Assessment report

Orphacol

cholic acid

Procedure No.: EMEA/H/C/001250//0000

Note

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

<table>
<thead>
<tr>
<th>Invented name of the medicinal product</th>
<th>Orphacol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applicant:</td>
<td>Laboratoire CTRS</td>
</tr>
<tr>
<td>Active substance:</td>
<td>Cholic acid</td>
</tr>
<tr>
<td>Common name:</td>
<td>Cholic acid</td>
</tr>
<tr>
<td>Pharmaco-therapeutic group (ATC code):</td>
<td>Bile Acid Preparations (A05AA03)</td>
</tr>
<tr>
<td>Therapeutic indications:</td>
<td>Orphacol is indicated for the treatment of inborn errors in primary bile acid synthesis due to 3β-Hydroxy-Δ⁵-C₂₇-sterol oxidoreductase deficiency or Δ⁴-3-Oxosteroid-5β-reductase deficiency in infants, children and adolescents aged 1 month to 18 years and adults.</td>
</tr>
<tr>
<td>Pharmaceutical form:</td>
<td>Hard capsules</td>
</tr>
<tr>
<td>Strengths:</td>
<td>50 mg and 250 mg</td>
</tr>
<tr>
<td>Route of administration:</td>
<td>Oral route</td>
</tr>
<tr>
<td>Pack sizes:</td>
<td>Pack of 30, 60, 120 hard capsules</td>
</tr>
</tbody>
</table>
# Table of contents

1. **Background information on the procedure** ............................................... 6  
   1.1. Submission of the dossier ........................................................................... 6  
   1.2. Steps taken for the assessment of the product .............................................. 7  

2. **General conditions for the marketing authorisation** .............................. 8  
   2.1. Marketing authorisation holder .................................................................. 8  
   2.2. Conditions or restrictions regarding supply and use .................................. 8  
   2.3. Conditions or restrictions with regard to the safe and effective use of the medicinal product ................................................................. 8  
   2.4. Other conditions ....................................................................................... 8  
   2.5. Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States. ......................... 9  
   2.6. Specific obligations following the Marketing Authorisation .................... 9  

3. **Scientific discussion** ............................................................................ 10  
   3.1. Introduction .............................................................................................. 10  
   3.2. Quality aspects ....................................................................................... 12  
   3.2.1. Introduction .......................................................................................... 12  
   3.2.2. Active Substance ............................................................................... 13  
   3.2.3. Finished Medicinal Product .................................................................. 15  
   3.2.4. Discussion on chemical, pharmaceutical and biological aspects ............ 17  
   3.2.5. Conclusions on the chemical, pharmaceutical and biological aspects .... 17  
   3.3. Non-clinical aspects ............................................................................... 17  
   3.3.1. Introduction .......................................................................................... 17  
   3.3.2. Pharmacology ...................................................................................... 17  
   3.3.3. Pharmacokinetics ............................................................................... 19  
   3.3.4. Toxicology .......................................................................................... 22  
   3.3.5. Ecotoxicity/environmental risk assessment (ERA) .................................. 25  
   3.3.6. Discussion on non-clinical aspects ....................................................... 25  
   3.3.7. Conclusion on the non-clinical aspects ................................................. 26  
   3.4. Clinical aspects ....................................................................................... 27  
   3.4.1. Introduction .......................................................................................... 27  
   3.4.2. Pharmacokinetics ............................................................................... 27  
   3.4.3. Pharmacodynamics ............................................................................ 34  
   3.4.4. Discussion and conclusions on clinical pharmacology ......................... 35  
   3.5. Clinical efficacy ..................................................................................... 36  
   3.5.1. Dose response studies ........................................................................... 36  
   3.5.2. Main Case reports ............................................................................... 39  
   3.5.3. Discussion on clinical efficacy .............................................................. 55  
   3.5.4. Conclusions on the clinical efficacy ...................................................... 57  
   3.6. Clinical safety .......................................................................................... 57  
   3.6.1. Discussion on clinical safety ................................................................. 60  
   3.6.2. Conclusions on the clinical safety ......................................................... 61  
   3.7. Pharmacovigilance ............................................................................... 62
3.8. Benefit-Risk Balance ........................................................................................... 63
3.8.1. Discussion on the benefit-risk balance ............................................................ 66
3.9. Recommendation ............................................................................................... 68
List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>3β-HSD</td>
<td>3β-hydroxy-Δ^{5,27}-sterol oxidoreductase</td>
</tr>
<tr>
<td>Δ&lt;sup&gt;4&lt;/sup&gt;-3-oxoR</td>
<td>Δ&lt;sup&gt;4&lt;/sup&gt;-3-oxosteroid 5β-reductase</td>
</tr>
<tr>
<td>AGEPS-EPHP</td>
<td>Agence Générale des Équipements et Produits de Santé - Etablissement Pharmaceutique des Hôpitaux de Paris</td>
</tr>
<tr>
<td>ALT</td>
<td>alanine aminotransferase</td>
</tr>
<tr>
<td>AP</td>
<td>alkaline phosphatase</td>
</tr>
<tr>
<td>AST</td>
<td>asparagine aminotransferase</td>
</tr>
<tr>
<td>ATC</td>
<td>anatomical therapeutic chemical</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>ATU</td>
<td>Autorisation Temporaire d'Utilisation Nominative</td>
</tr>
<tr>
<td>BA</td>
<td>bile acid</td>
</tr>
<tr>
<td>BSEP</td>
<td>bile salt export pump</td>
</tr>
<tr>
<td>CA</td>
<td>cholic acid</td>
</tr>
<tr>
<td>CDCA</td>
<td>chenodeoxycholic acid</td>
</tr>
<tr>
<td>DCA</td>
<td>deoxycholic acid</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>F</td>
<td>Female</td>
</tr>
<tr>
<td>FAB-MS</td>
<td>fast atom bombardment mass spectrometry</td>
</tr>
<tr>
<td>FDA</td>
<td>U.S. Food and Drug Administration</td>
</tr>
<tr>
<td>FTR</td>
<td>fractional turnover rate</td>
</tr>
<tr>
<td>FXR&lt;sub&gt;α&lt;/sub&gt;</td>
<td>farnesoid X receptor alpha</td>
</tr>
<tr>
<td>GC-MS</td>
<td>gas chromatography-mass spectrometry</td>
</tr>
<tr>
<td>GCP</td>
<td>good clinical practices</td>
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<tr>
<td>GLC</td>
<td>gas liquid chromatography</td>
</tr>
<tr>
<td>GLDH</td>
<td>glutamate dehydrogenase</td>
</tr>
<tr>
<td>GGT</td>
<td>γ-glutamyl transferase</td>
</tr>
<tr>
<td>LRH&lt;sub&gt;1&lt;/sub&gt;</td>
<td>liver receptor homolog-1</td>
</tr>
<tr>
<td>LXR&lt;sub&gt;α&lt;/sub&gt;</td>
<td>liver X receptor alpha</td>
</tr>
<tr>
<td>M</td>
<td>male</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>n.a.</td>
<td>not available</td>
</tr>
<tr>
<td>n.r.</td>
<td>not reported</td>
</tr>
<tr>
<td>NAD</td>
<td>nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>OMIM</td>
<td>Online Mendelian Inheritance in Man</td>
</tr>
<tr>
<td>PFB-TMS</td>
<td>pentafluorobenzyl-trimethylsilyl</td>
</tr>
<tr>
<td>SHP</td>
<td>short heterodimer partner</td>
</tr>
<tr>
<td>SPC</td>
<td>Summary of Product Characteristics</td>
</tr>
<tr>
<td>UDCA</td>
<td>ursodeoxycholic acid</td>
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</tbody>
</table>
1. Background information on the procedure

1.1. Submission of the dossier

The Applicant Laboratoires CTRS submitted on 30 October 2009 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Orphacol, 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 September 2008.

Orphacol was designated as an orphan medicinal product EU/3/02/127 on 18 December 2002 in the following indication: treatment of inborn errors in primary bile acid synthesis. The calculated prevalence of this condition was 0.06 per 10,000 EEA population.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Orphacol 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/Human medicines/Rare disease designations.

The Applicant applied for the following indications: treatment of inborn errors in primary bile acid synthesis due to 3β-Hydroxy-Δ5-C27-steroid oxidoreductase deficiency or Δ4-3-Oxosteroid-5β-reductase deficiency in infants, children and adolescents aged 1 month to 18 years and adults.

The legal basis for this application refers to:

Article 10(a) of Directive 2001/83/EC – well established use application. The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on bibliographic literature substituting all non-clinical tests and clinical studies.

Information on Paediatric requirements

Not applicable. Paediatric requirements, as described in Regulation (EC) No 1901/2006, do not apply for medicinal products under Article 10(a) of Directive 2001/83/EC – well established use application.

Information relating to Orphan Market Exclusivity

Similarity

Not applicable.

Market Exclusivity

Not applicable.

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. Several products containing combinations of cholic acid with other active substances are marketed in a few EU countries. Due to the presence of other active substances, unsuitable strength and/or inappropriate
route of administration they are not suitable for the treatment of inborn errors of primary bile acid synthesis due to 3β-hydroxy-Δ5-C27-steroid oxidoreductase (3β-HSD) or Δ4-3-oxosteroid 5β-reductase (Δ4-3-oxoR) deficiency.

1.2. 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 30 October 2009.
- The procedure started on 18 November 2009.
- The Rapporteur’s first Assessment Report was circulated to all CHMP members on 05 February 2010. The Co-Rapporteur’s first Assessment Report was circulated to all CHMP members on 10 February 2010.
- During the meeting in March 2010, the CHMP agreed on the consolidated List of Questions to be sent to the Applicant. The final consolidated List of Questions was sent to the Applicant on 18 March 2010.
- The Applicant submitted the responses to the CHMP consolidated List of Questions on 23 July 2010.
- The Rapporteurs circulated the Joint Assessment Report on the Applicant’s responses to the List of Questions to all CHMP members on 03 September 2010.
- During the CHMP meeting on 23 September 2010, the CHMP agreed on a list of outstanding issues to be addressed in writing and in an oral explanation by the Applicant.
- The Applicant submitted the responses to the CHMP consolidated List of Outstanding Issues on 18 October 2010. The oral explanation was cancelled during the November CHMP.
- The Rapporteurs circulated the updated Joint Assessment Report on the Applicant’s responses to the List of Outstanding Issues to all CHMP members on 12 November 2010.
- During the December 2010 meeting the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation under exceptional circumstances to Orphacol on 16 December 2010. The Applicant provided the letter of undertaking on the specific obligations to be fulfilled post-authorisation on 14 December 2010.
- Following a request from the European Commission dated 15 March 2011 to further motivate its opinion dated 16 December 2010, the CHMP, in the light of the overall data originally submitted by the applicant and the scientific discussion within the Committee, revised its positive opinion for granting a Marketing Authorisation under exceptional circumstances to Orphacol on 14 April 2011.
2. General conditions for the marketing authorisation

2.1. Marketing authorisation holder

Laboratoires CTRS
69, rue d'Aguesseau
FR-92100 Boulogne-Billancourt
France

Manufacturer responsible for batch release

CRID Pharma
17, Parc des Vautes, 34983 Saint Gély du Fesc
France

2.2. Conditions or restrictions regarding supply and use

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

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

The MAH, in agreement with the competent authorities in the Member States, shall implement, prior to the launch, an educational programme for physicians aiming to provide educational material on correct diagnosis and therapeutic managements of the treatment of inborn errors in primary bile acid synthesis due to 3β-Hydroxy-Δ5-C27-steroid oxidoreductase deficiency or Δ4-3-Oxosteroid-5β-reductase deficiency and to inform on expected and potential risks associated with the treatment.

The physician educational programme should contain the following key elements:

- Prescription of a supratherapeutic dose (MedDRA term: drug toxicity)
- Risk of gallstones

2.4. Other conditions

Pharmacovigilance system

The MAH must ensure that the system of pharmacovigilance, as described in Module 1.8.1 of the Marketing Authorisation, is in place and functioning before and whilst the product is on the market.

Risk Management plan

The MAH commits to performing the studies and additional pharmacovigilance activities detailed in the Pharmacovigilance Plan, as agreed in version 1.3 of the Risk Management Plan (RMP) presented in Module 1.8.2 of the Marketing Authorisation application and any subsequent updates of the RMP agreed by the CHMP.

As per the CHMP Guideline on Risk Management Systems for medicinal products for human use, the updated RMP should be submitted at the same time as the next Periodic Safety Update Report (PSUR).

In addition, an updated RMP should be submitted:
• When new information is received that may impact on the current Safety Specification, Pharmacovigilance Plan or risk minimisation activities
• Within 60 days of an important (pharmacovigilance or risk minimisation) milestone being reached
• at the request of the EMA

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

The MAH, in agreement with the competent authorities in the Member States, shall implement, prior to the launch, an educational programme for physicians aiming to provide educational material on correct diagnosis and therapeutic managements of the treatment of inborn errors in primary bile acid synthesis due to 3β-Hydroxy-Δ⁵-C₂₇-steroid oxidoreductase deficiency or Δ⁴-3-Oxosteroid-5β-reductase deficiency and to inform on expected and potential risks associated with the treatment.

The physician educational programme should contain the following key elements:

• Prescription of a supratherapeutic dose (MedDRA term: drug toxicity)
• Risk of gallstones

2.6. **Specific obligations following the Marketing Authorisation**

This being an approval under exceptional circumstances and pursuant to Article 14(8) of Regulation (EC) No 726/2004 and Part II.6 of the Annex I to Directive 2001/83/EC, the Applicant agreed to put in place, as requested by the CHMP, the following specific obligation which will form the basis of the risk benefit balance of Orphacol.

<table>
<thead>
<tr>
<th>Area</th>
<th>Description</th>
<th>Due date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>CTRS commits to monitor the safety and efficacy in patients treated with Orphacol from a patient surveillance database for which the protocol has been endorsed by the CHMP and is documented in the Orphacol RMP.</td>
<td>- first PSUR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Annual Re-assessment</td>
</tr>
<tr>
<td></td>
<td>The objectives of this surveillance programme is to monitor accumulating data on efficacy and safety in the treatment of inborn errors in primary bile acid synthesis due to 3β-Hydroxy-Δ⁵-C₂₇-steroid oxidoreductase deficiency or Δ⁴-3-Oxosteroid-5β-reductase deficiency with Orphacol in infants, children, adolescents and adults.</td>
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<tr>
<td></td>
<td>Reports on recruitment progress of the patient surveillance database will be analysed and reported to the CHMP at the time of PSURs (for safety) and of the Annual Re-assessments (for efficacy and safety). Progress and results from the database will form the basis of the annual reassessments of the benefit/risk profile of Orphacol.</td>
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3. Scientific discussion

3.1. Introduction

Problem statement

Orphacol is for use in the treatment of inborn errors of primary bile acid synthesis due to 3β-HSD deficiency or Δ4-3-oxoR deficiency. Both deficiencies 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/3/02/127) with a calculated prevalence of 0.06 per 10,000 EEA population.

In each of these two deficiencies, primary bile acid synthesis is absent or markedly impaired. Cholestasis and liver injury are the results of failure to synthesise adequate amounts of normal primary bile acids, associated with increased production of unusual primitive hepatotoxic and cholestatic bile acid metabolites (Clayton et al. 1987; Stieger et al. 1997). The liver disease associated with 3β-HSD and Δ4-3-oxoR deficiencies due to the production of hepatotoxic bile acid precursors is progressive and, if untreated, leads to death due to cirrhosis and liver failure. The only therapeutic option in severely affected cases is liver transplant. Currently there is no causal treatment. Treatment is limited to correcting the biochemical abnormalities, including the administration of bile acids and vitamin preparations. No medicinal product containing cholic acid as sole active substance has a marketing authorisation in the EU.

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.

About the product

The active substance of Orphacol is cholic acid. Cholic acid is a primary bile acid. Primary bile acids are biosynthesised in the liver and are key constituents of normal bile. The two primary bile acids synthesised by the liver, cholic acid and chenodeoxycholic acid, serve several important physiological functions (Setchell and O’Connell 2007). Cholic acid is the predominant primary bile acid in bile. It represents between approximately half and two thirds of the primary bile acids produced in adult. 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.

Cholic acid 50 and 250 mg capsules have been distributed in France from 1993 to October 2007 as a “préparation hospitalière” (hospital preparation). This preparation was used to treat a cohort of patients described by the Jacquemin group (Jacquemin et al. 2000; Potin et al. 2001; Gonzales et al. 2004; Gonzales et al. 2009). During this period, the Bicêtre Hospital pharmacy first and then the Agence Générale des Équipements et Produits de Santé - Établissement Pharmaceutique des Hôpitaux de Paris (AGEPS-EPHP) were successively both manufacturing site and batch release site of the finished product. Since October 2007, Laboratoires CTRS supplies cholic acid 50 and 250 mg capsules with the same composition and specifications as Named-Patient Compassionate Use in France under the invented name Orphacol. The Compassionate Use was authorised by the French National
Competent Authority (Afssaps) in October 2007. As of October 2009, 19 patients, aged from a few months to 28 years old, affected by 3\textbeta\textendash-HSD deficiency (17 patients) or Δ\textgreekalpha\textendash-3-oxoR deficiency (2 patients) are undergoing treatment with Orphacol. Three patients are EU residents but are receiving care through Bicêtre Hospital.

**Type of Application and aspects on development**

In accordance with Article 10a of Directive 2001/83/EC, as amended the application relies on well established medicinal use supported by bibliographic literature. According to Article 10a of Directive 2001/83/EC, as amended it is possible to replace results of pre-clinical and clinical trials by detailed references to published scientific literature (information available in the public domain) if it can be demonstrated that the active substance of a medicinal product has been in well-established medicinal use within the Community for at least 10 years, with a recognised efficacy and an acceptable level of safety. In this regard, the provisions of Annex I (Part II.1) to Directive 2001/83/EC shall apply.

The requirements of article 10a application are discussed below:

a) Factors which have been taken into account by the CHMP in order to establish a well-established use

- **Time over which the substance has been used**

This medicine has been used for these indications for over 10 years. In this application, the Applicant refers more than 30 publications dating back to 1987. The CHMP confirms that the requirement of an expiry of not less than one decade from the first systematic use of cholic acid in this condition in the EU is fulfilled. In this context, the CHMP takes duly account of the prevalence of the condition. The literature provided by the applicant showed that, where available to investigators, the clinical use of cholic acid has been documented since at least the mid-1990s through the work primarily conducted by the Jacquemin, Clayton and Setchell groups. The outcome for 22 identifiable 3\textbeta\textendash-HSD and Δ\textgreekalpha\textendash-3-oxoR deficiency patients treated with cholic acid has been presented. This patient population represents all patients who are reported in the literature at the time of the application submission. In view of the prevalence of the condition, it is considered that this reported population represents a substantial and relevant proportion of the overall estimated population in the EU. Therefore, cholic acid has extensively been used for the claimed therapeutic use in this condition.

- **Quantitative aspects of use of the substance**

As this is an orphan medicinal product for a rare condition the number of patients using the medicine is low. This is accepted by the CHMP. It should be noted that cholic acid 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 the at least the early part of the 20th century. The CHMP considers that this requirement is fulfilled.

- **The degree of scientific interest in the use of the substance (reflected in the published scientific literature)**

The Applicant has provided details of an adequate number of articles to fulfil this requirement. Of the 33 articles supporting this application on the treatment of inborn errors of primary bile acid synthesis, 18 articles are concerned with 3\textbeta\textendash-HSD deficiency and 8 concerned Δ\textgreekalpha\textendash-3-oxoR deficiency; 7 papers presented data for both indications.

- **Coherence of scientific assessments**

The literature provides a consistent view that cholic acid is the treatment of choice. Where available to investigators, its clinical use has been documented since at least the mid-1990s and its use can
therefore be considered as well-established. Overall there is coherence in the literature reviewed and analysed.

The CHMP therefore concludes that within the recognised limitation of an extremely rare and life-threatening condition that more than ten years have elapsed since the first systematic and documented use of cholic acid in the EU. The conclusion on systematic use takes into consideration the relevant proportion of patients covered by the presented data compared to the overall population affected by this condition, the consistency amongst the case reports, the representativeness of the patients for the overall patient population, as well as the duration over which such use has occurred. Therefore, despite the numbers are limited, it is the view of the CHMP that they are a reflection of the overall population, in which the applied product has systematically been used; hence the use of cholic acid can therefore be considered as well-established in the claimed indications.

b) The CHMP considers that the documentation submitted by the Applicant has covered all aspects of the quality, safety and efficacy and includes review of the relevant literature. The documentation, both favourable and unfavourable have been communicated.

c) Due to the rarity of the disease particular attention has been paid to any missing information and the CHMP considers that adequate justifications have been provided by the applicant, which demonstrate that an acceptable level of safety and efficacy can be supported although some studies are lacking.

d) The Applicant explained the relevance of data submitted concerning the product reviewed in the literature being different from the product intended for marketing. A judgement has been made that the product studied in the literature can be considered similar to the product intended for marketing. The formulations used are not always stated in the publications. A significant number of subjects were given capsules made at AGEPS (the French Hospital manufacturing facility). The formulation of the AGEPS product (qualitatively the same but with slight quantitative differences) was considered to be similar to the CRID Pharma product intended for marketing.

e) There is post-marketing experience with this active substance as this product has been used in patients for a number of years. It is noted that the medicinal product subject to this application is licenced for compassionate use since 2007 in France.

In accordance with Article 22 of Directive 2001/83/EC, 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 justified that he was unable to provide comprehensive data on the efficacy and safety under normal condition of use because the indications for which Orphacol are intended are encountered so rarely that he cannot reasonably be expected to provide comprehensive evidence. 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 3β-HSD deficiency or Δ⁴-3-oxoR deficiency by establishing a patient surveillance database.

3.2. Quality aspects

3.2.1. Introduction

Orphacol is presented as hard gelatine capsules containing 50 mg or 250 mg of cholic acid as the active substance.
Orphacol 50 mg strength is presented as a size 3 capsule with a blue cap and white body. The 250 mg strength is presented as a size 0 capsule with a green cap and white body.

Excipients used in the preparation of Orphacol are well known excipients used in the capsule preparations such as lactose monohydrate, colloidal anhydrous silica, magnesium stearate (present in capsule content), gelatin and titanium dioxide (E171), FD and C Blue 2 (indigo carmine, E132), yellow iron oxide (E172) (present in capsule shells).

The capsules are packed in polyvinyl chloride / aluminium (PVC/alu) blisters.

3.2.2. Active Substance

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

![Chemical structure of cholic acid](image)

It is a white to cream coloured 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 prove that only one crystal form is obtained by the utilised manufacturing process.

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 source 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. 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, 1H-NMR, 13C-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.

The particle size distribution of cholic acid is controlled by appropriate specification.

Potential impurities have been well discussed in relation to their origin (raw material, manufacturing process and degradation products) and potential carry-over into the final drug substance.

**Specification**

The drug substance specification includes tests for physical appearance, identification (FTIR and HPLC), 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. The HPLC method for identification, assay and impurities and GC method for residual solvents have been validated according to ICH Guideline Q3.

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 drug substance.

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

**Stability**

Three sets of stability studies have been performed. In the first set samples were stored at 25°C/65% RH, but were only tested annually not in line with ICH requirements. Therefore the second and third sets were performed according to ICH requirements (accelerated at 40°C/75% RH and long term at 25°C/65% RH). Data are available for 72 months from set 1 and 6 months and 24 months from sets 2 and 3 respectively.

The following tests were performed: Appearance, identification by HPLC, loss on drying, assay, related impurities and colour index. The parameters tested are acceptable.

The test methods are as described in section 3.2.S.4.2 except for assay which has been determined to date by area normalisation of the HPLC chromatogram as opposed to comparison with an external standard. Future testing is to be carried out by the external standard determination in addition to area normalisation. A comparison of the two methods has been provided and is acceptable.

The stability studies demonstrated that the drug 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 ± 2°C, 60%RH ± 5% RH and tested annually. In accordance with EU GMP guidelines\(^1\), any confirmed out of specification result, or significant negative trend, should be reported to the Rapporteur and the EMA.

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\(^1\) 6.32 of Vol. 4 Part I of the Rules Governing Medicinal Products in the European Union
3.2.3. Finished Medicinal Product

**Pharmaceutical Development**

The formulation is relatively simple and only limited information has been provided on pharmaceutical development.

The aim of pharmaceutical development was to obtain capsules containing 50 mg or 250 mg of cholic acid when filled by adjustment to the volume of size 3 or size 0 capsules respectively. The particle size of the active substance was important for the volume adjustment of the powder in the empty capsule. As a consequence a particle size specification is set for lactose monohydrate and it is comparable to that of cholic acid. The active substance needs to be milled prior to use as a number of large particles were observed in unmilled active substance which could affect blend homogeneity.

A number of blends of active substance and excipients were investigated and a final formulation which gave the required mean content was chosen for both strengths.

The clinical batches of the product were not manufactured by the proposed manufacturer. Active substance from a different supplier was also used; however the batch manufactured by the current manufacturer and with active substance from the current supplier has been used for compassionate use up until now. A bridging study has been performed in order to confirm similar or higher quality of current batches compared with those used in trials.

A suitable method for dissolution has been 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, as for infants and children who cannot swallow capsules, Orphacol may be prepared as a suspension for oral administration, palatability and compatibility of the formulation when mixed with some types of foods and drinks was investigated. This has been reflected in the SmPC.

**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 drug product only gelatin (component of the capsule shell) and lactose monohydrate (capsule filling) are of animal origin. For gelatin Ph Eur TSE Certificates of Suitability were provided. Declarations from lactose suppliers were provided stating that milk used for production of lactose is sourced from healthy animals under the same conditions as milk collected for human consumption.

Magnesium stearate used in the formulation is of vegetal origin.

**Manufacture of the product**

The manufacturing process is very simple consisting of premixing and mixing stages followed by lubrication and filling of capsules. The main parameters of importance are homogeneity of the final lubricated mix and homogeneity of the capsules, and validation of these parameters has been carried out during the product development.
The manufacturing process validation will be performed on three production scale batches. As the utilised manufacturing process is considered standard at the time of submission only process validation protocol was provided, and this was considered acceptable.

**Product Specification**

The drug product specification at release includes tests for appearance, disintegration in water, mean mass, mass uniformity, identification, assay (HPLC), impurities (HPLC), dissolution and microbiological testing (total aerobic viable count, yeasts and moulds, Escherichia Coli).

The shelf life specification is the same as the release specification with the exception of the absence of uniformity of dosage units and a slight widening of the assay specification.

Analytical methods have been sufficiently described, some of them are compendial methods described in the Ph Eur. Adequate validation data have been provided for non-compendial methods.

The HPLC method for identification and assay of cholic acid has been validated for specificity, linearity, stability of solutions, accuracy and precision.

The HPLC method for determination and assay of impurities has been validated for specificity, LOD/LOQ, linearity, stability of solutions, accuracy, precision.

Batch analysis results on commercial scale batches of each strength of the drug product demonstrated compliance with the proposed specification and confirmed consistency and uniformity of the product. 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 four industrial scale batches of the finished product. All batches were packed in aluminium/PVC blisters, which is the container closure system proposed for the commercial 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, up to 12 months from storage under intermediate conditions at 30°C/65%RH, and 6 months from accelerated conditions at 40°C/75% RH.

In addition stability data from forced degradation studies were provided. The following conditions were studies: acid degradation, basic degradation, oxidative degradation, light degradation, thermal degradation.

The overall stability data showed that Orphacol 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.

In accordance with EU GMP guidelines\(^2\), any confirmed out of specification result, or significant negative trend, should be reported to the Rapporteur and the EMA.

\(^2\) 6.32 of Vol. 4 Part I of the Rules Governing Medicinal Products in the European Union
3.2.4. Discussion on chemical, pharmaceutical and biological aspects

The active substance, cholic acid, is a well-known and well-characterized ingredient. 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. Its main use, up until now, has been as an intermediate in the production of ursodeoxycholic acid and dehydrocholic acid.

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

The active is shown to be stable, even when stored at elevated temperatures.

It is a hard gelatine capsule containing either 50 mg or 250 mg of the active substance.

The description and composition of the product are properly documented.

The drug product specification has been correctly discussed and the limits proposed for each test have been established taking into account the data of clinical and stability batches. In general, the specifications are acceptable.

Analytical methods used to control the quality of the finished product are well described and validated according ICH.

The results generated during the stability studies and statistical analyses support the proposed shelf life and storage conditions as defined in the SmPC.

3.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

The drug substance and the drug 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.

3.3. Non-clinical aspects

3.3.1. Introduction

The non-clinical data consists of literature references published from 1926 to 2009. Due to the bibliographic nature of this application and the date of origin of some of the submitted studies GLP aspects are not fully covered according to the present regulatory standards. This is however acceptable due to the accepted well established use of the product.

3.3.2. Pharmacology

Primary pharmacodynamic

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 Applicant has conducted an extensive literature review for the pharmacology of cholic acid.
The effects of cholic acid 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. All referenced studies are academic. 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 cholic acid (Li-Hawkins et al. 2002; Murphy et al. 2005; Shea et al. 2007). This action is mediated through the activation of the nuclear bile acid receptor FXR which in turn expresses the expression of SHP transcriptional repressor. SHP negatively interacts with other transcription factors; LHR-1 and HNF-4 α which in turn bind to the promoter region of the CYP7A1 and CYP8B1 gene, thus repressing primary bile acid synthesis (Goodwin et al. 2000; Lu et al. 2000; Brendel et al. 2002).

Mice deficient in HSD3β7 (the homolog of the human gene coding for 3β-HSD), saw survival rates increase after feeding with cholic acid and vitamin supplements (Shea et al. 2007). The 3-hydroxy 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. 3β,7α-dihydroxy-5-cholenoyltaurine (present in 3β-HSD deficiency) and 7α-hydroxy-3-oxo-4-cholenoyltaurine (present in Δ4-3-oxoR deficiency) are strong inhibitors of the canalicular, ATP-dependent bile acid transporter, BSEP. Patients with inborn errors such as 3β-HSD or Δ4-3-oxoR deficiency experience cholestasis as a result of inhibition of BSEP. BSEP expression can be up-regulated following addition of cholic acid.

No specific animal models addressing a Δ4-3-oxoR deficiency have been found.

It can be concluded that mutations in the 3β-HSD and cholesterol 7α-hydroxylase models have very similar phenotypes which can both be rescued by the addition of CA to the nursing mothers. Given that Δα-3-oxoR, cholesterol 7α-hydroxylase and 3β-HSD are part of the same bile acid biosynthesis pathway, it would be expected that a deficiency within Δα-3-oxoR would result in a similar phenotype to the aforementioned mutational models, and may also be rescued by cholic acid. Moreover, long term efficacy (although limited) has been observed in patients with Δα-3-oxoR deficiency.

Secondary pharmacodynamic studies

No published studies were found on cholic acid’s secondary pharmacodynamic effects in animal studies. Cholic acid being an endogenous substance, no secondary pharmacological effects is expected. Data from repeated-dose toxicology suggest that there is no pharmacologic or toxic action of cholic acid outside the organs of the enterohepatic circulation.

Safety pharmacology programme

The Applicant has provided reference to a range of studies for the cardiovascular system of rat and cat, and the rat autonomic nervous system following administration of cholic acid. The full core battery of safety pharmacology studies as defined in Nfg on safety pharmacology studies for human pharmaceuticals (CPMP/ICH/539/00) consists of cardiovascular, respiratory and central nervous system studies. Cholic acid is bound to specific transporter and receptor proteins. In this case it is sufficient to evaluate safety pharmacology endpoints as a part of the toxicology studies and a reduced number of safety pharmacology studies. Organs of the enterohepatic circulation have been identified from repeated-dose toxicity studies to be the target organs for cholic acid toxicity. A negative chronotropic effect was observed in vivo in rats at intravenous cholic acid doses between 10 and 40 mg/kg (Joubert 1978). This effect was shown to be dose-dependent in vitro and causes both a vagally mediated and direct effect on heart rate. This effect was reduced with atropine or vagotomy. Ganglion blockade and decerebration further diminishes this effect (Joubert 1978). At low concentrations on cat cardiac muscle, cholic acid induces a mild positive inotropic effect and induces an endocardial endothelium-dependent and β-receptor-mediated positive inotropic response, while cholic
acid at higher concentrations or after prolonged single low concentration causes extensive morphological damage of the endocardial endothelium (Colpaert et al. 1992).

In an *in vivo* experiment of the rat heart bile acids decrease rat heart rate when administrated with adrenaline and noradrenaline. Increased vasoconstriction is also observed and there is an inversion of acetylcholine effects on the heart and blood vessels. Bile acids decreased the relaxing effect of isoprenaline on the intestine and reversed the effects of adrenaline and noradrenaline on the intestine. The contraction-inducing effects of acetylcholine and pilocarpine were increased after administration with cholic acid (Kadlubowski et al. 1984).

The heart is exposed to concentrations of cholic acid in the micromolar range under normal conditions, when cholic acid is confined to the enterohepatic circulation. This is 10 to 1000-fold below the concentrations used in the experiments described above. Only under conditions of cholestasis or continued cholic acid administration does its concentration in peripheral blood approach these concentrations. Such situations were considered unlikely to persist due to the self-limiting toxicity of cholic acid 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 found in the literature detailing possible pharmacodynamic drug interactions of cholic acid. Studies which describe the pharmacokinetic drug interactions with cholic acid are discussed in the section below.

### 3.3.3. Pharmacokinetics

**Absorption**

The absorption of cholic acid is well recognised and established. Following ingestion, absorption of cholic acid will first be by the small intestine, and is then transported to the liver by the blood for further processing. Cholic acid was shown to be mainly absorbed in the distal (ileal) rather than proximal segments of the small intestine in the guinea pig (Weiner and Lack 1962). Absorption studies showed that cholic acid pool sizes were shown to be 168 ± 21 and 106 ± 12 μmol/kg body weight for mice and rats, respectively. This is equivalent to 68.5 ± 8.6 and 43.2 ± 4.9 mg/kg for mice and rats respectively (Hulzebos et al. 2001). Absorption studies have proposed the pool sizes to be 150 mg/kg for rats and 69 mg/kg for mice. The Fractional Turnover Rate (FTR) values were 0.44 ± 0.03 and 0.88 ± 0.10 for mice and rats and the corresponding synthesis rates were 29.8 ± 6.5 mg/kg and 38 ± 0.4 mg/kg respectively (Hulzebos et al. 2001).

**Distribution**

No publications were reported on kinetic parameters, bioequivalence and/or bioavailability. It is expected that the vast majority of cholic acid is present within the organs of the enterohepatic circulation, while a smaller fraction is present in peripheral blood; rhesus monkey studies provided further evidence of this (Little et al. 1975). Cholic acid is tightly bound to albumin with around 95% of total cholic acid bound. Placental transfer studies in a number of species (rat, sheep and rhesus monkeys) gives contradictory evidence to whether cholic acid transfers between mother and foetus. Bile acids and cholic acid are normally present in fetal serum (Little et al. 1975; Hassan and Subbiah
1980; Campos et al. 1986; Perez et al. 1994) so fetal exposure to endogenous levels of cholic acid and the potential placental transfer can be considered to be physiological (Hassan and Subbiah 1980).

**Metabolism**

Cholic acid and other bile acids are found as natural constituents of the bile. Cholic acid, chenodeoxycholic acid and deoxycholic acid constitute 95% of biliary bile acids in humans. Lithocholic acid and ursodeoxycholic acid are minor constituents. The bile acids exist largely as glycine and taurine conjugates the salts of which are called bile salts (Brunton et al. 2005). Once secreted, the bile acids are deconjugated and primary bile acids are partially converted into secondary bile acids through the action of intestinal bacteria. Between 90 and 95% of bile acids are reabsorbed, mainly from the lower half of the small intestine and undergo enterohepatic circulation, small quantities occur in the stools and very little is normally excreted in the urine. The bile acids that return to the liver via the enterohepatic route are reconjugated before re-secretion into bile. The self-regulating pathway for bile acid synthesis is sensitive to the enterohepatic circulation and to bile acid feeding (Setchell and O’Connell 2007).

The primary bile acids in humans, cholic acid and chenodeoxycholic acid, are synthesised in hepatocytes from cholesterol by a sequence of reactions modifying the steroid nucleus and the side chain (Setchell and O’Connell 2007). The enzymes that catalyse these reactions are located in various subcellular compartments. Considerable substrate promiscuity for the enzymes takes place under normal conditions, and this is exaggerated in pathologic conditions that interfere with the integrity of the biosynthetic pathway, as in 3β-HSD deficiency and Δ4-3-oxoR deficiency. Overall, it is now recognised that there are two main pathways leading to primary bile acid synthesis, termed the classical or neutral and the acidic pathways.

The first and rate-limiting reaction in bile acid synthesis through the classic pathway involves the introduction of a hydroxyl group at position C-7 of the cholesterol nucleus by cholesterol 7α-hydroxylase. Bile acid synthesis is largely regulated through negative feedback by bile acids returning via the portal vein during their enterohepatic recycling.

Following the synthesis of 7α-hydroxycholesterol, modifications to the steroid nucleus take place that result in oxidoreduction and hydroxylation, consequently preparing the sterol intermediates for direction into either the cholic acid or chenodeoxycholic acid pathways:

- **7α-hydroxycholesterol** is converted to 7α-hydroxy 4-cholesten-3-one; a reaction catalysed the microsomal nicotinamide adenine dinucleotide (NAD)-dependent 3β-hydroxy-Δ5-C27-sterol oxidoreductase enzyme, also referred to as 3β-hydroxy-Δ5-C27-steroid dehydrogenase/isomerase (3β-HSD).

- 7α-hydroxy 4-cholesten-3-one is converted to 7α,12α-dihydroxy-4-cholesten-3-one by a liver-specific microsomal cytochrome P450 12α-hydroxylase. 12α-Hydroxylation through the above reaction directs the (dihydroxy-) intermediates into the cholic acid pathway; intermediates that do not undergo this reaction will be converted to chenodeoxycholic acid.

- 7α,12α-Dihydroxy-4-cholesten-3-one and 7α-hydroxy 4-cholesten-3-one both undergo reduction by the soluble Δ4-3-oxosteroid 5β-reductase (Δ4-3-oxoR, also referred to as 5β-reductase).

- The resulting 3-oxo-5β (H) sterols and 3-hydroxysteroid dehydrogenase, which comprises a group of isozyms. This is the final modification of the steroid nucleus and results in the formation of the intermediates, 5β- cholestane-3α,7α,12α-triol and 5β-cholestane-3α,7α-diol (bile alcohols).
The bile alcohols then undergo a sequence of oxidation reactions of the side chain and its consequent shortening by three carbon atoms. These reactions are catalysed first by mitochondrial and then peroxisomal enzymes and lead to the final products cholic acid andchenodeoxycholic acid.

The primary bile acids are then conjugated to glycine and taurine, forming glycocholic acid and taurocholic acid as well as glycochenodeoxycholic acid and taurochenodeoxycholic acid. Upon secretion into the intestine, bile acids are largely reabsorbed in the ileum to return to the liver via the portal blood (Ross 2008). 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 Cholic acid is converted to deoxycholic acid, while chenodeoxycholic acid is converted to lithocholic acid by this reaction (Ross 2008).

Overall, cholic acid metabolism has been extensively reviewed and is considered well established.

**Excretion**

Excretion studies in rats have shown that cholic acid is almost exclusively excreted in the faeces and is in the form of metabolites (Lindstedt and Norman 1955). No radioactivity was found in urine and only minor amounts of cholic acid were found in the unconjugated form. There appears to be bi-directional placenta transfer of bile acids as observed in sheep (Perez et al. 1994; Campos et al. 1986) and rhesus monkeys (Little et al. 1975). Contrary to these studies, no significant increase was seen in fetal bile acids despite a 30-fold increase in maternal plasma bile acids following bile duct ligation in placenta transfer studies in rats, thus highlighting potential differences in the PK between species (Hassan and Subbiah 1980). [14C] cholate was only found in fetal rhesus monkey plasma, bile, liver and intestine (Little et al. 1975).

There was no literature found on excretion of milk in animals, mice. Feeding pregnant and nursing mice carrying a targeted inactivation of the HSD3B7 gene 0.1% or 0.5% cholic acid 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. This strongly indicates that cholic acid is secreted in milk in mice (Shea et al. 2007). Human data suggests that cholic acid is secreted in milk (Forsyth et al. 1983).

**Pharmacokinetic drug interaction**

Pharmacokinetic drug interaction in rats showed that cholestyramine, a bile acid sequestrant, removes cholic acid from the pool and stimulates its biosynthesis rate (Hulzebos et al. 2001).

Cyclosporine A and verapamil (strongly interacting with MDR1) interfere with the transport of cholic acid. Cyclosporine A inhibits the uptake of cholic acid into rat hepatocytes in vitro and leads to increased serum cholic acid concentrations in vivo (Azer and Stacey 1994). In isolated and cultured rat liver slices, both CsA and verapamil interfered with the transport of cholic acid. The in vivo significance of the verapamil interaction remains unclear from these experiments (Barth et al. 2006)

Phenobarbital treatment increases the transport capacity ($V_{max}$) for bile salt in the ileum of the rat. Cronholm et al. 1974 found that rats treated with phenobarbital for 10 days had a 4-fold smaller cholic acid pool compared to control rats. Additionally, the daily production of cholic acid was decreased in phenobarbital treated rats compared to control rats. Phenobarbital has an antagonistic effect to the desired action of cholic acid in patients and may potentially endanger the established metabolic control. Therefore use of Phenobarbital in patients treated with cholic acid has been contraindicated.
3.3.4. Toxicology

Nonclinical toxicology of cholic acid was reviewed in a series of publications based on single-dose, repeat-dose, in vitro genotoxicity, carcinogenicity and embryo-fetal development studies. These studies were conducted in six different species (mouse, rat, gerbil, sheep and chick), via five routes of administration (oral – via feeding [p.o.], subcutaneous [s.c.], intravenous [i.v.], intraperitoneal [i.p.], and intrarectal). The majority of studies were performed in the 1970s or earlier, before the implementation of Good Laboratory Practices (GLP) regulations. All referenced studies are academic, and none notes adherence to GLP.

**Single-dose toxicity**

Single-dose toxicity studies reported from the literature were carried out in intravenously in mice (Rothlin and Schalch 1944), rats (Joubert 1978) and rabbits (Gillert 1926) and additionally by oral administration to mice (Informatics Inc. 1973). In the mouse, the LD50 is estimated to 350 mg/kg (i.v.) while in the rabbit study the lethal dose and sub-lethal dose was considered to be 50 mg/kg (i.v.).

**Repeat dose toxicity**

No new repeated-dose toxicity studies were conducted for this application. Studies from published literature have been summarised and reviewed in mice (Fickert et al. 2001; Beasancon et al. 1970; Wang et al. 1999), rats (Delzenne et al. 1992; Bray and Gallagher 1969; Visek et al. 1965; Gambal and Quackenbush 1960; Lacassagne et al. 1967) and gerbils (Bergmann and van der Linden 1971). The studies reviewed are of small size and systematic dosing data is not available. Frequently reported effects of cholic acid have included decreased body weight (Bray and Gallagher 1969) or retarded gains in weight (Lacassagne et al. 1967), increased liver weight (Fickert et al. 2001; Delzenne et al. 1992; Lacassagne et al. 1967 and Bergmann and van der Linden 1971), development of gallstones (Tepperman et al. 1964; Wang et al. 1999; Beasancon et al. 1970 and Bergmann and van der Linden 1971) and diarrhoea (Visek et al. 1965; Gambal and Quackenbush 1960). Other effects have also been reported such as rectal bleeding (Baijal et al. 1998) and spleen hypertrophy (Lacassagne et al. 1967).

In Mongolian gerbils fed chow supplemented with 1% cholesterol and 0.5% cholic acid developed steatosis of the liver, accumulation of lipids in the reticuloendothelial system, high serum cholesterol levels and accentuated hypoplastic changes of the thyroid gland (Bergmann and van der Linden 1971). In the bile, a marked rise of the cholesterol concentration, a slight rise of the cholic acid and deoxycholic acid and a decrease of thechenodeoxycholic acid concentration were observed. After 4 months on the diet, cholesterol gallstones were found in the distended gallbladders. Discontinuance of the diet showed the gerbil to be rather unable to free itself from excess cholesterol with a very slow and incomplete return to normal of liver histology which was in contrast to that seen in rodents. It is considered that the gerbil is not a suitable model to draw direct conclusion to humans given that gerbils are vegetarians and are probably physiologically poorly adapted to metabolise cholesterol. The rat however like humans is an omnivore and so is considered more similar in term of cholesterol metabolism. Given the differences in cholesterol metabolism and excretion between gerbils and rats and the physiological dissimilarities of the gerbil to human, the likelihood that the toxicities seen with the Bergman and van der Linden 1971 study is unlikely to be relevant to humans.

**Genotoxicity**

*In vitro*
A number of studies have shown conflicting evidence as to whether cholic acid is genotoxic and whether it has mutagenic potential. The Salmonella typhimurium and Ames test has been used to evaluate the mutagenic potential of cholic acid. Three studies used a fluctuation test with metabolic activation in strains TA98 and TA100 and cholic acid was found to be negative in two (Venitt et al. 1987; Mori et al. 1991) but positive in another (Watabe and Bernstein 1985). The positive results seen in the fluctuation assay by Watabe and Bernstein have largely been discredited by Venitt et al., (1987) and Mori et al., (1991).

In mammalian cells in vitro, a clear dose-dependent genotoxic effect induced by chenodeoxycholic acid and deoxycholic acid was observed (Jenkins et al. 2007). 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 the OECD 471 guideline and ICH S2 (R1), the dose of cholic acid to be administered to the patients is intended to restore a concentration that is equivalent to that physiologically present in humans. Therefore any perceived genotoxic risk from cholic acid and deoxycholic acid to the patients would be equivalent to that of a normal healthy adult that produces these bile acids intrinsically. Overall, Cholic acid showed non significant mutagenic activity in a battery of genotoxicity tests performed in vitro.

In vivo

No in vivo data has been reported. 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 cholic acid has been shown to not interact with DNA via adduct formation (Scates et al. 1995).

Carcinogenicity

No long-term oral carcinogenicity studies of cholic acid or other bile acids have been found in the literature. All studies were instead reviewed in carcinogenicity models where a known carcinogen was given to initiate the formation of tumours or pre-neoplastic lesions.

In short or medium term studies animal models (rats) indicate that administered bile acids show liver carcinogenic promotion potential and that this is carcinogen dependent. No carcinogenic effects were seen under cholic acid 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 of treatment of patients with 3β-HSD and Δ4-3-oxoR deficiencies, the restoration of normal functional enterohepatic circulation and normal absorption of fat and fat-soluble vitamins together with the long-term survival and the avoidance of liver disease (in itself a risk for carcinogenicity) 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

Cholic acid has been demonstrated be present in foetal blood and to pass the placenta in rat (Hassan and Subbiah 1980) and sheep (Perez et al. 1994; Campos et al. 1986) studies. With regards to effects on the reproduction and development of the animals, in hamsters cholic acid showed toxic effects upon the histology of the liver in both mothers and offspring (Siviero et al. 2008). However, the hamsters received food supplemented with 0.5% (60 mg/day) cholic acid; given the endogenous cholic acid pool
sizes for both mice and rats, it is likely that this corresponds to at least the endogenous pool size fed per day. Both a bolus injection (Perez et al. 1994) into pregnant sheep and the continuous infusion (Campos et al. 1986) of 934 mg cholic acid per day into foetal lambs resulted in the early delivery of fetuses. However, at physiological concentrations cholic acid as an endogenously present molecule is non-embryotoxic. Verrett et al. (1980) reported no teratogenic effects in chickens when 25 mg of cholic acid was injected in a single volume of 100 μL or less per egg. No publications were reported on prenatal and postnatal development and on juvenile studies. Clinical data has shown there to be four successful pregnancies in patients who had been receiving Orphacol, all producing healthy babies. No nonclinical studies were identified investigating the effects of the use of cholic acid during lactation and paediatric development. From a mouse study examining deficiency of the HSD3B7 gene, there is evidence that cholic acid 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 and no non-rodent data was available. Due to the large amount of clinical data available on cholic acid, the absence of animal data is acceptable.

Target organs for toxicity for cholic acid are in the enterohepatic circulation, the liver and administration causes diarrhoea. In combination with a high-cholesterol containing diet, cholic acid administration leads to increased liver weight and the formation of gallstones in rodents. 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. These toxicities were observed at up to 11 to 22 times the endogenous cholic acid pool, although the comparison of administered doses remain approximate as cholic acid was supplemented to the diet of experimental animals rather than given in defined gavaged amounts. The lack of toxicokinetic data further limits the interpretation of these results as no correlation exists between dose/systemic concentrations and toxicities arising.

**Local Tolerance**

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

**Other toxicity**

**Metabolites**

Deoxycholic acid is the main metabolite of cholic acid. Patients with 3β-HSD deficiency and Δ⁴-3-oxoR deficiency and subjects with a normal bile acid metabolism have shown that upon treatment with cholic acid, serum and bile predominantly contain cholic acid and deoxycholic acid, while chenodeoxycholic acid and its metabolites appear to be reduced. Under cholic acid treatment, patients are therefore exposed to higher than normal deoxycholic acid concentrations, although the exact quantifications of these concentrations have not been described.

In single- and repeat-dose studies, deoxycholic acid showed lethal effects, gastrointestinal and hepatic toxicities at approximately half the doses needed for cholic acid to produce the same effects. It is therefore considered that deoxycholic acid is more toxic than cholic acid and may in fact be the causative agent of some of cholic acid’s toxicity.

Mutagenicity data from bacterial test for deoxycholic acid is ambiguous but deoxycholic acid was genotoxic in an *in vitro* micronucleus assay. Additionally, Rosignoli et al 2008 investigated the
genotoxic potential of BA (focusing on chenodeoxycholic acid and deoxycholic acid) on human colonocytes and colon tumour cells HT 29 by a comet assay. In both cell types a clear dose-dependent genotoxic effect induced by the two bile acids was observed, with deoxycholic acid being more genotoxic. Viability of cells appeared to be greater than 75%. Use of a nuclease III modified comet assay suggested that the DNA damage could be mediated by reactive oxygen species production but was somewhat protected by inclusion of anti-oxidants.

Short term carcinogenicity studies suggest that deoxycholic acid like cholic acid has carcinogenicity promoting properties. In rat liver, deoxycholic acid (75-150 mg/kg) exerted promoting activity as evidenced by significantly increased values of γ-glutamyl transpeptidase-positive (γ-GT+) liver foci compared with the corresponding controls given the carcinogen, diethylaminozoxy (DEN) alone. Deoxycholic acid (20 mg/kg) enhanced the development and growth of azoxymethane (AOM) -induced aberrant crypt foci in rat colons (Tsuda et al. 1984; Shiota et al. 1999). In a parallel study, deoxycholic acid in the absence of AOM did not significantly induce aberrant crypt foci. However, Rosignoli et al 2008 concluded that deoxycholic acid may act not only as promoters but also initiators of the multistage process of carcinogenesis. The potential carcinogenic effects of deoxycholic acid is highlighted as a potential risk, and long-term monitoring of the risk for carcinogenicity is included as an element in the Risk Management Plan.

**Impurities**

A study in oesophageal cells has demonstrated no genotoxicity for methyl cholate at concentrations of 25 to 200 µM test (Macdonald et al. 1978). This is in excess of methyl cholate concentrations found at maximal daily dose of Orphacol.

At least 15 patients reported by Gonzales et al. (2009), have been treated, most of them for more than 10 years, with cholic acid 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 cholic acid. Methyl cholate has been shown in vitro to inhibit cholesterol synthesis. According to specified limits of methyl cholate it is accepted that cholesterol synthesis of patients would not be adversely affected at this concentration.

**3.3.5. Ecotoxicity/environmental risk assessment (ERA)**

Given that cholic acid 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).

**3.3.6. Discussion on non-clinical aspects**

The pharmacological effects of cholic acid on the metabolism and secretion of bile acids are well documented in numerous scientific publications, including studies that document the effects of cholic acid in mice with deficiencies in bile acid metabolism comparable to that of 3β-HSD deficiency but not Δ^4-3-oxoR deficiency. Metabolism of bile acids is tightly controlled via a negative feedback regulation of bile acid synthesis. The pharmacokinetics of cholic acid is well recognised and established, although no publications were found in kinetic parameters, bioequivalence and/or bioavailability. The Applicant provided a satisfactory analysis to support this application. Target organs for toxicity for cholic acid are in the enterohepatic circulation, the liver and administration causes diarrhoea. In combination with a
high-cholesterol containing diet, cholic acid administration leads to increased liver weight and the formation of gallstones in rodents.

A number of studies have shown conflicting evidence as to whether cholic acid is genotoxic and whether it has mutagenic potential. The *Salmonella typhimurium* and Ames test has been used to evaluate the mutagenic potential of cholic acid. The positive results seen in the fluctuation assay by Watabe and Bernstein have largely been discredited by Venitt *et al*., (1987) and Mori *et al*., (1991). In mammalian cells *in vitro*, a clear dose-dependent genotoxic effect induced by CDCA and DCA was observed. 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.

While no long-term carcinogenicity studies have been preformed, cholic acid was shown to enhance carcinogenesis of target organs following administration of known carcinogens. The main metabolite, deoxycholic acid, was shown to be more toxic than cholic acid and may be the causative agent of some of cholic acid’s toxicity. The mutagenic potential of deoxycholic acid is ambiguous with one study suggesting that it may act not only as promoters but also initiators of the multistage process of carcinogenesis. The potential carcinogenic effects of deoxycholic acid has been highlighted as a potential risk, and long-term monitoring of the risk for carcinogenicity is included as an element in the Risk Management Plan for Orphacol.

A study in oesophageal cells has demonstrated no genotoxicity for methyl cholate at concentrations of 25 to 200 µM. This is in excess of methyl cholate concentrations found at maximal daily dose of Orphacol. At least 15 patients reported by Gonzales *et al*. (2009), have been treated, most of them for more than 10 years, with cholic acid 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 cholic acid. Methyl cholate has been shown *in vitro* to inhibit cholesterol synthesis. According to the specified limits of methyl cholate, it is accepted that cholesterol synthesis of patients would not be adversely affected at this concentration.

**3.3.7. Conclusion on the non-clinical aspects**

The Applicant submitted an application for a well established use product, and as such submitted no new non-clinical data. The extensive literature review of the pharmacology, pharmacokinetics and toxicology of cholic acid is considered appropriate and acceptable to support the non-clinical aspect of Orphacol.

With regard to the nonclinical aspects the majority of cited literature are dated in the 1970’s or earlier and pre-date implementation of GLP requirements. The GLP status of the studies reviewed from published literature cannot be verified. In view of the available data from use of cholic acid 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 studies reviewed have however not been conducted to the same level of detail as for a pharmaceutical agent, as cholic acid is a physiological substance in animals and humans.

The intravenous LD$_{50}$ of cholic acid in mice is 350 mg/kg body weight. Parenteral administration may cause haemolysis and cardiac arrest. Administered orally, bile acids and salts generally have only a minor toxic potential. The oral LD$_{50}$ in mouse is 1520 mg/kg. In repeated-dose studies, frequently reported effects of cholic acid have included decreased body weight, diarrhoea and liver damage with
elevated transaminases. Increased liver weight and gallstones have been reported in repeated dose studies in which cholic acid was co-administered with cholesterol.

Cholic acid showed non significant mutagenic activity in a battery of genotoxicity tests performed in vitro. Data reviewed suggest that cholic acid 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. As conservative approach, given the limited data provided the long-term monitoring of the risk for carcinogenicity is included as an element in the Risk Management Plan.

Animal studies showed that cholic acid did not induce any teratogenic effect or foetal toxicity. No data on the effects of cholic acid on fertility are available. At therapeutic doses, no effect on fertility is anticipated. Cholic acid and its metabolites are excreted in human milk, but at therapeutic doses of Orphacol, no effects on the breastfed newborns/infants are anticipated. Orphacol can be used during breast-feeding.

3.4. Clinical aspects

3.4.1. Introduction

To support the application, from clinical perspective, the Applicant has submitted a total of 38 patient reports involving confirmed or suspected 3β-HSD and 11 involving Δ4-3-oxoR deficiencies. Of the 38 patients with 3β-HSD deficiency, 21 were treated with cholic acid, 8 were treated with other bile acids and 9 did not receive bile acids. Of the 11 patients with Δ4-3-oxoR deficiency, 7 were treated with cholic acid, 3 were treated with other bile acids and 1 patient received no treatment.

In accordance with Article 22 of Directive 2001/83/EC, 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 provided adequate justification for being unable to provide comprehensive data on the efficacy and safety because the indications for which Orphacol are intended are encountered so rarely that the Applicant cannot reasonably be expected to provide comprehensive evidence. It would also be contrary to generally accepted principles of medical ethics to collect such information.

GCP

Due to the bibliographic nature of this application and the date of origin of some of the submitted case study reports GCP aspects are not fully covered according to the present regulatory standards. This is however acceptable due to the accepted well established use of the product.

3.4.2. Pharmacokinetics

No studies have been performed to determine the pharmacokinetic characteristics of Orphacol. However, the Applicant has provided a literature review of studies on bile acid kinetics. Cholic acid, a primary bile acid, is an endogenous molecule and present in normal human bile, blood and other biological fluids. Its metabolites, the secondary and tertiary bile acids, are equally present in normal human bile, blood and biological fluids and fulfill important biological functions. Therefore, cholic acid has generally been studied in the literature in terms of its kinetics and dynamics, and to a lesser degree as an exogenous pharmacological agent. Its pharmacokinetic characteristics, including its metabolism, are different from that of a conventional synthetic small molecule pharmaceutical in that once administered, any exogenously administered cholic acid will behave like an endogenous molecule.
in all respects. Data on the kinetics and dynamics of endogenous cholic acid thus provides relevant information on the clinical pharmacology of Orphacol.

As a bile acid, cholic acid is subject to highly efficient first-pass hepatic extraction and enterohepatic recirculation. An accurate determination of the absorption kinetics would thus require blood sampling from the portal vein, which is only ethically possible in the context of a surgical intervention (Angelin et al. 1982). Sampling of peripheral plasma is a suitable method only for relative bioavailability and bioequivalence studies of bile acids with a low endogenous concentration (Setchell et al. 2004), which are not of relevance to this application.

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 cholic acid 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 between 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.

Based on literature review, the normal concentration of cholic acid (as its glycocholic acid and taurocholic acid conjugates) in gallbladder bile is in the range of 4–74 mmol/L, with slight variations depending on the technique used (Perwaiz et al. 2001). Cholic acid makes up about 35% (range: 22–44%) of gallbladder and hepatic bile (Ahlberg et al. 1981).

Absorption

The physico-chemical properties of a bile acid fundamentally govern its intestinal absorption: hydrophobic acids, including cholic acid and chenodeoxycholic acid, are absorbed more rapidly by passive non ionic diffusion than hydrophilic bile acids such as ursodeoxycholic acid. Once absorbed, exogenously administered bile acids become part of the bile acid pool. The cholic acid pool size has been measured in healthy adult volunteers using a stable isotope dilution technique and capillary gas-liquid chromatography (GLC) combined with electron impact mass spectrometry (MS) (Stellaard et al.1984; Everson 1987; Koopman et al. 1988) and by a microscale stable isotope dilution technique with GLC/electron capture negative chemical ionization-MS applied to the pentafluorobenzyl-trimethylsilyl (PFB-TMS) derivative of cholic acid (Hulzebos et al. 2001), both with administration of the labelled cholic acid in a bicarbonate solution. In a healthy adult of 60 kg body weight, the pool size of cholic acid (and its conjugates) is approximately 600-750 mg.

Bioequivalence

Orphacol has been developed based on formulations established by the Bicêtre Hospital pharmacy and the Paris Hospitals Central Pharmacy (Agence Générale des Équipements et Produits de Santé, AGEPS) for the treatment of inborn errors in primary bile acid synthesis due 3β-HSD or Δ4-3-oxoR deficiency (Potin et al. 2001). Orphacol is formulated in two different strengths, 50 mg capsules and 250 mg capsules. The patients described in the studies of the Jacquemin group (Jacquemin et al. 2000; Gonzales 2006; Gonzales et al. 2009) have been treated with these formulations, produced by Bicêtre Hospital pharmacy and the AGEPS.

The most important data supporting this application were generated by the Jacquemin group in the Paediatric Hepatology Unit at Bicêtre Hospital (Jacquemin et al. 1994; Jacquemin et al. 2000; Potin et
al. 2001; Gonzales 2006; Gonzales et al. 2009). This hospital is part of the Assistance Publique – Hôpitaux de Paris (AP-HP), the hospital group reuniting all public hospitals in the Paris area. The cholic acid formulation used to treat this patient population was initially produced by the Bicêtre Hospital Pharmacy and then by the AGEPS, the AP-HP Central Pharmacy. CRID Pharma took over the production of cholic acid capsules from AGEPS in 2007 for the ongoing compassionate use programme in France and this MAA. The demonstration of comparability between the AGEPS and CRID products, including bridging data on dissolution and particle size distribution, was considered satisfactory.

**Similarity: Bicêtre Hospital to AGEPS**

The change in production site from Bicêtre Hospital to AGEPS occurred during the observation period of the main supporting publications (Gonzales 2006; Gonzales et al. 2009). A partial analysis is possible by comparing the data reported for 6 patients that had been treated exclusively with the Bicêtre Hospital product after 5 years of treatment, and 3 patients that were exclusively treated with AGEPS product during the first 5 years (Gonzales et al. 2009). All patients were treated for 3β-HSD deficiency. Outcomes after 5 years of treatment with cholic acid preparations made by Bicêtre Hospital and AGEPS are similar. All patients survived without the need for a liver transplant; clinical symptoms, in particular hepatomegaly, resolved, and urinary 3β-hydroxylated-Δ5 bile acids were strongly reduced or absent.

**Similarity: AGEPS to Orphacol**

As the end of the observation period reported in these publications coincides with the change to the CRID product, i.e. Orphacol, no published data on the pharmacodynamics of the CRID product are available. However, the change from the drug product produced by AGEPS and used to treat the patient population studied by (Gonzales et al. 2009) to the drug product produced by CRID for the compassionate use did not lead to the need to adjust the dose in any patient nor to any reported adverse events. In addition, pharmaceutical similarity has been demonstrated. The AGEPS hospital preparation and Orphacol can therefore be considered to be similar.

No data on the cholic acid formulations used in other studies are available in the literature.

**Similarity: Unpublished Data**

The Applicant has recovered the hospital records of the patients treated under compassionate use with Orphacol in France and compared the treatment response to its two predecessor formulations used to treat the patients. Data demonstrate that changes in the preparation of Orphacol had no significant effects on the treatment outcome.

Patients were analysed in two groups: one that has received all three cholic acid preparations (Group A, 10 patients), and a second group that has been treated only with AGEPS and Orphacol (Group B, 5 patients). The comparison of treatment results across treatment periods with the consecutive cholic acid formulations (Bicêtre vs. AGEPS vs. Orphacol) was made by deriving for every patient the means of the parameter of interest during the treatment period, and then comparing the mean of means of all patients during the treatment periods by ANOVA. There was no statistically significant difference in treatment outcome as measured by liver function tests (AST, ALT, total bilirubin, prothrombin time). The response to treatment was not significantly different between treatment periods in terms of serum AST and ALT. All values and standard deviations were in the normal range (AST: 9 to 60 IU/L, ALT: 10 to 40 IU/L). Among 10 patients that changed from the Bicêtre hospital preparation to the AGEPS product (Group A), no dose adjustment was required. Among the 15 patients that changed from the AGEPS product to Orphacol (Groups A and B), 5 dose increases were made in the 12 months following the change. A close analysis of these dose changes showed that they were all made to compensate for growth and/or weight gain of the patients and not to insufficient efficacy or safety. Four of the dose
increases had no close temporal relationship to patient visits, and the fifth patient had normal liver function tests when the change was made.

Overall it is accepted that formulations used in the publications are unknown and that it is therefore not possible to compare Orphacol with such formulations in terms of quality and clinical similarity. The Applicant has provided relevant information regarding the pharmacodynamic and clinical aspects of some patients who were followed-up during the course of treatment under compassionate use when the old formulation was used. Some of these patients were switched to the new formulation. This switch is reported not to have resulted in any dose adjustment and there were no reported adverse events during the follow-up of these patients. Taking into account the demonstrated pharmaceutical comparability, it is considered that enough information has been provided demonstrating clinical similarity between the investigational formulation and the formulation that is intended for marketing.

**Influence of food**

In response to a standardised meal portal venous bile acids concentrations increased two- to six fold, with a peak seen 15–60 min after the meal. The maximum postprandial portal venous bile acid concentration averaged 43.04 ± 6.12 μmol/L, and the corresponding concentration in peripheral serum was 5.22 ± 0.74 μmol/L.

The effect of food on the bioavailability of cholic acid has not been studied. Differences in the daily administered dose through interactions with different foods (in terms of composition and potentially quantity) are expected to be evened out through the long half life of bile acids in the enterohepatic circulation. It is recommended that the product should be taken with food. This recommendation is empirically based and follows the treatment modalities established at Bicêtre Hospital. There is a theoretical possibility that administration with food may increase cholic acid bioavailability and improve tolerability.

**Distribution**

Due to the enterohepatic distribution of bile acids, volume of distribution is not commonly used. Bile acid kinetics is more widely reported in terms of pool size and fractional turnover rate (Crosignani et al. 1996). The kinetics of bile acid distribution between the systemic vascular and non-vascular compartments is not fully understood and the total volume of distribution of bile acids outside the enterohepatic pool has not been accurately defined (Kaye et al. 1973; Crosignani et al. 1996).

The approximate volume of distribution of endogenous bile acids in humans with normal liver function can be calculated from pool size and serum levels. When calculated this way, the volume of distribution of cholic acid is approximately 3400 L (Crosignani et al. 1996). When determined conventionally and normalised for body surface area, the volume of distribution is 1.879 ± 0.054 L/m² (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 over the day in function of meal intake and bile secretion (Everson 1987). In untreated patients with 3β-HSD and Δ4-3-oxoR deficiencies, total serum bile acid levels are usually in the normal range or diminished (Gonzales et al. 2009). However, due to the metabolic defect in these patients, primary bile acids including cholic acid are absent or present only at trace concentrations. This is resolved upon treatment with primary bile acids, where the administered bile acid(s) and their secondary metabolites predominate (Ichimiya et al. 1991; Clayton et al. 1996; Gonzales et al. 2009).
Plasma has a very high binding capacity for bile acids and the binding shows rapid equilibration. Albumin in serum is capable of binding about 95% of the total cholic acid concentration present via the primary site for cholic acid. Cholic acid binding to whole serum can be accounted for by albumin.

**Elimination**

Cholic acid is synthesized in the liver and metabolised in the intestine. Patients under cholic acid therapy are not expected to differ qualitatively in their metabolism from normal subjects as their defect is in primary bile acid synthesis, which takes place in the liver. The metabolism to secondary bile acids takes place in intestine and through intestinal bacteria and is therefore unaffected by hepatic enzyme defects in 3β-HSD deficiency or Δ⁴-3-oxoR deficiency. The same metabolic steps in the intestine can be expected to also apply to exogenously applied cholic acid in patients under cholic acid therapy. Quantitatively, the secondary bile acids derived from chenodeoxycholic acid are expected to be low or absent in patients treated with cholic acid in monotherapy. Indeed, this pattern has been found in patients under cholic acid therapy, whose urine and biliary bile contained predominantly cholic acid and deoxycholic acid (Gonzales et al. 2009).

Clearance of cholic acid was quantified 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 [¹⁴C] cholic acid 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 h. Intravenous and oral clearance of cholic acid was quantified and found to be 271 ± 15 ml · min⁻¹ · m⁻² and 1248 ± 104 ml · min⁻¹ · m⁻², respectively (Gilmore and Thompson 1980). In patients with various hepatic diseases (cirrhoses, icteric and anicteric liver disease, hepatitis), these clearances were significantly reduced.

The half-life of cholic acid is approximately 27 hours (Crosignani et al. 1996). Approximately 5% of the bile acid pool enters the colon and provides a substrate for the extensive microbial population that deconjugates and oxidizes hydroxyl groups, 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 hr and was independent of the 24-hr urine volume (Alme et al. 1977). Cholic acid made up 2-7% of the total bile acids. The rest of the total urinary bile acids was made up of a broad spectrum of other bile acid metabolites. Other sources report a substantially lower urinary excretion of bile acids of <1 μmol/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 cholic acid and its metabolites. Overall, in subjects with normal liver function, bile acids including cholic acid are excreted in the urine in negligible amounts (Alme et al. 1977). In case of cholestasis, when plasma bile acid concentrations are increased, renal excretion is also strongly increased. This is the case in untreated patients with 3β-HSD or Δ⁴-3-oxoR deficiencies. Bile salts, including chololate, 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.
Pharmacokinetics in target population

No population pharmacokinetic studies are available for cholic acid in the treatment of 3β-HSD and Δ⁴-3-oxoR deficiencies. Such studies are considered to be not technically possible at the current state of scientific knowledge. No difference in intestinal absorption of cholic acid between patients and healthy subjects is expected, as the enzymatic defects in patients concern hepatic enzymes. In untreated patients with 3β-HSD and Δ⁴-3-oxoR deficiencies, total serum bile acid levels are usually in the normal range or diminished (Gonzales et al. 2009), but increases have also been reported (Ichimiya et al. 1991). However, due to the metabolic defect in these patients, primary bile acids including cholic acid are absent or present only at trace concentrations. This is resolved upon treatment with primary bile acids, where the administered bile acid(s) and their secondary metabolites predominate (Ichimiya et al. 1991; Clayton et al. 1996; Gonzales et al. 2009). Catabolic metabolism of cholic acid takes place in the intestine by intestinal bacteria. No difference between patients and normal subjects is expected in this regard, as the enzyme defect in 3β-HSD deficiency and Δ⁴-3-oxoR deficiency affects hepatic enzymes and not the intestinal microflora. Elimination of bile acids occurs almost exclusively by faecal excretion. No difference between patients and healthy subjects is to be expected.

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 cholic acid, regardless of possible inter- and intrasubject variations in pharmacokinetics.

Special populations

Impaired renal function

There are no reports on the use of cholic acid for the therapy of 3β-HSD deficiency or a Δ⁴-3-oxoR deficiency in patients with renal impairment in the literature. Bile acid metabolites are excreted only under conditions of cholestasis, which is resolved by cholic acid treatment. No data are available for patients with end-stage renal disease. Bile acids including cholic acid are excreted in the urine in negligible amounts in humans with normal liver function (Alme et al. 1977). Therefore, a strong reduction or even absence of renal clearance is expected to have negligible influence on plasma levels of bile acids, including of cholic acid and its metabolites, as long as patients are well controlled through bile acid therapy.

Impaired hepatic function

Cholic acid is synthesized in the liver and metabolised in the intestine. Patients who receive cholic acid therapy are not expected to differ qualitatively in their bile acid catabolism from normal subjects as their defect is in hepatic primary bile acid synthesis. The metabolism of primary to secondary bile acids takes place in intestine through intestinal bacteria and is therefore unaffected by the hepatic enzyme defects in 3β-HSD deficiency or Δ⁴-3-oxoR deficiency. The majority of patients treated with cholic acid presented with some degree of hepatic impairment at the time of diagnosis which resolved under therapy. The evaluation of the safety of cholic acid includes patients with this metabolic impairment.

No data on cholic acid treatment are available in patients with 3β-HSD deficiency or Δ4-3-oxoR deficiency with hepatic impairment unrelated to their primary disease. Theoretically, decreased hepatic clearance of bile acids and/or cholestasis may occur, leading to increased serum levels of bile acids, including of cholic acid. This suggests that the cholic acid dose would have to be reduced carrying the risk of a de-repression of the biosynthesis of primary bile acid synthesis and hence the biosynthesis of the hepatotoxic and cholestatic bile acid metabolites (3β,7α-dihydroxy- and 3β,7α,12α-trihydroxy-5-cholenoic acids in 3β-HSD deficiency and 3-oxo-7α-hydroxy-4-cholenoic and 3-oxo-7α,l2α-dihydroxy-
4-cholenoic acids in Δ4-3-oxoR deficiency). Overall, in the absence of clinical experience in patients with hepatic impairment from causes other than 3β-HSD or Δ4-3-oxoR deficiency, no recommendations on dosage adjustment can be made. Patients with hepatic impairment should be monitored closely.

**Familial hypertriglyceridemia**

Type IV hyperlipoproteinemia, in particular familial hypertriglyceridemia (FHT), has been associated with defective bile acid absorption (Angelin et al. 1978). Type IV hyperlipoproteinemia is not a rare condition: between 1 and 10% of the Western population could be affected, depending on the source. Therefore, the possibility exists that there may be patients with inborn errors in primary bile acid synthesis and FHT. Overall, patients with newly diagnosed or a family history of familial hypertriglyceridemia are expected to poorly absorb cholic acid in the intestine. The cholic acid dose for patients with familial hypertriglyceridemia will have to be established and adjusted, but an elevated dose, notably higher than the 500 mg daily limit for adult patients, may be required and safe.

**Pharmacokinetic interaction studies**

Numerous products have been shown to influence bile flow and composition (Okolicsanyi et al. 1986). The relevant interactions for cholic acid are expected to be those that are also relevant for the other bile acids that are used as medicinal products, chenodeoxycholic acid and ursodeoxycholic acid.

Colesevelam hydrochloride, colestipol hydrochloride and cholestyramine sequester bile acids (Okolicsanyi et al. 1986; Dr. Falk Pharma UK Ltd 2005; sigma-tau Arzneimittel GmbH 2008). Simultaneous administration of medicinal products containing bile acid sequestrants and cholic acid is expected to reduce the efficacy of both. Antacids containing aluminium hydroxide or aluminium oxide bind bile acids equally (Dr. Falk Pharma UK Ltd 2005; sigma-tau Arzneimittel GmbH 2008). It is therefore recommended to separate the administration of bile acid sequestrants or aluminium-containing antacids and cholic acid by at least five hours to avoid an interaction in the gastrointestinal tract.

Non-clinical data indicate that ciclosporin may alter the pharmacokinetics of cholic acid by blocking its uptake into hepatocytes and thus increasing its serum concentration. This interaction is also included in the product information of ursodeoxycholic acid (Dr Falk Pharma UK Ltd 2005). As a decreased uptake of cholic acid into hepatocytes will decrease its efficacy, co-administration with oral or intravenous ciclosporin should be avoided. If administration of ciclosporin is considered necessary, serum and urine bile acid levels should be closely monitored and the cholic acid dose adjusted accordingly.

Data in primates and humans indicate that phenobarbital administration leads to an increase in the pool size and turnover of cholic acid. It was found that administration of 90 mg or 180 mg per day phenobarbital for 6 months increases cholesterol 7a-hydroxylase activity by 20% (Coyne et al. 1976). This is an antagonistic effect to the desired action of cholic acid in patients and will endanger the established metabolic control. As therapeutic alternatives to phenobarbital are available, its use in patients treated with cholic acid is contraindicated.

The interactions of cholic acid with intestinal and hepatic transporter proteins, as well as with various hepatic cytochromes (principally CYP7A1, but also CYP7B1, CYP27A1 and CYP8B1) have been well characterised. No interactions of cholic acid with typical drug-metabolising cytochromes (as listed in the CPMP "Note for Guidance on the Investigation of Drug Interactions", CPMP/EWP/560/95) have been described in the literature.
3.4.3. Pharmacodynamics

Mechanism of action

No pharmacodynamic studies in patients with 3β-HSD or Δ4-3-oxoR deficiency are described in the literature. In patients with 3β-HSD or Δ4-3-oxoR deficiency, orally administered cholic acid acts to replace the missing endogenous primary bile acids both in terms of their physiological functions and their metabolic regulation.

Primary pharmacology

Bile acids have a multitude of functions as shown in Table 1 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 1 Physiological functions of bile acids

<table>
<thead>
<tr>
<th>A. In the liver</th>
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<tbody>
<tr>
<td>1. Generate bile flow through upregulation of bile acid transporters</td>
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<td>2. Induce biliary lipid secretion</td>
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<tr>
<td>3. Modulate cholesterol homeostasis through regulation of synthesis and catabolism</td>
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<tr>
<td>4. Regulation of lipoprotein production</td>
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<tr>
<td>B. In bile</td>
</tr>
<tr>
<td>1. Desaturate bile cholesterol – reduce lithogenic potential</td>
</tr>
<tr>
<td>2. Transport cholesterol</td>
</tr>
<tr>
<td>3. Buffer Ca²⁺ ion</td>
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<tr>
<td>C. In the intestine</td>
</tr>
<tr>
<td>1. Form micelles (fat digestion)</td>
</tr>
<tr>
<td>2. Accelerate lipid transport, including fat-soluble vitamin (A, D, E) absorption</td>
</tr>
<tr>
<td>3. Modulate motility</td>
</tr>
<tr>
<td>4. Modulate GI hormone output</td>
</tr>
<tr>
<td>5. Induce ion (and water) secretion</td>
</tr>
</tbody>
</table>

The effects of oral cholic acid 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 investigated subjects had an overtly normal bile acid metabolism. The administered dose was 15 mg/kg per day in two of the studies and 4.7-11.6 mg/day in another, while it was 750 mg/day in the fourth, which is a comparable dose. In all studies, cholic acid administration shifted the relative composition of bile or intestinal luminal content in the direction of cholic acid 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). Given the available data on the regulation of primary bile acid synthesis, it is apparent that the reduction in chenodeoxycholic acid and its metabolites is due to a repression of primary bile acid synthesis by the administered cholic acid.

In contrast to the changes in bile composition observed in the above studies, cholic acid treatment of patients with primary biliary cirrhosis did not alter the relative concentrations of bile acids in serum (Güldütuna et al. 1993). Absolute bile acid concentrations were however increased to more than two-fold normal level. This is in line with the strong increase of primary bile acid pool size upon oral administration of cholic acid or chenodeoxycholic acid to healthy volunteers (Woollett et al. 2004).

In the case of children affected by primary bile acid synthesis deficiency, the rationale for therapeutic approach is that oral administration of cholic acid, the substance missing in an affected patient, will inhibit endogenous production of hepatotoxic bile acids precursors produced as a consequence of the inborn error by down-regulating cholesterol 7α-hydroxylase, the rate limiting enzyme in bile acid synthesis (Setchell and O'Connell 2007). In addition, cholic acid will provide a stimulus for bile flow.
facilitating the hepatic clearance of toxic bile acid precursors and toxic substances, including bilirubin. Furthermore, cholic acid therapy will improve growth by facilitating fat-soluble vitamins and fat absorption.

**Secondary pharmacology**

The aim of a cholic acid therapy is to restore a normal bile acid physiology. Once a stable enterohepatic circulation of bile acids is established, patients with $3\beta$-HSD deficiency or $\Delta^4$-3-oxoR deficiency treated with cholic acid do not differ physiologically from normal healthy subjects in a significant way. The only difference is that the amount of primary bile acids that is endogenously synthesized in hepatocytes of normal healthy subjects to continuously replenish the bile acid pool is replaced by orally administered cholic acid, i.e. one of the two physiological primary bile acids.

No human or animal studies were identified describing secondary pharmacological effects of cholic acid. No secondary pharmacological effects in the classical sense are expected for this endogenous substance, but there may be important functions of bile acids next to their role in the absorption of nutrients in the intestine and the autoregulation of their biosynthesis from cholesterol that are the primary pharmacodynamic action of cholic acid in the treatment of inborn errors of primary bile acid synthesis due to $3\beta$-HSD or $\Delta^4$-3-oxoR deficiency. Safety pharmacology studies demonstrated effects on cardiac function only at 100 to 1000-fold the endogenous cholic acid concentration. Repeated-dose toxicology studies do not provide evidence for pharmacologic or toxic action of cholic acid outside the organs of the enterohepatic circulation. The absence of studies investigating secondary pharmacological effects of cholic acid is therefore considered acceptable.

3.4.4. Discussion and conclusions on clinical pharmacology

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 with Orphacol could have been useful. However, as cholic acid has been used for many years as a therapeutic agent this is not seen as necessary. Its pharmacokinetic characteristics, including its metabolism, are different from that of a conventional synthetic small molecule pharmaceutical in that once administered, any exogenously administered cholic acid will behave like an endogenous molecule in all respects. Therefore data on the kinetics and dynamics of endogenous cholic acid provides relevant information on the clinical pharmacology of Orphacol. In patients with $3\beta$-HSD deficiency or $\Delta^4$-3-oxoR deficiency, orally administered cholic acid acts to replace the missing endogenous primary bile acids both in terms of their physiological functions and their metabolic regulation.

Cholic acid, a primary bile acid, is partially absorbed in the ileum. The remaining part is transformed by reduction of the 7α-hydroxy group to deoxycholic acid by intestinal bacteria. Deoxycholic acid is a secondary bile acid. More than 90 % of the primary and secondary bile acids are reabsorbed in the ileum by a specific active transporter and are recycled to the liver by the portal vein; the remainder is excreted in the faeces. A small fraction of bile acids is excreted in urine. No pharmacokinetic study data for Orphacol are available. This is acceptable as cholic acid has been used for many years as a therapeutic agent.

The preparations of cholic acid used in many of the submitted case reports were not Orphacol. However, the Applicant has provided adequate explanation and evidence which show similarity between the formulation used in the past in some publications and the formulation that is intended for marketing.
Cholic acid is the predominant primary bile acid in man. In patients with inborn deficiency of 3β-Hydroxy-Δ5-C27-steroid oxidoreductase and Δ4-3-Oxosteroid-5β-reductase, 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. The dose of cholic acid should be adjusted individually. Limited data are available for patients with minor to severe hepatic impairment related to 3β-HSD or Δ4-3-oxoR deficiency. Patients are expected to present with some degree of hepatic impairment at diagnosis, which improves under cholic acid therapy. The dose of cholic acid should be adjusted individually. No experience exists in patients with hepatic impairment from causes other than 3β-Hydroxy-Δ5-C27-steroid oxidoreductase deficiency or Δ4-3-Oxosteroid-5β-reductase deficiency 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 hypertriglyceridemia are expected to poorly absorb cholic acid in the intestine. The cholic acid dose for patients with familial hypertriglyceridemia will have to be established and adjusted as described, but an elevated dose, notably higher than the 500 mg daily limit for adult patients, may be required and safe.

The effect of food on cholic acid bioavailability has not been studied. Empirically it is recommended to take the product with food. There is a theoretical possibility that administration with food may increase cholic acid bioavailability and improve tolerability.

Phenobarbital antagonises the effect of cholic acid. Use of phenobarbital with cholic acid is therefore contraindicated. Alternative treatments should be used. Ciclosporin alters the pharmacokinetics of cholic acid 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 cholic acid dose adjusted accordingly. Bile acid sequestrants and certain antacids bind bile acids and lead to their elimination. Administration of these medicinal products is expected to reduce the effect of cholic acid. The dose of bile acid sequestrants or antacids must be separated from the dose of cholic acid by an interval of 5 hours, regardless of which medicinal product is administered first.

3.5. Clinical efficacy

The Applicant submitted a total of 38 patient reports involving confirmed or suspected 3β-HSD and 11 involving Δ4-3-oxoR deficiencies. Of the 38 patients with 3β-HSD deficiency, 21 were treated with cholic acid, 8 were treated with other bile acids and 9 did not receive bile acids. Of the 11 patients with Δ4-3-oxoR deficiency, 7 were treated with cholic acid, 3 were treated with other bile acids and 1 patient received no treatment.

3.5.1. Dose response studies

No dose response study has been performed or reported in the literature regarding the use of cholic acid. The following analysis relevant to dosing recommendations was provided.
Bile acid dosing for all patients has been established empirically for each patient based on the normalisation of urine and serum bile acids as measured by FAB-MS, electrospray tandem-MS (LC-MS/MS) and/or GC-MS. Specifically, patients with 3β-HSD deficiency were monitored for the levels of 3β,7α-dihydroxy- and 3β,7α,12α-trihydroxy-5-cholenoic acids and patients with Δ^4-3-oxoR deficiency for the levels of 3-oxo-7α-hydroxy-4-cholenoic and 3-oxo-7α,12α-dihydroxy-4-cholenoic acids as well as of allo bile acids. Dosing information for 13 long-time surviving patients with 3β-HSD deficiency is available (Gonzales et al. 2009). Cholic acid was administered either in combination with ursodeoxycholic acid for 6 months in the context of a therapeutic protocol for 6 patients, or alone as replacement of ursodeoxycholic acid for 7 patients. All 13 patients are now treated with cholic acid alone. One patient (Su) (Table 4) was treated with cholic acid monotherapy, but the duration of treatment is not available (Subramaniam et al. 2010).

Patient Wi (Table 4) was treated with cholic acid, then chenodeoxycholic acid, and subsequently with a combination of cholic acid, chenodeoxycholic acid and ursodeoxycholic acid, but no data about dosing schedule and dosage are available. Patients MU2 and AB were last treated with cholic acid but no information about dosage and duration are available (Table 4).

Combination treatment associating cholic acid and chenodeoxycholic acid was reported to be administered to patient Kob (Table 4), at doses of 120 mg and 400 mg per day respectively, and to 5 patients at a dose of 7 mg/kg per day each (Kobayashi et al. 2000; Subramaniam et al. 2010).

For patient AB (Table 4), combination treatment was given in equal doses of cholic acid and chenodeoxycholic acid. Latest data available for patient AB indicate that she is now under cholic acid monotherapy; however no further details about dosage are provided. For patients described by (Gonzales et al. 2009), dose of ursodeoxycholic acid in mg/kg is not available, and comparison with dose of cholic acid is difficult.

Average weight-based daily doses for cholic acid in monotherapy were 12.9 mg/kg (range: 2.3-18.9 mg/kg) at the initiation of treatment and of 5.5 mg/kg (range: 2.5 – 9.8 mg/kg) after long-term maintenance (Table 2), with the patients increasing in age and weight over the time of treatment.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at start of CA (years)</th>
<th>Calculated weight* (kg)</th>
<th>Abs. Daily Dose (mg)</th>
<th>Weight-based Daily Dose (mg/kg)</th>
<th>Age at latest reporting (years)</th>
<th>Calculated weight* (kg)</th>
<th>Abs. Daily Dose (mg)</th>
<th>Weight-based Daily Dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.9</td>
<td>18</td>
<td>250</td>
<td>13.8</td>
<td>16.8</td>
<td>70</td>
<td>500</td>
<td>7.1</td>
</tr>
<tr>
<td>B</td>
<td>4.3</td>
<td>17</td>
<td>250</td>
<td>14.7</td>
<td>17.3</td>
<td>66</td>
<td>350</td>
<td>5.3</td>
</tr>
<tr>
<td>C</td>
<td>7.8</td>
<td>17</td>
<td>250</td>
<td>15</td>
<td>20.7</td>
<td>66</td>
<td>250</td>
<td>3.8</td>
</tr>
<tr>
<td>D1</td>
<td>4.3</td>
<td>18</td>
<td>250</td>
<td>14</td>
<td>17.2</td>
<td>66</td>
<td>300</td>
<td>4.5</td>
</tr>
<tr>
<td>D2</td>
<td>0.7</td>
<td>8</td>
<td>50</td>
<td>6.4</td>
<td>4.1</td>
<td>18</td>
<td>100</td>
<td>5.6</td>
</tr>
<tr>
<td>E1</td>
<td>5.3</td>
<td>17</td>
<td>250</td>
<td>15</td>
<td>18.1</td>
<td>72</td>
<td>500</td>
<td>6.9</td>
</tr>
<tr>
<td>E2</td>
<td>13.1</td>
<td>44</td>
<td>625</td>
<td>14.2</td>
<td>26</td>
<td>54</td>
<td>500</td>
<td>9.3</td>
</tr>
<tr>
<td>F</td>
<td>2.3</td>
<td>15</td>
<td>250</td>
<td>17</td>
<td>13.1</td>
<td>37</td>
<td>250</td>
<td>6.8</td>
</tr>
<tr>
<td>G1</td>
<td>2.3</td>
<td>13</td>
<td>250</td>
<td>18.9</td>
<td>12.6</td>
<td>43</td>
<td>300</td>
<td>7</td>
</tr>
<tr>
<td>G2</td>
<td>11.7</td>
<td>42</td>
<td>500</td>
<td>11.9</td>
<td>21.9</td>
<td>51</td>
<td>500</td>
<td>9.8</td>
</tr>
<tr>
<td>H</td>
<td>0.3</td>
<td>5</td>
<td>80</td>
<td>14.7</td>
<td>9.8</td>
<td>26</td>
<td>100</td>
<td>3.8</td>
</tr>
<tr>
<td>I1</td>
<td>5.2</td>
<td>20</td>
<td>250</td>
<td>12.5</td>
<td>10.2</td>
<td>37</td>
<td>100</td>
<td>2.7</td>
</tr>
<tr>
<td>I2</td>
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<td>5</td>
<td>50</td>
<td>10</td>
<td>4.6</td>
<td>20</td>
<td>50</td>
<td>2.5</td>
</tr>
<tr>
<td>Kob</td>
<td>23</td>
<td>52</td>
<td>120</td>
<td>2.3</td>
<td>23</td>
<td>52</td>
<td>120</td>
<td>2.3</td>
</tr>
<tr>
<td>Su</td>
<td>11</td>
<td>36</td>
<td>144</td>
<td>4</td>
<td>11</td>
<td>36</td>
<td>288</td>
<td>8</td>
</tr>
</tbody>
</table>

Avg.      | 255                        | 12.3                    |                      |                                 | 280                          | 5.7                     |

CA: cholic acid; * was calculated with available data on absolute and weight-based daily dose.

At the beginning of treatment (Gonzales et al. 2009; Subramaniam et al. 2010) the dose of cholic acid had to be increased once in 6 patients, twice in 1 patient, and several times in 1 patient in order to
achieve optimal response to treatment. The daily dose of cholic acid was increased when the concentrations of atypical metabolites in urine increased, an indication of poor feedback inhibition on cholesterol 7α-hydroxylase. Oppositely, the dose of cholic acid was decreased in 4 patients because of signs of overdose (increases in serum GGT and ALT; pruritus, diarrhoea and increased total serum bile acids in one patient). Good response to treatment without dose adjustment was obtained for 2 patients.

In a study by Riello et al. (2010) patients with 3β-HSD deficiency were treated with a combination of chenodeoxycholic and ursodeoxycholic acid. Data provides valuable insight on dose titration in function of body weight and metabolic response that are equally applicable to cholic acid therapy. Patients were started on 5 mg/kg each of ursodeoxycholic acid and chenodeoxycholic acid (3 patients) or 10 mg/kg each (1 patient) or 7.5 mg/kg each (1 patient). As patients were all infants or children, their rapid growth led to a drop of the relative (weight-based) dose while the absolute dose was constant. In most cases (4/5), this led to increases in urinary 3β-hydroxylated-Δ5 bile acid metabolites and the need to adjust the absolute dose so that the previous weight-based dose was restored. Under the dose of 5/5 mg/kg of ursodeoxycholic acid / chenodeoxycholic acid, the levels of urinary 3β-hydroxylated-Δ5 bile acid metabolites dropped again (data for 3 patients). One patient was temporarily well-controlled on a 3/3 mg/kg dose, but subsequently (after 20 months) needed uptitration to 5/5 mg/kg for metabolic control. This illustrates the need for regular control of metabolic control by urine analysis of 3β-hydroxylated-Δ5 bile acid metabolites. The same titration principle applies in cholic acid monotherapy.

Data on the starting doses in Δ4-3-oxoR deficiency are similar to the 3β-HSD deficiency. Bile acid dosing for all patients has been established empirically based on metabolic response also taking into consideration in this case that most patients additionally received ursodeoxycholic or chenodeoxycholic acid (Table 3).

**Table 3**  Bile acid treatment of patients with Δ4-3-oxoR deficiency – daily doses

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at reporting (years)</th>
<th>Estimated weight* (kg)</th>
<th>Reported BA/ daily dose</th>
<th>Absolute Daily Dose**</th>
<th>Weight-based Daily Dose**</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 (J)</td>
<td>12</td>
<td>40</td>
<td>CA 250 mg</td>
<td>250 mg</td>
<td>6.25 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>UDCA 4 mg/kg</td>
<td>160 mg</td>
<td>4 mg/kg</td>
</tr>
<tr>
<td>15 (J)</td>
<td>12</td>
<td>40</td>
<td>CA 200 mg</td>
<td>200 mg</td>
<td>5 mg/kg</td>
</tr>
<tr>
<td>MS</td>
<td>0.7–10</td>
<td>8.5–35</td>
<td>CA 8 mg/kg</td>
<td>68–280 mg</td>
<td>8 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CDCA 8 mg/kg</td>
<td>68–280 mg</td>
<td>8 mg/kg</td>
</tr>
<tr>
<td>BH</td>
<td>0.5</td>
<td>8</td>
<td>CA 8 mg/kg</td>
<td>64 mg</td>
<td>8 mg/kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CDCA 8 mg/kg</td>
<td>64 mg</td>
<td>8 mg/kg</td>
</tr>
<tr>
<td>2a/SG</td>
<td>0.1–1.5</td>
<td>5–12</td>
<td>CA 100 mg/UDCA 100 mg</td>
<td>100 mg/100 mg</td>
<td>20–8.3 mg/kg/20–8.3 mg/kg</td>
</tr>
<tr>
<td>2b/JG</td>
<td>0.1–1.5</td>
<td>5–12</td>
<td>CA 100 mg/UDCA 100 mg</td>
<td>100 mg/100 mg</td>
<td>20–8.3 mg/kg/20–8.3 mg/kg</td>
</tr>
<tr>
<td>3</td>
<td>0.1–1.5</td>
<td>3.5–12</td>
<td>CA 100 mg/UDCA 100 mg</td>
<td>100 mg/100 mg</td>
<td>28.6–8.3 mg/kg/28.6–8.3 mg/kg</td>
</tr>
</tbody>
</table>

CA: cholic acid, UDCA: ursodeoxycholic acid, CDCA: chenodeoxycholic acid; BA: bile acid. Estimated weight based on standard growth tables. Figures in italics are estimates, calculated from the available data. *Estimated weight was extracted from standard height/growth tables. **Data in italics were calculated with estimated weight and available data, either for absolute daily dose or for weight-based daily dose.

Younger and lighter patients appear to require and tolerate higher doses per body weight than older children. This is in agreement with the observation that infants and children have about 40% larger cholic acid pool sizes than adults on a per m² basis, and the cholic acid turnover rate decreases each by about 40% between infants and children, and between children and adults (Heubi et al. 1982).
Overall, no formal dose response studies have been performed. The posology is based on the previous clinical experience with patients' response to bile acids including cholic acid. The individual dose for each patient was established based on the normalisation of urine and serum bile acids as measured by FAB-MS, LC-MS/MS and/or GC-MS. The lowest dose of cholic acid that leads to an elimination of the metabolites from the urine should be chosen for maintenance. The starting daily cholic acid dose in 3β-HSD deficiency was on average 12.3 mg/kg (see Table 2); similarly, a starting daily dose of 4 mg cholic acid which was increased to 8 mg/kg after one month was reported, as was a starting daily dose of the combination of 7 mg/kg each of cholic acid and chenodeoxycholic acid (Subramaniam et al. 2010). Data on the starting doses in Δ4-3-oxoR deficiency are similar (Table 3), taking into consideration that in addition most patients received ursodeoxycholic or chenodeoxycholic acid. This therapeutic approach, based on the effect of treatment following the use of various dosing regimen, is endorsed and leads to the recommendation that therapy should be initiated with a starting dose of 5-15 mg/kg per day, with regular monitoring thereafter. Urinary bile acid analyses by FAB-MS, LC-MS/MS and/or GC-MS are complemented by serum bile acid and liver function tests to verify the normalisation of liver biology, and to monitor for possible overdosage indicated by elevated serum bile acids and GGT and/or transaminases.

3.5.2. Main Case reports

Overview of cases reports for 3β-HSD deficiency

The cases reports investigating the efficacy of bile acid therapy, including cholic acid therapy, in the treatment of 3β-HSD deficiency originate principally from the Jacquemin Group at Bicêtre Hospital, Le-Kremlin Bicêtre, France (Jacquemin et al. 1994; Jacquemin et al. 2000; Potin et al. 2001; Gonzales 2006; Gonzales et al. 2009) and the Setchell group at Cincinnati Children's Hospital Medical Center, Cincinatti, USA (Setchell et al. 1990; Balistreri 1991; Setchell et al. 1991; Witzleben et al. 1992; Balistreri 1999; Cheng et al. 2003; Bove et al. 2004; Setchell 2004; Setchell and Heubi 2006; Heubi et al. 2007), with additional reports from the United Kingdom (Clayton et al. 1987; Clayton 1991; Horslen et al. 1992; Akobeng et al. 1999), Sweden (Ichimiya et al. 1990; Ichimiya et al. 1991; Fischler et al. 2007), the Netherlands (Vanderpas et al. 1987) and Japan (Kobayashi et al. 2000; Yamato et al. 2001). These studies provide data on a group of 38 confirmed and suspected patients, 21 of which have been treated with cholic acid.

The large majority of the patients for which detailed data on cholic acid treatment are available come from the population studied at Bicêtre Hospital. The remainder of the European patient population comes from centres in the United Kingdom and Sweden. Most patients described in the literature have undergone treatment with several different bile acids and/or bile acid combinations during the establishment of their diagnosis and over the history of the establishment of cholic acid as the treatment of choice (Kobayashi et al. 2000; Gonzales et al. 2009). The identification of cholic acid as the treatment of choice was made empirically, taking into account the knowledge of bile acid physiology and toxicology and the availability of preparations of different bile acids.

Demographics of patient with 3β-HSD deficiency

The exact number of 3β-HSD deficiency patients described in the literature is difficult to determine. This number can be estimated to be 43 patients, including the 38 patients described in Table 4, one patient described in Bove et al. 2004 and four patients described in Cheng et al. 2003. Out of the 38 patients, 21 were treated with cholic acid, 8 were treated with other bile acids, and 9 were not treated with bile acids.
Table 4 Demographic features of reported cases of 3β-HSD deficiency

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at onset (months)</th>
<th>Sex</th>
<th>Origin</th>
<th>Mol. Defect</th>
<th>Age at initiation of CA</th>
<th>Age at death</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (A)</td>
<td>4</td>
<td>M</td>
<td>Portugal</td>
<td>Exon 6 c.1024delT p.F342SfsX74</td>
<td>4.4</td>
<td>--</td>
<td>(Jacquemin et al. 1994; Cheng et al. 2003; Gonzales 2006; Gonzales et al. 2009)</td>
</tr>
<tr>
<td>2 (B)</td>
<td>1</td>
<td>F</td>
<td>Chile</td>
<td>Exon 2 c.292insC p.R98PfsX5</td>
<td>4.9</td>
<td>--</td>
<td>(Jacquemin et al. 1994; Cheng et al. 2003; Gonzales 2006; Gonzales et al. 2009)</td>
</tr>
<tr>
<td>3 (C)</td>
<td>47</td>
<td>F</td>
<td>Portugal</td>
<td>Exon 1 c.16C&gt;T p.Q6X</td>
<td>8.3</td>
<td>--</td>
<td>(Jacquemin et al. 1994; Cheng et al. 2003; Gonzales 2006; Gonzales et al. 2009)</td>
</tr>
<tr>
<td>4 (D1)</td>
<td>6</td>
<td>F</td>
<td>Italy</td>
<td>Exon 2 c.292insC p.R98PfsX5</td>
<td>4.8</td>
<td>--</td>
<td>(Jacquemin et al. 1994; Cheng et al. 2003; Gonzales 2006; Gonzales et al. 2009)</td>
</tr>
<tr>
<td>5 (D2)</td>
<td>6</td>
<td>F</td>
<td>Italy</td>
<td>Exon 2 c.292insC p.R98PfsX5</td>
<td>0.7</td>
<td>--</td>
<td>(Jacquemin et al. 1994; Gonzales 2006; Gonzales et al. 2009)</td>
</tr>
<tr>
<td>6 (E1)</td>
<td>11</td>
<td>M</td>
<td>France</td>
<td>Splice junction Intron 5 c.695-1G&gt;A and</td>
<td>5.8</td>
<td>--</td>
<td>(Jacquemin et al. 1994; Cheng et al. 2003; Gonzales 2006; Gonzales et al. 2009)</td>
</tr>
<tr>
<td>7 (E2)</td>
<td>9</td>
<td>F</td>
<td>France</td>
<td>Splice junction Intron 2 c.322+1G&gt;T Exon 4 c.439G&gt;A p.E147K</td>
<td>13.6</td>
<td>--</td>
<td>(Jacquemin et al. 1994; Cheng et al. 2003; Gonzales 2006; Gonzales et al. 2009)</td>
</tr>
<tr>
<td>8 (F)</td>
<td>21</td>
<td>M</td>
<td>France (Roma)</td>
<td>Exon 4 c.439G&gt;A p.E147K</td>
<td>2.3</td>
<td>--</td>
<td>(Cheng et al. 2003; Gonzales 2006; Gonzales et al. 2009)</td>
</tr>
<tr>
<td>9 (G1)</td>
<td>24</td>
<td>F</td>
<td>France (Roma)</td>
<td>Exon 4 c.439G&gt;A p.E147K</td>
<td>2.3</td>
<td>--</td>
<td>(Cheng et al. 2003; Gonzales 2006; Gonzales et al. 2009)</td>
</tr>
<tr>
<td>10 (G2)</td>
<td>29</td>
<td>F</td>
<td>France (Roma)</td>
<td>Exon 4 c.439G&gt;A p.E147K</td>
<td>11.7</td>
<td>--</td>
<td>(Cheng et al. 2003; Gonzales 2006; Gonzales et al. 2009)</td>
</tr>
<tr>
<td>11 (H)</td>
<td>2</td>
<td>F</td>
<td>France</td>
<td>Exon 1 c.114insCC p.L39PfsX15 and Exon 1 c.73delGTG p.delV25</td>
<td>1.3</td>
<td>--</td>
<td>(Cheng et al. 2003; Gonzales 2006; Gonzales et al. 2009)</td>
</tr>
<tr>
<td>12 (I1)</td>
<td>6</td>
<td>M</td>
<td>France-Senegal</td>
<td>Exon 1 c.45-46delAG p.T15TfsX27 and Intron 3 c.432-2delA</td>
<td>5.2</td>
<td>--</td>
<td>(Gonzales 2006; Gonzales et al. 2009)</td>
</tr>
<tr>
<td>13 (I2)</td>
<td>1</td>
<td>F</td>
<td>France-Senegal-Algeria</td>
<td>–</td>
<td>0.3</td>
<td>--</td>
<td>(Gonzales 2006; Gonzales et al. 2009)</td>
</tr>
<tr>
<td>MU2 (Sib of JU/MU1) b</td>
<td>3</td>
<td>M</td>
<td>Saudi Arabian</td>
<td>1057, ΔCT</td>
<td>n.a.</td>
<td>--</td>
<td>(Clayton 1991; Kobayashi et al. 2000)</td>
</tr>
<tr>
<td>AB Kob</td>
<td>3</td>
<td>F</td>
<td>Caucasian</td>
<td>–</td>
<td>0.7</td>
<td>--</td>
<td>(Clayton et al. 2003; Witzleben et al. 1992; Bove et al. 2004)</td>
</tr>
<tr>
<td>Wi</td>
<td>n.a.</td>
<td>M</td>
<td>n.a.</td>
<td>–</td>
<td>10.5</td>
<td>--</td>
<td>(Gonzales et al. 2009)</td>
</tr>
<tr>
<td>Jc a</td>
<td>n.a.</td>
<td>M</td>
<td>n.a.</td>
<td>1057, ΔCT</td>
<td>n.a.</td>
<td>--</td>
<td>(Gonzales et al. 2009)</td>
</tr>
<tr>
<td>Patient</td>
<td>Age at onset (months)</td>
<td>Sex</td>
<td>Origin</td>
<td>Mol. Defect</td>
<td>Age at initiation of CA</td>
<td>Age at death</td>
<td>References</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------</td>
<td>-----</td>
<td>--------</td>
<td>-------------</td>
<td>------------------------</td>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>Kc a</td>
<td>50.4 (4.2 years)</td>
<td>M</td>
<td>Diagnosed at Cincinnati</td>
<td>60, ΔAG</td>
<td>n.a.</td>
<td>--</td>
<td>(Cheng et al. 2003)</td>
</tr>
<tr>
<td>Lc a</td>
<td>8</td>
<td>M</td>
<td>Diagnosed at Cincinnati</td>
<td>60, ΔAG</td>
<td>n.a.</td>
<td>Transplanted</td>
<td>(Cheng et al. 2003)</td>
</tr>
<tr>
<td>Y</td>
<td>2</td>
<td>M</td>
<td>Diagnosis at Cincinnati Japan</td>
<td>UDCA + CDCA</td>
<td>n.a.</td>
<td>--</td>
<td>(Yamato et al. 2001)</td>
</tr>
<tr>
<td>Mc</td>
<td>162 (13.5 years)</td>
<td>F</td>
<td>Diagnosed at Cincinnati</td>
<td>G19S, 140, ΔTC</td>
<td>UDCA</td>
<td>Transplanted</td>
<td>(Cheng et al. 2003)</td>
</tr>
<tr>
<td>Fi</td>
<td>3 weeks</td>
<td>M</td>
<td>Sweden</td>
<td>UDCA</td>
<td>S162R</td>
<td>UDCA</td>
<td>Survived until age 26, then treated</td>
</tr>
<tr>
<td>FF</td>
<td>2</td>
<td>M</td>
<td>Arabic</td>
<td>UDCA</td>
<td>--</td>
<td>--</td>
<td>(Clayton 1991; Horslen et al. 1992)</td>
</tr>
<tr>
<td>Ak1</td>
<td>9</td>
<td>F</td>
<td>Asia</td>
<td>UDCA</td>
<td>--</td>
<td>--</td>
<td>(Akobeng et al. 1999)</td>
</tr>
<tr>
<td>Ak2</td>
<td>n.a.</td>
<td>M</td>
<td>Jordan</td>
<td>UDCA</td>
<td>--</td>
<td>--</td>
<td>(Akobeng et al. 1999)</td>
</tr>
<tr>
<td>Va</td>
<td>13</td>
<td>M</td>
<td>Jewish-Moroccan</td>
<td>UDCA</td>
<td>--</td>
<td>--</td>
<td>(Vanderpas et al. 1987)</td>
</tr>
<tr>
<td>Se</td>
<td>120 (10 years)</td>
<td>M</td>
<td>n.a.</td>
<td>UDCA</td>
<td>--</td>
<td>--</td>
<td>(Setchell et al. 1990; Setchell et al. 1991)</td>
</tr>
<tr>
<td>A1</td>
<td>2.5</td>
<td>M</td>
<td>Italy</td>
<td>UDCA, then UDCA+ UDCA+ CDCA+ CDCA</td>
<td>--</td>
<td>--</td>
<td>(Riello et al. 2010)</td>
</tr>
<tr>
<td>A2</td>
<td>Birth b</td>
<td>M</td>
<td>Italy</td>
<td>UDCA+ CDCA</td>
<td>--</td>
<td>--</td>
<td>(Riello et al. 2010)</td>
</tr>
<tr>
<td>B3</td>
<td>Birth b</td>
<td>F</td>
<td>Italy (Moroccan)</td>
<td>UDCA+ CDCA</td>
<td>--</td>
<td>--</td>
<td>(Riello et al. 2010)</td>
</tr>
<tr>
<td>B4</td>
<td>Infancy (not specified)</td>
<td>M</td>
<td>Italy (Moroccan)</td>
<td>UDCA+ CDCA</td>
<td>--</td>
<td>--</td>
<td>(Riello et al. 2010)</td>
</tr>
<tr>
<td>B5</td>
<td>First year of life (not specified)</td>
<td>F</td>
<td>Italy (Moroccan)</td>
<td>UDCA+ CDCA</td>
<td>--</td>
<td>--</td>
<td>(Riello et al. 2010)</td>
</tr>
<tr>
<td>JU Sib of MU1/MU2</td>
<td>n.a.</td>
<td>F</td>
<td>Saudi Arabian</td>
<td>n.a.</td>
<td>--</td>
<td>19 months</td>
<td>(Clayton et al. 1987)</td>
</tr>
<tr>
<td>MU1 Sib of JU/MU2</td>
<td>1.5</td>
<td>M</td>
<td>Saudi Arabian</td>
<td>n.a.</td>
<td>--</td>
<td>3 years 9 months</td>
<td>(Clayton et al. 1987; Cheng et al. 2003)</td>
</tr>
<tr>
<td>Sib A</td>
<td>24</td>
<td>M</td>
<td>Portugal</td>
<td>n.a.</td>
<td>--</td>
<td>3 years (liver failure)</td>
<td>(Gonzales 2006)</td>
</tr>
<tr>
<td>Sib C</td>
<td>n.a.</td>
<td>F</td>
<td>Portugal</td>
<td>n.a.</td>
<td>--</td>
<td>6 years (liver failure)</td>
<td>(Gonzales 2006)</td>
</tr>
<tr>
<td>D0/Sib D1&amp;D2</td>
<td>n.a.</td>
<td>F</td>
<td>Italy</td>
<td>n.a.</td>
<td>--</td>
<td>3 y 11 mo (fibrogenic cholestasis; liver biopsy at 2.5 y: very active micronodular cirrhosis with hepatic cholestasis)</td>
<td>(Gonzales 2006)</td>
</tr>
<tr>
<td>E3/Sib E1&amp;E2</td>
<td>n.a.</td>
<td>F</td>
<td>France</td>
<td>n.a.</td>
<td>--</td>
<td>n.r.</td>
<td>(Gonzales 2006)</td>
</tr>
<tr>
<td>E4/Sib E1&amp;E2</td>
<td>n.a.</td>
<td>M</td>
<td>France</td>
<td>n.a.</td>
<td>--</td>
<td>n.r.</td>
<td>(Gonzales 2006)</td>
</tr>
<tr>
<td>Sib 1 of Fi</td>
<td>36</td>
<td>F</td>
<td>Sweden</td>
<td>n.a.</td>
<td>--</td>
<td>11 years, liver disease</td>
<td>(Fischler et al. 2007)</td>
</tr>
<tr>
<td>Sib 2 of Fi</td>
<td>n.a.</td>
<td>M</td>
<td>Sweden</td>
<td>n.a.</td>
<td>--</td>
<td>6 months</td>
<td>(Fischler et al. 2007)</td>
</tr>
</tbody>
</table>

CA: cholic acid; n.a.: not available; n.r.: not reported; Sib: sibling; *Patients only described in (Cheng et al. 2003) *Identified as MU1 with favourable outcome in (Cheng et al. 2003), but must be MU2 as MU1 died at 3 years, 9 months without treatment (Clayton et al. 1987).
This population represents the entire population reported in the literature. It thus represents the known spectrum of disease manifestations. Generally, patients presented in infancy or early childhood with jaundice, cholestasis, steatorrhea and hepatomegaly together with symptoms of fat-soluble vitamin deficiency such as rickets (vitamin D) or bleeding (vitamin K). Bilirubin and transaminases were several-fold above the normal range, while GGT was normal. Urinary bile acid excretion was strongly increased and consisted of (sulphated) \(3\beta,7\alpha\)-dihydroxy-5-cholenoic acid and \(3\beta,7\alpha,12\alpha\)-trihydroxy-5-cholenoic acid while the primary bile acids at low or undetectable levels. In liver histology, hepatocellular and canalicular cholestasis with giant-cell transformation, fibrosis and inflammation was reported in the majority of the patients. It should be noted that the patients on the low end of the ranges or with milder disease symptoms were frequently younger siblings. The baseline characteristics of the patients are summarised in Table 5.

**Table 5 Clinical and biochemical features of reported cases of \(3\beta\)-HSD deficiency before initiation of any bile acid treatment**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number of patients evaluated</th>
<th>Value [Range] / Number of patients</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at investigation</strong></td>
<td>28</td>
<td>4.0 [0.1-26] years</td>
<td>-</td>
</tr>
<tr>
<td><strong>Jaundice</strong></td>
<td>30</td>
<td>21</td>
<td>-</td>
</tr>
<tr>
<td><strong>Pruritus</strong></td>
<td>27</td>
<td>absent (15); n.r. (12)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Steatorrhea</strong></td>
<td>30</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td><strong>Hepatomegaly</strong></td>
<td>30</td>
<td>19</td>
<td>-</td>
</tr>
<tr>
<td><strong>Hepatosplenomegaly</strong></td>
<td>30</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td><strong>Vitamin A deficiency</strong></td>
<td>25</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td><strong>Rickets</strong></td>
<td>27</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td><strong>Vitamin D deficiency</strong></td>
<td>25</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td><strong>Vitamin E deficiency</strong></td>
<td>25</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td><strong>Vitamin K deficiency</strong></td>
<td>25</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td><strong>Bilirubin (total)</strong></td>
<td>19</td>
<td>73 [6-254] µmol/L</td>
<td>5.1–17.0 µmol/L</td>
</tr>
<tr>
<td><strong>Bilirubin (conjugated)</strong></td>
<td>16</td>
<td>57.6 [2-214] µmol/L</td>
<td>1.0–5.1 µmol/L</td>
</tr>
<tr>
<td><strong>ALT</strong></td>
<td>18</td>
<td>211.7 [16-1412] IU/L</td>
<td>7-56 IU/L</td>
</tr>
<tr>
<td><strong>AST</strong></td>
<td>18</td>
<td>222.4 [25-1469] IU/L</td>
<td>5-35 IU/L</td>
</tr>
<tr>
<td><strong>GGT</strong></td>
<td>15</td>
<td>24.9 [10-56] IU/L</td>
<td>8-78 IU/L</td>
</tr>
<tr>
<td><strong>Urinary Bile Acids</strong></td>
<td>28</td>
<td>Low or undetectable CA/CDCA</td>
<td>–</td>
</tr>
</tbody>
</table>

CA: cholic acid; CDCA: chenodeoxycholic acid; ALT: alanine transaminase; AST: aspartate transaminase; GGT: \(\gamma\)-glutamyltransferase; n.r.: not reported a number of patients where disease manifestation was reported; other patients had no manifestations or no data were reported

While this patient population represents all patients who are reported in the literature, it was questioned whether this could be considered representative of the true patient population in the European Union due to reporting bias and the strong representation of the Bicêtre Hospital population. Given the extreme rarity of the condition and the sophisticated technology needed for the diagnosis, it may not be properly diagnosed in all countries, from which then publications are missing. In addition it cannot be excluded that there are patients with milder clinical deficiencies who may survive without therapy (4 patients with \(3\beta\)-HSD deficiency were asymptomatic at the time of diagnosis through family screening - Riello et al. 2010; Subramaniam et al. 2010), similar to the late-detected cases reported by Kobayashi et al. 2000 and Fischler et al. 2007. These patients may not be detected or reported in the literature but could still benefit from cholic acid treatment to avoid long-term sub-clinical liver damage (Subramaniam et al. 2010).

The number of patients in the EU affected by \(3\beta\)-HSD deficiency and \(\Delta^4\)-3-oxoR deficiency is currently estimated to be a total 90 patients (approximately 75 patients with \(3\beta\)-HSD deficiency and approximately 15 patients with \(\Delta^4\)-3-oxoR deficiency) based on the observed incidence over the last 15 years in France and the United Kingdom (Gonzales et al. 2009; Subramaniam et al. 2010). Considering incidence, at Bicêtre Hospital in France, approximately 1 patient per year with \(3\beta\)-HSD deficiency (13
patients) or Δ4-3-oxoR deficiency (2 patients, twins) has been diagnosed over the last 15 years; 4 of these patients came from Member States other than France (i.e. Italy and Portugal). A very similar rate of diagnosis of 16 patients with 3β-HSD deficiency of domestic origin in 16 years has been reported from the United Kingdom by Subramaniam et al. 2010. The population treated at the reporting centres is not limited to the local population, but extends to other European and non-European countries. The Bicêtre Hospital population includes patients from France (including of Roma origin), Italy, Portugal, Algeria and one adopted child from Chile, while the UK population includes predominantly Middle Eastern and Asian patients. In addition, the dose range used in the reported treated patient population (Table 2) together with the age range of onset (Table 4) described indicate a wide range of severities of the enzymatic defect.

Therefore, within the limitations of the small sample size, the diversity of the population described combined with the wide spectrum of disease severity (showed by the range of dose used) 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.

**Treatments**

From the literature patients were treated with combinations of chenodeoxycholic acid, ursodeoxycholic acid and cholic acid. Treatment modalities were different for all patients due to the fact that cholic acid treatment was frequently unavailable for a certain period of time and that treatment algorithms were only established based on these patients and their individual responses. At the time of last reporting 19 patients were today under treatment with cholic acid alone, 2 patients were last reported to be treated with cholic acid in combination with chenodeoxycholic acid or with chenodeoxycholic acid and ursodeoxycholic acid, and 8 patients were under monotherapy with chenodeoxycholic acid or ursodeoxycholic acid alone (Table 6).
## Table 6: Bile Acid Treatment in Patients with 3β-HSD Deficiency

<table>
<thead>
<tr>
<th>Patient</th>
<th>BA/ daily dose</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3 (final)</th>
<th>Duration and status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (A)</td>
<td>UDCA 600 mg/m²</td>
<td>3 y 7 mo</td>
<td>UDCA 600 mg/m² + CA 13.8 mg/kg</td>
<td>CA 7.1 mg/kg (500 mg)</td>
<td>12.9, ongoing</td>
</tr>
<tr>
<td>2 (B)</td>
<td>UDCA 600 mg/m²</td>
<td>9 months</td>
<td>UDCA 600 mg/m² + CA 14.7 mg/kg</td>
<td>CA 5.3 mg/kg (350 mg)</td>
<td>12.9, ongoing</td>
</tr>
<tr>
<td>3 (C)</td>
<td>UDCA 600 mg/m²</td>
<td>3 y 7 mo</td>
<td>UDCA 600 mg/m² + CA 15 mg/kg</td>
<td>CA 3.8 mg/kg (250 mg)</td>
<td>12.9, ongoing</td>
</tr>
<tr>
<td>4 (D1)</td>
<td>UDCA 600 mg/m²</td>
<td>1 y 3 mo</td>
<td>UDCA 600 mg/m² + CA 14 mg/kg</td>
<td>CA 4.5 mg/kg (300 mg)</td>
<td>12.9, ongoing</td>
</tr>
<tr>
<td>5 (D2)</td>
<td>UDCA 600 mg/m²</td>
<td>2 months</td>
<td>CA 56.4 mg/kg</td>
<td>n.r.</td>
<td>CA 6 mg/kg (100 mg)</td>
</tr>
<tr>
<td>6 (E1)</td>
<td>UDCA 600 mg/m²</td>
<td>16 months</td>
<td>UDCA 600 mg/m² + CA 15 mg/kg</td>
<td>CA 6.9 mg/kg (500 mg)</td>
<td>12.8, ongoing</td>
</tr>
<tr>
<td>7 (E2)</td>
<td>UDCA 600 mg/m²</td>
<td>2 y 9 mo</td>
<td>UDCA 600 mg/m² + CA 14.2 mg/kg</td>
<td>CA 9.3 mg/kg (500 mg)</td>
<td>12.9, ongoing</td>
</tr>
<tr>
<td>8 (F)</td>
<td>UDCA 600 mg/m²</td>
<td>2 months</td>
<td>CA 17 mg/kg</td>
<td>n.r.</td>
<td>CA 6.8 mg/kg (250 mg)</td>
</tr>
<tr>
<td>9 (G1)</td>
<td>UDCA 600 mg/m²</td>
<td>2 months</td>
<td>CA 18.9 mg/kg</td>
<td>n.r.</td>
<td>CA 7 mg/kg (300 mg)</td>
</tr>
<tr>
<td>10 (G2)</td>
<td>UDCA 600 mg/m²</td>
<td>1 month</td>
<td>CA 11.9 mg/kg</td>
<td>n.r.</td>
<td>CA 9.8 mg/kg (500 mg)</td>
</tr>
<tr>
<td>11 (H) a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>CA 3.8 mg/kg (100 mg)</td>
</tr>
<tr>
<td>12 (I1)</td>
<td>UDCA 600 mg/m²</td>
<td>5 months</td>
<td>CA 12.5 mg/kg</td>
<td>n.r.</td>
<td>CA 2.7 mg/kg (100 mg)</td>
</tr>
<tr>
<td>13 (I2)</td>
<td>UDCA 600 mg/m²</td>
<td>1 month</td>
<td>CA 10 mg/kg</td>
<td>n.r.</td>
<td>CA 2.5 mg/kg (50 mg)</td>
</tr>
<tr>
<td>Patient</td>
<td>Treatment 1</td>
<td>Treatment 2</td>
<td>Treatment 3 (final)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
<td>-------------</td>
<td>---------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BA/ daily dose</td>
<td>duration</td>
<td>BA/ daily dose</td>
<td>duration</td>
<td>BA/ daily dose</td>
</tr>
<tr>
<td>J</td>
<td>UDCA 600 mg/m²</td>
<td>1 month</td>
<td>CA 5 mg/kg</td>
<td>6 months</td>
<td>CA / n.r.</td>
</tr>
<tr>
<td>MU2</td>
<td>CDCA 250 mg (18 mg/kg)</td>
<td>2 months</td>
<td>CDCA 125 mg (9 mg/kg)</td>
<td>6.9 years</td>
<td>CA / n.r.</td>
</tr>
<tr>
<td>AB</td>
<td>CDCA (15 mg/kg)</td>
<td>n.r.</td>
<td>CDCA 7 mg/kg + CA 7 mg/kg</td>
<td>&gt;1 year but n.r.</td>
<td>CA/n.r.</td>
</tr>
<tr>
<td>Jc</td>
<td>CA</td>
<td>n.r., likely ongoing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kc</td>
<td>CA</td>
<td>n.r., likely ongoing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lc</td>
<td>CA</td>
<td>n.r., transpl.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kob</td>
<td>UDCA 150 mg</td>
<td>4 mo</td>
<td>CDCA 400 mg</td>
<td>4 mo</td>
<td>CA 120 mg (2.3 mg/kg) then CA 120 mg + CDCA 400 mg</td>
</tr>
<tr>
<td>Wi</td>
<td>CA / n.r</td>
<td>n.r.</td>
<td>CDCA / n.r.</td>
<td>n.r.</td>
<td>CA, CDCA, UDCA / n.r.</td>
</tr>
<tr>
<td>Yb</td>
<td>UDCA 50 mg</td>
<td>2 mo</td>
<td>UDCA50 mg</td>
<td>8 mo</td>
<td>CDCA 8.3 mg/kg</td>
</tr>
<tr>
<td>Mc</td>
<td>UDCA</td>
<td>n.r., transpl.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fi</td>
<td>UDCA / n.r.</td>
<td>≥ 2 mo</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>FF</td>
<td>CDCA 125 mg (12 mg/kg)</td>
<td>n.r.</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ak1</td>
<td>CDCA 12 mg/kg</td>
<td>n.r.</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ak2</td>
<td>CDCA 9 mg/kg</td>
<td>n.r.</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Va</td>
<td>CDCA 750 mg</td>
<td>n.r.</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Se</td>
<td>CDCA 250 mg</td>
<td>≥ 2 months</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

CA: cholic acid; CDCA: chenodeoxycholic acid; UDCA: ursodeoxycholic acid; n.r.: not reported. * UDCA for 2 months then stopped; CA therapy [80 mg per day (14.7 mg/kg per day) ] was started at the age of 4 months. Bile acid therapy was then stopped for 1 month. UDCA therapy (600 mg/m²) was then reintroduced. CA therapy [50 mg per day (5.7 mg/kg per day)] in combination with UDCA (5.7 mg/kg per day) was resumed 4 months later (at the age of 11 months). UDCA was then stopped after 6 months of dual-agent therapy. CA dosage was increased to 60 then 70 mg per day (6 mg/kg per day) at the ages of 16 and 23 months respectively. CA dosage was again increased at the age of 5 years to 100 mg per day. in association with prednisolone 6 mg and vitamins A, E, K complementation; after a 6-months interruption.

Setchell 2004, who had at the time of publication about 20 years of experience in using the treatment with cholic acid, stresses the fact that monotherapy with cholic acid is now the strategy of choice for the treatment of 3β-HSD deficiency. The recent article of Gonzales et al. 2009 states that, at the present time, all new patients diagnosed with 3β-HSD deficiency are directly treated with cholic acid monotherapy and that all followed-up patients are treated with cholic acid alone. A similar statement is also provided by Setchell (Setchell 2004; Setchell and Heubi 2006) who add that the efficacy of cholic acid is greatest when cholic acid is given alone.

Overall, although patients were treated on different combinations, it appears that patients previously treated with ursodeoxycholic acid were transferred to cholic acid and newly diagnosed patients were...
commenced on cholic acid. Cholic acid monotherapy appears as the current treatment of choice for patients with 3β-HSD deficiency.

**Method of assessing efficacy for the 3β-HSD and Δ^4^-3-oxoR deficiencies**

Most patients described in the literature have undergone treatment with several different bile acids and/or bile acid combinations during the establishment of their diagnosis and over the history of the establishment of cholic acid as the treatment of choice (Kobayashi et al. 2000; Gonzales et al. 2009). The identification of cholic acid as the treatment of choice is made empirically, taking into account the knowledge of bile acid physiology and toxicology, and based on the availability of bile acids preparations. Therefore, these other active treatments represent internal controls. From a methodological point of view, many of the case reports can essentially be evaluated as open-label, non-randomised n-of-1 studies. Additionally, several cases of siblings that died early in infancy when diagnosis and treatment were not available are reported in the literature, and the symptoms of these siblings were highly suggestive of 3β-HSD or Δ^4^-3-oxoR deficiency (Clayton et al. 1987; Clayton et al. 1988; Fischler et al. 2007; Gonzales et al. 2009). The untreated siblings represent a historic control group.

A limitation of the unplanned, unsystematic and incomplete retrospective collection and documentation of treatment data in the literature is that a statistically rigorous meta-analysis of the results is impossible. However, few individual publications evaluate statistically the data of groups of patients in descriptive terms (Potin et al. 2001; Setchell 2004; Gonzales et al. 2009). The published data on the efficacy of cholic acid in the treatment of both deficiencies vary to a great degree in terms of the captured parameters, time points of their capture and their representation in the publications. A systematic review or statistically sound meta-analysis of the data is not possible. Nevertheless, a number of clinically relevant parameters are presented in some cases. The evaluation of the clinical efficacy of cholic acid takes the following endpoints into account:

- Overall survival on therapy (need for a liver transplantation is considered treatment failure)
- Liver function as assayed through biochemical test for transaminases and bilirubin
- Liver histology
- Serum and urinary bile acids

Liver function tests (ALT, AST, GGT, coagulation time) and histology are validated markers for hepatic function in these patients and provide a short and medium-term parameter of the efficacy. Liver function tests are objective markers not subject to investigator bias. These endpoints, with the exception of liver histology, are categorical or numerical and not subject to investigator evaluation bias. A reporting bias for unfavourable results cannot be excluded however all parameters for evaluation are causally linked, and therefore should evolve in concert over time, diminishing the impact and likelihood of reporting bias.

**Efficacy results in patients with 3β-HSD Deficiency**

**Survival**

The key clinical consideration in the treatment of patients with 3β-HSD deficiency is overall survival. Data from untreated siblings of patients with 3β-HSD deficiency show that this defect is lethal in infants if untreated (Table 2). An estimation of the rate of lethal disease is not possible due to reporting bias. Three children (including patients JU and MU1, siblings of MU2) died before they could be effectively treated (gastrointestinal haemorrhage at 19 months and acute abdominal haemorrhage following liver biopsy at 3.75 years of age, whereas the third died from end-stage liver disease at the
age of 4.6 years) (Clayton et al. 1987; Subramaniam et al. 2010). All siblings of patients A, C and D1 and D2 died from liver failure prior to or at the age of 6 (Gonzales 2006). Patient Fi had two older siblings who died from progressive cholestatic liver disease, at 11 years for the girl and at the age of 6 months for the boy (Fischler et al. 2007). Patient Fi, however, survived to adulthood (26 years) without treatment. It was suggested that low level of sulphation of the abnormal trihydroxy bile acid formed as a result of enzyme deficiency may be of importance for survival (Fischler et al. 2007).

Patients treated with cholic acid, alone or in combination withchenodeoxycholic acid or ursodeoxycholic acid, have survived long-term with at least 12.9 years of survival clearly documented in the literature (Gonzales et al. 2009; Subramaniam et al. 2010). According to the literature, patient J stopped his treatment at the age of 5 years (Gonzales 2006) and has not resumed treatment for at least 3 years (patient J is not mentioned in (Gonzales et al. 2009). Survival of patient MU2 for at least 16 years is clear from the fact that his case is described in 1987 and 2003 (Clayton et al. 1987; Cheng et al. 2003). Exceptions are patients Lc and Mc who did not respond to cholic acid and ursodeoxycholic acid, respectively, probably because of extensive liver damage before diagnosis. Bile acid therapy was unsuccessful. Both had to undergo orthotopic liver transplantation (Cheng et al. 2003). Overall, among the 22 patients reported in the literature as treated with cholic acid in monotherapy, data describe successful long-term treatment of 15 patients (Gonzales et al. 2009; Subramaniam et al. 2010). Patient MU2 was lost to follow-up, but available data suggest successful treatment with cholic acid. Only one treatment failure occurred under cholic acid therapy (Patient Mc). The patient had to undergo liver transplantation (Table 4).

**Liver Function**

Cholic acid treatment has avoided the need for a liver transplant in at least 3 patients. Two patients did not respond to primary bile acid therapy, 1 under cholic acid and the other under ursodeoxycholic acid, both probably because of extensive liver damage before diagnosis. Both had to undergo orthotopic liver transplantation (Cheng et al. 2003). Among all cholic-acid treated patients reported in the literature the above-mentioned patient is the only one who needed a liver transplant; all other patients recovered under cholic acid therapy.

Liver function tests have consistently been reported as improved on treatments containing cholic acid. Unfortunately, the data reported in the literature use very uneven reporting time points and sampling intervals, or are represented only as graphical data with no access to exact numerical values. The tabulations in the following compile the available data. Data are mainly extracted from Gonzales 2006; Gonzales et al. 2009.

Liver transaminases are consistently reported as decreasing under cholic acid treatment. They can be extremely elevated without any treatment (up to 30-fold ULN), but decrease to the normal range within 1-8 months of cholic acid treatment (Potin et al. 2001; Setchell 2004). Values for ALT and GGT are more consistently reported in the publications at time of diagnosis, however, the data reported for follow-up are limited and uneven reporting time points and sampling intervals are used (Table 7).

<table>
<thead>
<tr>
<th>Table 7</th>
<th>Long-term development of ALT values (IU/L) under cholic acid treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>A</td>
</tr>
<tr>
<td>First assessment*</td>
<td>248</td>
</tr>
<tr>
<td>Patients on initiation of CA*</td>
<td>34</td>
</tr>
<tr>
<td>Long-term (&lt;5 years)*</td>
<td>12</td>
</tr>
<tr>
<td>Last assessment (&gt;5 years)**</td>
<td>13</td>
</tr>
</tbody>
</table>
Patients were on UDCA treatment at initiation of CA. 1CA was administered in combination with UDCA for 6 months, then alone; 2CA was administered as replacement of UDCA therapy; CA: Cholic acid; UDCA: ursodeoxycholic acid; Normal range: 5-50 IU/L; * In Gonzales E. 2006; ** In Gonzales et al. 2009

Setchell 2004 reports data for 25 patients. For these patients, cholic acid therapy allowed a return to normal values for both AST and ALT in about 5 months and over up to 5 years of treatment. Transaminases returned to normal for patient AB described in (Clayton 1991) within one year under treatment with cholic acid in combination with chenodeoxycholic acid. An important and rapid improvement in transaminase level is also reported for patient Wi under treatment with cholic acid in combination with chenodeoxycholic and ursodeoxycholic acid (Witzleben et al. 1992).

GGT is reported as being in the normal range or slightly elevated in patients with 3β-HSD Deficiency. Under therapy with cholic acid, transient increases in GGT have been reported (Gonzales E. 2006 and Gonzales et al. 2009). These may be indicative of a supratherapeutic bile acid dose, as they returned to normal after reduction in cholic acid dose.

Total bilirubin and direct bilirubin are consistently reported to decrease under cholic acid treatment. They can be extremely elevated at baseline in patients with 3β-HSD deficiency (up to 15-fold ULN), but usually decrease to the normal range during treatment. There is no clear indication of the time for normalisation available from the literature. Values for total bilirubin are most consistently reported in the publications (Table 8).

### Table 8 Long-term developments of total bilirubin values (μmol/L) under cholic acid treatment

| Patient | A | B | C | D1 | D2 | E1 | E2 | F | G1 | G2 | H2 | I1 | I2 | I2
|---------|---|---|---|----|----|----|----|---|----|----|----|----|----|---
| First assessment* | 80 | 74 | 115 | 36 | 14 | 23 | 25 | 100 | 6 | 17 | 254 | 134 | 13 |   
| Patients on initiation of CA* | 22 | 26 | 18 | 47 | 14 | 31 | 15 | 15 | 4 | 8 | 86 | 59 | 8 |   
| Long-term (<5 years)* | 6 | 4 | 5 | 37 | 13 | 6 | 6 | 25 | 7 | 5 | 4 | 12 | 6 |   
| Last assessment (>5 years)** | 17 | 8 | 12 | 17 | 13 | 6 | 17 | 17 | 5 | 7 | 10 | 6 |   

Patients were on UDCA treatment at initiation of CA. 1CA was administered in combination with UDCA for 6 months, then alone; 2CA was administered as replacement of UDCA therapy; CA: Cholic acid; UDCA: ursodeoxycholic acid
* In Gonzales E. 2006; ** In Gonzales et al. 2009

Plasma bilirubin returned to normal for patient AB described in (Clayton 1991) within one year under treatment with cholic acid in combination with chenodeoxycholic acid. For patient Kob, total bilirubin levels was reported to be reduced under treatment with cholic acid alone and further reduced under treatment with cholic acid in combination with chenodeoxycholic acid (Kobayashi et al. 2000). A rapid improvement in bilirubin level is reported for patient Wi under treatment with cholic acid in combination with chenodeoxycholic and ursodeoxycholic acid (Witzleben et al. 1992).

### Liver Histology

While liver histology data before the initiation of treatment are available from all bile-acid treated patients, follow-up data are limited to 10 patients described in Gonzales 2006; Gonzales et al. 2009, 4 patients described in Subramaniam et al. 2010 and patient Wi described by Witzleben et al. 1992; the latter 5 patients have been treated with cholic acid in combination with other bile acids. All show a resolved liver pathology. Mild fibrosis remained in 9 patients.

### Serum and Urinary Bile Acids
While urinary and/or serum bile acid data before the initiation of treatment are available from almost all bile-acid treated patients, follow-up data are limited to patients described in Gonzales et al. 2009 and in Kobayashi et al. 2000.

In patients described in Gonzales et al. 2009, urinary atypical 3βOH−Δ5 bile acids initially were present at high levels, and strongly decreased under cholic acid therapy, without completely disappearing (Table 9). Total urinary bile acids were initially very high, consistent with cholestasis, but with low levels of the normal primary bile acids, cholic acid and chenodeoxycholic acid. The excretion was mainly made of atypical metabolites characteristic of 3β-HSD deficiency.

Under cholic acid therapy, there was a significant decrease in 3βOH−Δ5 bile acids and of total urinary bile acid excretion, indicating an improvement of cholestasis. In 5 patients, evaluation of these parameters under ursodeoxycholic acid alone showed an improvement but full metabolic control could not be achieved under ursodeoxycholic acid alone (Jacquemin et al. 1994). In patient Kob, ursodeoxycholic acid (150 mg/day) treatment did not lead to a significant decrease in the amount of abnormal bile acids in urine. In the same patient, chenodeoxycholic acid treatment (400 mg/day) lowered the amount of abnormal bile acids in the urine, but further reduction was achieved under combined therapy of chenodeoxycholic acid (400 mg/day) and cholic acid (120 mg/day).

Table 9

<table>
<thead>
<tr>
<th>Patient</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D1</th>
<th>D2</th>
<th>E1</th>
<th>E2</th>
<th>F</th>
<th>G1</th>
<th>G2</th>
<th>H</th>
<th>I1</th>
<th>I2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>50</td>
<td>36</td>
<td>15</td>
<td>55</td>
<td>16</td>
<td>25</td>
<td>50</td>
<td>54.5</td>
<td>65</td>
<td>73</td>
<td>17</td>
<td>23</td>
<td>15</td>
</tr>
<tr>
<td>CA 5 years</td>
<td>2.5</td>
<td>0.6</td>
<td>4.25</td>
<td>8.5</td>
<td>1.5</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>19.5</td>
<td>1.5</td>
<td>0.8</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CA last visit</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.5</td>
<td>0.75</td>
<td>0</td>
<td>1</td>
<td>0.04</td>
<td>0</td>
<td>1</td>
<td>1.9</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

CA: cholic acid; UDCA: ursodeoxycholic acid. All patients were on UDCA treatment at initiation of CA. For patients A, B, C, D1, E1 and E2, CA was administered in combination with UDCA for 6 months, then alone; For patients D2, F, G1, G2, H, I1 and I2, CA was administered as replacement of UDCA therapy.

Disappearance of the atypical bile acids from urine under cholic acid therapy in 25 patients described by Setchell 2004 was achieved within one week. Total serum bile acid concentrations remained normal under cholic acid treatment, with cholic acid comprising 40% and its metabolite deoxycholic acid comprising 35% of the total serum bile acids (Gonzales et al. 2009).

**Vitamin Malabsorption**

Various combinations of deficiencies in fat-soluble vitamins have been reported at baseline in 13 patients described in Gonzales et al. 2009. They were detected biochemically or manifested as coagulopathy (Vitamin K), lack of deep tendon reflexes (Vitamin E) and rickets (Vitamin D). These deficiencies have been treated by parenteral administration or oral supplementation of vitamins. Levels of vitamin A were reported to be above normal for 5 patients.

In patients described by Gonzales et al. 2009, levels of serum vitamin E remained normal under cholic acid treatment after vitamin E supplements were stopped. Patient A was reported to no longer require vitamin K supplementation on ursodeoxycholic acid therapy. No details are provided as whether supplementation was stopped under cholic acid for the other patients.

**Persistence of Efficacy and/or Tolerance Effects**

Long-term efficacy data for 13 patients with 3β-HSD deficiency are available. Treatment has been closely documented for 5 to more than 12 years in patients described by Gonzales et al. 2009. There is no report in the literature on a loss of efficacy. A need to increase the dose over time would be indicative of loss of efficacy with insufficient control of serum and urine bile acid concentrations or
transaminase levels. No such event was reported in the literature. No patients have been reported to have ended cholic acid treatment.

**Lack of Efficacy**

Kobayashi et al. 2000 report in a patient with 3β-HSD deficiency that cholic acid (120 mg/day in a 22-year-old Japanese patient) lacked efficacy in lowering the patient's urinary abnormal bile acids. Total bilirubin was however found to be decreased. The authors however noted that the amount of cholic acid administered was probably insufficient. Combined therapy with cholic acid (120 mg/day) and chenodeoxycholic acid (400 mg/day) restored total bilirubin levels to within 2-fold ULN and reduced abnormal bile acids. One treatment failure in 1 patient under cholic acid treatment is also reported by Cheng et al. 2003 but was attributed to pre-existing liver damage. The patient had to undergo liver transplantation after an unspecified treatment time.

**Overview of cases reports for the Δ⁴-3-oxoR deficiency**

Studies investigating the efficacy of bile acid therapy, including cholic acid therapy, in the treatment of Δ⁴-3-oxoR deficiency originate principally from three sources: the Jacquemin Group at Bicêtre Hospital, Le-Kremlin Bicêtre, France (Potin et al. 2001; Gonzales et al. 2004; Gonzales et al. 2009), the Clayton group at UCL Institute of Child Health, London, United Kingdom (Clayton et al. 1996; Lemonde et al. 2003; Palermo et al. 2008), and the Setchell group at Cincinnati Children's Hospital Medical Center, Cincinatti, USA (Setchell et al. 1988; Balistreri 1991; Setchell et al. 1991; Daugherty et al. 1993; Balistreri 1999; Bove et al. 2004). These studies provide data on a group of a total 11 patients, 7 of which have been treated with cholic acid.

**Demographics of patient with Δ⁴-3-oxoR Deficiency**

The literature contains reports on a total of 7 patients with Δ⁴-3-oxoR deficiency who have been treated with cholic acid and of 3 patients who have been treated with other bile acids. 1 patient received no treatment. The demographic characteristics of these patients are summarised in Table 10.

**Table 10** Demographic Features of Reported Cases of Δ⁴-3-oxoR Deficiency

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at onset (months)</th>
<th>Gender</th>
<th>Origin</th>
<th>Mol. Defect</th>
<th>Age at initiation of CA</th>
<th>Age at death</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 (J)</td>
<td>1</td>
<td>F</td>
<td>France</td>
<td>P133R, P261C</td>
<td>0.7 (8 months)</td>
<td>--</td>
<td>(Potin et al. 2001; Gonzales et al. 2004; Gonzales et al. 2009)</td>
</tr>
<tr>
<td>15 (J)</td>
<td>1</td>
<td>F</td>
<td>France</td>
<td>P133R, P261C</td>
<td>0.7 (8 months)</td>
<td>--</td>
<td>(Potin et al. 2001; Gonzales et al. 2004; Gonzales et al. 2009)</td>
</tr>
<tr>
<td>MS</td>
<td>0.75</td>
<td>F</td>
<td>Italy</td>
<td>P198L, R261C</td>
<td>0.7 (8.3 months)</td>
<td>--</td>
<td>(Clayton et al. 1996; Lemonde et al. 2003; Palermo et al. 2008)</td>
</tr>
<tr>
<td>BH</td>
<td>0</td>
<td>M</td>
<td>United Kingdom / Pakistan</td>
<td>frame shift</td>
<td>n.a. (2 months?)</td>
<td>--</td>
<td>(Lemonde et al. 2003)</td>
</tr>
<tr>
<td>2a/S Ga</td>
<td>0</td>
<td>F</td>
<td>USA</td>
<td>n.a.</td>
<td>4 weeks</td>
<td>--</td>
<td>(Setchell et al. 1988; Balistreri 1991; Setchell et al. 1991; Daugherty et al. 1993; Balistreri 1999; Bove et al. 2004)</td>
</tr>
<tr>
<td>2b/J Ga</td>
<td>0</td>
<td>M</td>
<td>USA</td>
<td>n.a.</td>
<td>4 weeks</td>
<td>--</td>
<td>(Setchell et al. 1988; Balistreri 1991; Setchell et al. 1991; Daugherty et al. 1993; Balistreri 1999; Bove et al. 2004)</td>
</tr>
</tbody>
</table>
CA: cholic acid; n.a.: not available; Sib: sibling; *matched through comparison of data in (Setchell et al. 1991; Daugherty et al. 1993). Patients below the line have not been treated with cholic acid; b treated with ursodeoxycholic acid, c treated with chenodeoxycholic acid.

The baseline characteristics of all patients, i.e. the clinical presentation before the initiation of any therapy, are summarised in Table 11.

### Table 11 Clinical and biochemical features of reported cases of Δ^4-3-oxoR deficiency before initiation of any bile acid treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number of patients evaluated</th>
<th>Value [Range] / Number of patients</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at investigation</td>
<td>9</td>
<td>2.4 months</td>
<td>-</td>
</tr>
<tr>
<td>Jaundice</td>
<td>9</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>Pruritus</td>
<td>9</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Steatorrhoea</td>
<td>9</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>9</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Hepatosplenomegaly</td>
<td>9</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin A deficiency</td>
<td>9</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Rickets</td>
<td>9</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin D deficiency</td>
<td>9</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin E deficiency</td>
<td>9</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin K deficiency</td>
<td>9</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Bilirubin (total)</td>
<td>9</td>
<td>313 [193-342] µmol/L</td>
<td>5.1–17.0 µmol/L</td>
</tr>
<tr>
<td>Bilirubin (conjugated)</td>
<td>8</td>
<td>193.25 [142-239] µmol/L</td>
<td>1.0–5.1 µmol/L</td>
</tr>
<tr>
<td>ALT</td>
<td>815.9 [32-1702] IU/L</td>
<td>7-56 IU/L</td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>850 [103-2279] IU/L</td>
<td>5-35 IU/L</td>
<td></td>
</tr>
<tr>
<td>GGT</td>
<td>76 [48-130] IU/L</td>
<td>8-78 IU/L</td>
<td></td>
</tr>
<tr>
<td>Urinary Bile Acids</td>
<td></td>
<td>Low or undetectable CA/CDCA</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>high 7α-hydroxy-3-oxo-4-cholenoic &amp; 7α,12α-dihydroxy-3-oxo-4-cholenoic</td>
<td></td>
</tr>
</tbody>
</table>

CA: cholic acid; CDCA: chenodeoxycholic acid; ALT: alanine aminotransferase; AST: asparagine aminotransferase; GGT: γ-glutamyl transferase

This population represents the entire population reported in the literature. It thus represents the known spectrum of disease manifestations. Generally, patients presented at the neonatal period or in early infancy with jaundice, cholestasis, hepatomegaly and steatorrhoea, together with symptoms of fat-soluble vitamin deficiency such as rickets (vitamin D). Pruritus was absent. Bilirubin and transaminases were more than 10-fold above the normal range, while GGT was normal or slightly elevated. Urinary bile acid excretion was strongly increased and consisted of 7α-hydroxy-3-oxo-4-cholenoic and 7α,12α-dihydroxy-3-oxo-4-cholenoic acid, while the primary bile acids were at low or undetectable levels. In liver histology, hepatocellular and canalicular cholestasis, giant hepatocytes, necrosis, fibrosis, extramedullary haematopoiesis and inflammation were reported in the majority of the patients.

Efficacy of cholic acid treatment Δ^4-3-oxoR Deficiency has been assessed using the same methods and parameters as for 3β-HSD deficiency as detailed above.
Treatments

Treatment modalities of all patients were different due to the fact that treatment algorithms were only established based on these patients and their individual responses. The treatment with bile acids of all patients are summarised in Table 12.

Table 12 Bile acid treatment in patients with Δ⁴-3-oxoR deficiency

<table>
<thead>
<tr>
<th>Patient</th>
<th>BA/ daily dose</th>
<th>Treatment 1 duration</th>
<th>BA/ daily dose</th>
<th>Treatment 2 duration</th>
<th>BA/ daily dose</th>
<th>Treatment 3 (final) Duration and status</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 (J1)</td>
<td>UDCA 600 mg/m²</td>
<td>4 months</td>
<td>CA 10 mg/kg</td>
<td>14 months</td>
<td>CA 250 mg/kg</td>
<td>&gt;9 years, ongoing</td>
</tr>
<tr>
<td></td>
<td>UDCA 15 mg/kg</td>
<td></td>
<td>UDCA 15 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 (J2)</td>
<td>UDCA 600 mg/m²</td>
<td>4 months</td>
<td>CA 10 mg/kg</td>
<td>14 months</td>
<td>CA 200 mg/kg</td>
<td>&gt;9 years, ongoing</td>
</tr>
<tr>
<td></td>
<td>UDCA 15 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>UDCA 12 mg/kg</td>
<td>1 month?</td>
<td>UDCA 20 mg/kg</td>
<td>4 months</td>
<td>CA 8 mg/kg</td>
<td>9.3 years, then stopped</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CA 200 mg/kg</td>
<td></td>
<td>CDCA 8 mg/kg</td>
<td></td>
</tr>
<tr>
<td>BH</td>
<td>CA 8 mg/kg</td>
<td>6 weeks</td>
<td>CA 8 mg/kg</td>
<td>Not specified</td>
<td>CA 8 mg/kg</td>
<td>&gt;10 years confirmed, likely &gt;15 years and ongoing</td>
</tr>
<tr>
<td></td>
<td>CDCA 8 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td>CDCA 8 mg/kg</td>
<td></td>
</tr>
<tr>
<td>2a/SG</td>
<td>UDCA n.r.</td>
<td>4 days</td>
<td>UDCA 100 mg</td>
<td>16 days</td>
<td>CA 100 mg/kg</td>
<td>&gt;10 years confirmed, likely &gt;15 years and ongoing</td>
</tr>
<tr>
<td></td>
<td>(100 mg?)</td>
<td></td>
<td>CDCA 100 mg</td>
<td></td>
<td>UDCA 100 mg</td>
<td></td>
</tr>
<tr>
<td>2b/JG</td>
<td>UDCA n.r.</td>
<td>4 days</td>
<td>UDCA 100 mg</td>
<td>16 days</td>
<td>CA 100 mg/kg</td>
<td>&gt;10 years confirmed, likely &gt;15 years and ongoing</td>
</tr>
<tr>
<td></td>
<td>(100 mg?)</td>
<td></td>
<td>CDCA 100 mg</td>
<td></td>
<td>UDCA 100 mg</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>CA 100 mg/kg</td>
<td>&gt;7 years confirmed, likely &gt;12 years and ongoing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>UDCA 100 mg</td>
<td>3.5 months; stopped</td>
</tr>
<tr>
<td>5</td>
<td>--</td>
<td>--</td>
<td>UDCA 5 mg/kg</td>
<td>4.5 months</td>
<td>UDCA 10 mg/kg</td>
<td>Not specified (ongoing). Awaiting liver transplant.</td>
</tr>
<tr>
<td>6</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>CDCA 12 mg/kg</td>
<td></td>
</tr>
</tbody>
</table>

CA: cholic acid; CDCA: chenodeoxycholic acid; UDCA: ursodeoxycholic acid; n.r.: not reported

Efficacy results in patients with Δ⁴-3-oxoR Deficiency

Survival

The key clinical consideration in the treatment of patients with inborn errors of primary bile acid synthesis due to Δ⁴-3-oxoR deficiency is overall survival. Data from an untreated sibling of patients with Δ⁴-3-oxoR deficiency show that this defect can be lethal at the infant age if untreated (Table 10).

On the other hand, the patients reported in the literature that were treated with cholic acid, alone or in combination with chenodeoxycholic acid or ursodeoxycholic acid, have survived long-term. Patients 14 (J) and 15 (J) are alive to date after 12 years of treatment, at least 10.7 of which are documented in the literature (Gonzales et al. 2009). Survival of patients 2a/2b for at least 14 years and of patient 3 for 12 years is clear from the fact that their cases are discussed by (Setchell et al. 1988; Bove et al. 2004) without any report to the contrary. Patient MS has been reported to have stopped her treatment the age of 10 after 9-10 years of treatment and has not resumed treatment for at least 3 years; she was reported alive and healthy at the age of 13 (Palermo et al. 2008).

On the other hand, the patients reported in the literature that were treated with cholic acid, alone or in combination with chenodeoxycholic acid and ursodeoxycholic acid, have survived long-term. Patients 14 (J) and 15 (J) are alive to date after 12 years of treatment, at least 10.7 of which are documented in the literature (Gonzales et al. 2009). Survival of patients 2a/2b for at least 14 years and of patient 3 for 12 years is clear from the fact that their cases are discussed by (Setchell et al. 1988; Bove et al. 2004) without any report to the contrary. Patient MS has been reported to have stopped her treatment the age of 10 after 9-10 years of treatment and has not resumed treatment for at least 3 years; she was reported alive and healthy at the age of 13 (Palermo et al. 2008).

The sole exception is patient BH who did not respond to a combination treatment with chenodeoxycholic acid and was reported surviving with a liver transplant (Lemonde et al. 2003). This therefore represents the only documented case (out of 7) where a bile acid therapy containing cholic acid did not lead to progression-free survival. However, in this patient no efforts to optimise therapy are reported. The patient also presented with very high ALT and elevated GGT, indicating substantial
hepatic damage already at the initiation of treatment. The patient may also not have tolerated the chenodeoxycholic acid included in the regimen.

Data from four patients (14 (J), 15 (J), MS and RM) show that treatment with ursodeoxycholic acid monotherapy is ineffective in halting disease progression. Patient 5 improved substantially during a total of 8 months of ursodeoxycholic acid and subsequently stopped treatment (Ueki et al. 2008). This patient is different from the others in that only a heterozygous mutation in the coding region of the \( SRD5B1 \) gene was found; no data on the level of expression of the \( \Delta^4-3\text{-oxoR} \) protein is available. The patient’s mother, with the same defective allele, survived to adulthood. Taken together, these data indicate a milder phenotype in this patient.

Patient 6, also with a heterozygous mutation in the coding region of the \( SRD5B1 \) gene, showed initial improvement on chenodeoxycholic acid monotherapy, but liver function subsequently deteriorated and the patient was referred for liver transplantation (Ueki et al. 2008). As for patient BH, this may indicate a poor tolerance of chenodeoxycholic acid.

**Liver Function**

Among all 7 cholic-acid treated patients reported in the literature, 1 patient needed a liver transplant; all other patients recovered under cholic acid or combination therapy.

Liver function tests have consistently been reported as improved in patients with \( \Delta^4-3\text{-oxoR} \) deficiency on treatments containing cholic acid. Unfortunately, the data reported in the literature use very uneven reporting time points and sampling intervals. Liver transaminases are consistently reported to decrease under cholic acid treatment. They can be extremely elevated without any treatment (up to 30-fold ULN), but drop to the normal range within about 12 months. Values for ALT are most consistently reported in the publications (Table 13).

<table>
<thead>
<tr>
<th>Table 13</th>
<th>Long-term development of ALT values (IU/L) under cholic acid treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2(^a)</td>
</tr>
<tr>
<td>1 wk / no treatment</td>
<td>103</td>
</tr>
<tr>
<td>4 wk / initiation of CA treatment</td>
<td>106 (^a)</td>
</tr>
<tr>
<td>7 wk</td>
<td>167 (^b)</td>
</tr>
<tr>
<td>12 wk</td>
<td>139 (^b)</td>
</tr>
<tr>
<td>8-9 mo</td>
<td>74 (^b)</td>
</tr>
<tr>
<td>13 mo</td>
<td>74 (^b)</td>
</tr>
<tr>
<td>15 mo</td>
<td>69 (^b)</td>
</tr>
<tr>
<td>Long-term (&lt; 5 years)</td>
<td>55 (^b)</td>
</tr>
<tr>
<td>&gt; 5 years</td>
<td>–</td>
</tr>
</tbody>
</table>

CA: Cholic acid; wk: week; mo: month \(^a\) patient on UDCA treatment; \(^b\) patient on combination treatment with ursodeoxycholic acid; \(^c\) patient on combination treatment with chenodeoxycholic acid. Normal range: 5-50 IU/L

There is a transient increase in transaminases observed at the initiation of treatment with cholic acid and chenodeoxycholic acid. This increase may have occurred without having been reported in all patients. GGT is reported as being in the normal range or slightly elevated (patient MS) before treatment. Under therapy with cholic acid (patient 14 (J)) or a combination of cholic acid and chenodeoxycholic acid (patient MS), transient increases in GGT have been reported. These may be indicative of a supratherapeutic bile acid dose.

Total bilirubin and direct bilirubin are consistently reported to decrease under cholic acid treatment. They can be extremely elevated at baseline in patients with \( \Delta^4-3\text{-oxoR} \) deficiency without any treatment (up to 18-fold ULN), but drop to the normal range within about 12 months. Values for total bilirubin are most consistently reported in the publications (Table 14).
Table 14  Long-term developments of total bilirubin values (μmol/L) under cholic acid treatment

<table>
<thead>
<tr>
<th></th>
<th>2a</th>
<th>2b</th>
<th>3</th>
<th>14</th>
<th>15</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 wk / no treatment</td>
<td>86</td>
<td>103</td>
<td>274</td>
<td>314</td>
<td>313</td>
<td>316</td>
</tr>
<tr>
<td>4 wk / initiation of CA treatment</td>
<td>342&lt;sup&gt;a&lt;/sup&gt;</td>
<td>342&lt;sup&gt;a&lt;/sup&gt;</td>
<td>308&lt;sup&gt;b&lt;/sup&gt;</td>
<td>304&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>7 wk</td>
<td>342&lt;sup&gt;b&lt;/sup&gt;</td>
<td>393&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>12wk</td>
<td>393&lt;sup&gt;b&lt;/sup&gt;</td>
<td>325&lt;sup&gt;b&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8-9 mo</td>
<td>393&lt;sup&gt;b&lt;/sup&gt;</td>
<td>136&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N&lt;sup&gt;b&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>13 mo</td>
<td>393&lt;sup&gt;b&lt;/sup&gt;</td>
<td>171&lt;sup&gt;b&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>15 mo</td>
<td>34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>–</td>
<td>–</td>
<td>N&lt;sup&gt;c&lt;/sup&gt;</td>
<td>–</td>
</tr>
<tr>
<td>Long-term (&lt;5 years)</td>
<td>17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>–</td>
</tr>
<tr>
<td>&gt; 5 years</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6</td>
<td>N&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

CA: Cholic acid; wk: week; mo: month  a patient on UDCA treatment;  b patient on combination treatment with ursodeoxycholic acid;  c patient on combination treatment with chenodeoxycholic acid. N: Normal range (5-17 μmol/L)

Liver Histology

While liver histology data before the initiation of treatment are available from all bile-acid treated patients, follow-up data are limited to only 5 patients. All show a resolved liver pathology (Table 15) except for fibrosis (mild or septal) that remained in all patients as a result of the earlier hepatic injury, except in patient 3 who had been treated from 1 week after birth.

Table 15  Liver histology under cholic acid treatment – main observations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Before treatment</th>
<th>First evaluation under CA therapy</th>
<th>Long-term evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 (J)</td>
<td>canalicular cholestasis</td>
<td>resolved&lt;sup&gt;a&lt;/sup&gt;</td>
<td>no&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>giant hepatocytes</td>
<td>n.r.</td>
<td>n.r.</td>
</tr>
<tr>
<td></td>
<td>portal inflammation</td>
<td>resolved</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>septal fibrosis</td>
<td>progressed</td>
<td>reduced</td>
</tr>
<tr>
<td>15 (J)</td>
<td>canalicular cholestasis</td>
<td>resolved&lt;sup&gt;a&lt;/sup&gt;</td>
<td>no&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>giant hepatocytes</td>
<td>n.r.</td>
<td>n.r.</td>
</tr>
<tr>
<td></td>
<td>portal inflammation</td>
<td>n.r.</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>septal fibrosis</td>
<td>stabilised</td>
<td>reduced</td>
</tr>
<tr>
<td>2a/SG</td>
<td>hepatocellular and canalicular cholestasis</td>
<td>resolved&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>giant hepatocytes, extramedullary haematopoiesis</td>
<td>resolved</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>inflammation</td>
<td>resolved mild fibrosis</td>
<td>n.a.</td>
</tr>
<tr>
<td>2b/JG</td>
<td>hepatocellular and canalicular cholestasis</td>
<td>resolved&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>giant hepatocytes, extramedullary haematopoiesis</td>
<td>resolved</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>inflammation</td>
<td>resolved mild fibrosis</td>
<td>n.a.</td>
</tr>
<tr>
<td>3</td>
<td>hepatocellular and canalicular cholestasis</td>
<td>normalised&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td>giant hepatocytes extramedullary haematopoiesis</td>
<td>normalised</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

<sup>a</sup> at 14 months;  <sup>b</sup> at 5 years;  <sup>c</sup> at 8-9 months;  n.a.: not available;  n.r.: not reported

Serum and Urinary Bile Acids

While urinary and/or serum bile acid data before the initiation of treatment are available from all bile-acid treated patients, follow-up data are limited to patients 14 (J), 15 (J), 2a and 2b. Unfortunately, data are not presented in a comparable way, as the bases of reporting are different (percent values vs. absolute values). Total urinary bile acids were initially very high. In these patients, urinary Δ<sup>4</sup>-3-oxo bile acids initially were present at high levels, and strongly decreased under cholic acid and ursodeoxycholic acid therapy, without completely disappearing. Under cholic acid therapy, urinary total bile acids were further decreased. Data on serum bile acids are available for patients 2a and 2b; allo- and Δ<sup>4</sup>-3-oxo bile acids initially were present at high levels (2a: 52.18 and 2b: 37.26 μmol/L) and...
completely disappeared under combination therapy with cholic acid and ursodeoxycholic acid. In patients 14 (J)/15 (J), total serum bile acid concentrations remained normal (< 10 µmol/L) under cholic acid and were essentially made of cholic acid (40%) and its metabolite deoxycholic acid (35%) and of ursodeoxycholic acid in patient 14 (J).

**Vitamin Malabsorption**

Deficiencies in fat-soluble vitamins, whether biochemically or manifesting as coagulopathy (Vitamin K), lack of deep tendon reflexes (vitamin E) and rickets (vitamin D) have been reported at baseline in patients 14 (J), 15 (J), 2a, 2b, MS, BH and RM in various combinations. These deficiencies have been treated by parenteral administration or oral supplementation of the vitamins. Patients 14 (J) and 15 (J) are reported to no longer require vitamin E supplementation on cholic acid therapy, with and without additional ursodeoxycholic acid, respectively.

**Persistence of Efficacy and/or Tolerance Effects**

Long-term efficacy data for 6 patients with \(\Delta^4\)-3-oxoR deficiency are available. Treatment has been closely documented for more than 15 months in patients 2a, 2b and 3, for 9-10 years in patient MS and for 10.7 years for patients 14 (J) and 15 (J). There is no report in the literature on a loss of efficacy. Further, the literature does not report an increase of the required dose over time; rather, there is evidence that the doses necessary for metabolic control decrease over time, although this may be related to the age of the patient. The patients, with the exception of patient MS, have not been reported to have ended cholic acid treatment, and there is confirmation available that patients 14 (J) and 15 (J) are on continued treatment. Patient MS’ decision to stop treatment appears unrelated to a loss of efficacy of treatment.

**Reports of Lack of Efficacy**

Gonzales et al. 2009 report that in one patient with \(\Delta^4\)-3-oxoR deficiency, monotherapy with cholic acid was insufficient to return serum liver function tests close to the normal range. Additional ursodeoxycholic acid normalized liver enzyme levels.

Clayton et al. 1996 report that ursodeoxycholic acid lacked efficacy in one patient (MS), while treatment with a combination of cholic acid and chenodeoxycholic acid showed efficacy.

Lemonde et al. 2003 report that a combination of cholic acid (8 mg/kg per day) and chenodeoxycholic acid (8 mg/kg per day) lacked in efficacy in an infant patient (BH). After six weeks of treatment, some liver function tests (bilirubin, ALT, prothrombin time) improved and others (albumin, GGT) deteriorated. The patient developed pruritus and had high plasma chenodeoxycholic acid levels. Chenodeoxycholic acid was stopped, while cholic acid presumably was continued. The authors do not report any attempts to optimise the cholic acid dose. Subsequently, liver function tests deteriorated, even after reintroduction of chenodeoxycholic acid, and the patient eventually underwent a liver transplant.

In the same paper, (Lemonde et al. 2003) report that ursodeoxycholic acid (60 mg per day) and chenodeoxycholic acid (30 mg/day) lacked in efficacy in an infant patient (RM). Liver function tests continued to deteriorate during treatment, and the patient underwent an unsuccessful liver transplant.

### 3.5.3. Discussion on clinical efficacy

**Clinical efficacy in 3\(\beta\)-HSD deficiency**

The literature provides consensus that cholic acid is the treatment of choice for 3\(\beta\)-HSD deficiency. Where available to investigators, its clinical use has been documented since at least the mid-1990s and its use can therefore be considered as well-established.
The treatment of 21 identifiable 3β-HSD deficiency patients with cholic acid has been documented in the literature. Detailed long-term data are available for 14 of them. Out of the total of 21 treated patients, a single patient with significant pre-existing liver damage needed a liver transplant. A generally favourable outcome is reported for 7 patients and long-term survival for 5 to more than 12 years without complications or need for additional care is reported for the remaining patients. This is in contrast to the reports of 10 older, untreated siblings of these patients, each of whom died as children before either a definite diagnosis of the disease or a treatment could be established. One patient survived without treatment to the age of 26 years, at which time he needed treatment. These survival data are supported by biochemical and histological data.

Treatment with cholic acid represses the biosynthesis of cholestatic and hepatotoxic bile acid metabolites via repression of cholesterol 7α-hydroxylase. Metabolites are cleared due to the choleretic effect of cholic acid and rapidly disappear from the patients’ urine (Table 9). As a result, biochemical liver parameters such as transaminases (Table 7) and bilirubin (Table 8) slowly normalise and histological liver damage is stopped or even improves. Finally, the administered cholic acid restores a normal enterohepatic circulation and absorption of fat and fat-soluble vitamins occurs at a normal level, resolving growth deficits or outright rickets. This pattern is consistently seen in all patients where detailed or partial data are available.

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 cholic acid 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. Complete treatment failures of patients without advanced hepatic damage would also be of high scientific interest, as they would indicate a new disease mechanism or a gap in the understanding of primary bile acid metabolism.

Chenodeoxycholic acid and ursodeoxycholic acid each have been used in combination with cholic acid or alone. Ursodeoxycholic acid therapy (alone or in combination with cholic acid or chenodeoxycholic acid) is usually effective to reduce strongly elevated urine bile acids and liver function tests, but is unable to normalise them fully. Unlike primary bile acids, ursodeoxycholic acid does not repress the synthesis of bile acid metabolites via repression of cholesterol 7α-hydroxylase. The authors of a study where patients were treated with a combination of chenodeoxycholic acid and ursodeoxycholic acid point out that despite achieving good control of 3β-HSD deficiency the optimum treatment was likely cholic acid or chenodeoxycholic acid monotherapy but had no access to suitable dosage forms for this treatment (Riello et al. 2010).

Clinical efficacy in Δ⁴-3-oxoR deficiency

All but one of the reported successful treatment regimes for patients with Δ⁴-3-oxoR deficiency included cholic acid. One patient was treated with cholic acid monotherapy, while all other patients were treated with a 1:1 (w/w) combination with another bile acid, usually ursodeoxycholic acid. On therapy containing cholic acid, 6 out of the 7 patients reported in the literature have survived long-term without additional interventions; the 7th patient was reported surviving after a liver transplant. Liver function tests returned to normal within about 12 months. Liver histology improved over 12-15 months, with mild to septal fibrosis remaining as a result of the hepatic injury. Vitamin malabsorption was completely corrected in at least 2 of the 7 patients. Δ⁴-3-oxo bile acid metabolites in urine and serum were strongly reduced under bile acid therapy, providing the metabolic basis for the observed pathologic improvements. Apart from their congenital defect, these patients are reported to be well and thriving.
The therapeutic dose of cholic acid for each patient needs to be established empirically based on the metabolic response in terms of liver function tests and urinary bile acids. Daily doses are reported as 5-8.3 mg/kg, with younger patients requiring higher doses than older patients. The association of cholic acid with ursodeoxycholic acid at the same dose is described as the appropriate therapy for \(\Delta^4\)-3-oxoR deficiency in a number of reviews (Balistreri 1999; Bove et al. 2004) with the aim of facilitating the excretion of the cholestatic 3-oxo-\(\Delta^4\) metabolites by ursodeoxycholic acid. On the other hand, cholic acid monotherapy has been successful for one patient (Potin et al. 2001; Gonzales et al. 2004; Gonzales et al. 2009); there is no evidence in the literature that it has been tried in all patients. The combination of chenodeoxycholic acid and cholic acid has led to equivocal results: while one patient has survived long-term, another has responded poorly and progressed to a liver transplant. Until data on a larger group of patients are available, the appropriate treatment modality needs to be determined empirically for each patient.

Two patients have been treated with treatment regimens not containing cholic acid (Ueki et al. 2008), providing additional comparative efficacy data. One patient stopped treatment with ursodeoxycholic acid after an 8-month treatment course resolved the disease symptoms, while the other was referred for a liver transplant after chenodeoxycholic acid treatment failed to permanently restore normal liver function. Both of these patients were heterozygous for mutant alleles of \(SRD5B1\), and no data on comparative expression levels of \(\Delta^4\)-3-oxoR protein are available. In addition to this limitation, data on the treatment in the publication are sparse.

3.5.4. Conclusions on the clinical efficacy

Clinical experience has been reported in the literature from small cohorts of patients and single case reports; absolute patient numbers are small due to the rarity of the conditions. This rarity also made the conduct of controlled clinical studies impossible. Overall, cholic acid treatment results for 38 patients with 3\(\beta\)-Hydroxy-\(\Delta^5\)-C\(27\)-steroid oxidoreductase deficiency are reported in the literature. Detailed long-term data on treatment with cholic acid monotherapy are available for 14 patients observed for up to 12.9 years. Cholic acid treatment results for seven patients with \(\Delta^4\)-3-Oxosteroid-5\(\beta\)-reductase deficiency for up to 14 years are reported in the literature. Detailed medium- to long-term data are available for 5 of these patients, of whom 1 has been treated with cholic acid monotherapy. 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 may reflect the restoration of an effective feedback mechanism of bile acid synthesis.

3.6. Clinical safety

The safety analysis of cholic acid is based on published literature data including data gathered in subjects without inborn errors.

Patient exposure

Data on the cholic acid treatment and outcome of 34 patients with 3\(\beta\)-HSD deficiency or \(\Delta^4\)-3-oxoR deficiency are available in the literature. Additionally, literature data was also reported on the short- or medium-term treatment with cholic acid of 253 subjects who were either healthy volunteers or affected by hepatic or biliary disorders other than inborn errors of primary bile acid synthesis (Table 16).
Table 16  Subject demographics and duration of exposure: all subjects treated with cholic acid

<table>
<thead>
<tr>
<th>Deficiency</th>
<th>N</th>
<th>N CA single treatment</th>
<th>N CA combination treatments</th>
<th>N ≤ 12 m treatment</th>
<th>N &gt; 12 m treatment</th>
<th>N ≥ 5 y treatment</th>
</tr>
</thead>
<tbody>
<tr>
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<td>253</td>
<td>116</td>
<td>137</td>
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<td>3β-HSD</td>
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<td>20</td>
<td>7</td>
<td>8</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>Δ⁴-3-oxoR</td>
<td>7</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>287</td>
<td>137</td>
<td>150</td>
<td>262</td>
<td>25</td>
<td>17</td>
</tr>
</tbody>
</table>

*As of latest reported observation (long term treatment), b Duration of treatment and safety data documented in the literature, if no data available set to ≤12 m, except for 6 patients with 3β-HSD deficiency reported to have been treated for a median of 3.5 years (range 1-17 years) (Subramaniam et al. 2010), these patients were classified as > 12 months; N: number of patients/volunteers, CA: cholic acid, m: month, y: year

The populations studied differ clearly between the healthy volunteers with a normal primary bile acid metabolism and subjects with inborn errors in primary bile acid metabolism. The latter are characterised by an onset in early infancy or childhood in the majority of cases and the patient population is largely paediatric. Treatment of these patients has continued into adolescence and adulthood, consequently there is also a substantial adult patient set in this population. The healthy volunteer population is composed almost exclusively of adult and elderly patients.

Distribution of sex was balanced between the patient populations. The male population was slightly larger than the female due to a larger number of male healthy volunteers. Distribution of race was a reflection of the patient population. Race was usually not reported in the studies of healthy volunteers; these studies were however all conducted in Western Europe or the United States of America.

Patients were treated with cholic doses between less than 5 and more than 20 mg/kg per day. All healthy volunteers in pharmacokinetic studies were treated with fewer than 5 single doses under 5 mg/kg. Subjects in pharmacodynamic studies were treated with doses between 4.7 and 15 mg/kg per day for between 1 to 6 months.

All reported adverse events in the publications have been included in the evaluation. Additionally, the publications have been critically reviewed for additional information that may indicate adverse events.

Adverse events

Very few adverse events to cholic acid 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.

In patients with 3β-HSD deficiency (13 patients) and Δ⁴-3-oxoR deficiency (2 patients), (Gonzales et al. 2009) observed no serious adverse events in a cumulative duration of treatment of more than 180 patient-years. Signs of acute and chronic cholic acid 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 cholic acid treatment for more than five years. Within this patient population (10 patients with 3β-HSD deficiency, 2 patients with Δ⁴-3-oxoR deficiency), Potin et al. 2001 noted that cases of nausea and vomiting occurred, which were not considered related to cholic acid.
Setchell 2004 reports that no adverse events have been associated with the use of cholic acid in any patient treated thus far. Setchell and Heubi 2006 report that no adverse events have been associated with the use of cholic acid in any patient treated for errors in primary bile acid synthesis, with treatment longer than 12 years in the longest treated patient. However, in both publications, the authors do not qualify their observation in relation to the number of patients nor the exact defect of primary bile acid synthesis treated, but it is clear that the observation includes patients with 3β-HSD deficiency and Δ^4-3-oxoR deficiency.

One publication (Setchell et al. 1991) reports of twins with Δ^4-3-oxoR deficiency treated with bile acid preparation in which chenodeoxycholic acid was suspected to lead to debilitating diarrhoea. The patients were being treated with cholic acid (100 mg/day) and chenodeoxycholic acid (100 mg/day) when the diarrhoea occurred. Diarrhoea resolved when chenodeoxycholic acid was replaced by ursodeoxycholic acid, indicating that the adverse event was due to either chenodeoxycholic acid or to its association with cholic acid.

In the 5 studies with 48 subjects with a normal primary bile acid metabolism treated with oral cholic acid for 1 to 6 months, no subject developed an adverse event. However in 1 study with 6 patients with primary biliary cirrhosis all patients initially had elevated liver function tests (AST, ALT, GGT, AP, GLDH) as part of the condition. After initial improvement, the results of liver function tests deteriorated after 6 to 8 weeks of cholic acid therapy and the changes were correlated (r = 0.92) with an increase in α-dihydroxy-bile acids (chenodeoxycholic acid and deoxycholic acid) in the serum.

**Serious adverse event/deaths/other significant events**

No deaths or serious adverse events under cholic acid therapy of 3β-HSD deficiency or Δ4-3-OxoR deficiency have been reported in the literature.

**Laboratory findings**

Transient increases in AST and ALT have been observed at the initiation of the treatment with cholic acid or other bile acids, starting from elevated levels. The transaminases values subsequently normalise over the course of a few months.

Gonzales et al. 2009 reported episodes of overdose in 4 patients having 3β-HSD deficiency or Δ^4-3-oxoR deficiency. In one patient with 3β-HSD deficiency, accidental intake of a high cholic acid dose (53 mg/kg in one dose) was followed by the transient appearance of pruritus and diarrhoea, as well as an increase in transaminases, GGT and total serum bile acids (50 μmol/L, normal range: <10 mol/L). In the other four cases of overdose (one in the above mentioned patient), increased serum transaminase and GGT activities, as well as abnormal serum bile acid levels have been observed, which resolved after reducing the daily dose.

In the case of an overdose, treatment should be continued at the recommended dosage after normalisation of clinical signs and/or biological abnormalities.

**Safety in special population**

**Pregnancy**

Four pregnancies in two women treated with cholic acid for 3β-HSD have been reported (Gonzales et al. 2009). No adverse effect of cholic acid therapy on these four pregnancies was observed and they resulted in normal babies. Bile acid profiles as monitored by GC-MS were persistently good throughout pregnancy.
**Lactation**

Orphacol provides physiological levels of cholic acid to patients who are otherwise unable to produce it. Bile salts, including cholate, are a normal component of human breast milk (Forsyth et al. 1983). Due to the low concentration of cholic acid (<1 μmol/L) in milk, the amount ingested by the child is unlikely to make a significant contribution to the concentration of cholic acid in its duodenum. At these physiological levels cholic acid is almost certainly innocuous. Therefore, Orphacol can be used during lactation.

**Elderly**

There are no reports on the use of cholic acid for the therapy of 3β-HSD deficiency or a Δ^{4}-3-oxoR deficiency in elderly patients in the literature. All currently reported patients are less than 30 years old, the majority less than 20. There is therefore currently no experience on the safety of cholic acid in this population.

**Infants and Children**

Cholic acid therapy has been used for infants from one month of age, and for children and adolescents.

**Hepatic impairment**

The majority of patients treated with cholic acid presented with some degree of hepatic impairment at the time of diagnosis, which resolved under therapy. The evaluation of the safety of cholic acid includes patients with this metabolic impairment. In the absence of clinical experience in patients with hepatic impairment from causes other than 3β-HSD or Δ^{4}-3-oxoR deficiency, no recommendations on dosage adjustment can be made. Patients with hepatic impairment should be monitored closely.

**Renal impairment**

There are no reports on the use of cholic acid for the therapy of 3β-HSD deficiency or a Δ^{4}-3-oxoR deficiency in patients with renal impairment in the literature. The dose of cholic acid should be adjusted individually. Bile acid metabolites are excreted only under conditions of cholestasis, which is resolved by cholic acid treatment.

**Safety related to drug-drug interactions and other interactions**

No safety issues related to drug-drug interactions or other interactions have been presented by the Applicant. Due to the limited number of data and the lack of systematic controlled studies no conclusions can be drawn on the lack of reports regarding the safety aspects of drug-drug or other interaction.

**Discontinuation due to adverse events**

In the literature reported no patient interrupted or stopped treatment due to adverse events.

**3.6.1. Discussion on clinical safety**

Data on the treatment and outcome of 28 patients with 3β-HSD deficiency or Δ^{4}-3-oxoR deficiency are available in the literature. Additionally, literature data on the short- or medium-term treatment with cholic acid of 141 subjects who were either healthy volunteers or affected by hepatic or biliary disorders other than inborn errors of primary bile acid synthesis are reported. While some of the patients described in the submitted case reports have been under medical supervision, they have not
been evaluated in a systematic manner, and the reporting of adverse events is comparable to spontaneous reporting. No clear, pre-defined observation period on which an incidence calculation may be based is available. In some cases, information on the number of patients and the total time of observation is not available.

Very few adverse events under cholic acid treatment are reported in the literature. The only documented adverse reactions to cholic acid 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. All other adverse events that have been reported appear linked to an overdosage of cholic acid, were not serious, reversible and did not interfere with therapy. No patient interrupted or stopped treatment due to adverse events.

The development of gallstones requiring cholecystectomy has been observed 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 cholic acid, has been linked to biliary cholesterol super-saturation and may accelerate gallstone development (Thomas 2008). On the other hand, gallstones also occurred in two other 3β-HSD deficiency patients before any cholic acid treatment. The development of gallstones on cholic acid treatment is considered a potential risk that will be monitored as part of the Risk Management Plan. It is also reported as adverse reaction reported after long-term therapy in section 4.8 of the SmPC.

The adverse events observed after acute overdosage of cholic acid are in line with the safety observations from toxicology studies and with the class effects of bile acids. The principally observed adverse reaction in these cases is diarrhoea and gastrointestinal irritation. Chronic overdose, presumably due to prescription of an excessive dose, was shown to be related to transient increases in serum GGT and transaminase activities. Note that in untreated patients, GGT is low; therefore an increase of GGT is a marker of hepatic damage that allows distinction of a lack of efficacy from treatment-emergent adverse reactions. As all (paediatric) patients appear to require lower doses as they become older, they should be monitored regularly for appropriate metabolic response, and the dose adjusted as required. The SmPC contains a warning 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 SmPC also reflects that more frequent investigations should be undertaken to monitor therapy during periods of fast growth.

The potential risk of increased carcinogenicity associated with the long-term administration of cholic acid is largely outweighed by the increased life expectancy and the avoidance of liver disease.

3.6.2. Conclusions on the clinical safety

The clinical safety of cholic acid in the treatment of 3β-HSD deficiency or Δ4-3-oxoR deficiency, given at appropriate doses appears satisfactory. No serious adverse events have been reported in the literature. No patient interrupted or stopped treatment due to adverse events. The adverse events that have been reported are linked to an overdosage of cholic acid i.e. pruritus and diarrhoea. These events were not serious, reversible and did not interfere with the therapy. Based on the available data no frequency can be calculated. Patients presenting with pruritus and/or persistent diarrhoea should be investigated for a potential overdose by a serum and/or urine bile acid assay. Chronic 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 cholic acid treatment is considered a potential risk that will be monitored as part of the Risk Management Plan.

### 3.7. Pharmacovigilance

**Detailed description of the Pharmacovigilance system**

The CHMP considered that the Pharmacovigilance system as described by the Applicant fulfills the legislative requirements and provides adequate evidence that the Applicant has the services of a qualified person responsible for pharmacovigilance and has the necessary means for the notification of any adverse reaction suspected of occurring either in the Community or in a third country.

**Risk Management Plan**

The MAA submitted a risk management plan, which included a risk minimisation plan.

#### Table Summary of the risk management plan

<table>
<thead>
<tr>
<th>Safety concern</th>
<th>Proposed pharmacovigilance activities (routine and additional)</th>
<th>Proposed risk minimisation activities (routine and additional)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Important identified risks</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prescription of a supratherapeutic dose (MedDRA term: drug toxicity)</td>
<td>Routine pharmacovigilance Patient Surveillance Database</td>
<td>Labelling in Section 4.2 of the SmPC, requiring 3-monthly monitoring during the first year, 6-monthly during the subsequent three years and at least annually thereafter. Restricted prescription for paediatric patients. Educational material through Orphacol Educational Web Site</td>
</tr>
<tr>
<td><strong>Important potential risks</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallstones</td>
<td>Routine pharmacovigilance Patient Surveillance Database</td>
<td>Labelling in Section 4.8 of the SmPC Educational material through Orphacol Educational Web Site</td>
</tr>
<tr>
<td>Carcinogenicity</td>
<td>Routine pharmacovigilance Long-term surveillance through Patient Surveillance Database</td>
<td>None</td>
</tr>
<tr>
<td><strong>Important missing information</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long-term data in large patient population</td>
<td>Routine pharmacovigilance Long-term surveillance through Patient Surveillance Database</td>
<td>Labelling in Section 5.1 of the SmPC</td>
</tr>
<tr>
<td>Medication error in infants and children</td>
<td>Routine pharmacovigilance Exploration of liquid formulation development</td>
<td>Labelling in Section 4.2. and 6.6 of the SmPC</td>
</tr>
</tbody>
</table>

The CHMP, having considered the data submitted in the application, is of the opinion that the following risk minimisation activity is necessary for the safe and effective use of the medicinal product: the MAH, in agreement with the competent authorities in the Member States, shall implement, prior to the launch, an educational programme for physicians aiming to provide educational material on correct diagnosis and therapeutic managements of the treatment of inborn errors in primary bile acid.
synthesis due to 3β-Hydroxy-Δ5-C27-steroid oxidoreductase deficiency or Δ4-3-Oxosteroid-5β-reductase deficiency and to inform on expected and potential risks associated with the treatment. The physician educational programme should contain the following key elements:

- Prescription of a supratherapeutic dose (MedDRA term: drug toxicity)
- Risk of gallstones

**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.

### 3.8. Benefit-Risk Balance

**Benefits**

*Beneficial effects*

There is a consensus in the literature that cholic acid is the treatment of choice for 3β-HSD deficiency. Where available to investigators, its clinical use has been documented since at least the mid-1990s and its use can therefore be considered as well-established. Oral administration of cholic acid, the substance missing in patients with 3β-HSD and Δ4-3-oxoR deficiencies, 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 stopped or even improves. Finally, the administered cholic acid restores a normal enterohepatic circulation and absorption of fat and fat-soluble vitamins occurs at a normal level, resolving growth deficits or outright rickets. This pattern is consistently seen in all patients where detailed or partial data are available.

No medicinal product containing cholic acid as the sole active substance has a marketing authorisation in any European Union member state. Several products containing combinations of cholic acid with other active substances are marketed in a few EU countries. Due to the presence of other active substances or unsuitable strength they are not suitable for the treatment of inborn errors of primary bile acid synthesis due to 3β-HSD deficiency or Δ4-3-oxoR deficiency.

*Uncertainty in the knowledge about the beneficial effects*

Clinical experience has been reported in the literature from small cohorts of patients and single case reports; absolute patient numbers are small due to the rarity of the conditions. This rarity and ethical considerations also made the conduct of controlled clinical studies impossible. Cholic acid treatment results for approximately 60 patients with 3β-HSD deficiency reported in the literature. Detailed long-term treatment data with cholic acid monotherapy are available for 14 patients observed for up to 12.9 years. Cholic acid treatment results for seven patients with Δ4-3-oxoR deficiency for up to 14 years are reported in the literature. Detailed medium- to long-term data are available for 5 of these patients, of whom 1 has been treated with cholic acid monotherapy. The scarcity of these uncontrolled data makes difficult to ascertain the the beneficial effects of cholic acid. However, the CHMP also recognising the limitations to conduct “standard” controlled clinical studies in a rare condition with an ethically acceptable comparator - concludes that the available clinical data presented in this application are
sufficient to support demonstration of efficacy of cholic acid in the two claimed indications, as measured by objective improvement in metabolic and pathologic parameters in patients studied.

As the majority of the case reports come from France (for 3β-HSD Deficiency) and France the UK (for Δ4-3-oxoR Deficiency) the representativeness of the results was discussed. Within the limitations of the small sample size, the diversity of the population described combined with the spectrum of disease severity (showed by the range of dose used) was considered to be sufficiently representative of the EU patient population.

The choice of cholic acid dosage is empirically derived for all patients based on the normalisation of urine and serum bile acids. No dose response study has been conducted. Hence it is not possible to ascertain whether the proposed dosing regimen is optimal in the patient population. Cases of overdosage suggest that the doses, at least initially, were excessive. Nevertheless, it is consistently documented that this therapeutic approach based on the effect of treatment following the use of various dosing regimen showed that patients are controlled satisfactorily over several years. Therefore, it is concluded that the proposed dosing regimen is adequate and in line with the established systematic use of the compound in this rare condition.

**Risks**

**Unfavourable effects**

In general, adverse effects seen with cholic acid therapy appear to be not serious, reversible and are mainly related to elevated hepatic enzymes and appear to be dose-related. Cases of diarrhoea and pruritus have also been reported. 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 cholic acid is an endogenous molecule present in normal human bile, blood and other biological fluids. Once administered cholic acid will behave like an endogenous molecule in all respects. At physiological concentrations, cholic acid is non-toxic, thereby providing a safe dose range for use in human. Substantial knowledge of human cholic acid physiology in healthy subjects as well as patients with chronic liver diseases exists. It should be also noted that cholic acid is used in foods as a food additive (E 1000) and that 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.

**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 demonstrate that the clinical safety of cholic acid in the treatment of 3β-HSD deficiency or Δ4-3-oxoR deficiency, is satisfactory in view of the established safety profile of this endogenous molecule.

It is also recognised that only a few patients described in the submitted case reports had been under medical supervision. These patients appear not to have been evaluated in a systematic manner, and the reporting of adverse events is comparable to spontaneous reporting rather than in a clinical study. No clear pre-defined observation period on which an incidence calculation may be based is available. The CHMP duly considered the known safety profile of cholic acid. The adverse events reported (i.e. pruritus and diarrhoea) were reversible and did not lead to the interruption of the treatment as reported consistently in the literature.
Benefit-Risk Balance

Importance of favourable and unfavourable effects

In spite of the empirical use of bile acids including cholic acid, in 3β-HSD and Δ⁴-3-oxoR deficiencies, no suitable medicinal product is currently available for the affected 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, 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. In addition, blood cholic acid concentration was normal and fat-soluble vitamins were restored to their normal range.

The adverse events reported i.e. pruritus and diarrhoea are linked to an overdosage and reversible. Transient increases transaminase activities have been also observed in chronic overdosage. The development of gallstones requiring cholecystectomy has been observed in a single patient with 3β-Hydroxy-Δ⁴-C₂₇-steroid oxidoreductase deficiency. Overall the safety profile of cholic acid is acceptable.

Benefit-risk balance

Oral cholic acid appears an effective and rather well tolerated long-term treatment for inborn errors of bile acid synthesis due to 3β-HSD deficiency or Δ⁴-3-oxoR deficiency, allowing patients to lead a normal life. The use of cholic acid to treat these conditions has been well established and documented in the literature over a period of almost 20 years through the work primarily conducted by the Jacquemin, Clayton and Setchell groups. Due to the extreme rarity of the diseases as well as the specialised knowledge and the sophisticated tools needed for their diagnosis, the number of cases reported in the literature remains very limited. However, observations demonstrate objectively measurable significant improvements in metabolic and pathologic parameters. While all children diagnosed with 3β-HSD deficiency or Δ⁴-3-oxoR deficiency have died due to liver failure before the introduction of bile acid therapy, all children that have been treated with cholic acid to date are alive, have not needed a liver transplantation (with exceptions of one child each with 3β-HSD deficiency and Δ⁴-3-oxoR deficiency) and are in general good health, leading normal lives. With a single exception, all successful long-term treatment regimes for both disorders have included cholic acid.

While ursodeoxycholic acid and/or chenodeoxycholic acid have been used initially instead of or in combination with cholic acid, there is now a consensus in the literature that cholic acid monotherapy is the most efficacious therapy in 3β-HSD deficiency. In patients with Δ⁴-3-oxoR deficiency combination treatment with ursodeoxycholic acid may be necessary in some patients with to maintain serum liver tests within the normal range and ensure a good histological outcome. It may confer additional benefit in some cases of sub-optimal therapeutic response with cholic acid alone.

The only documented adverse reactions to cholic acid are pruritus and diarrhoea. They may be indicative of an overdose. Nevertheless these events were not serious, reversible and no patient interrupted or stopped treatment due to these events. Treatment with cholic acid at appropriate doses produced no adverse events, as reported consistently in the literature.

Overall, the data provided allows the CHMP to conclude on an acceptable level of safety of cholic acid when used with the recommended dosing regimen and are up to the required standard. 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
surveillance database; these data will be reviewed annually pursuant to article 14(8) of Regulation (EC) No 726/2004.

Overall based on the data presented the benefit-risk of Orphacol is considered positive. The CHMP evaluated the risks due to uncertainties and considers that these risks are not substantial in the light of the demonstrated benefits.

Overall based on the data presented the benefit-risk of Orphacol is considered positive.

3.8.1. Discussion on the benefit-risk balance

The liver disease associated with 3β-HSD and Δ4-3-oxoR deficiencies due to the production of hepatotoxic bile acid precursors is progressive and, if untreated, leads to death due to cirrhosis and liver failure. The only therapeutic option in severely affected cases is liver transplant. Currently there is no causal treatment. Treatment is limited to correcting the biochemical abnormalities, including the administration of bile acids and vitamin preparations. No medicinal product containing cholic acid as sole active substance has a marketing authorisation in the EU.

All children that have been treated with cholic acid to date are alive, have not needed a liver transplantation (with exceptions of one child each with 3β-HSD deficiency and Δ4-3-oxoR deficiency) and are in general good health, leading normal lives. With a single exception, all successful long-term treatment regimes in the literature for both disorders have included cholic acid.

In accordance with Article 22 of Directive 2001/83/EC and 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 condition of use because the indications for which Orphacol are intended are encountered so rarely that he cannot reasonably be expected to provide comprehensive evidence. It would also be contrary to generally accepted principles of medical ethics to collect such information. The CHMP agrees on these justifications and considers that the criteria defined in the above-mentioned provisions are met:

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

The indications for which Orphacol is intended, treatment of inborn errors of primary bile acid synthesis due to 3β-HSD deficiency or Δ4-3-OxoR deficiency, are rarely encountered. Indeed, cholic acid has been designated as Orphan Medicinal Product (EU/3/02/127) by the European Commission for the treatment of inborn errors of primary bile acid synthesis on 18 December 2002. A total maximum of about 90 patients – about 75 patients with 3β-HSD deficiency and approximately 15 patients with Δ4-3-OxoR deficiency – are estimated to live in the European Union, based on the observed incidence over the last 15 years. Given the rarity of the diseases, the CHMP considers that the Applicant cannot be reasonably expected to provide comprehensive nonclinical and clinical evidence. Patients are so rarely identified (e.g. approximately 1 patient/year in France for both indications combined) that conduct of a controlled clinical trial would be unachievable.

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

Acquisition of complete pharmacokinetic information is considered not possible in regards to medical ethics consideration. As a bile acid, cholic acid is subject to highly efficient first-pass hepatic extraction and enterohepatic recirculation. An accurate determination of the absorption kinetics would thus require blood sampling from the portal vein, which is only ethically possible in the context of a surgical
intervention (Angelin et al. 1982). Sampling of peripheral plasma is a suitable method only for relative bioavailability and bioequivalence studies (Setchell et al. 2005), which are not of relevance to this application. A secondary measure is enrichment of the applied bile acid in the duodenal bile, which requires duodenal intubation or the less invasive, but also less reliable, use of the Enterotest string sampling method. Therefore, a pharmacokinetic study in adult healthy volunteers would in principle be possible, but would either be highly invasive, or be fraught with substantial imprecision of the data.

It would be contrary to medical ethics principles to collect evidence of clinical efficacy of cholic acid in the 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. This would be the case in both parallel group and sequential (crossover) studies. A number of studies has 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. The data on the efficacy and safety of cholic acid for the treatment of inborn errors in bile acid synthesis due to 3β-HSD deficiency or Δ⁴-3-oxoR deficiency reported in the literature is compelling, but was in no case generated in the context of a formal clinical study. However, the data contain many elements that would be utilised in a formal clinical study in these very rare disorders, such as those discussed in the CHMP “Guideline on Clinical Trials in Small Populations” (CHMP/EWP/83561/2005). Taken together, the combined evaluation of the single case studies provides significant evidence to support the efficacy and safety of cholic acid.

The Applicant demonstrated that he was unable to provide comprehensive data on the efficacy and safety under normal conditions of use. This is largely because the indications for which Orphacol is intended are encountered so rarely that the Applicant cannot reasonably be expected to provide comprehensive evidence and it would be contrary to medical ethics principles to collect such information. The CHMP endorses that due to the rarity of 3β-HSD deficiency and particularly Δ⁴-3-oxoR deficiency, the provision of comprehensive data, particularly randomised controlled clinical trials is not feasible. The CHMP also endorses 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 sufficiently comprehensive to support demonstration of efficacy and safety of cholic acid in the claimed indications. A marketing authorisation under exceptional circumstances for Orphacol is acceptable in regards to the fulfilled criteria of rarity of the disease and medical ethics.

As specific obligation the Applicant has committed to monitor the safety and efficacy in patients treated with Orphacol from a patient surveillance database for which the protocol has been endorsed by the CHMP and is documented in the RMP. The objectives of this surveillance programme is to monitor accumulating data on efficacy and safety in the treatment of inborn errors in primary bile acid synthesis due to 3β-Hydroxy-Δ⁵-C₂₇-steroid oxidoreductase deficiency or Δ¹-3-Oxosteroid-5β-reductase deficiency with Orphacol in infants, children, adolescents and adults. Reports on recruitment progress of the patient surveillance database will be analysed and reported to the CHMP at the time of PSURs (for safety) and of the Annual Re-assessments (for safety and efficacy). Progress and results from the database will form the basis of the annual reassessments of the benefit/risk profile of Orphacol.

Overall, the safety and efficacy of the product was demonstrated based on the literature data presented and analysed. The Applicant demonstrated that the use of cholic acid for the treatment of 3β-HSD and Δ⁴-3-oxoR deficiencies has been well-established within the Community for at least 10
years, with consistently recognised efficacy and an acceptable level of safety. Due to the rarity of 3β-HSD and Δ⁴-3-oxoR deficiencies, particular attention has been paid to missing information. Justifications have been provided by the applicant, which demonstrate that there is an acceptable level of safety and efficacy can be supported although some studies are lacking (Annex I, Part II, 1.c of Directive 2001/83/EC). These arguments have been agreed to by the CHMP.

The applicant has provided enough evidence to support a positive benefit/risk balance for the medicinal product. Based on the rarity of the disease and ethical considerations, the CHMP concludes that the criteria for a Marketing Authorisation under exceptional circumstances are met (Annex I, Part II, 6. Directive 2001/83/EC).

In conclusion, the CHMP considers that a Marketing Authorisation under exceptional circumstances can be granted subject to the setting of a specific obligation to monitor the safety of the medicinal product.

3.9. Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Orphacol was favourable in the treatment of inborn errors in primary bile acid synthesis due to 3β-Hydroxy-Δ⁵-C27-steroid oxidoreductase deficiency or Δ4-3-Oxosteroid-5β-reductase deficiency in infants, children and adolescents aged 1 month to 18 years and adults and therefore recommended the granting of the marketing authorisation under exceptional circumstances.
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