron capture negative chemical ionization-MS technique (Hulzebos *et al.* 2001). In a healthy adult of 60 kg body weight, the pool size of CA (and its conjugates) is approximately 600-750 mg.

Bioequivalence

Study CAC-003-001 title: Comparative bioavailability of three formulations of CA in healthy male subjects using a multiple dose repeated measures approach

The study was a single-centre Phase I study of multiple oral doses without wash-out to compare the bioavailability of a 250 mg oral capsule (Pharmacy capsule formulation used in the pivotal clinical study CAC-91-10-10) to a 250 mg capsule (cGMP capsule: intended commercial formulation of Kolbam) and to an oral solution or suspension (250 mg) at steady state using a fully randomised replicate dose design. The objective of this study was to evaluate the bioavailability and pharmacokinetics of multiple oral doses of CA in 18 healthy male volunteers. In addition, the safety of CA was assessed following multiple dose administration of CA.

The CA concentration-time profiles for the 3 formulations were similar. Comparison of Treatment B (cGMP capsule formulation) to Treatment A (Pharmacy formulation) demonstrated only slight differences in bioavailability. Comparison of the two oral 250 mg capsule formulations with the oral 250 mg solution (Treatment C) revealed significantly greater bioavailability for the capsules relative to the solution. In terms of CA, Treatment B (cGMP formulation) had slightly greater bioavailability (10%) when compared to Treatment A (Pharmacy formulation) with the 90% C.I. = 1.03 – 1.175 for AUCtau; whereas for 'total CA' the differences in AUCtau was only 5.7% with 90 % C.I. = 1.007 – 1.11. Assessment of the CA Cmax ratios for the cGMP capsule versus the Pharmacy capsule revealed a 90% CI that fell outside the 0.80 to 1.25 CI limits. A similar lack of bioequivalence for the cGMP capsule versus the Pharmacy capsule was seen for Total CA Cmax. Assessment of the CA and 'total CA' AUCtau ratios for the two oral capsules versus the oral solution revealed lack of bioequivalence with the 90% CIs falling outside the acceptable limits of 0.80 to 1.25. Assessment of the CA and 'total CA' Cmax Based upon the steady state levels of all 'active CA analytes' i.e., C ratios for the oral capsules versus the oral solution demonstrated bioequivalence for the Pharmacy capsule versus the solution (90% CI = 0.857 to 1.019) but not for the cGMP capsule versus the solution (90% CI = 1.059 to 1.26).

Based upon the steady state levels of all 'active CA analytes' i.e., Cmin and AUCtau values for 'total CA' the MAH concluded that these two capsule formulations (Treatment A and Treatment B) are equally bioavailable. With regards to CA levels, Treatment B (cGMP formulation) resulted in only a slightly greater level of CA (AUCtau ratio = 1.10), but a 17.9% and 23.7% greater Cmax value for 'total CA' and CA respectively when compared to Treatment A. These differences in Cmax levels are not clinically relevant since CA safety and efficacy does not depend upon rate of absorption and in clinical practice dose is based on titration to effect in the individual patient.

The recommended initial dosage for CA in treatment of patients with inborn errors of bile acid metabolism is a range from 10-15 mg/kg/day. Then, patients are appropriately managed by titration of the dose over time to achieve optimal ALT and suppression of toxic atypical bile acid intermediates. Therefore, the observed differences in the PK parameters of the two formulations are not of clinical significance in terms of safety or efficacy, and the data from prior use of the Pharmacy formulation is considered similar to the current cGMP formulation.

Influence of food

The effect of food on the bioavailability of CA has not been studied. Because of the hydrophobic nature of CA, it is theoretically possible that the presence of food may enhance the absorption, and thus the bioavailability, if both are administered concurrently. The day-to-day variation in the amount of a dose absorbed is expected to be mitigated by the long residence of bile acids in the enterohepatic circulation. It is also possible that tolerability may be improved when CA is administered with food (gastroesophageal reflux and diarrhoea have been reported uncommonly in clinical studies). It

therefore recommended administering CA capsules together with food as reflected in section 4.2 of the SmPC.

Distribution

The volume of distribution of exogenously administered CA has not been characterized because of its enterohepatic distribution along with other bile acids. Bile acid kinetics have been reported in terms of pool size and fractional turnover rate (Crosignani *et al.* 1996). Conventional calculation of volume of distribution has been reported as 1.879 ± 0.054 L/m^2 (Gilmore and Thompson, 1980). The bile acid pool is largely confined within the enterohepatic circulation and there is poor systemic distribution, resulting in low serum bile acid concentrations (about 5 µM) (Gilmore and Thompson, 1980; Everson, 1987; Crosignani *et al.* 1996). Serum bile acid concentrations fluctuate during the day as a function of meal intake and bile secretion (Everson, 1987). In untreated patients with inborn errors of bile acid synthesis, total serum bile acid concentrations are usually in the normal range or lower (Gonzales *et al.* 2009). However due to the metabolic defect in these patients, primary bile acids, including CA, are absent or present only at trace concentrations. This is resolved after treatment with primary bile acids, when the administered bile acids and their secondary metabolites predominate (Ichimiya *et al.* 1991; Clayton *et al.* 1995; Gonzales *et al.* 2009). Plasma has a very high binding capacity for bile acids and the binding shows rapid equilibrium. Serum albumin binds approximately 95% of the total CA concentration via the primary site for CA.

Elimination

The metabolism of exogenously administered CA in patients with inborn errors of bile acid synthesis is not expected to differ qualitatively that in normal subjects. The metabolism of CA to secondary bile acids takes place in the intestine by intestinal bacteria and is not affected by hepatic enzyme defects, i.e. is the same in normal subjects and patients with enzyme defects. In patients with inborn errors of bile acid synthesis who are administered CA, urine and bile contained predominantly CA and deoxycholic acid (Gonzales *et al.* 2009).

Cholic acid clearance has been reported in healthy volunteers and cirrhotic patients (Kaye *et al.* 1973). In both groups, during fasting, the curve for disappearance of radioactivity from the serum during the first 100 min after [^{14}C] CA injection was double-exponential in form. During the early phase, clearance was significantly more rapid, and concentrations of conjugated and free bile acid were significantly lower in healthy volunteers than in cirrhotic patients. Radioactivity disappeared from the systemic circulation of healthy volunteers within 3 hours. Intravenous and oral clearance of CA was quantified and found to be 271 ± 15 ml/min/m^2 and 1248 ± 104 ml/min/m^2, respectively (Gilmore and Thompson, 1980). In patients with various hepatic diseases (cirrhosis, icteric and anicteric liver disease, hepatitis), these clearances were significantly reduced.

The half-life of CA is approximately 27 hours (Crosignani *et al.* 1996). Approximately 5% of the bile acid pool enters the colon and provides a substrate for intestinal microbes, leading to formation of the secondary bile acids deoxycholic and lithocholic acids that are the major bile acids in faeces. The daily excretion of urinary bile acids was determined to be 7.0 ± 0.8 µmol per 24 hours and was independent of the 24-hour urine volume (Alme *et al.* 1977). In this study, CA comprised 2-7% of the total bile acids. The remainder of the total urinary bile acids constituted a broad spectrum of other bile acid metabolites. Other studies reported a lower urinary excretion of bile acids (<1µmol/day) (Bernstein *et al.* 2005). Given a daily total synthesis rate of approximately 10 µmol/kg body weight per day which quantitatively replaces the amount lost by excretion, renal excretion is therefore a minor (≤1%) elimination route for CA and its metabolites. Overall, in subjects with normal liver function, bile acids

including CA are excreted in the urine in negligible amounts (Alme *et al.* 1977). In the context of cholestasis, when plasma bile acid concentrations are increased, renal excretion is also significantly increased. This is the case in untreated patients with 3ß-HSD and Δ^4-3-oxoR deficiencies. Bile salts, including cholate, are a normal component of human breast milk (Forsyth *et al.* 1983).

Dose proportionality and time dependencies

This has not been specifically studied or discussed. No discussion is required as this issue does not generate safety concerns.

PK in target population

No population PK studies have been provided for CA in the treatment of the claimed bile acids deficiencies. Cholic acid dosage is established individually for each patient based on their pharmacodynamic response, both at the initiation and regularly during maintenance of treatment. Therefore such studies are not considered as providing any additional information for the dosage recommendations or the safe and effective use of CA, regardless of possible inter- and intrasubject variations in pharmacokinetics.

Special populations

Impaired renal function

There are no reports on the use of CA for the treatment of inborn errors of bile acid metabolism in patients with renal impairment in CAC-91-10-10 or in the published literature. Under normal physiological conditions, excretion of bile acids occurs almost exclusively in the faeces. Accordingly, bile acids including CA are excreted in the urine in negligible amounts (Alme *et al*. 1977) and compromise of renal function would not be expected to result in systemic accumulation and toxicity. In case of cholestasis, such as in patients with untreated inborn errors of bile acid metabolism, serum concentrations of bile acids are increased and renal excretion is proportionately increased. For patients with inborn errors of bile acid metabolism that are successfully treated with CA, the development of renal failure may have little to no impact on systemic bile acid concentrations. However, these patients should be carefully monitored and the dose of CA titrated individually.

Impaired hepatic function

Cholic acid is synthesized endogenously in the liver and metabolized to secondary bile acids in the intestine by intestinal bacteria. Metabolism to secondary bile acids is therefore unaffected by the hepatic enzyme defects in patients with inborn errors of bile acid metabolism. Patients who receive treatment with exogenous CA are not expected to differ qualitatively in their bile acid catabolism from normal subjects as their metabolic defect is in primary bile acid (CA) synthesis.

Reduced clearance of bile acids, including CA and glycocholic acid, in patients with hepatic impairment of various etiologies has been reported by multiple investigators (de Caestecker *et al.* 1995; Engelking *et al.* 1979; Gilmore and Thompson, 1980; Luey and Heaton, 1979;
Gilmore and Thompson, 1981). The degree of reduction in clearance appears related to the severity of the hepatic impairment, i.e. greater reduction in icteric liver disease and less in anicteric disease. The decrease in clearance results in elevated serum concentrations of bile acids.

The majority of patients with inborn errors of bile acid metabolism that have been treated with CA presented with some degree of hepatic impairment at the time of diagnosis (CAC-91-10-10; Gonzalez

et al. 2009). In most patients, the hepatic impairment improved or resolved with treatment. Accordingly, the evaluation of the safety of CA includes patients with this metabolic impairment.

No data regarding CA treatment were available in patients with inborn errors of bile acid metabolism that have hepatic impairment unrelated to their primary disease (CAC-91-10-10; Gonzalez *et al.* 2009). If such patients are encountered, decreased hepatic clearance of bile acids and/or cholestasis could occur, leading to increased serum concentrations of CA and symptoms/signs of toxicity. The CA dose should be reduced which could result in inadequate suppression of the biosynthesis of the hepatotoxic and cholestatic bile acid metabolites and recurrence of the manifestations of the primary disease. As such, in the absence of clinical experience in patients with hepatic impairment from causes other than the genetic bile acid enzyme deficiencies, no recommendations on dosage adjustment can be made. It is essential that patients with hepatic impairment unrelated to their primary disease that are treated with CA be monitored closely.

Familial hypertriglyceridemia

Type IV hyperlipoproteinemia, in particular familial hypertriglyceridemia, has been associated with defective bile acid absorption (Angelin *et al*. 1978). The possibility exists that there may be patients with both inborn errors in primary bile acid synthesis and familial hypertriglyceridaemia (estimated to occur at the rate of 1 in 5,000,000 to 1 in 7,000,000 live births). Patients with newly diagnosed or a family history of hypertriglyceridemia are expected to poorly absorb CA in the intestine. The CA dose for patients with familial hypertriglyceridemia will have to be established and adjusted, but an elevated dose may be required and safe.

Pharmacokinetic interaction studies

No drug-drug interaction studies with CA and concomitantly administered medications have been carried out. The relevant drug interactions for CA are expected to be those that are relevant for the other bile acids, chenodeoxycholic acid and ursodeoxycholic acid.

Drug interactions with CA mainly relate to agents capable of interrupting the enterohepatic circulation of bile acids, such as the sequestering agents cholestyramine, colestipol, or colesevalem (Gallaher and Schneeman, 1986). Administration of CA concomitantly with bile acid sequestrants would be expected to reduce the efficacy of both. Aluminium-based antacids have been shown to adsorb bile acids *in vitro* and may be expected to reduce the bioavailability of CA in the same manner as the bile acid sequestering agents (Mangnall *et al.* 1986). Because of this, if either bile acid sequestrants or aluminium containing antacids and CA are required to be taken concomitantly, it is recommended that the substance must be taken at least 5 hours before or after CA in order to minimise the interaction.

In humans, phenobarbital has been shown to increase the pool size and turnover of CA (Miller and Nestel, 1973). Thus, phenobarbital antagonises the effect of CA and may endanger the established metabolic control.

Ciclosporin alters the pharmacokinetics of CA by inhibition of the hepatic uptake and hepatobiliary secretion of bile acids (Azer and Stacey, 1994), and also alters its pharmacodynamics by inhibiting cholesterol 7α-hydroxylase. As decreased uptake of CA into hepatocytes will decrease its efficacy, concomitant administration with ciclosporin should be avoided, if possible.

Oestrogens, oral contraceptives, and clofibrate (and perhaps other lipid-lowering substances) increase hepatic cholesterol secretion and hence may counteract the effectiveness of CA (Okolicsanyi *et al*. 1986). Any medicinal products implicated in drug-induced cholestasis through inhibition of transporters could reduce the effectiveness of CA treatment on co-administration.

2.4.3. Pharmacodynamics

Mechanism of action

No pharmacodynamics studies have been conducted with CA by the applicant. Treatment with exogenous CA is intended to replace this physiological bile acid in cases of inborn errors of bile acid synthesis. Cholic acid and CDCA are the primary bile acids in man on which essential physiological functions depend. The purpose of substituting missing CA is to restore the main functions of this bile acid consisting of lipid transport in the form of mixed micelles, the activation of co-lipase and fat digestion and absorption, the absorption of fat-soluble vitamins, and the induction of bile flow, thus preventing cholestasis. Primary bile acids down-regulate their own biosynthesis via activation of farnesoid X receptor, which represses transcription of the CYP7A1 gene encoding cholesterol 7α-hydroxylase, the rate-limiting enzyme of bile acid synthesis (Gonzales *et al*. 2009). The rationale for CA therapy is improvement of bile flow and fat absorption and restoration of a physiologic feedback inhibition on bile acid synthesis, lowering the production of toxic bile acid precursors.

Primary pharmacology

Bile acids have a multitude of functions as shown in Table 2 below. The individual contribution of different primary bile acids and their various conjugates have only been partially elucidated. Cholic acid and its conjugates appear have a major function in most, if not all of the functions (Balistreri, 1991).

Table 2 Physiological functions of bile acids

A. In the liver
1. Generate bile flow through upregulation of bile acid transporters 2. Induce biliary lipid secretion 3. Modulate cholesterol homeostasis through regulation of synthesis and catabolism 4. Regulation of lipoprotein production
B. In bile
1. Desaturate bile cholesterol – reduce lithogenic potential 2. Transport cholesterol 3. Buffer Ca 2+ ion
C. In the intestine
1. Form micelles (fat digestion) 2. Accelerate lipid transport, including fat-soluble vitamin (A, D, E) absorption 3. Modulate motility 4. Modulate GI hormone output 5. Induce ion (and water) secretion

The effects of oral CA administration on the bile acid composition of bile or luminal content have been studied in healthy volunteers and in patients with gallstones or liver disease, all of whom had overtly normal bile acid metabolism. The administered dose was 15 mg/kg per day in two of the studies, 4.7-11.6 mg/day in one study, and 750 mg/day in one study, all of which are similar doses. In all studies, CA administration shifted the relative composition of bile or intestinal luminal content towards CA and its metabolite deoxycholic acid, while reducing the relative amount of chenodeoxycholic acid (Toouli *et al.* 1975; Einarsson and Grundy, 1980; Ahlberg *et al.* 1981; Woollett *et al.* 2004). Considering the available data on the regulation of primary bile acid synthesis, it is apparent that the reduction in CDCA and its metabolites is due to suppression of primary bile acid synthesis by the exogenously administered CA.

In contrast, exogenous CA treatment of patients with primary biliary cirrhosis did not alter the relative concentrations of bile acids in serum (Guldutuna *et al.* 1993). However, absolute bile acid concentrations did increase to more than twice the normal level. This is consistent with the significant

increase of primary bile acid pool size after oral administration of CA or CDCA to healthy volunteers (Woollett *et al.* 2004).

In children affected by primary bile acid synthesis deficiency, the rationale for the therapeutic approach is that oral administration of CA will inhibit endogenous production of hepatotoxic bile acid precursors produced as a consequence of the inborn error by down-regulating cholesterol 7ɒ-hydroxylase, the rate limiting enzyme in bile acid synthesis (Setchell and O'Connell, 2007). In addition, CA administration provides a stimulus for bile flow which facilitates the hepatic clearance of toxic bile acid precursors and other toxic substances, including bilirubin. In addition, CA treatment improves growth by facilitating intestinal absorption of fat-soluble vitamins and fats.

Secondary pharmacology

No human studies have described secondary pharmacological effects of CA treatment. Given that CA is a naturally occurring molecule whose absence is replaced at physiologic levels in patients with primary bile acid synthesis deficiencies, no typical secondary pharmacological effects would be expected. In general, exogenous administration of CA to animals in repeat toxicology studies identified organs of enterohepatic circulation as the targets for CA toxicity and did not provide any evidence of secondary pharmacological effects. Safety pharmacology studies demonstrated effects on cardiac function only at 100 to 1000-fold the endogenous CA concentration. Moreover, the use of CA (up to 15 mg/kg body weight/day) to treat patients with inborn errors of bile acid synthesis has not been associated with any serious or persisting adverse effects on organ function. The absence of studies investigating secondary pharmacological effects of CA is therefore considered acceptable.

2.4.4. Discussion on clinical pharmacology

The pharmacological data have been mainly compiled from published literature. During the procedure the applicant provided upon request of the CHMP a more in depth literature review for the pharmacokinetics and pharmacodynamics allowing a greater characterisation of CA than initially provided. Cholic acid is an endogenous product and has been used in humans over a considerable number of years. Since 1992 CA has been administered to infants with inborn errors of bile acid synthesis and been shown to be an effective treatment. The extensive literature review of the pharmacokinetics and pharmacodynamics of CA was considered appropriate and acceptable to support the pharmacological aspect of Kolbam.

Cholic acid has been studied in the literature in terms of its kinetics and dynamics, and to a lesser degree as an exogenous pharmacological agent. In this regard a pharmacokinetic study could have been useful. Since the pharmacological effects of bile acids such as CA are mainly limited to the enterohepatic circulation rather than plasma, serial sampling of the bile pool would give the most meaningful pharmacokinetic data. However, the procedure is highly invasive and not ethical to perform on a serial basis. For this reason pharmacokinetic studies such as these have not been performed for treatment with oral CA capsules. The pharmacokinetic characteristics of Kolbam, including its metabolism, are different from that of a conventional synthetic small molecule pharmaceutical in that once administered, any exogenously administered CA will behave like an endogenous molecule in all respects. Therefore data on the kinetics and dynamics of endogenous CA provided relevant information on the clinical pharmacology of Kolbam.

Distribution and pharmacological effects of bile acids such as CA are mainly limited to the enterohepatic circulation, which includes the intestine, portal vein, liver and biliary tract. Orally administered CA is well absorbed by passive diffusion along the length of the gastrointestinal tract.

Once absorbed, exogenous CA will enter into the body's bile acid pool and is likely to undergo multiple cycles of enterohepatic circulation. Cholic acid will pass to the liver in the portal blood, in which it is moderately bound to albumin. In the liver, CA is efficiently extracted from portal blood by multiple mechanisms, including passive diffusion and transporters. Within the liver, CA is amidated in species-specific proportions, with glycine and/or taurine, into a more hydrophilic, conjugated form. Conjugated CA is secreted into bile and will pass in due course into the small intestine where, in association with other components of bile, it will perform its principal digestive function. Conjugated CA is efficiently absorbed in the ileum via transporters, passed back to the liver and enters another cycle of enterohepatic circulation. Any conjugated CA not absorbed in the ileum will pass into the lower intestine where it may be subject to bacterial metabolism, principally deconjugation and 7-dehydroxylation. Deconjugated CA and deoxycholic acid, the product of 7-dehydroxylation, are passively absorbed in the lower intestine and carried back to the liver in portal blood, where reconjugation and, in some species, 7-hydroxylation will take place. In this manner the vast majority of the bile acid pool is conserved and will cycle multiple times during feeding. Any CA not absorbed will be excreted in the faeces, either unchanged or following dehydroxylation via bacterial metabolism.

The preparations of CA have changed over the 18 years of the trial period. Originally batches were extemporaneously manufactured without excipients as capsules containing only the active ingredient. From approximately 1997 the formulation changed until patients were gradually transitioned to the to-be-marketed formulation beginning 2010. In order to show similarity between the formulations used in the past in the pivotal trial and the formulation that is intended for marketing the applicant conducted a bioavailability Study CAC-003-001. This was a single-centre Phase I study of multiple oral doses to compare the bioavailability of a 250 mg oral capsule (Pharmacy capsule formulation used in the pivotal clinical study CAC-91-10-10 to a 250 mg capsule (intended commercial formulation of Kolbam) and to an oral solution or suspension (250 mg). The two oral capsule formulations had similar PK profiles however the strict criteria for bioequivalence were not met. In addition, despite having similar PK profiles, neither of the capsule formulations met the criteria for bioequivalence to the oral solution. The recommended initial dosage for CA in treatment of patients with inborn errors of bile acid metabolism is a range from 10-15 mg/kg/day. Thereafter, patients are appropriately managed by titration of the dose over time to achieve an optimal clinical response. Therefore, the CHMP concluded that the observed differences in the PK parameters of the two formulations are not of clinical significance in terms of safety or efficacy. The applicant has provided acceptable explanation which shows similarity between the formulation used in the past in the pivotal trial and the formulation that is intended for marketing.

CA is the predominant primary bile acid in man. In patients with inborn errors of primary bile acid synthesis, the biosynthesis of primary bile acids is reduced or absent. In the absence of treatment, unphysiologic cholestatic and hepatotoxic bile acid metabolites are predominant in the liver, serum and urine. The rational basis for treatment consists of restoration of the bile acid-dependent component of bile flow enabling restoration of biliary secretion and biliary elimination of toxic metabolites; inhibition of the production of the toxic bile acid metabolites by negative feedback on cholesterol 7α-hydroxylase, which is the rate-limiting enzyme in bile acid synthesis; and improvement of the patient's nutritional status by correcting intestinal malabsorption of fats and fat-soluble vitamins.

No data are available for patients with renal impairment. Bile acids including CA are excreted in the urine in negligible amounts and compromised renal function would not be expected to result in systemic accumulation and toxicity. For patients with inborn errors of bile acid metabolism that are successfully treated with CA, development of renal failure may have little or no impact on systemic bile acid concentrations. However, these patients should be carefully monitored and the dose of CA titrated individually as reflected in section 4.2 of the SmPC.

The majority of patients with inborn errors of bile acid metabolism that have been treated with CA presented with some degree of hepatic impairment at the time of diagnosis; in most patients, the hepatic impairment improved or resolved with treatment. The dose of CA should be adjusted individually. No experience exists in patients with hepatic impairment from causes other than their primary disease and no dose recommendation can be given. Patients with hepatic impairment should be monitored closely.

Patients with newly diagnosed or a family history of familial hypertriglyceridaemia are expected to poorly absorb CA from the intestine. The CA dose for patients with familial hypertriglyceridaemia will have to be established and adjusted as necessary; an elevated dose may be required in order to suppress urinary bile acids.

Phenobarbital antagonises the effect of CA. Use of phenobarbital with CA is therefore contraindicated. Ciclosporin alters the pharmacokinetics of CA by inhibition of the hepatic uptake and hepatobiliary secretion of bile acids, as well as its pharmacodynamics by inhibition of cholesterol 7α-hydroxylase. Co-administration should be avoided. If administration of ciclosporin is considered necessary, serum and urine bile acid levels should be closely monitored and the CA dose adjusted accordingly. Drug interactions with CA mainly relate to medicinal products capable of interrupting the enterohepatic circulation of bile acids, such as the sequestering agents cholestyramine, colestipol, or colesevalem. Aluminium-based antacids have been shown to adsorb bile acids in vitro and may be expected to reduce the levels of CA in the same manner as the bile acid sequestering agents. Should the use of a preparation containing one of these substances be necessary, it must be taken at least 5 hours before or after CA. Any medicinal products implicated in drug-induced cholestasis through inhibition of transporters could reduce the effectiveness of CA treatment on coadministration. In these cases, serum/bile levels of CA should be closely monitored and the dose adjusted accordingly as reflected in the product information.

2.4.5. Conclusions on clinical pharmacology

Overall, the clinical pharmacology data submitted were considered satisfactory by the CHMP.

2.5. Clinical efficacy

The clinical programme comprised one main study (Study CAC-91-10-10) supported by a second study (Study CAC-001-01) which was a subset of the main study. In addition, the findings of these studies were supplemented by study CAC-002-001 (continuation study of studies CAC-91-10-10 or CAC-001-01) and clinical studies reported in the literature.

2.5.1. Dose response study

No formal dose ranging studies have been conducted. The dose of CA administered to patients with genetic defects in bile acid synthesis was based upon the need to adequately down-regulate endogenous bile acid synthesis that otherwise will continue to lead to the accumulation of atypical bile acids that are intrinsically hepatotoxic, and to completely replace these atypical bile acids with a normal primary bile acid pool size. After absorption from the distal intestine, bile acids are returned to the liver and secreted into bile. This enterohepatic circulation leads to the accumulation of a bile acid pool that is about 2-4 g in normal adults. Because the pool cycles 10 to 12 times/day, stimulated by postprandial gallbladder contraction, the effective pool size is much greater (Heubi *et al.* 2007). Conservation of the bile acid pool occurs by an efficient reabsorption, principally from the small intestine, and an effective hepatic extraction from the portal venous circulation so that each day less

than 5% is lost in the stool. This bile acid loss is compensated for by hepatic synthesis of newly formed bile acids. Although the term and pre-term neonate are born with a relatively reduced, size-corrected bile acid pool, rapid expansion of the pool in the first few months of life ensures adequate intraluminal concentrations for fat and fat-soluble vitamin absorption and promotion of bile flow (Heubi *et al.* 2007). Therefore, in order to substitute a new pool of bile acids in patients that do not synthesise bile, it is necessary to at least administer an amount equivalent to, or above the daily synthesis rate of the primary bile acids. As the fractional absorption of CA is not following oral dosing, and as there will be some conversion by intestinal bacterial metabolism to the secondary bile acid, deoxycholic acid (Hoffman & Cummings, 1982; Lindstedt, 1957; Duane, 1997), the optimal therapeutic target dose of 10-15 mg/kg/day is considered to be required to accomplish the primary goal of down-regulating endogenous bile acid synthesis, and substituting a normal bile acid pool (Danielsson *et al.* 1963; Tauber *et al.* 1996; LaRusso *et al.* 1975; Ahlberg *et al.* 1981; Einarsson *et al.* 1974). This has been the dose range used for approximately 18 years in Study CAC-91- 10-10 conducted at CCHMC. Additionally, urinary bile acids were monitored by fast atomic bombardment mass spectrometry FAB-MS, and the administered CA dose could be titrated against the extent of disappearance of atypical bile acid metabolites and the improvement in serum liver function tests, documentation of liver enzymes (AST, ALT, GGT), and bilirubin levels analysis.

2.5.2. Main study

Title of Study CAC-91-10-10: Investigation in the pathogenesis of liver disease in patients with inborn errors of bile acid metabolism (including data from Protocol CAC-92-8-19 'investigations of the potential benefit of bile acid therapy for patients with peroxisomal disorders affecting bile acid metabolism')

Methods

Study CAC-91-10-10 was a Phase 3, open-label, single arm, non-randomized, non-comparative, compassionate treatment study investigating CA in the treatment of patients with inborn errors of bile acid metabolism. The sub-study CAC-92-8-19 was also a Phase 3, open-label, single arm, non-randomized, non-comparative, compassionate treatment study, extending the investigation of CA treatment to patients with peroxisomal disorders, a conglomerate of defects affecting later stage bile acid metabolism.

Study Participants

Inclusion criteria

Patients with inborn errors of bile acid synthesis affecting early synthesis steps (CAC-91-10-10)

- Infants < age 3 months
- Children presenting for evaluation of cholestasis defined as a conjugated bilirubin > 2 mg/dl or increased serum bile acids
- Older patients of any age with cholestatic liver disease if urine screens suggested that they had inborn errors of bile acid metabolism
- Confirmation of a diagnosis of an inborn error of bile acid synthesis by urine FAB-MS analysis

Patients with peroxisomal disorders (CAC-92-8-19)

- Infants and children with peroxisomal disorder, with neurologic evaluation performed and serum analysed for the presence of long-chain fatty acids (most patients)
- Confirmation of abnormality of bile acid synthesis by urine FAB-MS analysis

Exclusion criteria

No exclusion criteria were stipulated for the study. Patients with other organ dysfunction were not excluded (if inclusion criteria were otherwise met).

Treatments

In the original study protocol approved on 3 Jan 1992, a combination of ursodeoxycholic acid (URSO) and CA was stipulated as study medication. Since URSO does not down regulate cholesterol and bile acid synthesis, it was initially combined with CA, which as a primary bile acid has the required inhibitory effect on cholesterol synthesis. However, analysis of urinary bile acids by FAB-MS or gas chromatography mass spectrometry (GC-MS) indicated incomplete down regulation of endogenous bile acid synthesis under combined URSO/CA therapy and the investgators suggested that URSO could possibly interfere with intestinal absorption of CA. After administration of CA alone, a greater down-regulation of bile acid synthesis was obtained as evidenced from a strong decrease or almost complete disappearance of bile acid precursors (Jacquemin *et al*. 2001). Similarly, in a report on 15 children with genetic defects in primary bile acid synthesis (Gonzalez *et al.* 2009), marked reductions in Δ4-3-oxo bile acids and in total urinary bile acid excretion after CA therapy were described, while optimal suppression of metabolite synthesis was not achieved with URSO and CA therapy. The authors concluded that URSO in the long-term treatment of a bile acid synthesis defects was seldom useful.

Hence, monotherapy with CA was considered the most appropriate therapeutic strategy to treat inborn errors in bile acid synthesis, because it provides a stimulus for bile flow and secretion, it inhibits endogenous production and accumulation of potentially hepatotoxic and cholestatic bile acid precursors, and it facilitates fat absorption without persisting disadvantageous or toxic side effects at therapeutic doses. In accordance with this evolving awareness for CA efficacy, URSO was removed as study medication from 12 June 2001. Cholic acid and URSO at a dose of 15 mg/kg each was to be administered orally, once a day. Following termination of URSO as study medication, only CA at a dose of 15 mg/kg was to be administered.

The study drug consisted of 250 mg capsule. A liquid formulation, consisting of 15 mg/mL CA in a sodium bicarbonate solution, was prepared for those patients who could not be administered capsules. Different sources for the drug substance were used over the long period of the study. The formulations used in the trial have changed over time. As of July 2008 the CCMHC prepared the CA capsules as well as the liquid formulation. Patients were gradually transitioned to the to-be-marketed formulation beginning 2010.

Objectives

The study objectives were to evaluate the therapeutic efficacy and safety of CA during provision of compassionate treatment to patients with identified inborn errors of bile acid metabolism.

Outcomes/endpoints

Therapeutic efficacy was evaluated by assessing the effects of the administration of CA on

(1) suppression of synthesis of atypical bile acids as measured by urine bile acid analysis using mass spectrometry,

(2) serum transaminases and bilirubin, and

(3) height/weight gain

(4) change in liver histology (for patients in whom biopsy was performed).

Safety and tolerability were assessed by monitoring the incidence and severity of adverse events (AEs).

Sample size

The original purpose of the study was provision of compassionate treatment; therefore, no formal sample size calculations were performed.

Randomisation

All patients enrolled in the study comprised a single treatment group; no randomisation was done.

Blinding (masking)

The study was open-label; no blinding was involved.

Statistical methods

Analysis Sets

ITT: All subjects identified as having an inborn error in bile acid synthesis were offered participation in the study and were included in the ITT population.

Safety: All subjects for whom it could be determined that they received at least one dose of CA were included in the Safety population

mITT: If a subject had both a pre- and a post-treatment assessment for any of the main endpoints (LFT, FAB-MS, or height/weight), that subject was included in the mITT population.

The mITT set was the main analysis set. If a subject had both a pre- and a post-treatment assessment for any of the main endpoints (LFT, FAB-MS, or Height/Weight assessment), that subject was included in the mITT population.

Given the variable distribution of pre- and post-treatment visits, it was decided that one pre- and one post-treatment visit should be selected and used to assess the impact of CA on the main endpoints. The primary analysis was the worst pre-treatment to the best post-treatment response, per efficacy outcome. Sensitivity analyses of the worst pre-treatment to the worst post-treatment and the best pre-treatment to the best post-treatment were presented.

The level of urinary bile excretion was scored using the scale of: 0, normal; 1, slight; 2, significant; or 3, marked. A Cochran-Mantel-Haenszel Chi-Square test, with modified ridit scoring, was used to test the impact of treatment with CA.

LFTs (transaminases) were evaluated in terms of elevation above the upper normal limit (ULN: below ULN; ≥1x - <2x ULN; ≥2x - <3x ULN; and ≥3x ULN. A Cochran-Mantel-Haenszel Chi-Square test, with

modified ridit scoring, was used to test the impact of treatment with CA on LFTs. Pre-treatment vs. post-treatment height and weight percentiles as well as bilirubin values were compared using a t-test.

Safety parameters were analysed using descriptive statistics.

Results

Participant flow

Patient disposition is summarized below.

Table 3 Patient Disposition

Disposition	Sub-Group	Total	91-10-10	92-8-19
		N (%)	N (%)	N (%)
Screened and invited to treatment		85 (100)	63 (74)	22 (26)
Treated		79 (93)	59 (75)	20 (25)
Final Study Disposition	No evidence of treatment	6 (7)	4 (6)	2 (9)
	Patient Terminated: Liver Transplant	4 (5)	4 (6)	0 (--)
	Patient Terminated: Worsening Cholestasis	1 (1)	1 (2)	0 (--)
	Patient has expired	16 (19)	9 (14)	7 (32)
	Patient is Lost To Follow-Up	13 (15)	9 (14)	4 (18)
	Patient is cared for by an outside clinician and efforts to retrieve data have been unsuccessful	4 (5)	2 (3)	2 (9)
	Treatment with cholic acid ongoing as of December 31, 2009	41 (48)	34 (54)	7 (32)

A total of 16 patients expired according to the final patient disposition information, however, based on available safety information a total of 21 patients died during the study period. A total of 13 patients were lost to follow-up, and there were 4 patients where data retrieval was unsuccessful. The remaining patients continued to receive study medication at the end of the study period.

Recruitment

The study was conducted in one single site at the Cincinnati Children's Hospital Medical Center from 3 January 1992 to 31 December 2009.

Conduct of the study

Studies CAC-91-10-10 and CAC-92-8-19 were initially initiated in 1992 as academic trials and were not intended to be used for a MAA. The studies started before ICH GCP was published. In consequence, the full requirements of GCP with regard to study conduct and documentation were often not met. For example, while the original protocols were reviewed and approved by the IRB, amendments to the studies were mainly only reported to the IRB via letters or changes to the informed consent documentation (ICD). No formal protocol amendment was provided until June 2010, after the data cut-off point for the final CAC-91-10-10 CSR. This casted doubt on the reliability of the data presented and triggered a GCP inspection by the CHMP. The potential to retrospectively implement any corrective and preventative actions remained limited, however, every effort was made by the applicant to address and rectify as far as possible any finding and deficiencies identified by the inspection. An extensive re-monitoring was undertaken at the study site from November 2012 to January 2013, to ensure that a revised CSR contains an accurate and complete picture of all data. The comprehensive re-review

included patient charts, analytical screening logs, adverse events, laboratory findings, study medication/treatment, and urine FAB-MS data (see GCP section).

In the original protocols (CAC-91-10-10 and the sub-study CAC-92-8-19) specified URSO in combination with CA as study medication. In June 2001, the use of URSO as study medication was stopped. Study CAC-91-10-10 started with three visits to CCHMC, one baseline visit, one visit after 1 month on treatment and a third visit after 6 months on treatment. In June 2004, the number of visits to CCHMC was reduced to two and the on-treatment visit was scheduled at 3-6 months after treatment start. The original CAC-92-8-19 protocol defined two visits to CCHMC, however, there were also some scheduled assessments after 6 months on treatment. In general, the number of pre-and post-treatment visits available per patient is highly variable and often did not follow the protocol outline. This non-compliance with the protocol was more prevalent once the study was extended to patients outside of CCHMC. For these patients no CCHMC visits were required, however, monthly on-treatment urine and serum monitoring was requested per protocol for the first 3 months and every 6 months afterwards.

Urine FAB-MS analysis was to be confirmed by serum FAB-MS analysis if urine FAB-MS results were inconclusive. However, with increasing study duration, it became apparent that urine FAB-MS analysis was very accurate and as a consequence, serum FAB-MS analysis was often omitted. Since CA exerts its activity in bile and not serum, therapeutic monitoring of serum is also secondary and not physiologically relevant. Similarly, the highly invasive procedure to determine the bile acid pool was often not performed, when it was recognized that due to the advances in the development of analytical procedures permitting non-invasive sample collection (i.e. FAB-MS analysis of urine), it was no longer ethical to perform bile acid pool assessments.

Laboratory tests were to include bilirubin, ALT, AST, alkaline phosphatase, cholesterol, albumin, prothrombin time, MEG-X. In addition, determination of partial thromboplastin time, prealbumin and transferrin was to be performed. However, only transaminases (ALT/AST) and bilirubin values were collected in the study database and are presented in this report. Bilirubin was mostly used as a diagnostic factor as elevations in direct or conjugated bilirubin are noted in patients with cholestasis. However, bilirubin monitoring was not used as standard of care in patients with inborn errors of bile acid synthesis, since it may not necessarily improve with treatment.

A full nutritional evaluation was to be performed for patients included in studies CAC-91-10-10 and CAC-92-8-19 and a neurologic examination/history assessment was to be done in CAC-92-8-19 patients. However, these assessments were performed infrequently.

Baseline data

Demographics characteristics

Demographic characteristics for the ITT, safety and mITT sets are summarised below.

Table 4 Demographics characteristics v

Characteristic		ITT (N = 85)	Safety (N = 79)	mITT (N = 70)
Gender [N (%)]	Female	31 (36)	29 (37)	25 (36)
	Male	50 (59)	48 (61)	45 (64)
	Unknown	4 (5)	2 (3)	0 (0)
Race [N (%)]	Arabic	1 (1)	1 (1)	1 (1)
	Asian Indian	2 (2)	2 (3)	2 (3)
	Black/African American	1 (1)	1 (1)	1 (1)
	Egyptian	1 (1)	1 (1)	1 (1)
	Haitian	1 (1)	1 (1)	1 (1)
	Hispanic	10 (12)	10 (13)	10 (14)
	Middle Eastern	7 (8)	7 (9)	6 (9)
	Persian/Iranian	1 (1)	1 (1)	1 (1)
	Portuguese	2 (2)	2 (3)	2 (3)
	UNK	29 (34)	23 (29)	16 (23)
	White	30 (35)	30 (38)	29 (41)
Age at diagnosis [years]	Mean ± SD	2 ± 4 (n = 74)	2 ± 4 (n = 71)	2 ± 4 (n = 62)
	Min, Max	0, 13 (n = 74)	0, 13 (n = 71)	0, 13 (n = 62)
Age group at diagnosis [N (%)]	<3 months	NA	23 (27)	NA
	3-6 months		19 (22)	
	7-12 months		13 (15)	
	13-36 months		12 (14)	
	>36 months		18 (21)	
Age at treatment start [years]	Mean ± SD	3 ± 4 (n = 77)	3 ± 4 (n = 77)	3 ± 4 (n = 68)
	Min, Max	0, 16 (n = 77)	0, 16 (n = 77)	0, 16 (n = 68)
Baseline height percentile	Mean ± SD	33 ± 31 (n = 16)	33 ± 31 (n = 16)	34 ± 31 (n = 15)
	Min, Max	0, 92 (n = 16)	0, 92	0, 92 (n = 15)
Baseline weight percentile	Mean ± SD	39 ± 36 (n = 16)	39 ± 36 (n = 16)	35 ± 35 (n = 15)
	Min, Max	0, 98 (n = 16)	0, 98 (n = 16)	0, 98 (n = 15)

N = number of patients, n = number of patients with data available, NA = not assessed, SD = standard deviation.

Disease characteristics

A summary of the primary diagnosis by type of disorder is provided for patients in the ITT, safety and mITT sets in the Table 5 below.

Table 5 Primary diagnosis by disorder type

Type of Disorder Primary Diagnosis	**ITT (N = 85) N (%)**	**Safety (N = 79) N (%)**	**mITT (N = 70) N (%)**
Single Enzyme Defect	**54 (64)**	**50 (63)**	**43 (61)**
3ß-hydroxy-5-C27-steroid oxidoreductase (3ß-hydroxy-5-C27-steroid dehydrogenase/isomerase or 3ß-HSD or HSD3ß7)	35 (41)	35 (44)	32 (46)
Δ4-3-oxosteroid 5ß-reductase (Δ4-3-oxo-R or AKR1D1)	10 (12)	9 (11)	6 (9)
Sterol 27-hydroxylase (CTX)	5 (6)	3 (4)	3 (4)
2- (or a-) methylacyl-CoA racemase (AMACR)	1 (1)	1 (1)	1 (1)
Cholesterol 7 a-hydroxylase (CYP7A1)	1 (1)	1 (1)	0 (0)
Smith-Lemli-Opitz	1 (1)	1 (1)	1 (1)
Unknown	1 (1)	0 (0)	0 (0)
Peroxisomal Disorder	**31 (36)**	**29 (37)**	**27 (39)**
Peroxisomal Biogenesis Disorder: Zellweger's	12 (14)	11 (14)	9 (13)
Peroxisomal Biogenesis Disorder: Neonatal adrenoleukodystrophy	8 (9)	8 (10)	8 (11)
Peroxisomal Biogenesis Disorder: Type unknown	6 (7)	5 (6)	5 (7)
Peroxisomal Biogenesis Disorder: Refsum's	4 (5)	4 (5)	4 (6)
Peroxisomal Biogenesis Disorder: Generalized peroxisomal disorder	1 (1)	1 (1)	1 (1)

N = number of patients

Numbers analysed

The number of patients included in each analysis set is summarised below.

Table 6 Analysed data sets

Disposition	Sub-Group	N	Percent
ITT		85	100
Protocol	91-10-10	63	74
	92-8-19	22	26
Safety		79	93
Protocol	91-10-10	59	75
	92-8-19	20	25
mITT		70	82
Protocol	91-10-10	50	71
	92-8-19	20	29

N = number of patients

Outcomes and estimation

Urinary Bile acids by FAB-MS

A comparison of worst pre-treatment urine bile acid scores with best post-treatment scores after the initiation of CA treatment shows a highly statistically significant decrease in atypical bile acids ($p<0.0001$) for both the ITT and mITT sets, evidenced by an increase in the number of patients with lower degrees of atypical bile acids or a normal FAB-MS spectrum. Thus, while 50% of the patients presented with marked elevation of atypical bile acids at pre-treatment, only about 10% of patients showed marked increases following treatment with CA. The opposite was true for the number of patients with FAB-MS spectra equal to those of normal, healthy subjects; the incidence of normal spectra increased from less than 15% to 60%-70%.

Table 7 Impact of cholic acid treatment on urinary bile acid excretion – worst to best analysis

Visit	Normal n (%)	Slight n (%)	Significant n (%)	Marked n (%)	CMH p-value
ITT (N = 85)					
Pre-TMT	11 (12.9)	13 (15.3)	21 (24.7)	40 (47.1)	<.0001
Post-TMT	54 (63.5)	11 (12.9)	11 (12.9)	9 (10.6)	
mITT (N = 70)					
Pre-TMT	10 (14.3)	11 (15.7)	16 (22.9)	33 (47.1)	<.0001
Post-TMT	51 (72.9)	9 (12.9)	4 (5.7)	6 (8.6)	

CMH = Cochran-Mantel-Haenszel, N = number of patients, n = number of patients in the specified category, TMT = treatment.

A statistically significant improvement in urinary bile acid scores was also observed for the best pre-treatment to best post-treatment analysis for both the ITT and mITT sets (p <0.0001), despite slightly lower levels of marked bile acid abnormalities and higher numbers of normal FAB-MS spectra at pre-treatment (Table 8 below).

Table 8 Impact of cholic acid Treatment on Urinary Bile acid Excretion – Best to Best Analysis

Visit	Normal n (%)	Slight n (%)	Significant n (%)	Marked n (%)	CMH p-value
ITT (N = 85)					
Pre-TMT	20 (23.5)	15 (17.6)	21 (24.7)	29 (34.1)	<.0001
Post-TMT	54 (63.5)	11 (12.9)	11 (12.9)	9 (10.6)	
mITT (N = 70)					
Pre-TMT	18 (25.7)	13 (18.6)	14 (20.0)	25 (35.7)	<.0001
Post-TMT	51 (72.9)	9 (12.9)	4 (5.7)	6 (8.6)	

CMH = Cochran-Mantel-Haenszel, N = number of patients, n = number of patients in the specified category, TMT = treatment.

The comparison of worst pre-treatment and worst post-treatment urine bile acid values also showed a trend towards improved atypical bile acid scores, however, statistical significance was not reached in either the ITT or mITT analysis.

The impact of CA treatment on urine bile acid secretion analyzed by type of defect is presented for the ITT and mITT sets based on the worst pre-treatment to best post-treatment analysis in the Table below and based on the best pre-treatment to best post-treatment analysis in Table 9.

Table 9 Impact of cholic acid treatment on urinary bile acid excretion by type of defect – Worst to Best Analysis

Type of Defect	Post-TMT	Normal n (%)	Slight n (%)	Significant n (%)	Marked n (%)	CMH p-value
ITT (N = 85)						
Single Enzyme Defect	Pre-TMT	2 (3.7)	3 (5.6)	11 (20.4)	38 (70.4)	<.0001
	Post-TMT	31 (57.4)	6 (11.1)	8 (14.8)	9 (16.7)	
Peroxisomal Disorder	Pre-TMT	9 (29.0)	10 (32.3)	10 (32.3)	2 (6.5)	0.0003
	Post-TMT	23 (74.2)	5 (16.1)	3 (9.7)	0 (0.0)	
mITT (N = 70)						
Single Enzyme Defect	Pre-TMT	1 (2.3)	3 (7.0)	8 (18.6)	31 (72.1)	<.0001
	Post-TMT	28 (65.1)	6 (14.0)	3 (7.0)	6 (14.0)	
Peroxisomal Disorder	Pre-TMT	9 (33.3)	8 (29.6)	8 (29.6)	2 (7.4)	<.0001
	Post-TMT	23 (85.2)	3 (11.1)	1 (3.7)	0 (0.0)	

CMH = Cochran-Mantel-Haenszel, N = number of patients, n = number of patients in the specified category, TMT = treatment.

Table 10 Impact of cholic acid treatment on urinary bile acid excretion by type of defect – Best to Best Analysis

Type of Defect	Post-TMT	Normal n (%)	Slight n (%)	Significant n (%)	Marked n (%)	CMH p-value
ITT (N = 85)						
Single Enzyme Defect	Pre-TMT	4 (7.4)	7 (13.0)	15 (27.8)	28 (51.9)	<.0001
	Post-TMT	31 (57.4)	6 (11.1)	8 (14.8)	9 (16.7)	
Peroxisomal Disorder	Pre-TMT	16 (51.6)	8 (25.8)	6 (19.4)	1 (3.2)	0.0586
	Post-TMT	23 (74.2)	5 (16.1)	3 (9.7)	0 (0.0)	
mITT (N = 70)						
Single Enzyme Defect	Pre-TMT	2 (4.7)	7 (16.3)	10 (23.3)	24 (55.8)	<.0001
	Post-TMT	28 (65.1)	6 (14.0)	3 (7.0)	6 (14.0)	
Peroxisomal Disorder	Pre-TMT	16 (59.3)	6 (22.2)	4 (14.8)	1 (3.7)	0.0286
	Post-TMT	23 (85.2)	3 (11.1)	1 (3.7)	0 (0.0)	

CMH = Cochran-Mantel-Haenszel, N = number of patients, n = number of patients in the specified category, TMT = treatment.

No statistically significant changes in urine bile acid scores were seen in the worst pre-treatment to worst post-treatment analysis by defect type for the ITT and mITT sets.

The impact of CA treatment on urinary bile acid excretion is presented by primary diagnosis for the worst pre-treatment to best post-treatment mITT analysis in the Table 11 below.

Table 11 Impact of cholic acid treatment on urinary bile acid excretion by primary diagnosis – Worst to Best Analysis – mITT (N = 70)

Primary Diagnosis	Visit	Normal n (%)	Slight n (%)	Significant n (%)	Marked n (%)	CMH p-value
Single Enzyme Defects						
2- (or a-) methylacyl-CoA racemase (AMACR)	Pre-TMT	0 (0.0)	- (--)	1 (100.0)	- (--)	0.3173
	Post-TMT	1 (100.0)	- (--)	0 (0.0)	- (--)	
3ß-hydroxy-Δ^5-C27-steroid oxidoreductase (3ß-hydroxy-Δ^5-C27-steroid dehydrogenase/ isomerase or 3ß-HSD or HSD3ß7)	Pre-TMT	0 (0.0)	2 (6.3)	5 (15.6)	25 (78.1)	<.0001
	Post-TMT	20 (62.5)	4 (12.5)	3 (9.4)	5 (15.6)	
Δ^4-3-oxosteroid 5ß-reductase (Δ^4-3-oxo-R or AKR1D1)	Pre-TMT	0 (0.0)	0 (0.0)	1 (16.7)	5 (83.3)	0.0123
	Post-TMT	3 (50.0)	2 (33.3)	0 (0.0)	1 (16.7)	
Sterol 27-hydroxylase (CTX)	Pre-TMT	1 (33.3)	- (--)	1 (33.3)	1 (33.3)	0.1213
	Post-TMT	3 (100.0)	- (--)	0 (0.0)	0 (0.0)	
Smith-Lemli-Opitz	Pre-TMT	0 (0.0)	1 (100.0)	- (--)	- (--)	0.3173
	Post-TMT	1 (100.0)	0 (0.0)	- (--)	- (--)	
Combined selected single enzymes[a]	Pre-TMT	1 (20.0)	1 (20.0)	2 (40.0)	1 (20.0)	0.0182
	Post-TMT	5 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Peroxisomal Biogenesis Disorder						
Generalized Peroxisomal Disorder	Pre-TMT	1 (100.0)	- (--)	- (--)	- (--)	NE
	Post-TMT	1 (100.0)	- (--)	- (--)	- (--)	
Neonatal Adrenoleukodystrophy	Pre-TMT	3 (37.5)	3 (37.5)	1 (12.5)	1 (12.5)	0.0383
	Post-TMT	7 (87.5)	1 (12.5)	0 (0.0)	0 0.0)	
Refsum's	Pre-TMT	0 (0.0)	2 (50.0)	2 (50.0)	- (--)	0.0126
	Post-TMT	4 (100.0)	0 (0.0)	0 (0.0)	- (--)	
Type unknown	Pre-TMT	3 (60.0)	1 (20.0)	- (--)	1 (20.0)	0.4386
	Post-TMT	4 (80.0)	1 (20.0)	- (--)	0 (--)	
Zellweger's	Pre-TMT	2 (22.2)	2 (22.2)	5 (55.6)	- (--)	0.0205
	Post-TMT	7 (77.8)	1 (11.1)	1 (11.1)	- (--)	

[a] The single enzyme defects AMACR, CTX, Smith-Lemli-Opitz, CYP7A1, and Unknown were combined in this subgroup. CYP7A1, and Unknown defect types were not represented in the mITT set. CMH = Cochran-Mantel-Haenszel, N = number of patients, n = number of patients in the specified category, NE = not estimable, TMT = treatment.

Similar results were seen for the worst pre-treatment to best post-treatment ITT.

Table 12 presents the overall worst pre-treatment to best post-treatment analysis of changes in urinary bile acid excretion in patients with and without concomitant treatment with URSO. Any patient who received URSO at any time point during the study duration was included in the URSO 'yes' subgroup.

Table 12 Impact of cholic acid treatment on urinary bile acid excretion by URSO (yes/no) – Worst to Best Analysis

URSO use	Post-TMT	Normal n (%)	Slight n (%)	Significant n (%)	Marked n (%)	CMH p-value
ITT (N = 85)						
No	Pre-TMT	8 (16.0)	11 (22.0)	11 (22.0)	20 (40.0)	<.0001
	Post-TMT	31 (62.0)	5 (10.0)	9 (18.0)	5 (10.0)	
Yes	Pre-TMT	3 (8.6)	2 (5.7)	10 (28.6)	20 (57.1)	<.0001
	Post-TMT	23 (65.7)	6 (17.1)	2 (5.7)	4 (11.4)	
mITT (N = 70)						
No	Pre-TMT	7 (17.9)	9 (23.1)	7 (17.9)	16 (41.0)	<.0001
	Post-TMT	29 (74.4)	3 (7.7)	3 (7.7)	4 (10.3)	
Yes	Pre-TMT	3 (9.7)	2 (6.5)	9 (29.0)	17 (54.8)	<.0001
	Post-TMT	22 (71.0)	6 (19.4)	1 (3.2)	2 (6.5)	

CMH = Cochran-Mantel-Haenszel, N = number of patients, n = number of patients in the specified category, TMT = treatment.

Statistically significant improvements in urine bile acids were also shown for the best to best analysis for all subgroups and sets analysed. No statistically significant changes were seen in the worst to worst analysis.

Liver function tests (serum transaminases)

The results for the analysis of changes from worst pre-treatment to best post-treatment LFT values is presented for the ITT and mITT sets in Table 13. Results for the best to best analysis are summarized in Table 14.

Table 13 Impact of cholic acid treatment on LFT elevation – Worst to Best Analysis

LFT Test	Visit	Below ULN n (%)	≥1 to <2 x ULN n (%)	≥2 to <3 x ULN n (%)	≥3 x ULN n (%)	CMH p-value
ITT (N = 85)						
ALT	Pre-TMT	16 (21.6)	16 (21.6)	9 (12.2)	33 (44.6)	<.0001
ALT	Post-TMT	55 (74.3)	13 (17.6)	2 (2.7)	4 (5.4)	
AST	Pre-TMT	10 (13.9)	15 (20.8)	8 (11.1)	39 (54.2)	<.0001
AST	Post-TMT	41 (56.2)	17 (23.3)	5 (6.8)	10 (13.7)	
mITT (N = 70)						
ALT	Pre-TMT	14 (20.9)	16 (23.9)	8 (11.9)	29 (43.3)	<.0001
ALT	Post-TMT	49 (73.1)	13 (19.4)	2 (3.0)	3 (4.5)	
AST	Pre-TMT	9 (13.8)	14 (21.5)	7 (10.8)	35 (53.8)	<.0001
AST	Post-TMT	38 (57.6)	15 (22.7)	4 (6.1)	9 (13.6)	

P-value is from a Cochran-Mantel-Haenszel Chi-Square test with modified ridit scoring CMH = Cochran-Mantel-Haenszel, N = number of patients, n = number of patients in the specified category, TMT = treatment.

Table 14 Impact of cholic acid treatment on LFT elevation – Best to Best Analysis

LFT Test	Visit	Below ULN n (%)	≥1 to <2 x ULN n (%)	≥2 to <3 x ULN n (%)	≥3 x ULN n (%)	CMH p-value
ITT (N = 85)						
ALT	Pre-TMT	37 (50.0)	23 (31.1)	1 (1.4)	13 (17.6)	0.0019
ALT	Post-TMT	55 (74.3)	13 (17.6)	2 (2.7)	4 (5.4)	
AST	Pre-TMT	27 (37.5)	21 (27.8)	8 (11.1)	17 (23.6)	0.0187
AST	Post-TMT	41 (56.2)	17 (23.3)	5 (6.8)	10 (13.7)	
mITT (N = 70)						
ALT	Pre-TMT	31 (46.3)	23 (34.3)	1 (1.5)	12 (17.9)	0.0011
ALT	Post-TMT	49 (73.1)	13 (19.4)	2 (3.0)	3 (4.5)	
AST	Pre-TMT	24 (36.9)	18 (27.7)	7 (10.8)	16 (24.6)	0.0138
AST	Post-TMT	38 (57.6)	15 (22.7)	4 (6.1)	9 (13.6)	

P-value is from a Cochran-Mantel-Haenszel Chi-Square test with modified ridit scoring
CMH = Cochran-Mantel-Haenszel, N = number of patients, n = number of patients in the specified category, TMT = treatment.

The comparison of worst pre-treatment and worst post-treatment values also showed a trend towards lower LFT scores, however statistical significance was not reached in either the ITT or mITT analyses.

The impact of CA treatment on ALT and AST analyzed by type of defect is presented based on the worst pre-treatment to best post-treatment analysis in Table 20 and based on the best pre-treatment to best post-treatment analysis in Table 15.

Table 15 Impact of cholic acid treatment on LFT elevation by type of defect – Worst to Best Analysis

Type of Defect	LFT Test	Visit	Below ULN n (%)	≥1 to <2 x ULN n (%)	≥2 to <3 x ULN n (%)	≥3 x ULN n (%)	CMH p-value
ITT (N = 85)							
Single Enzyme Defect	ALT	Pre-TMT	11 (24.4)	10 (22.2)	5 (11.1)	19 (42.2)	<.0001
	ALT	Post-TMT	40 (88.9)	3 (6.7)	0 (0.0)	2 (4.4)	
Peroxisomal Disorder	ALT	Pre-TMT	5 (17.2)	6 (20.7)	4 (13.8)	14 (48.3)	0.0002
	ALT	Post-TMT	15 (51.7)	10 (34.5)	2 (6.9)	2 (6.9)	
Single Enzyme Defect	AST	Pre-TMT	8 (18.6)	11 (25.6)	4 (9.3)	20 (46.5)	<.0001
	AST	Post-TMT	37 (82.2)	6 (13.3)	0 (0.0)	2 (4.4)	
Peroxisomal Disorder	AST	Pre-TMT	2 (6.9)	4 (13.8)	4 (13.8)	19 (65.5)	0.0056
	AST	Post-TMT	4 (14.3)	11 (39.3)	5 (17.9)	8 (28.6)	
mITT (N = 70)							
Single Enzyme Defect	ALT	Pre-TMT	9 (22.5)	10 (25.0)	4 (10.0)	17 (42.5)	<.0001
	ALT	Post-TMT	35 (87.5)	3 (7.5)	0 (0.0)	2 (5.0)	
Peroxisomal Disorder	ALT	Pre-TMT	5 (18.5)	6 (22.2)	4 (14.8)	12 (44.4)	0.0003
	ALT	Post-TMT	14 (51.9)	10 (37.0)	2 (7.4)	1 (3.7)	
Single Enzyme Defect	AST	Pre-TMT	7 (18.4)	10 (26.3)	3 (7.9)	18 (47.4)	<.0001
	AST	Post-TMT	34 (85.0)	4 (10.0)	0 (0.0)	2 (5.0)	
Peroxisomal Disorder	AST	Pre-TMT	2 (7.4)	4 (14.8)	4 (14.8)	17 (63.0)	0.0073
	AST	Post-TMT	4 (15.4)	11 (42.3)	4 (15.4)	7 (26.9)	

P-value is from a Cochran-Mantel-Haenszel Chi-Square test with modified ridit scoring.
CMH = Cochran-Mantel-Haenszel, N = number of patients, n = number of patients in the specified category, TMT = treatment.

Table 16 Impact of cholic acid Treatment on LFT Elevation by Type of Defect – Best to Best Analysis

Type of Defect	LFT test	Visit	Below ULN n (%)	≥1 to <2 x ULN n (%)	≥2 to <3 x ULN n (%)	≥3 x ULN n (%)	CMH p-value
ITT (N = 85)							
Single Enzyme Defect	ALT	Pre-TMT	27 (60.0)	12 (26.7)	0 (--)	6 (13.3)	0.0022
	ALT	Post-TMT	40 (88.9)	3 (6.7)	0 (--)	2 (4.4)	
Peroxisomal Disorder	ALT	Pre-TMT	10 (34.5)	11 (37.9)	1 (3.4)	7 (24.1)	0.1074
	ALT	Post-TMT	15 (51.7)	10 (34.5)	2 (6.9)	2 (6.9)	
Single Enzyme Defect	AST	Pre-TMT	23 (53.5)	12 (27.9)	1 (2.3)	7 (16.3)	0.0033
	AST	Post-TMT	37 (82.2)	6 (13.3)	0 (--)	2 (4.4)	
Peroxisomal Disorder	AST	Pre-TMT	4 (13.8)	8 (27.9)	7 (24.1)	10 (34.5)	0.5002
	AST	Post-TMT	4 (14.3)	11 (39.3)	5 (17.9)	8 (28.6)	
mITT (N = 70)							
Single Enzyme Defect	ALT	Pre-TMT	22 (55.0)	12 (30.0)	0 (--)	6 (15.0)	0.0018
	ALT	Post-TMT	35 (87.5)	3 (7.5)	0 (--)	2 (5.0)	
Peroxisomal Disorder	ALT	Pre-TMT	9 (33.3)	11 (40.7)	1 (3.7)	6 (22.2)	0.0882
	ALT	Post-TMT	14 (51.9)	10 (37.0)	2 (7.4)	1 (3.7)	
Single Enzyme Defect	AST	Pre-TMT	20 (52.6)	10 (26.3)	1 (2.6)	7 (18.4)	0.0020
	AST	Post-TMT	34 (85.0)	4 (10.0)	0 (--)	2 (5.0)	
Peroxisomal Disorder	AST	Pre-TMT	4 (14.8)	8 (29.6)	6 (22.2)	9 (33.3)	0.4859
	AST	Post-TMT	4 (15.4)	11 (42.3)	4 (15.4)	7 (26.9)	

P-value is from a Cochran-Mantel-Haenszel Chi-Square test with modified ridit scoring.
CMH = Cochran-Mantel-Haenszel, N = number of patients, n = number of patients in the specified category, TMT = treatment.

No statistically significant changes in ALT and AST were seen in the worst pre-treatment to worst post-treatment analysis by defect type for the ITT and mITT sets.

The impact of CA treatment on ALT and AST is presented by primary diagnosis for the worst pre-treatment to best post-treatment mITT analysis in Table 17.

Table 17 Impact of cholic acid treatment on LFT elevation by primary diagnosis – Worst to Best Analysis – mITT (N = 70)

Primary Diagnosis	LFT test	Visit	Below ULN n (%)	≥1 to <2 x ULN n (%)	≥2 to <3 x ULN n (%)	≥3 x ULN n (%)	CMH p-value
Single Enzyme Defect							
2- (or a-) methylacyl-CoA racemase (AMACR)	ALT	Pre-TMT	0 (0.0)	- (--)	- (--)	1 (100.0)	0.3173
	ALT	Post-TMT	1 (100.0)	- (--)	- (--)	0 (0.0)	
	AST	Pre-TMT	0 (0.0)	- (--)	- (--)	1 (100.0)	0.3173
	AST	Post-TMT	1 (100.0)	- (--)	- (--)	0 (0.0)	
3ß-hydroxy-Δ5-C27-steroid oxidoreductase (3ß-hydroxy-Δ5-C27-steroid dehydrogenase/ isomerase or 3ß-HSD or HSD3ß7)	ALT	Pre-TMT	7 (22.6)	9 (29.0)	4 (12.9)	11 (35.5)	<.0001
	ALT	Post-TMT	27 (87.1)	3 (9.7)	0 (0.0)	1 (3.2)	
	AST	Pre-TMT	5 (16.7)	10 (33.3)	3 (10.0)	12 (40.0)	<.0001
	AST	Post-TMT	27 (87.1)	3 (9.7)	0 (0.0)	1 (3.2)	
Δ4-3-oxosteroid 5ß-reductase (Δ4-3-oxo-R or AKR1D1)	ALT	Pre-TMT	1 (20.0)	- (--)	- (--)	4 (80.0)	0.0719
	ALT	Post-TMT	4 (80.0)	- (--)	- (--)	1 (20.0)	
	AST	Pre-TMT	1 (20.0)	0 (0.0)	- (--)	4 (80.0)	0.1060
	AST	Post-TMT	3 (60.0)	1 (20.0)	- (--)	1 (20.0)	
Sterol 27-hydroxylase (CTX)	ALT	Pre-TMT	1 (33.3)	1 (33.3)	- (--)	1 (33.3)	0.1213
	ALT	Post-TMT	3 (100.0)	0 (0.0)	- (--)	0 (0.0)	
	AST	Pre-TMT	1 (50.0)	- (--)	- (--)	1 (50.0)	0.2207
	AST	Post-TMT	3 (100.0)	- (--)	- (--)	0 (0.0)	
Combined selected single enzymes[a]	ALT	Pre-TMT	1 (25.0)	1 (25.0)	- (--)	2 (50.0)	0.0455
	ALT	Post-TMT	4 (100.0)	0 (0.0)	- (--)	0 (0.0)	
	AST	Pre-TMT	1 (33.3)	0 (0.0)	- (--)	2 (66.7)	0.0736
	AST	Post-TMT	4 (100.0)	0 (0.0)	- (--)	0 (0.0)	
Peroxisomal Biogenesis Disorder							
Generalized Peroxisomal Disorder	ALT	Pre-TMT	0 (0.0)	1 (100.0)	- (--)	- (--)	0.3173
	ALT	Post-TMT	1 (100.0)	0 (0.0)	- (--)	- (--)	
	AST	Pre-TMT	- (--)	0 (0.0)	1 (100.0)	- (--)	0.3173
	AST	Post-TMT	- (--)	1 (100.0)	0 (0.0)	- (--)	
Neonatal Adrenoleukodystrophy	ALT	Pre-TMT	1 (12.5)	2 (25.0)	1 (12.5)	4 (50.0)	0.0252
	ALT	Post-TMT	4 (50.0)	3 (37.5)	1 (12.5)	0 (0.0)	
	AST	Pre-TMT	1 (12.5)	0 (0.0)	2 (25.0)	5 (62.5)	0.2583
	AST	Post-TMT	1 (12.5)	3 (37.5)	1 (12.5)	3 (37.5)	
Refsum's	ALT	Pre-TMT	0 (0.0)	1 (25.0)	1 (25.0)	2 (50.0)	0.1776
	ALT	Post-TMT	2 (50.0)	1 (25.0)	0 (0.0)	1 (25.0)	
	AST	Pre-TMT	- (--)	0 (0.0)	1 (25.0)	3 (75.0)	0.0396
	AST	Post-TMT	- (--)	2 (66.7)	1 (33.3)	0 (0.0)	
Type unknown	ALT	Pre-TMT	3 (60.0)	0 (0.0)	- (--)	2 (40.0)	0.3662
	ALT	Post-TMT	4 (80.0)	1 (20.0)	- (--)	0 (0.0)	
	AST	Pre-TMT	1 (20.0)	2 (40.0)	0 (0.0)	2 (40.0)	0.3242
	AST	Post-TMT	2 (40.0)	2 (40.0)	1 (20.0)	0 (0.0)	
Zellweger's	ALT	Pre-TMT	1 (11.1)	2 (22.2)	2 (22.2)	4 (44.4)	0.0212
	ALT	Post-TMT	3 (33.3)	5 (55.6)	1 (11.1)	0 (0.0)	
	AST	Pre-TMT	0 (0.0)	2 (22.2)	0 (0.0)	7 (77.8)	0.1693
	AST	Post-TMT	1 (11.1)	3 (33.3)	1 (11.1)	4 (44.4)	

[a] The single enzyme defects AMACR, CTX, Smith-Lemli-Opitz, CYP7A1, and Unknown were combined in this subgroup. CYP7A1, and Unknown defect types were not represented in the mITT set.
P-value is from a Cochran-Mantel-Haenszel Chi-Square test with modified ridit scoring.
CMH = Cochran-Mantel-Haenszel, N = number of patients, n = number of patients in the specified category, TMT = treatment.

The worst pre-treatment to best post-treatment ITT analyses of changes in LFTs provided similar results.

In the best pre-treatment to best post-treatment analysis only LFT changes for patients with defects in the single enzymes 3ß-hydroxy-Δ5-C27-steroid oxidoreductase were statistically significant. No statistically significant changes in LFTs were seen in the worst pre-treatment to worst post-treatment analysis.

Table 18 presents the worst pre-treatment to best post-treatment analysis of changes in ALT and AST for patients with and without concomitant treatment with URSO.

Table 18 Impact of cholic acid treatment on LFT elevation by URSO (yes/no) – Worst to Best Analysis

URSO use	LFT	Visit	Below ULN n (%)	≥1 to <2 x ULN n (%)	≥2 to <3 x ULN n (%)	≥3 x ULN n (%)	CMH p-value
ITT (N = 85)							
No	ALT	Pre-TMT	13 (31.0)	10 (23.8)	4 (9.5)	15 (35.7)	<.0001
	ALT	Post-TMT	31 (73.8)	7 (16.7)	2 (4.8)	2 (4.8)	
	AST	Pre-TMT	7 (17.5)	10 (25.0)	4 (10.0)	19 (47.5)	0.0002
	AST	Post-TMT	20 (48.8)	12 (29.3)	4 (9.8)	5 (12.2)	
Yes	ALT	Pre-TMT	3 (9.4)	6 (18.8)	5 (15.6)	18 (56.3)	<.0001
	ALT	Post-TMT	24 (75.0)	6 (18.8)	0 (0.0)	2 (6.3)	
	AST	Pre-TMT	3 (9.4)	5 (15.6)	4 (12.5)	20 (62.5)	<.0001
	AST	Post-TMT	21 (65.6)	5 (15.6)	1 (3.1)	5 (15.6)	
mITT (N = 70)							
No	ALT	Pre-TMT	11 (29.7)	10 (27.0)	4 (10.8)	12 (32.4)	<.0001
	ALT	Post-TMT	27 (73.0)	7 (18.9)	2 (5.4)	1 (2.7)	
	AST	Pre-TMT	6 (17.1)	9 (25.7)	4 (11.4)	16 (45.7)	0.0002
	AST	Post-TMT	19 (52.8)	10 (27.8)	3 (8.3)	4 (11.1)	
Yes	ALT	Pre-TMT	3 (10.0)	6 (20.0)	4 (13.3)	17 (56.7)	<.0001
	ALT	Post-TMT	22 (73.3)	6 (20.0)	0 (0.0)	2 (6.7)	
	AST	Pre-TMT	3 (10.0)	5 (16.7)	3 (10.0)	19 (63.3)	<.0001
	AST	Post-TMT	19 (63.3)	5 (16.7)	1 (3.3)	5 (16.7)	

P-value is from a Cochran-Mantel-Haenszel Chi-Square test with modified ridit scoring.
CMH = Cochran-Mantel-Haenszel, N = number of patients, n = number of patients in the specified category, TMT = treatment.

Bilirubin

The impact of CA treatment on bilirubin values is summarized in Table 19.

Table 19 Impact of cholic acid treatment on bilirubin - ITT

Parameter	Visit	N	Mean [mg/dL]	Standard deviation	Standard error of mean	T-Test p value
Bilirubin	Pre-TMT	2	0.8	0.7	0.46	0.880
	Post-TMT	15	0.7	0.4	0.10	
Direct bilirubin	Pre-TMT	90	3.5	7.8	0.83	<0.001
	Post-TMT	198	0.6	2.1	0.15	
Indirect bilirubin	Pre-TMT	5	0.5	0.9	0.40	0.463
	Post-TMT	17	0.2	0.2	0.04	
Total bilirubin	Pre-TMT	95	3.1	10.1	1.04	0.125
	Post-TMT	289	1.5	3.2	0.19	

N = number of laboratory tests, TMT = treatment.

Height and Weight

To assess the impact of CA treatment on height and weight, height and weight percentiles were used. The results for the analysis of changes in height/weight percentiles from the worst pre-treatment to best post-treatment assessment are presented for the ITT and mITT sets in Table 25. Results for the best to best analysis are summarized in Table 20.

Table 20 Impact of cholic acid treatment on height and weight percentiles – Worst to Best Analysis

Percentile	Visit	n	Mean	Standard error of mean	T-Test p-value
ITT (N = 85)					
Height	Pre-TMT	34	29.4	5.37	0.093
	Post-TMT	34	43.1	6.00	
Weight	Pre-TMT	67	20.7	3.51	<0.001
	Post-TMT	67	42.5	4.59	
mITT (N = 70)					
Height	Pre-TMT	33	30.1	5.49	0.096
	Post-TMT	33	44.0	6.10	
Weight	Pre-TMT	63	21.3	3.70	<0.001
	Post-TMT	63	42.3	4.80	

The p-values are from t-tests. The choice of p-value was based on the significance of the equality of variances test. If the test was not significant, the equal variances p-value is reported; otherwise, the unequal variances p-value is reported.
N = number of patients, n = number of patients in the specified category.

Table 21 Impact of cholic acid treatment on height and weight percentiles – Best to Best Analysis

Percentile	Visit	n	Mean	Standard error of mean	T-Test p-value
ITT (N = 85)					
Height	Pre-TMT	34	43.0	5.67	0.997
	Post-TMT	34	43.1	5.99	
Weight	Pre-TMT	67	43.9	4.14	0.816
	Post-TMT	67	42.5	4.59	
mITT (N = 70)					
Height	Pre-TMT	33	43.9	5.77	0.997
	Post-TMT	33	44.0	6.10	
Weight	Pre-TMT	63	43.8	4.31	0.813
	Post-TMT	63	42.3	4.80	

The p-values are from t-tests. The choice of p-value was based on the significance of the equality of variances test. If the test was not significant, the equal variances p-value is reported; otherwise, the unequal variances p-value is reported.
N = number of patients, n = number of patients in the specified category

The comparison of worst pre-treatment and worst post-treatment height and weight percentiles also showed no statistically significant changes in either the ITT or mITT analyses.

The impact of CA treatment on height and weight percentiles analysed by type of defect is presented based on the worst pre-treatment to best post-treatment analysis in the table below.

Table 22 Impact of cholic acid treatment on height and weight percentiles by type of defect – Worst to Best Analysis

Type of Defect	Percentile	Visit	n	Mean	Standard error of mean	T-Test p-value
ITT (N = 85)						
Single Enzyme Defect	Height	Pre-TMT	25	35.9	6.78	0.230
	Height	Post-TMT	25	48.0	7.36	
Peroxisomal Disorder	Height	Pre-TMT	9	11.3	3.47	0.073
	Height	Post-TMT	9	29.2	8.69	
Single Enzyme Defect	Weight	Pre-TMT	38	30.4	5.32	0.002
	Weight	Post-TMT	38	55.8	6.07	
Peroxisomal Disorder	Weight	Pre-TMT	29	7.9	2.76	0.009
	Weight	Post-TMT	29	25.0	5.63	

Type of Defect	Percentile	Visit	n	Mean	Standard error of mean	T-Test p-value
mITT (N = 70)						
Single Enzyme Defect	Height	Pre-TMT	24	37.2	6.94	0.234
	Height	Post-TMT	24	49.5	7.52	
Peroxisomal Disorder	Height	Pre-TMT	9	11.3	3.47	0.073
	Height	Post-TMT	9	29.2	8.69	
Single Enzyme Defect	Weight	Pre-TMT	36	31.1	5.58	0.006
	Weight	Post-TMT	36	54.9	6.38	
Peroxisomal Disorder	Weight	Pre-TMT	27	8.3	2.95	0.014
	Weight	Post-TMT	27	25.6	6.04	

The p-values are from t-tests. The choice of p-value was based on the significance of the equality of variances test. If the test was not significant, the equal variances p-value is reported; otherwise, the unequal variances p-value is reported.
N = number of patients, n = number of patients in the specified category

No statistically significant improvements in height or weight percentiles were shown in the best to best or worst to worst ITT and mITT analyses for either patients with single enzyme defects or patients with peroxisomal disorders

The impact of CA treatment on height and weight percentiles is presented by primary diagnosis for the worst pre-treatment to best post-treatment mITT analysis in Table 23.

Table 23 Impact of cholic acid treatment on height and weight percentiles by primary diagnosis – Worst to Best Analysis – mITT (N = 70)

Primary Diagnosis	Percentile	Visit	n	Mean	Standard error of mean	T-Test p-value
Single Enzyme Defects						
2- (or a-) methylacyl-CoA racemase (AMACR)	Height	Pre-TMT	1	63.6	.	
	Height	Post-TMT	1	83.1	.	
	Weight	Pre-TMT	1	5.9	.	
	Weight	Post-TMT	1	87.0	.	
3ß-hydroxy-Δ5-C27-steroid oxidoreductase (3ß-hydroxy-Δ5-C27-steroid dehydrogenase/ isomerase or 3ß-HSD or HSD3ß7)	Height	Pre-TMT	19	38.9	8.28	0.374
	Height	Post-TMT	19	49.6	8.56	
	Weight	Pre-TMT	27	35.7	6.94	0.019
	Weight	Post-TMT	27	60.0	7.27	
Δ4-3-oxosteroid 5ß-reductase (Δ4-3-oxo-R or AKR1D1)	Height	Pre-TMT	1	1.0	.	
	Height	Post-TMT	1	1.0	.	
	Weight	Pre-TMT	4	27.8	13.01	0.638
	Weight	Post-TMT	4	37.2	13.80	
Sterol 27-hydroxylase (CTX)	Height	Pre-TMT	3	29.5	12.05	0.371
	Height	Post-TMT	3	53.7	20.83	
	Weight	Pre-TMT	3	12.7	6.51	0.421
	Weight	Post-TMT	3	39.8	29.61	
Smith-Lemli-Opitz	Height	Pre-TMT	0	.	.	
	Height	Post-TMT	0	.	.	
	Weight	Pre-TMT	1	1.0	.	
	Weight	Post-TMT	1	1.0	.	
Combined selected single enzymes[a]	Height	Pre-TMT	4	38.1	12.05	0.301
	Height	Post-TMT	4	61.1	16.46	
	Weight	Pre-TMT	5	9.0	4.30	0.201
	Weight	Post-TMT	5	41.5	21.19	
Peroxisomal Biogenesis Disorder						
Generalized Peroxisomal Disorder	Height	Pre-TMT	1	23.8	.	
	Height	Post-TMT	1	46.3	.	
	Weight	Pre-TMT	1	2.7	.	
	Weight	Post-TMT	1	15.8	.	
Neonatal Adrenoleukodystrophy	Height	Pre-TMT	4	3.5	2.45	0.518
	Height	Post-TMT	4	9.4	8.16	
	Weight	Pre-TMT	8	8.7	7.70	0.531
	Weight	Post-TMT	8	18.1	12.54	
Refsum's	Height	Pre-TMT	1	9.5	.	

Primary Diagnosis	Percentile	Visit	n	Mean	Standard error of mean	T-Test p-value
	Height	Post-TMT	1	56.1	.	
	Weight	Pre-TMT	4	14.7	11.12	0.642
	Weight	Post-TMT	4	22.0	9.78	
Type unknown	Height	Pre-TMT	2	13.5	7.84	0.333
	Height	Post-TMT	2	47.9	26.03	
	Weight	Pre-TMT	5	11.6	5.94	0.086
	Weight	Post-TMT	5	41.6	14.16	
Zellweger's	Height	Pre-TMT	1	27.4	.	
	Height	Post-TMT	1	27.4	.	
	Weight	Pre-TMT	9	3.9	1.50	0.096
	Weight	Post-TMT	9	25.9	11.64	

[a]The single enzyme defects AMACR, CTX, Smith-Lemli-Opitz, CYP7A1, and Unknown were combined in this subgroup. CYP7A1, and Unknown defect types were not represented in the mITT set.
The p-values are from t-tests. The choice of p-value was based on the significance of the equality of variances test. If the test was not significant, the equal variances p-value is reported; otherwise, the unequal variances p-value is reported.
N = number of patients, n = number of patients in the specified category

The worst pre-treatment to best post-treatment ITT analyses of changes in height and weight percentiles provided similar results.

No statistically significant changes in height and weight percentiles were seen in the best pre-treatment to best post-treatment or worst pre-treatment to worst post-treatment analyses.

Table 24 presents the worst pre-treatment to best post-treatment analysis of changes in height and weight percentiles for patients with and without concomitant treatment with URSO.

Table 24 Impact of cholic acid treatment on height and weight percentiles by URSO (yes/no) – Worst to Best Analysis

URSO use	Percentile	Visit	n	Mean	Standard error of mean	T-Test p-value
ITT (N = 85)						
No	Height	Pre-TMT	18	31.9	7.16	0.174
	Height	Post-TMT	18	47.4	8.59	
	Weight	Pre-TMT	37	16.7	4.65	0.032
	Weight	Post-TMT	37	33.2	5.96	
Yes	Height	Pre-TMT	16	26.5	8.28	0.332
	Height	Post-TMT	16	38.1	8.41	
	Weight	Pre-TMT	30	25.6	5.29	0.001
	Weight	Post-TMT	30	53.9	6.68	
mITT (N = 70)						
No	Height	Pre-TMT	18	31.9	7.16	0.174
	Height	Post-TMT	18	47.4	8.59	
	Weight	Pre-TMT	34	17.9	5.01	0.068
	Weight	Post-TMT	34	32.8	6.32	
Yes	Height	Pre-TMT	15	27.9	8.72	0.347
	Height	Post-TMT	15	39.8	8.81	
	Weight	Pre-TMT	29	25.4	5.48	0.002
	Weight	Post-TMT	29	53.4	6.90	

The p-values are from t-tests. The choice of p-value was based on the significance of the equality of variances test. If the test was not significant, the equal variances p-value is reported; otherwise, the unequal variances p-value is reported.
N = number of patients, n = number of patients in the specified category

No statistically significant changes in height and weight percentiles were seen in the best to best or worst to worst analyses stratified by URSO treatment

Liver Histology

Pre- and post-treatment liver biopsies were analyzed qualitatively for the presence of inflammation, fibrosis, necrosis, giant cells and cholestasis, and quantitatively for the degrees of the aforementioned histologic features. In a third step, any liver biopsy results were compared to those of the previous biopsy and the degree of change (improved or worse) was assessed. The results of the qualitative histopathology assessment are summarized in Table 25.

Table 25 Qualitative Histopathology Summary – ITT (N = 85)

Parameter	Visit	Patients with at least one report	Number of biopsies		
			Not assessed	Absent	Present
Inflammation: Periportal	Pre-TMT	25	10	5	19
	Post-TMT	26	10	11	16
Inflammation: Lobular	Pre-TMT	25	27	2	5
	Post-TMT	25	28	4	4
Inflammation: Not Specified	Pre-TMT	25	28	3	3
	Post-TMT	24	32	3	0
Fibrosis: Bridging	Pre-TMT	25	8	1	25
	Post-TMT	26	3	3	31
Fibrosis: Not Specified	Pre-TMT	25	25	3	6
	Post-TMT	24	26	3	6
Cholestasis	Pre-TMT	25	8	6	20
	Post-TMT	26	11	16	10
Giant Cells	Pre-TMT	25	13	3	18
	Post-TMT	25	18	6	12
Necrosis	Pre-TMT	25	17	3	14
	Post-TMT	25	29	5	2

N = number of patients, TMT = treatment.

The results for the quantitative histology analysis are summarized in Table 26.

Table 26 Quantitative Histopathology Summary – ITT (N = 85)

Parameter	Visit	Patients with at least one report	Number of biopsies			
			Not assessed	Absent	Trace	Moderate
Inflammation: Periportal	Pre-TMT	20	1	5	14	4
	Post-TMT	19	1	10	13	2
Inflammation: Lobular	Pre-TMT	5	--	2	5	0
	Post-TMT	7	--	4	3	1
Inflammation: Not Specified	Pre-TMT	3	--	4	2	1
	Post-TMT	2	--	3	0	0
Fibrosis: Bridging	Pre-TMT	18	1	0	13	9
	Post-TMT	21	1	3	16	11
Fibrosis: Not Specified	Pre-TMT	5	2	2	3	--
	Post-TMT	6	1	3	5	--
Cholestasis	Pre-TMT	18	4	6	8	7
	Post-TMT	18	2	16	6	1
Giant Cells	Pre-TMT	15	3	3	10	3
	Post-TMT	14	3	6	6	3
Necrosis	Pre-TMT	12	2	2	13	--
	Post-TMT	6	0	5	2	--

N = number of patients, TMT = treatment.

Supportive studies

Study CAC-001-01

Title of Study CAC-001-01: An open-label, single-center, nonrandomized study to compare the therapeutic efficacy of to be marketed (TBM) CA capsules with that of the currently used (CU) formulation of CA capsules used to treat children with inborn errors of bile acid synthesis.

Methods

This was a Phase 3, open-label, single-centre**,** nonrandomized study in a small population of patients who were previously receiving established doses of the CU cholic acid capsules prepared at the CCHMC Pharmacy. The study was designed to compare the efficacy of the current treatment with the efficacy of the same treatment provided in the TBM cholic acid capsule. Patients served as their own controls. The study did not compare CA therapy with placebo.

Study Participants

Inclusion Criteria

- The patient must have stable transaminase levels within 2 times the upper limits of the normal range.
- The patient must have a diagnosis of an inborn error of bile acid synthesis.
- The patient and/or parent/legal guardian must have signed the written informed consent document before study start.
- Assent must be obtained from eligible children greater than age 7 years
- The patient must be currently receiving CU cholic acid therapy under IND 45,470.
- The patient must be willing and able to comply with all study assessments and procedures.

- The patient must be able to make two visits (Visit 1 and Visit 2) to the study site (CCHMC).

Exclusion Criteria

- The patient is not currently receiving CA therapy for inborn errors of bile acid synthesis under IND 45,470.
- The patient is unable or unwilling to comply with study requirements.

Treatments

The treatment consisted of a cross-over from the Currently (previously) Used (CU) cholic acid capsules prepared by the CCHMC Investigational Pharmacy to those manufactured by the proposed commercial contract manufacturer, i.e., the To Be Marketed (TBM) capsules.

Objectives

The primary objective of this study was to evaluate the therapeutic efficacy of TBM cholic acid capsules compared with the effect of the CU formulation of CA prepared in the CCHMC Pharmacy. Therapeutic efficacy was evaluated by assessing the effects of the administration of TBM cholic acid capsules on (1) serum transaminases and (2) suppression of synthesis of atypical bile acids as measured by urine and serum bile acid analysis using mass spectrometry. These findings were compared with the effects of the currently used formulation of CA.

The secondary objective of the study was to assess the safety and tolerability of TBM cholic acid capsules. Safety and tolerability were assessed by monitoring vital signs, physical examination findings, clinical laboratory results, and from the incidence and severity of adverse events which were compared with baseline data.

Outcomes/endpoints

Efficacy and safety measurements consisted of the effect of CU cholic acid capsules (baseline visit) compared with the effect of the TBM formulation of CA (Visit 2 after 30 days of study drug administration) on:

1. Serum transaminases
2. regulation of synthesis of atypical bile acids in blood and urine as determined by mass spectrometry (FAB-MS, and LC-MS/MS)
3. The safety and tolerability of TBM cholic acid capsules were assessed by the incidence and severity of adverse events (AEs) and serious adverse events (SAEs)

Sample size

No sample size calculations were done; the planned sample size (n=25)

Randomisation

Since the study treatment consisted of a cross-over from the CU cholic acid capsules to the TBM capsules, all patients enrolled in the study comprised a single treatment group.

Blinding (masking)

The study was open-label; no blinding was involved.

Statistical methods

Efficacy:

1. Descriptive statistics for the treatment periods (e.g., liver function tests, urine FAB-MS, GCMS, serum LC-MS) were presented for all baseline data (Visit 1; CU formulation) and compared with results from the final visit (Visit 2; TBM formulation).
2. The primary parameters (maintenance of baseline ALT/AST values and regulation of atypical metabolites) were compared between the treatment groups.

Safety:

The incidence and severity of adverse events, clinical laboratory test results, vital signs, and physical examination findings are presented.

Results

Participant flow

All 16 patients enrolled have completed each phase of the study, no patients were discontinued or withdrew, and all enrolled patients were included in both the efficacy and safety analyses.

Recruitment

The study was conducted at the Cincinnati Children's Hospital Medical Center from 28 April to 23 August 2010

Conduct of the study

The original planned enrolment for the study was 25 patients. However, as above, only 16 subjects would agree to participate in the study and travel to the study site.

Baseline data

Study demographics included an age range of from 0.6 – 20 years of age; 11 males and 5 females; and an ethnicity breakdown of 9 white/non-Hispanic, 5 white/Hispanic, 2 black/non-Hispanic; and included a diagnosis breakdown of 11 3ß-HSD, 3 CTX and 2 5ß-reductase defects.

Numbers analysed

All 16 patients enrolled were analysed.

Outcomes and estimation

Therapeutic equivalence was assessed at the end of 30 days of treatment using a T-test comparison for the baseline and 30 day results (e.g. liver function tests, urine and serum bile acids by FAB-MS, GC-MS, LC-MS/MS) and safety was assessed by the incidence and severity of adverse events, clinical

laboratory test results, vital signs, and physical examination. There were no clinically significant changes in laboratory values, in physical exam, vital signs at the follow-up visit, except for one subject who had increases in AST and ALT. Although, due to the extreme rarity of these bile metabolism defects, it is not possible to conduct such a study with a large sample of patients, the applicant judged that the results were adequate to demonstrate the therapeutic efficacy of TBM cholic acid capsules compared with that of the CU formulation of cholic acid capsules as therapeutic equivalence was observed in 15 out of 16 subjects (93.75%, 95% confidence interval: 69.8%, 99.8%).

Study CAC-002-001

Title of Study CAC-002-001: An open-label, single-centre, nonrandomized continuation study of CA capsules in subjects with inborn errors of bile acid synthesis.

Methods

This was a Phase 3, open-label, single arm, non-randomized, non-comparative study investigating CA in the treatment of subjects with inborn errors of bile acid metabolism. The study was a continuation study that included eligible subjects who had previously received CA in studies CAC-91-10-10 or CAC-001-01 and newly diagnosed subjects.

Prior to enrolment, new subjects were diagnosed at CCHMC for inborn errors of bile acid synthesis using urine FAB-MS analysis. Upon enrolment, the subject underwent baseline assessments including standard of care assessments and LFTs. Further safety and efficacy assessments were collected every 6-12 months or when clinically indicated. For subjects continuing treatment from previous studies, laboratory assessments and urine bile acid analysis was performed every 6-12 months or when clinically feasible. Standard of care physical examinations could also be performed.

The objective was to evaluate the therapeutic efficacy and safety of CA in subjects with identified inborn errors of bile acid metabolism. The study was conducted at the Cincinnati Children's Hospital Medical Center. The observation period for this interim report submitted during the procedure was 33 months from 1 January 2010 to 30 September 2012.

The intent-to-treat (ITT) population comprised all enrolled subjects. A subject was considered enrolled if he or his caregiver had provided consent and had undergone at least one round of study evaluations. The safety population was comprised of all subjects for whom it could be determined that they received at least one dose of CA. The efficacy evaluable population (EEP) comprised all subjects who, for any efficacy end-point, had both a baseline and at least one post-baseline assessment. For each individual efficacy analysis, only patients who had both a baseline and post baseline assessments contributed to that analysis. The ITT set was the main analysis set for efficacy analyses. The safety set was the main analysis set for safety analyses. Since patients did not follow a fixed visit schedule, analyses of changes from baseline to a given time point were not feasible. The primary efficacy analyses were the baseline to the worst post-baseline response, per efficacy outcome.

Results

A total of 41 patients took part in the continuation study. Of these 31 (76%) were on CA medication at study start, i.e. transitioned from study CAC-91-10-10. Another 10 patients (24%) were treatment naive, i.e. received their first dose of CA during study CAC-002-01. A total of 33 patients (80%) had at least one baseline and one post-baseline assessment for an efficacy endpoint and were included in the

EEP set. The majority of patients received CA treatment from both the CCHMC and the commercial formulation.

Because of its nature as a long-term, ongoing study, with some and non-rigorous determination of study visits and assessments, protocol violations were not used to determine the exclusion of patients from the analysis sets, but instead the availability of pre- and post-baseline assessments was used.

Overall, the majority of patients with single enzyme defects presented with 3β-HSD deficiency (21), followed by Δ^4-3-oxo-R (4) and CTX deficiency (4). Twelve patients had peroxisomal disorders.

Urine bile acids

A comparison of baseline urinary bile acid scores with worst post-baseline scores for the overall population that includes both patients on CA at study start and treatment naive patients showed no statistically significant changes. In the baseline to best post-baseline value analysis bile acid scores showed an increase in the percentage of patients with normal bile acid spectra and decreases in the percentages of patients with marked or significant elevations in atypical bile acids. The changes were not statistically significant in the ITT analysis, but reached statistical significance in the EE population.

Transaminases

No statistically significant changes were seen in the baseline to worst post-baseline analysis. In the baseline to best post-baseline value analysis the number of patients with ALT values <ULN increased, while the number of patients with values ≥2x ULN decreased, in both analysis sets. However, the changes were not statistically significant. In the baseline to worst post-baseline analysis, the number of patients ALT values <ULN decreased slightly, while the number of patients with ALT values ≥1x but <2 ULN increased. The changes were also not statistically significant. AST values improved with statistical significance from baseline to the best post-baseline assessment ($p < 0.05$).

Bilirubin

Mean total bilirubin values remained stable (mean change 0.1 mg/dL; range of changes -1.9 to 1.6 mg/dL) in the baseline to worst post-baseline value analysis and decreased by about 0.8 mg/dL (range -12.0 to 1.6 mg/dL) in the baseline to best post-baseline value analysis.

Height and weight percentiles

Both height and weight percentiles decreased slightly in the baseline to worst post-baseline value analysis and increased slightly in the baseline to best post-baseline value analysis. None of the changes were statistically significant.

Subgroup analyses

Treatment naive patients generally showed improvements in all efficacy parameters analysed. However, statistical significance could not be shown, most likely owing to the small number of treatment naive subjects (10 in the ITT set and 5 in the EEP set). Of note, total bilirubin values and height percentiles improved even in the baseline to worst post-baseline value analysis. Patients who had received CA treatment in the previous study showed continuous therapeutic efficacy and presented with significant improvements in some efficacy parameters, potentially indicating increasing efficacy during long-term treatment. The subgroup of patients receiving the commercial formulation tended to show larger improvements in the baseline to best post-baseline value analysis than patients receiving the CCHMC formulation, while no relevant differences were seen in the baseline to worst analysis. Patients with single enzyme defects responded better to treatment with CA than patients with peroxisomal disorders, as indicated by larger improvements in the baseline to best post-baseline value analyses.

Literature reports

A clinical study reported in the literature (Gonzales *et al.* 2009) enrolled 15 patients (eight of whom had been included in the patients diagnosed under the CCHMC and then transferred) followed up for at least 5 years at Hospital Bicetre, France. Thirteen of these patients were diagnosed with HSD3ß7 and 2 with Δ4-3-oxosteroid 5β-reductase deficiency by mass spectrometry and gene sequencing. All patients were treated with CA with the median duration of treatment 12.4 years (range 5.6 – 15 years), however some very early patients were treated with oral ursodeoxycholic acid as well. Therapy started at a median age of 3.9 years (range 0.3 – 13.1 years). The mean dose at the start of treatment was 13 mg/kg and the mean dose at last follow up was 6 mg/kg. During treatment clinical and laboratory findings gradually normalised, and atypical bile acid metabolites in the urine were reduced 500 fold in the HSD3ß7 patients and 30 fold in the Δ4-3-oxosteroid 5β-reductase deficiency patients. Liver biopsies were performed in 14 patients after at least 5 years treatment and all showed marked improvement, particularly those with HSD3ß7. The authors stated that CA was well tolerated in all patients.

A published clinical investigation on the use of CA in the treatment of bile acid metabolic disorders was performed at the Centre Hospitalier Universitaire Bicêtre, France (Potin *et al.* 2001). Ten children diagnosed with HSD3ß7 and two children with Δ4-3-oxosteroid 5β-reductase deficiency were included in a clinical trial to assess the effect of treatment with CA and with a combination of CA and ursodeoxycholic acid. Clinical and biological parameters such as weight, jaundice, hepatosplenomegaly, biochemical parameters, and haematology were recorded at inclusion and thereafter every 6 months. Until the nature of the enzyme deficiency was diagnosed, monotherapy with ursodeoxycholic acid was started to treat cholestasis. Once the diagnosis was confirmed, the children received a regimen of either, ursodeoxycholic acid monotherapy (18-30 mg/kg/day), or CA and ursodeoxycholic acid in combination (each 5-15 mg/kg/day), or CA monotherapy (5–15 mg/kg/day) depending on the clinical presentation. The children with HSD3ß7 deficiency had normalised liver enzymes after treatment with ursodeoxycholic acid followed by CA or CA and ursodeoxycholic acid combination. By contrast, in the children with Δ4-3-oxosteroid 5β-reductase deficiency, liver disease remained either unchanged or was aggravated during ursodeoxycholic acid monotherapy. Under CA and ursodeoxycholic acid combination therapy, the liver enzymes became normal within 10 months and remained stable during the following CA monotherapy for a mean period of 4.2 years. Cholic acid treatment was well tolerated in 11 of these 12 children. Only one patient developed pruritus under therapy, which completely disappeared after dose reduction from 15 mg/kg/day down to 5 mg/kg/day.

A paper by Pierre *et al.* 2008 describes the use of CA to treat two patients with Cerebrotendinous Xanthomatosis (CTX). These patients were siblings who were born of Asian parents. The eldest was male and was transferred to a specialist liver unit at 3 months of age with jaundice and hepatitis. The child was initially treated with ursodeoxycholic acid and the jaundice resolved by 5 months of age. CTX was diagnosed before 1 year of age as a result of abnormal bile alcohol glucuronides and reduced primary bile acids in the urine. Treatment was changed to CA 15 mg/kg daily at age 14 months, which resulted in a good biochemical response and a marked reduction in bile alcohol excretion. The patient remained clinically normal at 8 years of age. The younger sibling was a female and was diagnosed with CTX at a young age, and commenced treatment with CA at age 5 months. She never developed jaundice or abnormal liver function and remains free of liver symptoms at 7 years of age, though there was some evidence that neurodevelopment may have been slightly below average.

Daugherty *et al.* 1993 described the use of CA to treat three male patients with Δ4-3-oxosteroid 5ß-reductase deficiency. Two of these patients were identical twins, and all were jaundiced at birth. The twins started treatment with ursodeoxycholic acid on Day 29 after birth, and were switched 4 days

later to CDCA and CA therapy. The third child commenced treatment with ursodeoxycholic acid and CA from the first week. There was a positive response to ursodeoxycholic acid/chenodeoxycholic acid/cholic acid therapy as well as the improvement in histology at both the light and electron microscope level in these patients.

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

Study 91-10-10

Study 91-10-10 was an open-label, single arm, non-comparative, compassionate treatment study of CA in the treatment of inborn errors of bile acid metabolism. It is acknowledged by the CHMP that a controlled clinical study was not feasible in the condition at stake. Inborn errors in primary bile acid synthesis remain a condition that is debilitating in the long term and life threatening. It would be therefore contrary to principles of medical ethics to collect evidence of clinical efficacy of CA in a controlled clinical study. A controlled study implies withholding the sole existing treatment, CA, at least for a limited period of time. A number of studies have shown that an interruption of bile acid treatment of patients may lead to a rapid deterioration of liver function tests and increases in the production of hepatotoxic and cholestatic bile acids (Vanderpas *et al*. 1987; Koopman *et al*. 1988; Kobayashi *et al*. 2000; Yamato *et al*. 2001). Therefore, participation in a controlled clinical trial would expose patients to a risk of severe hepatic damage or even death.

Studies CAC-91-10-10 (and its substudy CAC-92-8-19) were initiated in 1992 as academic trials. The studies had started before ICH GCP was published. Therefore, the full requirements of GCP with regard to study conduct and documentation were often not met. This casted doubt on the reliability of the data presented and triggered a GCP inspection by the CHMP. The potential to retrospectively implement any corrective and preventative actions remained limited, however, every effort was made by the applicant to address and rectify as far as possible any finding and deficiencies identified by the inspection. During the procedure the applicant addressed the inspection findings and provided a revised CSR for which an extensive re-monitoring of the study data was performed resulting in an increased reliability of the data integrity. The comprehensive re-review included patient charts, analytical screening logs, adverse events, laboratory findings, study medication/treatment, and urine FAB-MS data. As a result of the re-monitoring, more patients could be included in the analysis. In addition, sensitivity analyses comprising additional data analysis sets and subpopulation analyses were performed to further investigate the robustness of the results. The results of the revised CSR are presented in this report. Despite a non-optimal documentation of study data due to the early stage academic nature of the study and the long-term study period, the results presented consistently showed improvements in the efficacy parameters analyzed and across the majority of subpopulations and analysis sets. Even given the sparseness of data documentation, GCP lapses and remaining uncertainties in data integration, the CHMP considered that observed consistency of the results and the maintenance of improvement in patients' symptoms provides evidence in support of the therapeutic efficacy of CA in the treatment of CTX, AMACR and CYP7A1 deficiencies.

Eighty-five (85) patients were diagnosed and enrolled from January 1992 to December 2009; 52 patients in study CAC-91-10-10 having single enzymatic defects (3β-HSD, Δ^4-3-oxoR, CTX, AMACR and CYP7A1 deficiencies) and 31 patients in study CAC-92-8-19 having peroxisomal disorders. Of the 52 patients, 6 died, 3 had no evidence of treatment, 4 terminated the study, 10 were lost to follow-up, and for 1 data retrieval was unsuccessful. Of the 85 patients, 79 have received study medication treatment, 49 in study CAC-91-10-10 (patients suffering from 3β-HSD, Δ^4-3-oxoR, CTX, AMACR and

CYP7A1 deficiencies) and 29 in study CAC-92-8-19. In this set of patients (49), about one quarter was below or at most 6 months of age at diagnosis, and about one third were between 7 and 36 months. On average, patients in this subgroup were 3 years at treatment start, minimum and maximum ages were 0 and 14 years, respectively. Of the patients who received treatment at least once, four discontinued the study because of liver transplantation and one due to worsening cholestasis. Only patients who received treatment and had at least one pre- and one post-treatment assessment for urine bile acids, LFTs, height and weight were included in the mITT set. This prerequisite was fulfilled for a total of 70 patients (82%), 42 patients from study 91-10-10 and 27 patients from study 92-8-19. While the patient population is the result of screening several thousands of patients with 'idiopathic liver disease' it may not be entirely representative of the true patient population in the European Union due the strong representation of a single centre, the Cincinnati Children's Hospital Medical Centre. However, data from the publication of study reports from European centres such as the Centre Hospitalier Universitaire Bicêtre, France gave European representation to the patient population. Within the limitations of a small sample size, the diversity of the population described combined with the wide spectrum of disease severity reflects the complete scientific knowledge on the patient population. Thus, the reported patient population can be considered to be reasonably representative of the entire patient population in the EU.

The demographic characteristics were comparable for the ITT, safety and mITT sets. Most patients in each set were White (≥35%), followed by Hispanic patients (≥12%). For 23% to 34% of patients the race was unknown. About 60% of patients included in each set were male, compared to about 36% of female patients. Patients were on average 2 years of age at diagnosis and started treatment at 3 years of age. However, the range of age at diagnosis varied from 0 to 13 years and for age at treatment start from 0 to 16 years. About half the patients (49%) were below 6 months of age at diagnosis. On average, the patients were in the 33% to 34% height percentile, i.e. about 33% to 34% of children are shorter at a comparable age. The mean weight percentile was 35% to 39%.

Overall, about two-thirds of the patients included presented with a single enzyme defect, while about one-third presented with a peroxisomal disorder. The majority of patients with single enzyme defects presented with *3β-HSD* deficiency, followed by Δ^4-3-oxo-R and CTX deficiency.

The treatment consisted of CA capsules, each containing 250 mg of CA, or a liquid preparation of 15 mg/mL CA, administered orally in a dose of about 15 mg/kg body weight/day. This dose has been used empirically based on metabolic response in terms of liver functions and urinary bile acids. The therapeutic target dose of 10-15 mg/kg/day was considered to be required to accomplish the goal of down-regulating endogenous bile acid synthesis, and substituting a normal bile acid pool. Thereafter the dose is adapted based on titration to the effect (metabolic response) observed in the individual patient. During the procedure the applicant further clarified that, in order to substitute a pool of bile acids in patients with defects in bile acid synthesis it is necessary to at least administer an amount equivalent to, or above the daily synthesis rate of the primary bile acids. As the fractional absorption of bile acids is not 100% and, as there is some conversion by intestinal bacteria to the secondary bile acid deoxycholic acid, a therapeutic dose of 15 mg/kg/day was chosen to enable substitution of a normal bile acid pool. In addition, dosing information for long-time surviving patients with 3ß-HSD deficiency was reported by Gonzalez *et al*. 2009. When CA treatment was established as monotherapy, the average weight-based daily doses were 12.9 mg/kg (range: 2.3-18.9 mg/kg) at the initiation of treatment and 5.5 mg/kg (range: 2.5-9.8 mg/kg) after long-term maintenance, with the patients increasing in age and weight over the time of treatment. The authors noted that at the beginning of treatment, the dose of cholic acid had to be increased from one to several times in 8 of the 13 patients in order to achieve optimal response to treatment (based on concentrations of atypical metabolites in urine). Conversely, the daily dose of CA was decreased in 4 patients because of signs of overdose. In

this study, data on the starting doses in Δ4-3-oxoR deficiency were similar, with the caveat that most of these patients were additionally receiving URSO or chenodeoxycholic acid. Younger and lighter patients appeared to require and tolerate higher doses per body weight than older children, which is in agreement with observations by Heubi *et al.* 1982 that infants and children have about 40% larger cholic acid pool sizes than adults on a per m^2 basis, and the CA turnover rate decreases by about 40% between infants and children, and between children and adults. Overall, the initial dose proposed for CA, 10 to 15 mg/kg per day, is considered consistent with the estimates of the amount required for bile pool replenishment and maintenance, and with the clinical experience in treatment of patients.

It is important to emphasize that the recommended dose range is for initial dosing only. While this initial dose range has proven to be appropriate for nearly all patients, the practice has always been to titrate patients to obtain the desired biologic effect in terms of suppression of abnormal bile acid synthesis and normalization of serum transaminases and, in addition, to monitor for possible overdosage indicated by clinical symptoms and increases in GGT and/or transaminases. Regarding dose recommendations based on the two CA capsule strengths, where the dose is not a multiple of 50, the nearest dose below the maximum of 15 mg/kg/day should be selected using a combination of the two capsule strengths, provided that it is sufficient to suppress urinary bile acids. If not, titration of the dose should commence using the next higher dose. Appropriate guidance for dose titration is provided in the SmPC.

Study CAC-001-01

The study was an open-label, non-randomised, single centre, cohort comparative study of two CA oral capsule formulations using each patient as his/her own control. Sixteen patients were enrolled and completed. Patients with inborn errors of bile acid synthesis who were being treated with CA capsules prepared by the CCHMC Pharmacy were switched from Study CAC-91-10-10 to the to be marketed commercial formulation CA capsules, each containing 50 or 250 mg of CA to be taken orally in a dose of 10-15 mg/kg body weight/day. Therapeutic equivalence was assessed at the end of 30 days of treatment using a T-test comparison for the baseline and 30 day results (e.g. liver function tests, urine and serum bile acids by FAB-MS, GC-MS, LC-MS/MS).

Efficacy data and additional analyses

Study 91-10-10

The mITT set (70 patients) was the main analysis set; of which 42 patients (60%) had one of the claimed single enzyme defects. If a subject had both a pre- and a post-treatment assessment for any of the main endpoints (LFT, FAB-MS, or height/weight assessment), that subject was included in the mITT population. Changes in urinary bile acids and in LFTs (AST/ALT) were analysed as main efficacy parameters. The applicant presented analysis by primary diagnosis for urinary bile acids, LFTs and height and weight analyses. A global analysis combining the single enzyme defects and the peroxisomal disorders patients was presented for all parameters studied; however analyses by primary diagnosis are the only relevant data in support of the claimed indications as peroxisomal disorder is a different condition to inborn errors of primary bile acid synthesis.

Urinary Bile acids by FAB-MS

A global analysis (combining single enzyme defects and peroxisomal disorders) of worst pre-treatment urine bile acid scores with best post-treatment scores after the initiation of CA treatment showed a statistically significant decrease in atypical bile acids ($p < 0.0001$, mITT). A statistically significant improvement in urinary bile acid scores was also observed for the best pre-treatment to best post-treatment analysis for both the ITT and mITT sets ($p < 0.0001$). Treatment with CA also statistically

significantly improved urine bile acid scores in patients with single enzyme defects considered as a group in the worst to best analysis (p <0.0001, mITT). Statistically significant improvements in urine bile acid scores were also shown in the best to best analysis. In accordance with the improvements seen in the analysis by type of disorder as groups, general improvements in the degree of atypical urine bile acids were seen in each individual enzyme defect group. However, the patient numbers per defect were often too low to enable statistically significant results. Statistically significant improvements were seen in primary diagnosis groups with at least six patients. When the single enzyme defect groups with less than six patients were combined, the improvement in urinary bile acids reached statistical significance also for this group. In the best pre-treatment to best post-treatment analysis changes in bile acids for patients with defects in the single enzymes *3β-HSD* or *Δ^4-3-oxo-R* showed statistical significance. Combined selected single enzymes showed a statistically significant improvement in the mITT analysis. No statistically significant changes were seen in the worst pre-treatment to worst post-treatment analysis.

Although measurements of urine bile acids were highlighted as being unverifiable in the GCP inspection, there was no specific concern highlighted in the GCP inspection to indicate that these measures were systematically incorrect and it is reassuring that the changes reported matches clinical expectation and other literature reports of cholic acid treatment.

Patients with and without concomitant URSO treatment showed statistically significant improvements in urinary bile acids from pre- to post-treatment, indicating that URSO had no impact on the overall efficacy of CA treatment (p <0.0001, mITT). Data were presented only for the global analysis combining single enzyme defects and peroxisomal disorders.

Liver function tests (serum transaminases)
In the global analysis, both ALT and AST values improved with statistical significance from the worst pre-treatment to the best post-treatment assessment (p <0.0001) in the ITT and mITT sets. Especially the number of patients with ALT and AST values < ULN increased notably, while the number of patients with values ≥3x ULN decreased. The results were comparable for ALT and AST. A statistically significant improvement in ALT and AST scores was also shown for the best pre-treatment to best post-treatment analysis in the ITT and mITT analyses, although more patients presented with values <ULN and less patients with values ≥3x ULN at pre-treatment, in accordance with the analysis type. In the worst to best analysis, treatment with CA improved LFT scores in patients with single enzyme defects as a group, reaching statistical significance for all analyses. There was a strong increase in ALT/AST values <ULN together with a strong decrease in values ≥3x ULN. Statistically significant improvements in LFT scores were also shown in the best to best ITT and mITT analyses for patients with single enzyme defects. Shifts towards improvements in ALT and AST values were shown for each primary diagnosis category; however, statistical significance was only reached in the *3ß-HSD*. The result can be explained with the generally low patient numbers per group. When the small groups of the single enzyme defects are combined, the worst to best analysis also shows a statistically significant reduction of ALT and a strong trend for AST.

In the global analysis, patients with and without concomitant URSO treatment showed statistically significant improvements in ALT and AST from pre- to post-treatment (p <0.003, mITT), indicating that URSO had no impact on the overall efficacy of CA treatment. The improvements were generally comparable for ALT and AST values. Data were presented only for the global analysis combining single enzyme defects and peroxisomal disorders.

Bilirubin
In the global analysis, mean bilirubin values decreased from pre-treatment to post-treatment assessments for each bilirubin category analysed. The decrease was statistically significant for direct

bilirubin, the category with most data available and particularly important for the diagnostic picture of inborn errors of bile acid synthesis, since direct bilirubin is elevated in cholestasis. No analysis by primary diagnosis was provided.

Height and weight

Both height and weight percentiles increased from the worst pre-treatment to the best post-treatment assessment in the ITT and mITT sets, but statistical significance was only reached for the changes in weight (global analysis). It has to be noted that the number of patients with data available was about twice as high for the weight compared to the height analysis. The results were comparable for the ITT and mITT analyses. No statistically significant changes in height or weight percentiles were shown in the best pre-treatment to best post-treatment analysis in the ITT and mITT analyses. Best pre-treatment and best post-treatment values were generally comparable. Treatment with CA improved height and weight percentiles in patients with single enzyme defects, but only the changes in weight were statistically significant for both the ITT and mITT sets. Shifts towards increases in height and weight were generally shown for each primary diagnosis category; however, statistical significance was only reached for the change in weight of patients with defects in *3ß-HSD*.

Both patients with and without concomitant URSO treatment showed statistically significant improvements in weight percentiles from pre- to post-treatment in the ITT analysis. Statistical significance was missed in the weight analysis in patients without concomitant URSO treatment in the mITT analysis. Changes in height percentiles were not statistically significant in either subgroup or analysis. No analysis by primary diagnosis was provided.

Liver histology

With the exception of bridging fibrosis and unspecified fibrosis, all parameters assessed were less frequently present in post-treatment biopsies than in pre-treatment biopsies. Particularly the numbers of patients with cholestasis, giant cells, and necrosis had improved from pre- to post-treatment. For the majority of parameters, the number of patients with trace or moderate levels of histopathologic signs decreased from pre- to post-treatment, while the number of patients with absent symptoms increased. No analysis by primary diagnosis was provided.

The text below discusses in general terms the evidence for efficacy of Kolbam in the treatment of inborn errors of primary bile acid synthesis.

The CHMP considered that the above discussed results demonstrated that treatment with CA acts by feedback regulation on the liver and down regulation of primary bile acid synthesis, leading to a reduction and/or disappearance of atypical hepatotoxic bile acids and their intermediates and normalization of serum liver enzymes levels in the single enzyme defects studied. The absolute number of patients remains limited due to the rarity of the conditions; moreover, the distributed nature of the study with many international patients under the direct care of their local physicians led to sporadic collection of data. The rarity of the condition, as well as ethical considerations, made the conduct of a controlled clinical study impossible. The uncontrolled data made difficult to ascertain the beneficial effects of CA. The patient numbers per defect were often too low to enable statistically significant results. However, treatment with CA statistically significantly improved urine bile acid scores in patients with single enzyme defects considered as a group; significance was achieved for patients with defects in the single enzymes *3β-HSD* or *Δ^4-3-oxo-R* in the best pre-treatment to best post-treatment analysis. Statistically significant improvements in LFT scores were also shown in the best to best ITT and mITT analyses for patients with single enzyme defects considered as a group. Shifts towards improvements in ALT and AST values were shown in individual defect groups; however, statistical significance was only reached in the 3ß-HSD patients.

In an uncontrolled trial, statistical tests of pre-treatment to post-treatment changes to draw inferences on efficacy are confounded by time and by concomitant therapeutic interventions and patient management. Hence, a close scrutiny of individual profiles of response and a clear biological and pharmacological rationale for the intervention, substantiated by basic science and literature data, is the basis on which to draw conclusions about therapeutic efficacy. The conclusion of therapeutic efficacy is made considering results across all single enzyme defects included in the trial. This rationale can then be applied to each single enzyme defect under consideration based on knowledge of disease pathology and cholic acid pharmacology, in addition to the available clinical trial and literature data. In particular, it is agreed with the applicant that in each of these enzyme deficiencies, the elevated transaminases and abnormal liver histology result from the abnormal bile acids that occur as a consequence of the blockage of the CA biosynthetic pathway by the enzyme defects. For each of these deficiencies, the therapeutic benefit of CA derives from the same mechanism, namely the ability to inhibit transcription of the 7-alpha hydroxylase (at the head of the pathway through an FXR-dependent mechanism) (Setchell and O-Connell, 2007) or provides benefit to patients by providing CA to patients. Whilst the magnitude of changes in metabolic and pathologic parameters may differ in each different phenotype (noting also that clinical prognosis differs by phenotype), it is evident that an effective cholic acid preparation will be therapeutically efficacious in each.

Overall, the clinical data presented in this study support demonstration of efficacy of CA for patients with single enzyme defects as measured by objective improvement in metabolic and pathologic parameters over the 17 years period of the study.

Study CAC-001-01

The study results showed that there were no clinically significant changes in laboratory values at the follow-up visit, except for one subject who had increases in AST and ALT. The applicant claimed that therapeutic equivalence was observed in 15 out of 16 subjects (93.75%, 95% CI 69.8%, 99.8%) with a return back to baseline LFT for the lone patient not meeting the therapeutic equivalence criteria immediately post treatment. While there is no evidence to show that the TBM CA formulation is therapeutically inferior to the CA used in the previous study, the data remains limited. In total 25 patients were planned to be enrolled; however only 16 patients enrolled and one of these patients was not treatment compliant. The applicant has concluded the 95% therapeutic equivalence CI ranged 69.8% to 99.8% which is limited. The duration of the trial was relatively short and some variability of the data was noted where AST may improve but ALT worsened. For urinary and serum atypical bile acid metabolites there was a general trend of improvement however some cases show increases in urinary atypical bile acid metabolites. Overall due to the lower power, the relatively short duration of study, the observed variability of data and the overall results of this trial the CHMP could not conclude that both formulations were therapeutically equivalent. However, as the treatment of patients with inborn errors of bile acid metabolism is appropriately managed by titration of the dose over time to achieve an optimal clinical response, the CHMP concluded that the observed differences were not of clinically significant in terms of efficacy or safety. Patients responded similarly when they switched treatments and importantly no new safety events arose.

Study CAC-002-001

During the procedure the applicant provided as supportive data the 33 months interim report of study CAC-002-001; the continuation study of 91-10-10 and also including newly diagnosed patients. The efficacy analysis of urinary bile acids and transaminases using the comparison from baseline to worst post-baseline value for the overall population that included both patients on CA at study start and treatment naive patients showed no statistically significant changes. Similar results were shown for the height and weight analysis. Mean total bilirubin values also remained stable in the baseline to worst

post-baseline value analysis. Since the overall population included a large proportion of patients already on CA treatment at study start (31 of 41 patients, 76%), the results showed the continuous efficacy of CA treatment without deterioration of the parameters analysed. Subgroup analyses stratifying patients by formulation, treatment status, and disorder type generally confirmed the results obtained for the overall population.

Literature reports

Clinical experience has been reported in the literature by the applicant from small cohorts of patients and single case reports; absolute patient numbers are small (<30) due to the rarity of the conditions. Reporting and publication bias are potential concerns given the small number of cases reported in the literature as well as their geographic concentrations. However, given the documented survival benefit of treatment with CA and bile acid therapy overall, complete treatment failures appear unlikely except for patients with advanced liver disease. Mixed treatment results may not have been considered for publication by investigators or scientific journals. Long-term data on treatment with CA monotherapy are reported in the literature presented for 3ß-HSD, Δ^4-3-oxo-R and CTX patients observed for more than 10 years in the EU. The CHMP considered that the available clinical data presented in this literature review support the demonstration of efficacy of CA reported in the pivotal study 91-10-10, as measured by objective improvement in metabolic and pathologic parameters in patients reported. In the reported patients oral cholic acid therapy has been shown to postpone or obviate the need for liver transplantation; restore normal laboratory parameters; improve histological lesions of the liver, and significantly improve all of the patient's symptoms. Mass spectrometry analysis of urine during cholic acid therapy shows the presence of cholic acid and a marked reduction and in some cases, complete elimination of the toxic bile acid metabolites. This reflects the restoration of an effective feedback mechanism of bile acid synthesis.

Discussion on the indication statement

The applicant initially applied for the treatment of nine indications being nine primary enzyme defects included in the inborn errors of primary bile acid synthesis condition. During the procedure the applicant was requested to restrict the indications to the specific defects that have been actually studied in the application. The applicant argued that in each of these enzymes deficiencies, the elevated transaminases and abnormal liver histology result from the abnormal bile acids that occur as a consequence of the blockage of the CA biosynthetic pathway by the enzyme defects. For each of these deficiencies, the therapeutic benefit of CA derives from precisely the same mechanism, namely the ability to inhibit transcription of the 7-alpha hydroxylase (at the head of the pathway through an FXR-dependent mechanism) (Setchell and O'Connell (2007)) or provides benefit to patients by providing CA to patients.

The CHMP acknowledged that it may not be possible to investigate the effects of CA in all the inborn errors of bile acid synthesis. However, specific enzymes deficiencies can only be claimed if they have been actually studied. The applicant agreed that only the specific enzymes deficiencies that have been studied in the 91-10-10 study could support the therapeutic indications. The following revised indication statement was proposed:

"cholic acid FGK is indicated for the treatment of inborn errors of primary bile acid synthesis responsive to treatment with cholic acid, in infants from one month of age for continuous lifelong treatment through adulthood, encompassing the following single enzyme defects:

- *3ß-hydroxy-Δ5-C27-steroid oxidoreductase (also known as 3ß-hydroxy- Δ5-C27-steroid dehydrogenase/isomerase or 3ß-HSD or HSD3ß7) deficiency*
- *Δ4-3-oxosteroid 5ß-reductase (Δ4-3-oxo-R or AKR1D1) deficiency*

- *Sterol 27-hydroxylase (presenting as cerebrotendinous xanthomatosis, CTX) deficiency*
- *2- (or α-) methylacyl-CoA racemase (AMACR) deficiency*
- *Cholesterol 7 α-hydroxylase (CYP7A1) deficiency"*

Furthermore, the CHMP concluded that each of the claimed indications covering a specific enzyme deficiency is a clinical distinct medical entity.

On 13 September 2013 the Commission granted a marketing authorisation for the orphan medicinal product Orphacol. In accordance with Articles 8(1) and 8(3) of Regulation (EC) No 141/2000, the applicant submitted a report on similarity of Kolbam to Orphacol and a report claiming the derogation of Orphacol's market exclusivity based on clinical superiority to Orphacol. The CHMP considered that Kolbam was similar to Orphacol with respect to two of the five therapeutic indications claimed i.e. 3β-HSD and Δ^4-3-oxoR deficiencies (see Appendix 1). The CHMP also concluded that Cholic acid was not considered safer, more effective or otherwise clinically superior to Orphacol (see Appendix 2).

Following the outcome of the clinical superiority report, the applicant withdrew from the application the indications 3β-HSD and Δ^4-3-oxoR deficiencies. The scope of the application was reduced to the following 3 indications:

- *Sterol 27-hydroxylase (presenting as cerebrotendinous xanthomatosis, CTX) deficiency*
- *2- (or α-) methylacyl-CoA racemase (AMACR) deficiency*
- *Cholesterol 7 α-hydroxylase (CYP7A1) deficiency"*

As a result, the report on the clinical superiority (Appendix 2) is not relevant for the purpose of this opinion as Kolbam is not anymore similar to Orphacol in the claimed indications.

MA under exceptional circumstances

In accordance with Article 14(8) of Regulation (EC) No 726/2004 and Annex I, of the Directive 2001/83/EC the applicant applied for a marketing authorisation under exceptional circumstances. The applicant argued that he was unable to provide comprehensive data on the efficacy and safety under normal conditions of use because the indications for which Kolbam are intended are encountered so rarely that he cannot reasonably be expected to provide comprehensive evidence. The applicant also argued that it would be contrary to generally accepted principles of medical ethics to collect such information.

The CHMP agreed on these justifications and considered that the criteria defined in the above-mentioned provision are met for the 3 indications claimed:

Inability to provide comprehensive efficacy and safety data due to rarity of the indication

The three indications for which Kolbam is intended are rarely encountered. Indeed, cholic acid has been designated as Orphan Medicinal Product (EU/3/02/683) by the European Commission for the treatment of inborn errors of primary bile acid synthesis on 09 December 2011. At the time of designation, inborn errors in primary bile acid synthesis re affected approximately 0.07 in 10,000 people in the EU. Over a 17 years period of study the applicant was able to recruit only 54 patients affected by the condition. Given the rarity of the diseases, the CHMP considered that the applicant cannot be reasonably expected to provide comprehensive non-clinical and clinical evidence. Patients are so rarely identified that conduct of a controlled clinical trial would be unachievable.

Inability to collect comprehensive information because it would be contrary to medical ethics

It would be contrary to medical ethics principles to collect evidence of clinical efficacy of cholic acid in the three intended indications in a controlled clinical study. A controlled study implies withholding the sole existing treatment, cholic acid, at least for a limited period of time. A number of studies have shown that an interruption of bile acid treatment of patients may lead to a rapid deterioration of liver function tests and increases in the production of hepatotoxic and cholestatic bile acids (Vanderpas et al. 1987; Koopman et al. 1988; Kobayashi et al. 2000; Yamato et al. 2001). Therefore, participation in a controlled clinical trial would expose patients to a risk of severe hepatic damage, or even death.

2.5.4. Conclusions on the clinical efficacy

In an uncontrolled trial, statistical tests of pre-treatment to post-treatment changes to draw inferences on efficacy are confounded by time and by concomitant therapeutic interventions and patient management. Hence, a close scrutiny of individual profiles of response and a clear biological and pharmacological rationale for the intervention, substantiated by basic science and literature data, is the basis on which to draw conclusions about therapeutic efficacy. The conclusion of therapeutic efficacy is made considering results across all single enzyme defects included in the trial. This rationale can then be applied to each single enzyme defect under consideration based on knowledge of disease pathology and cholic acid pharmacology, in addition to the available clinical trial and literature data. Whilst the magnitude of changes in metabolic and pathologic parameters may differ in each different phenotype (noting also that clinical prognosis differs by phenotype), it is evident that Kolbam will be therapeutically efficacious in the treatment of CTX, AMACR and CYP7A1 deficiencies.

The efficacy analysis from the pivotal Study 91-10-10 showed that the patient numbers in the 5 enzymes deficiencies studied were often too low to enable statistically significant results. When the individual single enzyme defect disorder types were assessed, general improvements in the degree of atypical urine bile acids and in LFTs were seen. Treatment with CA statistically significantly improved urine bile acid scores in patients with single enzyme defects considered as a group; significance was achieved for patients with defects in the single enzymes 3β-HSD or Δ^4-3-oxo-R in the best pre-treatment to best post-treatment analysis. Statistically significant improvements in LFT scores were also shown in the best to best ITT and mITT analyses for patients with single enzyme defects considered as a group. Shifts towards improvements in ALT and AST values were shown in individual defect groups; statistical significance was only reached in the 3ß-HSD patients.

Treatment with CA improved height and weight percentiles in patients with single enzyme defects, but only the changes in weight were statistically significant for both the ITT and mITT sets for the single enzyme defect considered as a group. Shifts towards increases in height and weight were generally shown in each individual group; however, statistical significance was only reached for the change in weight of patients with defects in *3ß-HSD*. While weight is not sole indicator of efficacy of CA treatment, it supports the results seen for the more direct measures of efficacy urinary bile acids and LFTs. Height, on the other hand, is a highly multifactorial parameter that may not be expected to change significantly. However, a trend for increased growth was also seen in the height analyses.

Ursodeoxycholic acid was originally the co-study medication together with CA, but was discontinued from June 2001. That URSO treatment had no impact on the overall efficacy of CA treatment was confirmed in subgroup analyses comparing patients with CA monotherapy to those who have at least once received URSO treatment. Patients with and without concomitant URSO treatment showed statistically significant improvements in urinary bile acids and in LFTs from worst pre- to best post-treatment visits. Although no analysis by primary diagnosis was provided these observations supports the efficacy of CA as monotherapy.

The clinical data from the literature review support the demonstration of efficacy of CA reported in the pivotal study CAC-91-10-10, as measured also by objective improvement in metabolic and pathologic parameters in patients reported. Cholic acid has been used since 1990 to treat patients who have inborn errors of bile acid synthesis (Setchell *et al*. 2006, Heubi *et al*. 2007).

Taken together, although the data remains limited, the results confirmed that oral CA treatment down-regulates the synthesis of atypical bile acids, provides an effective therapy by reducing the production of hepatotoxic bile acid intermediates, and has a palliative effect on the patient's clinical course that may translate into improved and prolonged life. The body of clinical data primarily from Study CAC-91-10-10 and its continuation study CAC-002-001 showed evidence relevant to the understanding of the therapeutic efficacy of CA in the treatment of inborn errors of the synthesis of primary bile acids. Overall, the CHMP is able to conclude that Kolbam has therapeutic efficacy in the treatment of patients with CTX, AMACR and CYP7A1 deficiencies.

On 13 September 2013 the Commission granted a marketing authorisation for the orphan medicinal product Orphacol. In accordance with Articles 8(1) and 8(3) of Regulation (EC) No 141/2000, the applicant submitted a report on similarity of Kolbam to Orphacol and a report claiming the derogation of Orphacol's market exclusivity based on clinical superiority to Orphacol. The CHMP considered that Kolbam was similar to Orphacol with respect to two of the five therapeutic indications claimed i.e. 3β-HSD and Δ^4-3-oxoR deficiencies (see Appendix 1). The CHMP also concluded that Cholic acid was not considered safer, more effective or otherwise clinically superior to Orphacol (see Appendix 2).

Following the outcome of the clinical superiority report, the applicant withdrew from the application the indications 3β-HSD and Δ^4-3-oxoR deficiencies. The scope of the application was reduced to the following 3 indications:

- *Sterol 27-hydroxylase (presenting as cerebrotendinous xanthomatosis, CTX) deficiency*
- *2- (or α-) methylacyl-CoA racemase (AMACR) deficiency*
- *Cholesterol 7 α-hydroxylase (CYP7A1) deficiency"*

As a result, the report on the clinical superiority (Appendix 2) is not relevant for the purpose of this opinion as Kolbam is not anymore similar to Orphacol in the claimed indications.

Based on the above, the CHMP endorsed that due to the rarity of the condition, the provision of comprehensive data, particularly randomised controlled clinical trials is not feasible. The CHMP also endorsed the argumentation on inability to collect comprehensive information because it would be contrary to medical ethics principles. Data submitted is limited as expected in such rare condition it is nevertheless considered that the clinical data presented in this application are sufficient to support demonstration of efficacy and safety of cholic acid in the three claimed indications. A marketing authorisation under exceptional circumstances for Kolbam is acceptable in regards to the fulfilled criteria of rarity of the disease and medical ethics.

In the context of a MA under exceptional circumstances a specific obligation is included in the terms of the Marketing Authorisation. The applicant will monitor the long term efficacy in patients treated with Kolbam from a patient registry for which details are reflected in the risk management plan. The registry will monitor accumulating data on efficacy in the treatment of inborn errors in primary bile acid synthesis due CTX, AMACR and CYP7A1 deficiencies with Kolbam in infants, children, adolescents and adults. Reports on recruitment progress of the registry will be reported at the time of PSURs and

Annual Re-assessments. Progress and results from the registry will form the basis of the annual reassessments of the benefit/risk profile of Kolbam.

2.6. Clinical safety

The safety data of CA were collected in Study CAC-91-10-10 and CAC-001-01. In addition safety data from study CAC-002-001 (continuation study from CAC-91-10-10 and CAC-001-01) and safety data reported in the literature were reported.

Patient exposure

When study CAC-91-10-10 was started in 1992, CA in combination with URSO was used as study medication. However, once the Investigators concluded that URSO was not effective in the treatment of defects in bile acid synthesis, URSO was removed as study medication. Most frequently patients have received CA treatment alone. Other frequent treatment regimens included CA and URSO either in combination or as single treatments following each other. Very few patients received CDCA or docosahexenic acid (DHCA). CDCA was used during the earlier years for treatment of bile acid defects, but is now contraindicated since it was found to be hepatotoxic (e.g. Chenodiol prescribing information). DHCA is a polyunsaturated fatty acid that is used in the treatment of patients with peroxisomal disorders to support brain development.

The majority of patients received orally CA doses of up to about 15 mg/kg body weight per day. Twelve patients received daily doses >20 mg/kg. Two patients received the highest doses with 61.5 and 58.1 mg/kg/day, respectively. The application of higher doses was generally based on rational decisions, e.g. due to increased urinary bile or transaminase values. The majority of patients included in the safety set had received treatment with CA; on average for a duration of 145 weeks. At least one patient had received CA for a maximum of 545 weeks. The mean treatment duration for URSO was 82 weeks, and in accordance with the deletion as study medication shorter than the overall treatment duration for CA. DHCA and CDCA were used by a limited number of patients only, but particularly DHCA was used for up to 259 weeks.

Adverse events

Study CAC-91-10-10

The table below presents the most frequent treatment-emergent AEs [TEAEs] (i.e. AEs reported by at least 2 patients) by MedDRA system organ class (SOC) and preferred term (PT). On the SOC level, general disorders and administration site conditions were the most frequent TEAEs, followed by gastrointestinal disorders and infections and infestations. On the preferred term level, disease progression was the most common TEAE followed by pyrexia and diarrhea.

Table 27 Treatment-emergent AEs by System Organ Class and Preferred Term (cut-off: at least 2 patients) - Safety Set

MedDRA[a] System Organ Class	Preferred Term	Events	N	Percent
Blood and lymphatic system disorders		**2**	**2**	**3**
Gastrointestinal disorders		**21**	**14**	**18**
	Abdominal pain	2	2	3
	Ascites	2	2	3
	Constipation	2	2	3
	Diarrhea	6	6	8
	Gastrointestinal hemorrhage	3	2	3

MedDRA[a] System Organ Class	Preferred Term	Events	N	Percent
General disorders and administration site conditions		**18**	**15**	**19**
	Disease progression	9	9	11
	Pyrexia	7	7	9
Hepatobiliary disorders		**5**	**5**	**6**
	Jaundice	2	2	3
Infections and infestations		**20**	**13**	**16**
	Bronchopneumonia	2	2	3
	Gastroenteritis viral	2	2	3
	Otitis media	2	2	3
	Upper respiratory tract infection	5	5	6
	Urinary tract infection	2	2	3
Injury, poisoning and procedural complications		**7**	**4**	**5**
	Fracture	6	4	5
Investigations		**4**	**3**	**4**
Metabolism and nutrition disorders		**9**	**8**	**10**
	Dehydration	3	3	4
	Vitamin E deficiency	2	2	3
Musculoskeletal and connective tissue disorders		**2**	**2**	**3**
Nervous system disorders		**12**	**8**	**10**
	Convulsion	7	5	6
	Lethargy	3	3	4
Psychiatric disorders		**1**	**1**	**1**
Reproductive system and breast disorders		**2**	**1**	**1**
Respiratory, thoracic and mediastinal disorders		**9**	**6**	**8**
	Cough	2	2	3
	Epistaxis	2	2	3
Skin and subcutaneous tissue disorders		**3**	**3**	**4**

[a] MedDRA version 11.0 MedDRA = medical dictionary for drug regulatory affairs, N = number of patients.

Causalities are summarized below. For only 2 patients the AEs were assessed by the investigator as treatment-related or relationship unknown. For the remaining patients the AEs were considered unrelated to study medication treatment. The most frequent alternative reason documented was deterioration of the disease under study.

Table 28 Summary of Patients with Treatment-Emergent AEs by Relationship to Study Medication - Safety Set

Relationship	N	Percent
Yes/Unknown	2	3
No[a]	33	42
Disease under study	7	9
Neurologic deterioration with treated disease	1	1
Unknown	1	1

[a]Please note that category 'No' is not cumulative; A patient could be listed in several categories. N = number of patients.

AEs assessed as treatment-related or without known relationship to study medication treatment are summarized by MedDRA SOC and PT below. Each particular AE considered treatment-related/unknown occurred in one patient only (malaise and jaundice in 1 patient; skin lesion in another patient).

Table 29 Treatment-related/unknown AEs by System Organ Class and Preferred Term – Safety Set

MedDRA[a] System Organ Class	Preferred Term	N	Percent
General disorders and administration site conditions		**1**	**1**
	Malaise	1	1
Hepatobiliary disorders		**1**	**1**
	Jaundice	1	1
Skin and subcutaneous tissue disorders		**1**	**1**
	Skin lesion	1	1

[a] MedDRA version 11.0; N = number of patients

The intensities of TEAEs are summarized below. TEAEs were predominantly mild or moderate in intensity. Only 10 patients (13%) experienced AEs of severe intensity. These were most frequently related to disease progression (9 patients). The only other severe AE occurring in at least two patients was dehydration.

Table 30 Number of Patients with Treatment-emergent AEs by Intensity – Safety Set

Severity	N	Percent
Mild	22	28
Moderate	15	19
Severe	10	13
Unknown	9	11

Patients could be included in more than one category. Each patient counted once per category.
N = number of patients.

Most patients with TEAEs recovered from the AEs; for 13 patients the outcome was unknown. Only three patients (4%) had not recovered from the AEs (1 patient with portal hypertension, 1 patient with ovarian cyst and ovarian failure, and 1 patient with convulsion). For seven patients death was documented as outcome, either due to the respective AE or due to other causes. For two patients non-TEAEs (both 'disease progression') were documented prior to the patient's death; a patient died due to the AE, the other patient died due to other causes. For the remaining 12 patients who died during the study, no AE was documented as potential cause of death.

Study CAC-001-01

There were 13 AEs reported for 9 of 16 patients in the study. AEs judged not related to study drug included 5 incidents of low vitamin A, D, or 25-OHD; 1 incident of nausea and 1 incident of vomiting (same patient), 1 abdominal muscle spasm, 1 nose bleed. There were 2 incidents of slightly elevated liver function tests (1 each ALT and AST for the same patient) judged to be unlikely to be related to the study drug, and 1 incident each of mild reflux and moderate diarrhoea (different patients) said to be possibly related to the study drug.

Serious adverse event/deaths/other significant events

Study CAC-91-10-10

Deaths

A total of 21 patients died during the study period (and an additional death occurred after the cut-off of the reporting period). Only for 10 of the expired patients an AE had been documented. Disease progression or an event secondary to worsening of the underlying condition was the most frequently noted AE. For none of these patients the death was considered related to study medication treatment.

Thirteen (13) patients were no longer being followed by CCHMC at the time of death. The patient's death was frequently reported only a long time after the death occurred, often through contacts with the primary treating physician. Efforts to retrieve additional information from the patient family or primary caregiver were unsuccessful. However, based on the severe, potentially fatal disease, worsening of the underlying condition was considered a reasonable assumption for most cases.

Fifteen of the 21 patients who died suffered from peroxisomal disorder, mainly Zellweger's syndrome. Of the six patients with single enzyme defects who died, four suffered from Δ^4-3-oxoR deficiency. There was a clear trend that worsening of the underlying condition leading to death occurred more often in patients with a peroxisomal disorder. This is not unexpected as patients with Zellweger's syndrome, neonatal adrenoleukodystrophy (NALD), and infantile Refsum's disease typically present with significant comorbidities often including CNS impairment which would not be treated by addressing the bile defect effects. Almost all patients who died had been diagnosed with a defect in bile acid synthesis prior to 1 year of age, four patients were 1 year of age at diagnosis and one patient was 3 years of age

Serious adverse events

All treatment-emergent SAEs documented during the study period are summarized below. SAEs occurred in a total of 16 patients. Disease progression was the most frequently reported SAE, followed by urinary tract infection, diarrhoea, and dehydration. All other SAEs occurred in single patients only. None of the SAEs were considered related to study medication treatment by the Investigator. Unless a patient died due to the SAE, the vast majority of patients recovered. Only for one patient each with serious gastric ulcer and disease progression, the SAE outcome was unknown.

Table 31 Treatment-emergent SAEs

MedDRA[a] System Organ Class	Preferred Term	N	Percent
Blood and lymphatic system disorders		**1**	**1**
	Coagulopathy	1	1
Gastrointestinal disorders		**4**	**5**
	Diarrhea	2	3
	Gastric ulcer	1	1
	Gastrointestinal hemorrhage	1	1
	Pneumoperitoneum	1	1
General disorders and administration site conditions		**7**	**9**
	Disease progression	7	9
Hepatobiliary disorders		**1**	**1**
	Jaundice	1	1
Infections and infestations		**4**	**5**
	Gastroenteritis	1	1
	Gastroenteritis viral	1	1
	Infection	1	1
	Nasopharyngitis	1	1
	Rotavirus infection	1	1
	Urinary tract infection	2	3
Investigations		**1**	**1**
	Nutritional condition abnormal	1	1
Metabolism and nutrition disorders		**2**	**3**
	Dehydration	2	3
Nervous system disorders		**1**	**1**
	Convulsion	1	1
Respiratory, thoracic and mediastinal disorders		**2**	**3**
	Epistaxis	1	1
	Respiratory disorder	1	1
	Respiratory distress	1	1

[a] MedDRA version 11.0. Subjects with multiple events for a given outcome were counted once only for each outcome.
N = number of patients.

Diarrhoea and Gastroenteritis

Diarrhoea is a known side effect of excessive dosing with CA. During the 18-year study period, diarrhoea was documented for six patients. One of these patients and another four patients complained of gastroenteritis or enteritis, however, for three the gastroenteritis was of viral cause. One case of viral gastroenteritis occurred prior to study medication start. All events of diarrhea and gastro-enteritis were of mild intensity, except for one case of moderately intense diarrhea and one case of severe diarrhea. There was no correlation between the severity of diarrhea and daily CA doses; patients with higher severity events had received regular CA doses only. None of the cases of enteritis or diarrhoea were assessed as related to study medication treatment. All patients with enteritis or diarrhoea recovered, with the exception of one patient who died due to other causes. Shortly prior to the death the patient had experienced severe coagulopathy, gastrointestinal haemorrhage, epistaxis, and diarrhea secondary to end stage liver disease.

Table 32 Patients with the Treatment-emergent AEs (Gastro-)enteritis and Diarrhea

Preferred Term [a]	Relationship	Serious	Outcome
Gastroenteritis viral[b]	No	No	Recovered
Gastroenteritis viral	No	Yes	Recovered
Gastroenteritis	No	Yes	Recovered
Gastroenteritis viral	No	No	Recovered
Enteritis	No	No	Recovered
Diarrhea	No	No	Recovered
Diarrhea	No	No	Died due to other causes
Diarrhea	No	No	Recovered
Diarrhea	No	Yes	Recovered
Diarrhea	No	No	Recovered
Diarrhea	No	Yes	Recovered

[a] MedDRA version 11.0
[a] Adverse event started on 26 Jan 1993, prior to the first study medication intake on 27 Jan 1993.

One case of moderate diarrhoea was reported in study CAC-001-01 which was possibly related to the treatment.

In general, the potential to produce diarrhoea with CA dosing is quite low in infants and children or even adults. Children clearly would not be prone to diarrhoea with CA until prepuberty or puberty when they would have body weights similar to adults. Bile acid diarrhoea is caused by the cathartic and other related effects on colonic epithelium that leads to water and electrolyte secretion. It was recognized by Mekhjian *et al*. 1971 that to induce water and electrolyte secretion, it was necessary to achieve a faecal level of at least 3 mmol/L of dihydroxy bile acids (chenodeoxycholic and deoxycholic acid) to produce this effect. They found that CA and URSO did not affect water and electrolyte secretion. In addition, it was later recognized that the levels necessary to produce diarrhoea had to be present in the water phase of stool and that a critical pH needed to be present to allow bile acids to have their effect on the colonic epithelium (McJunkin *et al*. 1981).

Normal children excrete bile acids at the rate of 90.8 mg/m^2/day with 0.50 mmol/L in the aqueous phase (Heubi *et al*. 1979). In children with a small bowel resection leading to loss of the ileum and its ability to conserve bile acids, the total faecal bile acids are 797 mg/m^2/day with 5.41 mmol/L in stool (Heubi *et al*. 1980) which indicates that in case of total interruption of the recovery of bile acids by the intestine, it is possible to have levels in the stool that would be capable of stimulating water and electrolyte secretion. However, if healthy humans receive exogenous bile acids by oral application, a

fixed size pool of bile acids is maintained because of a sensitive feedback inhibition system on the synthesis of bile acids. As such it would not be anticipated that humans have enough stool loss of bile acids, even with CA supplementation, to produce excessive stool bile acid levels or levels in the water component of the stool until a child achieved a much larger body size and bile acid pool. Although treatment doses of CDA may produce diarrhoea, CA would have to be converted to deoxycholic acid to have a cathartic effect. The conversion of CA to deoxycholic acid is likely to be relatively modest (no more than 1/2 of the dose) and not sufficient to produce enough deoxycholic acid to stimulate water and electrolyte secretion with resultant diarrhoea.

Study CAC-001-01

There were no deaths, and no adverse events were determined to be serious or significant.

Laboratory findings

Study CAC-91-10-10

Changes in liver function tests (serum transaminases) and bilirubin are analysed as efficacy endpoints. The results are presented in the efficacy section. No other clinical laboratory data were analysed.

Study CAC-001-01

There were 2 incidents of slightly elevated liver function tests (1 each ALT and AST for the same patient) judged to be unlikely to be related to the study drug

Safety in special populations

Pregnancy
Psychoyos *et al*. 1989 reported that HPLC analysis of the embryo-toxic fraction of human uterine fluid, collected between the 22nd and 25th day of the menstrual cycle, revealed the presence of CA at high concentrations. It was suggested that CA could be responsible for the embryotoxicity of the uterine environment, which follows the receptive period for implantation. However, Gonzales *et al.* 2009 reported four normal pregnancies in two patients resulting in the birth of four healthy infants while patients were treated with CA. This suggests that CA is unlikely to be embryotoxic and is safe when taken at therapeutic doses during pregnancy. GC-MS surveillance confirmed continued compliance with therapy by these two patients during pregnancy with maintenance of consistent bile acid metabolism.

Lactation
No data are available in lactating women. However no effects are anticipated on the breast-fed infant since the systemic exposure of the breast-feeding woman to CA are negligible given the low serum levels.

Elderly
Safety in patients over the age of 65 has not been established.

Children
Cholic acid can be recommended for infants over the age of one month, older children and adults based on the available clinical data. The recommended dosage is 10-15 mg/kg/day. There is insufficient data to establish the safety of efficacy of CA in children under the age of one month.

Hepatic impairment

No data regarding CA treatment are available in patients with inborn errors of bile acid metabolism that have hepatic impairment unrelated to their primary disease (CAC-91-10-10; Gonzalez *et al*. 2009). If such patients are encountered, decreased hepatic clearance of bile acids and/or cholestasis could occur, leading to increased serum concentrations of CA and symptoms/signs of toxicity (primarily pruritus and diarrhoea). Intuitively, the CA dose should be reduced which may reduce toxicity but could result in inadequate suppression of the biosynthesis of the hepatotoxic and cholestatic bile acid metabolites and recurrence of the manifestations of the primary disease. As such, in the absence of clinical experience in patients with hepatic impairment from causes other than the genetic bile acid enzyme deficiencies, no recommendations on dosage adjustment can be made. It is essential that patients with hepatic impairment unrelated to their primary disease that are treated with CA be monitored closely.

Renal impairment

No data are available for patients with renal impairment. Bile acids including CA are excreted in the urine in negligible amounts and compromised renal function would not be expected to result in systemic accumulation and toxicity. For patients with inborn errors of bile acid metabolism that are successfully treated with CA, development of renal failure may have little or no impact on systemic bile acid concentrations. However, these patients should be carefully monitored and the dose of CA titrated individually.

Overdose

Gonzales *et al*. 2009 reported apparent signs of CA overdose in four children (one of which was accidental administration of 56 mg/kg as a single dose) with clinical features including pruritus, diarrhoea, and elevation of serum GGT, ALT, and total serum bile acid concentration. However, these increases returned to normal after CA dose reduction. Moreover, CA is an endogenous primary bile acid and a normal end product of cholesterol metabolism in healthy humans and animals. As such, orally administered exogenous CA will be subject to established physiological mechanisms of absorption, distribution, metabolism and excretion and multiple feedback processes are constantly engaged in managing the enterohepatic recirculation of the bile acid pool. In the event of overdosage the patient should be monitored and treated symptomatically. There is no specific antidote.

Safety related to drug-drug interactions and other interactions

No safety data related to drug-drug interactions or other interactions have been presented.

Discontinuation due to adverse events

Treatment-emergent AEs that led to study discontinuation are presented below. A total of four patients discontinued the study due to AEs. The AE most frequently leading to study discontinuation was disease progression. All other AEs leading to study discontinuation occurred in single patients only. One patient who discontinued the study after TEAEs died, 1 patient recovered from the associated AE (disease progression), and for 2 patients who discontinued due to AEs (disease progression and cholestasis) the outcome was unknown. None of the AEs leading to study discontinuation was considered related to study medication treatment by the Investigator.

Table 33 Treatment-emergent AEs Leading to Study Discontinuation

MedDRA[a] System Organ Class	Preferred Term	N	Percent
Blood and lymphatic system disorders		**1**	**1**
	Coagulopathy	1	1
Gastrointestinal disorders		**1**	**1**
	Gastrointestinal hemorrhage	1	1
General disorders and administration site conditions		**2**	**3**
	Disease progression	2	3
Hepatobiliary disorders		**1**	**1**
	Cholestasis	1	1
Respiratory, thoracic and mediastinal disorders		**1**	**1**
	Epistaxis	1	1

[a] MedDRA version 11.0. Subjects with multiple events for a given outcome were counted once only for each outcome.
N = number of patients.

Study CAC-002-001

During the 33-month study period, approximately half of the patients experienced TEAEs. Vitamin D decreased was the most common TEAE followed by disease progression, hepatic enzyme increased and upper respiratory tract infection. Study drug-related AEs were only reported for 2 patients, none of the TEAEs was serious (mild peripheral neuropathy and mild nausea) and both resolved. Four patients discontinued the study due to TEAEs: disease progression in 3 patients and peripheral neurophathy in 1 patient. Two patients who discontinued due to disease progression died and the 2 others recovered. Treatment-emergent SAEs occurred in 7 patients. Disease progression was the most frequently reported treatment-emergent SAE, occurring in 2 patients with single enzyme defects and 2 patients with peroxisomal disorder. All other SAEs occurred only once. None of the SAEs was considered related to CA by the Investigator. A total of 3 patients died during the study period all due to AEs; two patients due to disease progression which was associated with a multisystem failure in one patient, and one patient due to thrombosis following liver transplantation. Two patients who died suffered from peroxisomal disorders, one patient from a single enzyme defect. After the data cut-off date of the reporting period, one additional patient died as a result of sepsis following a pamidronate infusion. None of the AEs leading to death was considered related to study treatment by the Investigator. Data on available laboratory values and physical examinations did not reveal any clinically significant changes.

Literature reports

Very few adverse events to CA are reported in the literature. No clear quantification of the frequency of a given adverse reaction is possible, as either no adverse reactions occurred, or the period of observation was undefined. Gonzales *et al*. 2009 observed no serious adverse events. Signs of acute and chronic CA overdose were observed, however, in 4 children with 3β-HSD deficiency. Chronic overdose, presumably due to prescription of an excessive dose, was shown to be related to transient increases in serum bile acids, GGT and transaminase activities. While not noted as an adverse event, 1 patient with 3β-HSD deficiency in this population was described to have developed gallstones requiring cholecystectomy during CA treatment for more than five years.

In Potin *et al.* 2001, ten children diagnosed with HSD3ß7 and two children with Δ^4*-3-oxo-R* deficiency were included in a clinical trial to assess the effect of treatment with CA and with a combination of CA and ursodeoxycholic acid. Cholic acid treatment was well tolerated in 11 of these 12 children. Only one

patient developed pruritus under therapy, which completely disappeared after dose reduction from 15 mg/kg/day down to 5 mg/kg/day.

2.6.1. Discussion on clinical safety

In study CAC-91-10-10, during the 18 years of the study period, approximately half of patients experienced TEAEs. Disease progression was the most common TEAE followed by pyrexia and diarrhea. TEAEs were predominantly mild or moderate in intensity. Study drug related AEs were only reported for two patients (malaise and jaundice in 1 patient; skin lesion in another patient) and none were serious. Four patients discontinued the study due to TEAEs. The AE most frequently leading to study discontinuation was disease progression. None of the AEs leading to study discontinuation was considered related to study treatment by the Investigator. Treatment-emergent SAEs occurred in 16 patients. Disease progression was the most frequently reported SAE, followed by urinary tract infection, diarrhoea and dehydration. No SAEs were considered related to the study drug. A total of 21 patients died during the study period. Thirteen (13) patients were no longer being followed by CCHMC at the time of the patient's death. There was a clear trend that worsening of the underlying condition leading to death occurred more often in patients with a peroxisomal disorder, as fifteen of the 21 patients who died suffered from peroxisomal disorder, mainly Zellweger's syndrome. Of the six patients with single enzyme defects who died, four suffered from *Δ^4-3-oxo-R* deficiency. Almost all patients who died had been diagnosed with a bile acid deficiency before the age of one. For none of the patients the death was considered related to study medication treatment. Based on the severe, potentially fatal disease, worsening of the underlying condition can be considered a reasonable assumption for most cases.

In study CAC-001-01; there were 13 AEs reported for 9 of 16 patients. The AEs reported were generally in line with those reported in the pivotal trial. There were 2 incidents of slightly elevated liver function tests (1 each ALT and AST for the same patient) judged to be unlikely to be related to the study drug, and 1 incident each of mild reflux and moderate diarrhoea (different patients) said to be possibly related to the study drug. There were no deaths, and no adverse events were determined to be serious or significant.

The safety data reported from study CAC-002-001 were also generally in line with those reported in the pivotal trial. The most common TEAEs were Vitamin D decreased, disease progression, hepatic enzyme increased and upper respiratory tract infection. Mild peripheral neuropathy and mild nausea were the two reported drug-related AEs. The main SAE reported was disease progression and none of the SAEs were considered drug-related. Three patients died of disease progression and thrombosis; these events were not related to the study treatment. Of the 29 patients affected by a single enzymatic defect (3β-HSD, Δ^4-3-oxo-R or CTX) that were treated in Study CAC-002-01 3 patients discontinued (all due to AEs), and one patient died.

The impossibility to conduct controlled studies was recognised by the CHMP in regards to the rarity of the condition and the ethical considerations. In the absence of controlled trial data, it remains difficult to ascertain causality regarding unfavourable effect. This is a methodologically driven outcome of a trial design which has no comparator arm hence impacting on the possibility to assess whether an adverse event is related to the study drug. However, this absence of the formal establishment of the causality does not prevent that the clinical safety data reported from the study showed that the clinical safety of Kolbam given at appropriate doses is satisfactory. The majority of the event observed in treated patients are mild or moderate in intensity (diarrhoea, reflux, nausea, malaise, jaundice and skin lesion), there were no serious event related to CA treatment, the events were transitory and

generally did not interfere with the therapy. Mainly events were related to the disease progression rather than being related to CA treatment.

The applicant provided a review of the literature in order to support the safety of CA. Very few adverse events under CA treatment were reported in the literature. The only documented adverse reactions to CA are pruritus and diarrhoea, which may be indicative of an overdose. No clear quantification of the frequency of a given adverse reaction is possible, as either no adverse reactions occurred, or the period of observation was undefined. No serious adverse events have been reported in the literature. All other adverse events that have been reported appeared linked to an over dosage of CA, were not serious, reversible and did not interfere with therapy. No patient interrupted or stopped treatment due to adverse events. Gonzales *et al*. reported apparent signs of CA overdose in four children (one of which was accidental administration of 56 mg/kg as a single dose) with clinical features including pruritus, diarrhoea, and elevation of serum GGT, ALT, and total serum bile acid concentration. However, these increases were transient and returned to normal after CA dose reduction. The SmPC contains guidance that patients should be intensively monitored for their biochemical response and liver functions tests during the initiation of therapy and at least annually thereafter, and that the dosage should be adjusted accordingly.

The majority of patients with inborn errors of bile acid metabolism that have been treated with CA presented with some degree of hepatic impairment at the time of diagnosis; in most patients, the hepatic impairment improved or resolved with treatment. The dose of CA should be adjusted individually. No experience exists in patients with hepatic impairment from causes other than their primary disease and no dose recommendation can be given. Patients with hepatic impairment should be monitored closely.

The development of gallstones requiring cholecystectomy was observed in the literature in a single patient with 3β-HSD deficiency. Development of gallstones has also been observed in cholic-acid-fed rodents on a high-cholesterol diet. An enrichment of bile with deoxycholic acid, as seen in patients under treatment with CA, may be linked to biliary cholesterol super-saturation and may accelerate gallstone development. The development of gallstones on CA treatment is considered a potential risk that will be monitored as part of the Risk Management Plan.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

Additional safety data needed in the context of a MA under exceptional circumstances

In the context of a MA under exceptional circumstances a specific obligation is included in the terms of the Marketing Authorisation. The applicant will monitor the long term safety in patients treated with Kolbam from a patient registry for which details are reflected in the risk management plan. The registry will monitor accumulating data on safety in the treatment of inborn errors in primary bile acid synthesis due CTX, AMACR and CYP7A1 deficiencies with Kolbam in infants, children, adolescents and adults. Reports on recruitment progress of the registry will be reported at the time of PSURs and Annual Re-assessments. Progress and results from the registry will form the basis of the annual reassessments of the benefit/risk profile of Kolbam.

2.6.2. Conclusions on the clinical safety

Data reported from the open label studies conducted showed that the clinical safety of CA in the treatment of CTX, AMACR and CYP7A1 deficiencies, given at appropriate doses appeared satisfactory.

The majority of the event observed in treated patients were mild or moderate in intensity (diarrhoea, reflux, nausea, malaise, jaundice and skin lesion), there were no serious event related to CA treatment, the events were transitory and generally did not interfere with the therapy. Mainly events were related to the disease progression rather than being related to CA treatment. The uncontrolled nature of the data rendered difficult to evaluate the causal association with CA however it does not prevent to conclude that the AEs reported with Kolbam therapy appeared generally to be not serious and mostly related to the underlying disease condition. There have been six patients with single enzyme defects who died over 18 years of the study period. Worsening of the underlying condition is considered a reasonable assumption for most cases although not all complete information were available despite all efforts made by the applicant to retrieve it.

The safety data from the case reports from the literature are generally consistent with the safety profile reported in the pivotal trial. The adverse events that have been reported are mainly linked to an over dosage of CA i.e. pruritus and diarrhoea. No serious adverse events have been reported in the literature. No patient interrupted or stopped treatment due to adverse events. Patients presenting with pruritus and/or persistent diarrhoea should be investigated for a potential overdose by a serum and/or urine bile acid assay. Overdose was also associated with elevated serum GGT and transaminases. The development of gallstones requiring cholecystectomy has been observed in a single patient with 3β-HSD deficiency. The development of gallstones on CA treatment is considered a potential risk that will be monitored as part of the Risk Management Plan.

The applicant as specific obligation will monitor the long term safety of CA in the treatment of CTX, AMACR and CYP7A1 by establishing a patient registry. The CHMP considered this registry as adequate in order to monitor the clinical safety of CA.

2.7. Pharmacovigilance

Detailed description of the pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

2.8. Risk Management Plan

The CHMP received the following PRAC Advice on the submitted Risk Management Plan:

PRAC Advice

Based on the PRAC review of the Risk Management Plan version 1.7, the PRAC considers by consensus that the risk management system for cholic acid (Kolbam) for the treatment:

> of inborn errors of primary bile acid synthesis, responsive to treatment with cholic acid, in infants from one month of age for continuous lifelong treatment through adulthood

is acceptable.

This advice is based on the following content of the Risk Management Plan:

Safety concerns

The applicant identified the following safety concerns in the RMP:

Summary of Safety Concerns	
Important identified risks	• Gastroesophageal reflux • Diarrhoea • Pruritus • Increased transaminases
Important potential risks	• Reproductive toxicity • Elevated systolic blood pressure • Carcinogenicity • Medication error • Formation of gallstones
Missing information	• Newborns less than 1 month of age • Elderly patients • Pregnant or lactating women • Patients with ethnic backgrounds other than Caucasian • Patients with pre-existing liver disease • Patients with hepatic encephalopathy • Patients needing urgent liver transplantation • Off-label use

The PRAC agreed.

Pharmacovigilance plan

On-going and planned studies in the PhV development plan

Activity / Study title (type of activity, study title category 1-3)*	**Objectives**	**Safety concerns addressed**	**Status Planned, started,**	**Date for submission of interim or final reports (planned or actual)**
An Open-Label, Single-Center, Non-Randomized Continuation Study of cholic acid Capsules in Subjects with Inborn Errors of Bile acid Synthesis (CAC-002-01) (Phase III clinical study, category 3)	To evaluate the therapeutic efficacy and safety of cholic acid in subjects with identified inborn errors of bile acid metabolism.	The study will assess the safety and tolerability of cholic acid capsules. The incidence and severity of adverse events compared with baseline, clinical laboratory test results, vital signs, physical examination findings and assessment of malabsorption (height, weight gain, normalization of steatorrhoea, Vitamins A, E, D and pro-thrombin time) will be assessed.	Ongoing: interim analysis conducted with data cut-off date of 30 September 2012	Interim CSR was submitted on 19 August 2013 (eCTD sequence 0006)
Patient registry (Registry, category 1)	To collect efficacy and safety information in “real life” clinical use.	This will provide additional safety information (e.g. adverse events and routine test results) including safety information in those patient groups considered to be missing information. Furthermore, the	Planned	Protocol to be submitted by February 28th 2015 latest. Safety data to be submitted on a yearly basis together with the

Activity / Study title (type of activity, study title category 1-3)*	Objectives	Safety concerns addressed	Status Planned, started,	Date for submission of interim or final reports (planned or actual)
		proposed registry would enable assessment of the extent of any offlabel use. The registry will collect outcome information and an additional source of efficacy data in 'real-life' clinical use.		annual reassessment report for KOLBAM. Reports on recruitment progress of the registry will be submitted with PSURs and annual reassessments.

*Category 1 are imposed activities considered key to the benefit risk of the product.
Category 2 are specific obligations
Category 3 are required additional PhV activity (to address specific safety concerns or to measure effectiveness of risk minimisation measures)

The PRAC, having considered the data submitted, was of the opinion that the proposed post-authorisation PhV development plan is sufficient to identify and characterise the risks of the product.

Risk minimisation measures

Safety concern	Routine risk minimisation measures	Additional risk minimisation measures
Important identified risks • Gastroesophageal reflux • Diarrhoea • Pruritus • Increased transaminases	SmPC text	Physician's educational material
Important potential risks • Reproductive toxicity • Elevated systolic blood pressure • Carcinogenicity • Medication error • Formation of gallstones	SmPC text	
Missing information • Newborns less than 1 month of age • Elderly patients • Pregnant or lactating women • Patients with ethnic backgrounds other than Caucasian • Patients with pre-existing liver disease • Patients with hepatic encephalopathy • Patients needing urgent liver transplantation • Off-label use	SmPC text	

The PRAC, having considered the data submitted, was of the opinion that the proposed risk minimisation measures were sufficient to minimise the risks of the product in the proposed indications. As additional risk minimisation measure an educational material has been deemed appropriate by the PRAC in order to convey information to the prescribers on the correct and safe use of the product.

The CHMP endorsed this advice without changes.

2.9. Significance of paediatric studies

The CHMP is of the opinion that study CAC-91-10-10, which is contained in the agreed Paediatric Investigation Plan P/206/2011 and has been completed after 26 January 2007, is considered as significant. This is the pivotal study for the intended indications as detailed in the above-mentioned PIP. The justification is that the data generated and results of this study taken together provided important and clinically relevant information for the paediatric population in line with the EU communication (Guideline on the format and content of applications for agreement or modification of a paediatric investigation plan and requests for waivers or deferrals and concerning the operation of the compliance check and on criteria for assessing significant studies (2008/C 243/01). This view is in line with the EMEA/PDCO opinion on a request for agreement of a Paediatric Investigation Plan on Kolbam (EMEA-000651-PIP01-09-M02), in which it is stated: “The PDCO considered that this study, which will be completed after the entry into force of the Regulation, could be significant with respect to Article 45 (3) of Regulation (EC) No 1901/2006, as amended.”

2.10. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the Guideline on the readability of the label and package leaflet of medicinal products for human use.

3. Benefit-Risk Balance

Benefits

Beneficial effects

Cholic acid has been shown to have a beneficial effect in patients with inborn errors of primary bile acid synthesis responsive to treatment with cholic acid. The clinical use of cholic acid has been documented since at least the mid-1990s. Oral administration of cholic acid replaces the substance missing in patients with these in born errors. Cholic acid inhibits the production of cholestatic and hepatotoxic bile acid metabolites by down-regulating cholesterol 7α-hydroxylase, the rate limiting enzyme in bile acid synthesis (Setchell and O’Connell 2007). Metabolites are cleared due to the choleretic effect of cholic acid and rapidly disappear from the patients’ urine. As a result, biochemical liver parameters such as transaminases and bilirubin more slowly normalise and histological liver damage is attenuated or even improves. Finally, the administered cholic acid restores a normal enterohepatic circulation and absorption of fat and fat-soluble vitamins occurs resolving growth deficits or outright rickets.

In study CAC-91-10-10 there are indications, consistent with clinical expectations and literature data, for decreased urinary bile acid excretion in patients with single enzyme defects (CTX and AMACR). General improvements in the degree of atypical urine bile acids were also seen as well as shifts towards improvements in ALT and AST values.

In an uncontrolled trial, statistical tests of pre-treatment to post-treatment changes to draw inferences on efficacy are confounded by time and by concomitant therapeutic interventions and patient management. Hence, a close scrutiny of individual profiles of response and a clear biological and pharmacological rationale for the intervention, substantiated by basic science and literature data, is the basis on which to draw conclusions about therapeutic efficacy. The conclusion of therapeutic efficacy is made considering results across all single enzyme defects included in the trial. This rationale can then be applied to each single enzyme defect under consideration based on knowledge of disease pathology and cholic acid pharmacology, in addition to the available clinical trial and literature data. In particular, it is agreed with the applicant that in each of these enzyme deficiencies, the elevated transaminases and abnormal liver histology result from the abnormal bile acids that occur as a consequence of the blockage of the CA biosynthetic pathway by the enzyme defects. For each of these deficiencies, the therapeutic benefit of CA derives from the same mechanism, namely the ability to inhibit transcription of the 7-alpha hydroxylase (at the head of the pathway through an FXR-dependent mechanism) (Setchell and O-Connell, 2007) or provides benefit to patients by providing CA to patients. Whilst the magnitude of changes in metabolic and pathologic parameters may differ in each different phenotype (noting also that clinical prognosis differs by phenotype), it is evident that Kolbam will be therapeutically efficacious in the treatment of CTX, AMACR and CYP7A1 deficiencies.

Overall, Kolbam is recognised as having beneficial effect in patients with inborn errors of primary bile acid synthesis that are due to CTX, AMACR and CYP7A1 deficiencies.

Uncertainty in the knowledge about the beneficial effects

The rarity and the requirement for long term treatment of this condition makes the conduct of an adequately powered and controlled clinical study not feasible. Inborn errors in primary bile acid synthesis remain a condition that is debilitating in the long term and life threatening. It would be contrary to principles of medical ethics to collect evidence of clinical efficacy of CA in a controlled clinical study.

The uncontrolled data makes difficult to ascertain the magnitude of the beneficial effects of CA. However, the clinical data presented in this application are sufficiently comprehensive to support demonstration of efficacy of CA in the claimed indications as measured by objective improvement in metabolic and pathologic parameters in patients studied. The applicant will monitor the long term efficacy in patients treated with cholic acid from a patient registry. The registry will monitor accumulating data on efficacy. Reports will be submitted as part of the PSURs and annual re-assessments.

Risks

Unfavourable effects

In general, adverse effects seen with CA therapy appear to be not serious, reversible, mainly related to elevated hepatic enzymes and appear to be dose-related. Cases of diarrhoea and pruritus have been reported in the literature. The development of gallstones requiring cholecystectomy has been observed in a single patient with 3β-HSD deficiency.

The CHMP took due account of the fact that CA is an endogenous molecule present in normal human bile, blood and other biological fluids. Once administered CA will behave like an endogenous molecule in all respects. At physiological concentrations, CA is non-toxic, thereby providing a safe dose range for use in human. Substantial knowledge of human CA physiology in healthy subjects as well as patients

treated at current pharmacological doses with chronic liver diseases exists. It should be also noted that CA is used in foods as a food additive (E 1000) and that historically medicinal products containing CA have been used for other indications, such as laxatives and choleretics since at least the early part of the 20th century.

Uncertainty in the knowledge about the unfavourable effects

In the absence of controlled trial data, it is difficult to comprehensively ascertain causality regarding unfavourable effects. This is a methodologically driven outcome of a trial design which has no comparator arm hence impacting on the possibility to assess whether an adverse event is related to the investigational drug. However, this absence of the formal establishment of the causality does not prevent that the clinical data reported to support this application showed that the safety of CA in the treatment of inborn error of primary bile acid synthesis that are due to CTX, AMACR and CYP7A1 deficiencies, is satisfactory in view of the results presented and the established safety profile of this endogenous molecule. The applicant will monitor the long term safety of cholic acid treatment as part of a patient registry. Reports will be submitted as part of the PSURs and Annual Re-assessments.

Benefit-risk balance

Importance of favourable and unfavourable effects

Effective treatment with oral CA therapy has been shown to restore normal laboratory parameters; improve histological lesions of the liver, and significantly improve patient symptoms. Analysis of urine during CA therapy showed a marked reduction, or even complete elimination of the toxic bile acid metabolites. This reflects restoration of an effective feedback control of bile acid synthesis and a metabolic equilibrium.

Kolbam has been shown to have a palliative effect on the patient's clinical course by decreasing urinary bile acid excretion in patients with CTX, AMACR deficiencies. Improvements in the degree of atypical urine bile acids were also seen as well as shifts towards improvements in ALT and AST values.

The adverse events reported were not serious, reversible and mainly linked to an over dosage. Transient increases transaminase activities have been also observed in chronic over dosage in the literature. The development of gallstones requiring cholecystectomy has been observed in a single patient with 3β-HSD deficiency. Therefore the safety profile of CA is acceptable.

Benefit-risk balance

Kolbam appeared as effective and rather well tolerated for the long-term treatment of inborn errors of primary bile acid synthesis that are due to CTX, AMACR and CYP7A1 deficiencies.

The conclusion of therapeutic efficacy is made considering results across all single enzyme defects included in the trial. This rationale can then be applied to each single enzyme defect under consideration based on knowledge of disease pathology and cholic acid pharmacology, in addition to the available clinical trial and literature data.

The adverse events observed under CA oral treatment in study CAC-90-10-10 appeared not serious, reversible, and mainly related to the disease progression rather than being related to CA treatment. Cases of diarrhoea and pruritus linked to an over dosage of CA were reported in the literature submitted. Overall, the safety profile of CA appears acceptable. It should be noted that in conjunction with the recommendation for a marketing authorisation under exceptional circumstances, the specific

obligation relates to the generation of follow-up safety data in a patient registry; these data will be reviewed annually pursuant to article 14(8) of Regulation (EC) No 726/2004.

The CHMP evaluated the risks due to uncertainties and considers that these risks are not substantial in the light of the demonstrated benefits. The benefits observed outweigh the risks involved with CA treatment and therefore, the benefit /risk balance is considered positive for the treatment of CTX, AMACR and CYP7A1 deficiencies.

Discussion on the benefit-risk balance

The liver disease associated with primary bile acids synthesis due to inborn error metabolism leading to the production of hepatotoxic bile acid precursors is progressive and, if untreated, leads to death from cirrhosis and liver failure. Currently there is no causal treatment. At the time the applicant submitted this application, the only therapeutic option in severely affected cases was liver transplant. Orphacol (cholic acid) was granted a MA on 13 September 2013 for the treatment of treatment of inborn errors in primary bile acid synthesis due to 3β-HSD deficiency or Δ^4-3-oxoR deficiency in infants, children and adolescents aged 1 month to 18 years and adults.

In accordance with Article 14(8) of Regulation (EC) No 726/2004 and Annex I, part II of Directive 2001/83/EC the applicant applied for a marketing authorisation under exceptional circumstances. The applicant justified that he was unable to provide comprehensive data on the efficacy and safety under normal conditions of use because the indications for which Kolbam are intended are encountered so rarely that he cannot reasonably be expected to provide comprehensive evidence. The applicant also argued that it would be contrary to generally accepted principles of medical ethics to collect such information as participation in a controlled trial would expose patients to a risk of liver failure or even death. The CHMP agreed on these justifications and considers that the criteria defined in the above-mentioned provisions were met. A marketing authorisation under exceptional circumstances for Kolbam is acceptable in regards to the fulfilled criteria of rarity of the disease (the indications for which the product is intended are so rare that the applicant cannot be reasonably expected to provide comprehensive evidence) and medical ethics (it would be contrary to generally accepted principles of medical ethics to collect such information).

Overall, the efficacy and safety of the product was demonstrated based on the results of open label study CAC-90-10-10 supported by literature data. The use of CA for the treatment for inborn errors of bile acid synthesis due to CTX, AMACR and CYP7A1 deficiencies has been recognised as effective and the safety profile was considered satisfactory. The open label design and limited sample size of this non-GCP compliant study limited the demonstration of efficacy as well as the causality assessment of the adverse events observed. However, the results presented were considered sufficient to support demonstration of efficacy of CA in the claimed indications. The results also showed that the safety profile of CA is acceptable. In conclusion, the data submitted are sufficient to conclude on a positive benefit–risk balance for an application made under exceptional circumstances.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that, within the meaning of Article 3 of Commission Regulation (EC) No. 847/2000, Kolbam is not similar to Orphacol for the following indications: Sterol

27-hydroxylase (presenting as cerebrotendinous xanthomatosis, CTX), 2- (or α-) methylacyl-CoA racemase (AMACR) and Cholesterol 7α-hydroxylase (CYP7A1) deficiencies (See Appendix 1).

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Kolbam in the treatment of inborn errors in primary bile acid synthesis that are due to CTX, AMACR and CYP7A1 deficiencies is favourable. Therefore, the CHMP recommends the granting of the Marketing Authorisation for Kolbam in the treatment of CTX, AMACR and CYP7A1 deficiencies under exceptional circumstances subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (See Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the Marketing Authorisation

- **Periodic Safety Update Reports**

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation. Subsequently, the marketing authorisation holder shall submit periodic safety update reports for this product in accordance with the requirements set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing Authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

If the dates for submission of a PSUR and the update of a RMP coincide, they can be submitted at the same time.

- **Additional risk minimisation measures**

Prior to launch in each Member State, the MAH shall agree the final educational material with the competent authority in that Member State. The MAH shall ensure that at launch all physicians expected to prescribe the product are provided with information on the correct and safe use of the product.

The physician's educational material should contain the following key elements:

- Summary of product characteristics
- Information on:
    - Calculation of the correct dose and the need to instruct caregivers on how to administer the product correctly
    - Symptoms and signs of an overdose and the management of this

Specific Obligation to complete post-authorisation measures for the marketing authorisation under exceptional circumstances

This being an approval under exceptional circumstances and pursuant to Article 14(8) of Regulation (EC) No 726/2004, the MAH shall conduct, within the stated timeframe, the following measures:

Description	Due date
To monitor the long term safety and efficacy in patients treated with Kolbam from a patient registry for which details are reflected in the risk management plan. The registry will monitor accumulating data on efficacy and safety in the treatment of inborn errors in primary bile acid synthesis in infants, children, adolescents and adults due to Sterol 27-hydroxylase (presenting as cerebrotendinous xanthomatosis, CTX), 2- (or α-) methylacyl-CoA racemase (AMACR) and Cholesterol 7α-hydroxylase (CYP7A1) deficiencies. Reports on recruitment progress of the registry will be submitted with PSURs and Annual Re-assessments. Progress and results from the registry will form the basis of the annual reassessments of the benefit/risk profile of Kolbam.	- PSURs - Annual Re-assessments

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

New Active Substance Status

Based on the CHMP review of data on the quality properties of the active substance, the CHMP considers that cholic acid contained in Kolbam is not qualified as a new active substance, as it was previously authorised as a medicinal product in the European Union.

Paediatric Data

Furthermore, the CHMP reviewed the available paediatric data of studies subject to the agreed Paediatric Investigation Plan P/206/2011 and the results of these studies are reflected in the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet.

In accordance with Article 45(3) of Regulation (EC) No 1901/2006, the significant study CAC-90-10-10 in the agreed paediatric investigation plan P/206/2011 has been completed after the entry into force of that Regulation.

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