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Orphacol® Patient Surveillance Database

Twelfth Report

Data Lock Point: 12 September 2025

CONFIDENTIAL

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SIGNATURE PAGE FOR CLINICAL STUDY REPORT

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.

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1 TITLE PAGE

Study title:	Orphacol® Patient Surveillance Database – Twelfth Annual Report
Name of Test Drug:	Orphacol® (cholic acid)
Indication studied:	Treatment of inborn errors in primary bile acid synthesis due to 3β-Hydroxy-Δ^5-C_{27}-steroid oxidoreductase deficiency or Δ^4-3-Oxosteroid 5β-reductase deficiency in infants, children and adolescents aged 1 month to 18 years and adults.
Study description:	Retrospective and prospective collection and evaluation of pathophysiological and treatment data
Sponsors:	Theravia
Clinical Phase:	Not applicable
Study dates:	20 November 2006 to 12 September 2025
Medical Officer:	Virginija Bambalaite, MD
Sponsor signatory:	Audrey Berrebi
GCP Statement:	Compliance with International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) was not possible for this data collection.
Date of report:	V1.0 on 28 October 2025

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2 SYNOPSIS

NAME OF SPONSOR Theravia NAME OF FINISHED PRODUCT Orphacol® NAME OF ACTIVE INGREDIENT Cholic acid	INDIVIDUAL STUDY TABLE REFERRING TO MODULE 5 OF THE CTD Volume: Page:	(FOR NATIONAL AUTHORITY USE ONLY)

Title of Study	Orphacol® Patient Surveillance Database – Twelfth Annual Report	
Study centre(s) with enrolled patients	18 centres: 4 in France, 4 in Germany, 6 in Italy, 1 in Spain, 1 in Switzerland, and 2 in United Kingdom (UK).	
Publication	N/A	
Study period	From: 20 November 2006 To: 12 September 2025	Phase of development: not applicable
Objectives	To increase the amount of available data on the treatment of inborn errors in primary bile acid synthesis due to 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase (3β-HSD) deficiency or Δ^4-3-oxosteroid 5β-reductase (Δ^4-3-oxoR) deficiency with Orphacol® in infants, children, adolescents and adults, and especially data on initial efficacy and safety of treatment with cholic acid (CA).	
Methodology	Retrospective and prospective collection and evaluation of pathophysiological and treatment data.	
Number of patients	A total of 52 patients have been considered in this twelfth report (41 patients with 3β-HSD deficiency and 11 patients with Δ^4-3-oxoR deficiency). Six new patients were enrolled since the previous report.	
Diagnosis and main criteria for inclusion	All patients treated with Orphacol® and having signed an informed consent form can be included in the patient database.	
Test product, dose and mode of administration	Cholic acid hard capsules, 50 mg or 250 mg.	
Duration of treatment	Indefinite. The longest CA treatment duration to the cut-off date is approximately 31 years and 11 months.	
Criteria for evaluation in this report	Primary: the primary criteria for efficacy evaluation were measurement of blood biochemistry parameters, in particular the levels of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT),	

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	gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin and Vitamin E.
Statistical methods	Descriptive statistics were used to describe the included patients. Blood biochemistry parameters were first described using best and worst values (as categorical and then as quantitative variables) in each treatment period. Then, mean values per patient of each blood biochemistry parameters were described in each treatment period, and comparisons between treatment periods using the Wilcoxon's test for paired data were performed (only for patients with 3β-HSD deficiency). Graphical representations (boxplot and individual time-response graphs) were generated.

EFFICACY RESULTS

3β-HSD deficient patients (n = 41)

Patients in this population were almost equally distributed between genders (46.3 % male vs 53.7 % female). A total of 46.3 % of the patients were born after 2005 and 48.8 % treated in French centres. The diagnosis was made in the first year of life for 28.9 % of patients. A further 26.3 % of patients were diagnosed between 1 and 3 years of age, 34.2 % between 3 and 10 years and 10.5 % between 10 and 15 years. At the time of data cut-off, most patients (85.4 %) had been treated with CA for more than 5 years, of whom 41.5 % had been treated for more than 15 years.

All descriptive analyses performed on categorical (using best-to-worst values) and quantitative variables (using patient's mean values) per treatment period showed a general normalisation of values in serum liver functions and Vitamin E, well shown by each parameter boxplot (see Figures 2 to 7 related to Table 21 and Table 22). All parameters tested (ASAT, ALAT, total bilirubin, GGT, ALP and Vitamin E) normalised when CA has been added to UDCA and with CA in monotherapy (during the first and second years of treatment) as well as after the second year.

The descriptive analyses for worst-to-worst data supported the results seen for CA treatment (individual treatment periods and pooled) for ASAT, ALAT, total bilirubin and Vitamin E (but not for GGT or ALP) although less clearly than the best-to-worst analysis.

Besides, all parameters had generally a significant shift towards normal values versus no treatment and versus UDCA (as per Wilcoxon's tests), the latest indicating that CA monotherapy was more

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effective than UDCA monotherapy (results included in Table 21 and Table 22). These multiple comparison tests results must be interpreted with caution considering their high number and the associated increased likelihood of having significant results just by chance.

Individual data displays for five patients were also graphically represented to show the evolution over time (and thus treatment periods) of serum transaminases and bilirubin parameters.

Δ^4-3-oxoR deficient patients (n = 11)

There were more males than females in this population (72.7 % vs 27.3%), more than half of the patients born after 2016 (63.6 %); 44.5 % of them are treated in French centres and the others in Germany and Italy. Their diagnosis was made in the first 6 months of life. At the time of data cut-off, 2 patients had been treated with CA for less than 5 years, 6 patients for 5 to 10 years, 1 patient for 10 to 15 years and 2 patients for more than 20 years.

Descriptive analyses were performed for the first time in patients with Δ^4-3-oxoR deficiency in the eighth report), as the sample size of 8 patients was considered sufficient to allow the analysis. Quantitative and categorical (best-to-worst data) analyses showed comparable results to those observed in patients with 3β-HSD deficiency, namely a normalisation of all parameters tested under CA monotherapy, especially after the first year. These parameters remained stable throughout CA monotherapy.

The descriptive analyses for worst-to-worst data supported the results seen for CA treatment although usually less clearly than the best-to-worst analysis for ASAT, ALP and vitamin E.

Due to the small sample size, no statistical comparison was performed in this population. The inclusion of new patients in the next years could allow such analysis and will reduce the variability of the mean observed for some parameters.

Individual data displays for four patients were also graphically represented to show the evolution of serum transaminases, bilirubin, GGT and ALP parameters over treatment periods.

SAFETY RESULTS

Twenty-five non-serious AEs, one AE reclassified as serious and three AEs for which the seriousness is unknown have been reported during the whole period captured in the database, including five more since the previous report. Among all AEs, two were considered related to Orphacol®, 23 were not related to Orphacol® and four were not assessable. No additional serious

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AEs have been entered in the database since last report, indicating good tolerance of the treatment. The detailed analysis and follow up of these ADRs/AEs are performed by the GRA, PV/MV department and will be summarized in the PSUR.

LIMITATIONS

The main limitation of this study is still its small sample size (in total and per subtypes) preventing any complex data analyses; and its observational and voluntary participation nature further impacting the data quality and the risk of missing data. However, these two drawbacks are quite common in rare diseases context. Although important efforts have been made by the Sponsor the last few years, it remains very challenging to open new centres, especially in certain countries (i.e. UK). Nevertheless, the Sponsor continues to involve as many sites as possible and collect as many data as possible to increase data quality and obtain valid and reliable results on Orphacol® treatment's effectiveness on a representative sample of patients. Efforts have been rewarded and opening of several new centres has been done during the reporting period in countries already participating in ORPHABASE: two in France, one in Italy and one in the UK. Opening of new sites in several countries is ongoing.

CONCLUSION

These results confirm previous observations, and that CA monotherapy is the treatment for patients with 3β-HSD deficiency with an extremely favourable benefit/risk ratio for patients. Although the number of patients with Δ^4-3-oxoR deficiency increases each year, the number of such patients included in the database remains insufficient for statistical analysis. Nevertheless, descriptive analyses showed a fast normalisation of liver parameters and Vitamin E while under CA monotherapy showing that this treatment is effective and safe in this patient population.

The results presented in this twelfth report support the value of the data contained in Orphabase for the detailed evaluation of the effectiveness and safety of Orphacol® therapy in 3β-HSD and Δ^4-3-oxoR deficiencies.

DATE OF THE REPORT: 28 October 2025.

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4 LIST OF ABBREVIATIONS & DEFINITION OF TERMS

3β-HSD	3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase
AEs	Adverse Events
ADRs	Adverse drug reactions
AGEPS	Agence Générale des Equipements et Produits de Santé
ALP	Alkaline phosphatase
ALAT	Alanine aminotransferase
Alk.	Alkaline
AP-HP	Assistance Publique – Hôpitaux de Paris
ASAT	Aspartate aminotransferase
CA	Cholic acid
CDCA	Chenodeoxycholic acid
CNIL	Commission nationale de l'informatique et des libertés
eCRF	Electronic Case Report Form
EM	Educational Material
EU	European Union
FAB-MS	Fast Atom Bombardment/Mass Spectrometry
GC-MS	Gas Chromatography/Mass Spectrometry
GCP	Good Clinical Practices
GDPR	General Data Protection Regulation
GEE	Generalized estimating equations
GGT	Gamma-glutamyl transferase
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
LLN	Lower limit of normal
MAA	Marketing authorisation application
N	Number of samples
PASS	Post-Authorisation Safety Study
PSUR	Periodic safety update report
SD	Standard deviation
SmPC	Summary of product characteristics
TFL	Tables, Figures, Listings
UDCA	Ursodeoxycholic acid
UK	United Kingdom
ULN	Upper limit of normal
yr	Year
Δ^4-3-oxoR	Δ^4-3-oxosteroid 5β-reductase

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5 ETHICS AND REGULATORY APPROVAL

5.1 INDEPENDENT ETHICS COMMITTEE APPROVAL

Relevant approvals of this non interventional Post-Authorisation Safety Study (PASS) were obtained in each involved country according to local regulations. Where relevant, ethics committee and/or data protection committee's approval(s) were thus obtained prior to patient enrolment.

5.2 ETHICAL CONDUCT OF THE STUDY

The study was performed in accordance with the current version of the declaration of Helsinki (75th WMA General Assembly, Helsinki, Finland, October 2024). The study was not planned to be conducted in agreement with the International Conference on Harmonisation (ICH) guidelines on Good Clinical Practice (GCP), due to a number of constraints that apply in this specific situation.

5.3 PATIENT INFORMATION AND CONSENT

All patients provided written consent to participate in the study prior to being enrolled.

The patient information sheet provided the details of the study (aims, methodology, potential risks, and anticipated benefits) and the investigator explained these to each patient. The patient was then allowed sufficient time to consider the information presented before not opposing to his/her data collection or signing and dating the informed consent form (depending on local regulations) to indicate that they fully understood the information, and willingly volunteered to participate in the study. The patient was given a copy of the information note or informed consent form (if applicable) for their information. The original copy of the informed consent was kept in a confidential file in the Investigators centre records. A sample of the patient information sheet and consent form can be found in Section 15.1.5.

Orphacol® is indicated for the treatment of inborn errors in primary bile acid synthesis due to 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase (3β-HSD) deficiency or Δ^4-3-oxosteroid 5β-reductase (Δ^4-3-oxoR) deficiency in infants, children, adolescents aged 1 month to 18 years and adults. Therefore, the patients included in the patient database will include infants, children and adolescents. The parents or guardians of the paediatric patients will sign an informed consent form on behalf of the patient. A new informed consent will be obtained by the physician when paediatric patients become adults. Some local Ethics Committees required the signature of an assent form for adolescent patients, which has been obtained accordingly where necessary.

5.4 REGULATORY APPROVAL

This non-interventional PASS is usually not subject to regulatory approval, but where applicable Theravia (formerly Laboratoires CTRS) has complied with local requirements to these regards. The principle of the study has been approved through the positive opinion of the Committee for Human Medicinal Products of the European Medicines Agency on the Risk Management Plan and the

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Specific Obligations of the Marketing Authorisation for Orphacol®, at the time of Marketing Authorisation for Orphacol® granted by the European Commission.

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Table 1 shows the principal study personnel involved in the study.

Table 1: Study Personnel

Title	Name and affiliation
Sponsor	Theravia
Medical Director	Virginija Bambalaite, Theravia
Sponsor Representative	Audrey Berrebi, Theravia
International Medical Advisor	Célia Montaut, Theravia
Project Manager	Nathalie Hantzperg Buzy-Debat, ICTA PM
Statistician	Edwige Yon, ICTA PM
Data Management	Anne-Marie Crespy, ICTA PM
Medical Writer	Joëlle Castellani, ICTA PM

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7 INTRODUCTION

7.1 TREATMENT OF INBORN ERRORS IN PRIMARY BILE ACID SYNTHESIS DUE TO 3B-HYDROXY-Δ5-C27-STEROID OXIDOREDUCTASE DEFICIENCY OR Δ4-3-OXOSTEROID 5B-REDUCTASE DEFICIENCY

Cholic acid (CA) is a primary bile acid. Primary bile acids are biosynthesised in the liver of healthy mammals and are key constituents of normal bile. Cholic acid is the predominant primary bile acid in human bile.

Orphacol® (CA) is indicated for the treatment of inborn errors in primary bile acid synthesis due to 3β-HSD deficiency or Δ^4-3-oxoR deficiency. Both deficiencies are extremely rare genetic disorders. A study involving 39 centres in 21 countries found that the minimum prevalence could be estimated at 0.99 and 0.14 per 10 million for respectively 3β-HSD deficiency and Δ^4-3-oxoR deficiency (Jahnel et al., 2017). Due to this rarity, the available clinical data on the efficacy and safety of CA in the treatment of 3β-HSD and Δ^4-3-oxoR deficiencies is more limited than for more common diseases.

The enzymes 3β-HSD and Δ^4-3-oxoR are involved in the synthesis of the primary bile acids CA and chenodeoxycholic acid (CDCA) (Subramaniam, Clayton, Portmann, Mieli-Vergani, & Hadzic, 2010). Deficiencies in these enzymes lead to accumulation of their substrates and production of alternative atypical bile acids which cause cholestasis and hepatotoxicity. The liver disease associated with deficiencies due to the production of hepatotoxic bile acid precursors is progressive and, if untreated, leads to death due to cirrhosis and liver failure, usually within the first years of life (Clayton et al., 1987; Gonzales et al., 2009; K. D. R. Setchell, 2004; Sundaram, Bove, Lovell, & Sokol, 2008). Oral administration of CA, the substance missing in an affected patient, replenishes the bile acid pool and stimulates bile flow, thereby facilitating the hepatic clearance of toxic bile acid metabolites and toxic substances, including bilirubin. CA administration also inhibits the production of these hepatotoxic bile acid precursors by down-regulating cholesterol 7α-hydroxylase, the rate limiting enzyme in bile acid synthesis. Furthermore, it will improve growth by facilitating the absorption of fat-soluble vitamins and fat (Gonzales et al., 2009; K. D. R. Setchell, 2004).

The patient population includes infants and toddlers (28 days – 23 months), children (2-11 years), adolescents (12-18 years) and adults (>18 years).

Cholic acid is formulated as 50 mg and 250 mg hard capsules.

The daily dose of CA is 5 to 15 mg/kg in adults and children. In adults, the daily dose should not exceed 500 mg, corresponding to the total amount of primary bile acids synthesised per day in a normal adult human. The dosage must be adjusted for each patient in a specialised unit according to blood and/or urine chromatographic bile acid profiles and liver function tests. Treatment is chronic and, in the absence of other treatment options that may arise in the future, for life.

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7.2 RATIONALE FOR THE STUDY

This patient surveillance database is a Post Authorisation Safety Study (PASS) - a specific obligation of the Marketing Authorisation for Orphacol®, and its protocol is part of the current product Risk Management Plan approved by the European Medicines Agency. Data collected are used as a basis for annual reassessment of the marketing authorisation and compilation of the Periodic Safety Update Reports (PSUR).

For the ongoing surveillance of patients being treated with Orphacol®, Theravia has developed a patient database. This database has two main intended functions: first, as a central tool for all volunteer health care professionals treating patients with Orphacol® to record and access information regarding patient clinical information. In addition, Theravia is providing educational materials on the safety of the product to health care professionals through the web site that serves as portal to the patient database. Second, the database is available to Theravia in order to have access to treatment, safety and efficacy data that can be communicated to regulatory authorities. Such data are, in principle, available from the regular (at least annual) clinical and laboratory investigations in patients, and the database serves to capture this information in a central location. These data are used to further increase the available safety and efficacy data and refine the therapeutic recommendations as required.

This protocol will be continued as long as deemed necessary by the regulatory authorities, Theravia and the participating treating physicians.

8 STUDY OBJECTIVES

The objective of this ongoing surveillance protocol is to increase the amount of available data on the treatment of inborn errors in primary bile acid synthesis due to 3β-HSD deficiency or Δ^4-3-OxoR deficiency with Orphacol® in infants, children, adolescents and adults, especially data on initial efficacy and safety of treatment with CA.

9 INVESTIGATIONAL PLAN

9.1 OVERALL STUDY DESIGN AND PLAN

This is a multi-centre, multi-national, retrospective and prospective observational study to collect data from patients with 3β-HSD and Δ^4-3-oxoR deficiencies receiving Orphacol®. These data originate from hospital and physician's records of the included patients, which were maintained by the treating physicians, and collected in the Orphacol® patient surveillance database. The records are the results of the examinations and laboratory exams that were made on an as-needed and as-scheduled basis. While the database is designed to capture a wide range of possible demographic, pathology and treatment data (including treatment with other bile acids that existed prior to Orphacol® availability) for any given examination point (see Section 9.5.1), not all of the possible data is generated at every examination. In addition, the number of examinations is different for each patient, depending on the development of the diagnosis and the duration of medical treatment. As a result, the data set for every included patient is different.

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This analysis focuses on the results of a set of laboratory parameters indicating hepatic damage, secretory function and nutritional status. The aim of the analyses is to demonstrate the impact of long-term CA treatment on hepatic and secretory functions as well as on nutritional status using descriptive and comparative analyses, and graphical representations.

The impact of CA treatment on the survival of patients with 3β-HSD and Δ^4-3-oxoR deficiencies without the need for a hepatic transplant has previously been demonstrated in the Orphacol® marketing authorisation application (MAA) dossier and is further supported by the long-term follow up data presented here.

9.1.1 STUDY TIMING

This analysis covers the entire period of treatment captured in the Orphacol® patient surveillance database, commencing at the earliest examinations of yet-to-be identified patients, up to and including the twelfth year of the marketing authorisation of Orphacol®. This approach was chosen to present the entire available data set and to set-up a baseline for future investigations into additional patients to be included in the database.

All patients went through different phases of treatment, as described in Section 9.4.3. The respective duration of these phases is shown in Table 4.

As noted in the previous paragraph, data were generated in an unsystematic, unscheduled fashion.

9.1.2 STUDY LOCATION

Data were collected in 18 expert centres at the following locations:

- Centres 001, 002, 003 and 020: France;
- Centre 004: Switzerland;
- Centre 015: Spain;
- Centres 007, 011, 016, 017, 018 and 021: Italy;
- Centres 008, 009, 010 and 014: Germany;
- Centres 012 and 022: United Kingdom (UK).

One more centre (023) in France was opened in which three patients were included on the 11th of September 2025 (the day before the database lock). Therefore, no data were yet entered.

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9.2 Discussion of Study Design

The collected data consists in demographics, treatment and outcome parameters including laboratory tests, hepatic histology, ultrasound investigation and safety (i.e. (serious) adverse events / adverse drug reactions, off-label doses, death). They are collected from the respective patient's hospital records. These data have not been generated in a systematic fashion but following patients' routine care and needs in accordance with the new gained knowledge on the pathologies and their treatment(s). The retrospective data collection concerns patients that have been diagnosed long time ago and treated with other options before they start Orphacol® treatment: either with other CA than Orphacol® or with other medications (like Ursodeoxycholic acid (UDCA), for example), and for whom legacy data is collected from the start of treatment until the date of signature of the ICF (i.e. the majority of the patients enrolled at the time of this twelfth report). The prospective data collection concerns the patients who continue the treatment with Orphacol®, as well as the new patients who start Orphacol® treatment right at the diagnosis and who are included in the database at the very beginning of their treatment, and for whom the data that are collected do not yet exist at the time of signature of the ICF.

For a discussion of the design of the parameters analyses see Section 9.7.

9.3 Selection of Study Population

9.3.1 Inclusion Criteria

All patients treated with Orphacol® and having signed an ICF (or their legal representant for minor patients) or who did not oppose to their data collection (depending on local regulations) could be included in the patient database.

9.3.2 Exclusion Criteria

No exclusion criteria.

9.3.3 Withdrawal of Patients from Therapy or Assessment

Patients were free to withdraw their data from the study at any time without giving a reason. Patients were advised that if they requested to withdraw their data from the study, at any time during the trial, then this would have no negative consequences. It was the treating physician's responsibility to decide if a patient should stop the treatment and document this in the database. When patients withdraw their consent, any collected data not yet analysed at the time of consent withdrawal should be deleted. For data integrity, data already processed for the annual reassessment and/or PSUR are kept in the database.

The data of patient 009001 were not collected anymore since he/she (or his/her legal representative) refused to sign the new ICF version (Consent form version 2.0) in January 2021. Data collected before the consent withdrawal will remain in the database and will be used for analysis (last data collected on 17 June 2019). End date of CA treatment was imputed to 01JAN2021.

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9.4 TREATMENTS

9.4.1 TREATMENTS ADMINISTERED

The patients were treated by the physician under their responsibility and as per the standard of care and the indications of the Orphacol® summary of product characteristics (SmPC).

9.4.2 DESCRIPTION OF INVESTIGATIONAL PRODUCTS

Cholic acid capsules for patients treated at Hôpital Bicêtre were initially (1993 to 1994) received from Cincinnati Children's Hospital Medical Center (Cincinnati, OH, USA). Subsequently (1994 to 2001), CA capsules were prepared by the Hôpital Bicêtre pharmacy. Both were simple preparations containing only CA and lactose in a gelatine hard capsule shell. Production of these capsules was then transferred to the Agence Générale des Equipements et Produits de Santé (AGEPS), the central pharmacy of the Assistance Publique – Hôpitaux de Paris (AP-HP; Nanterre, France), who supplied the patient population treated through Hôpital Bicêtre until October 2007. Production was then transferred to CRID Pharma (subsequently renamed Amatsi; St. Gély du Fesc, France) where adaptations to the formulations for an industrial process were made, including the addition of microcrystalline cellulose and magnesium stearate as excipients. This formulation, in use since October 2007, is identical to Orphacol® and has been used to supply patients in France and Switzerland under named-patient programme ("ATU – Autorisation Temporaire d'Utilisation") until the product received marketing authorisation. Orphacol® was approved on 13 September 2013 in the European Union under exceptional circumstances.

Marketed UDCA formulations were used in a number of patients, including but not limited to Delursan® 125 mg film-coated tablets (Aptalis Pharma, France), Ursolvan® 200 mg, hard capsules (Sanofi-Aventis France), Arsacol® 150 mg tablets (Zambon) in France and Ursofalk® 250 mg/5 ml oral suspension (Dr. Falk Pharma) in Switzerland and France.

9.4.3 METHOD OF ASSIGNING PATIENTS TO TREATMENT GROUPS

No formal treatment groups were established. However, many patients went through different phases of observation according to treatment's absence or types as decided by the treating physician. These phases also reflect the development of the approach to treating 3β-HSD and Δ^4-3-OxoR deficiencies. Patients may have gone through a part of or all of the following phases of treatment:

1. No treatment (before diagnosis)
2. UDCA (initial/emergency treatment)
3. CA and UDCA (standard treatment in the early- to mid-1990s)
4. CA (standard treatment since the mid-1990s); including patients treated with Orphacol®.

9.4.4 SELECTION OF DOSES IN THE STUDY

CA dose selection was individual for each patient (see Section 9.4.5) and was based according to the weight of the patients and the laboratory results at the different timepoints.

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UDCA was used without any dose optimisation, at doses between 100 mg and 600 mg/day, the dose being generally related to the age of the patient.

9.4.5 SELECTION AND TIMING OF DOSE FOR INDIVIDUAL PATIENTS

CA dose selection was individual for each patient based on urine bile acid analysis by gas chromatography coupled to mass spectroscopic (GC-MS) methods (or other suitable analytical techniques) and liver function tests. The target dose was between 5 and 15 mg/kg. An upper limit of 500 mg CA per day is corresponding to the total amount of primary bile acids synthesised per day in a normal adult human and is considered in the SmPC as a maximum recommended daily dose. CA capsules were administered following instructions in the SmPC.

9.4.6 PRIOR AND CONCOMITANT THERAPY

Prior and concomitant therapy was noted from patient records, when available. No formal limitations on concomitant therapy existed up to the MAA for Orphacol®, when a contraindication for concomitant use of phenobarbital was applied. This contraindication has been extended to primidone in May 2022.

9.4.7 TREATMENT COMPLIANCE

There is no direct means to determine treatment compliance from the available data due to a lack of treatment accountability or any adherence data, except when it is reported as a comment by the investigator.

9.5 EFFICACY AND SAFETY VARIABLES

9.5.1 EFFICACY AND SAFETY MEASUREMENTS ASSESSED

The following variables can be captured in the database for every investigation. However, not all data are available for every data point, as they were not all systematically investigated by the treating physicians. Note that this report focusses only on the liver-related laboratory parameters and Vitamin E as a laboratory marker of nutritional status.

Demographic and familial data:

1. Sex
2. Date of birth
3. Number of siblings living (today)
4. Number of siblings who have died, and those who have probably died from the same disease
5. Number of living siblings with the same disease
6. Parental consanguinity

Diagnostic Information:

1. Date
   Age (yyyy/mm)

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Height (cm)
Weight (kg)

2. Results:
Fast Atom Bombardment/Mass Spectrometry (FAB-MS) and/or GC-MS or any other suitable analytical technique to detect abnormal bile acid metabolites
If available: disease gene sequence data

3. Kind of Deficiency: [3β HSD], [Δ^4-3-oxoR]

4. Clinical Examination
   a) Icterus (jaundice)
   b) Pruritus
   c) Steatorrhea
   d) Ascites
   e) Hepatomegaly
   f) Splenomegaly
   g) Rickets

<u>Functional signs at diagnosis and after:</u>

- Blood Biochemistry:
  - Aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), bilirubin (total), serum-conjugated bilirubin, total cholesterol, triglycerides, Vitamin A, Vitamin D, Vitamin E, Vitamin K, bile acids (total)
  - Oncological exploration: serum alpha-fetoprotein
  - Serum iron
  - 25-OH serum Vitamin D
  - Cytology-blood cell count
  - Haemostasis: fibrinogen, quick time, Factor 11, Factor VII +X, Factor V

  Bile acids in serum
  - Primary bile acids: CA, chenodeoxycholic acid
  - Secondary bile acids: deoxycholic acid, lithocholic acid, UDCA, total biliary acid, ratio cholate/chenodeoxycholate

- Urinary Biochemistry
  - Creatinine

  Bile acids in urine
  - Primary bile acids: CA, chenodeoxycholic acid
  - Secondary bile acids: deoxycholic acid, lithocholic acid, UDCA, hyodeoxycholic acid, hyocholic acid, total secondary bile acids

<u>Histology / Medical imaging at diagnosis and after:</u>

- Abdominal scan

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Liver
Spleen
Hepatosplenomegaly
Adenopathy

- Abdominal ultrasound
  Liver
  Gallbladder
  Dilated bile ducts
  Hilum
  Spleen
  Kidneys
- Cardiac Doppler
- Excrement investigation
- Liver biopsy

Treatment and others

- CA: posology
- Associated or simultaneous treatment: drug and posology
- Other investigations or observations
- Adverse events

This report focuses on the data obtained in blood biochemistry, in particular regarding liver function and nutritional status, in relation to the phase of treatment.

9.5.2 APPROPRIATENESS OF MEASUREMENTS

The measurements capture all aspects of the pathology of 3β-HSD and Δ^4-3-oxoR deficiencies as described in the clinical literature (Clayton et al., 1987; Gonzales, 2006; Gonzales et al., 2009; K. D. Setchell et al., 1988). A number of more indirectly related factors can also be captured (see also Section 13).

The evaluated measurements allow for a quantitative assessment of the patient status in terms of hepatic function and damage through ASAT, ALAT, bilirubin, ALP and GGT values (Rigato, 2007). It should be noted that serum ALP can be confounded in children by bone growth and by the possibility of comorbid rickets in untreated patients.

Vitamin E provides a marker of the absorptions of fat and fat-soluble vitamins from food, which is impaired in the absence of primary bile acids.

It would have been desirable to include hepatic histological data and urine and/or serum bile acids measurements, but few data points are available, and evaluation of urine bile acids over the entire period was not possible due to limited availability of detailed values in the patient records.

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9.6 DATA QUALITY ASSURANCE

Primary data were obtained from the patient's hospital records. Data were entered into the electronic database (eCRF) directly by the site staff or contractor (in reports n°1-5, it was partially entered by Sponsor staff for the French sites, but this process has been stopped since the sixth report with the contract of a CRO for such activity). Queries on unclear data were addressed with the treating physician.

9.7 STATISTICAL METHODS PLANNED IN THE PROTOCOL AND DETERMINATION OF SAMPLE SIZE

9.7.1 STATISTICAL AND ANALYTICAL PLANS

Considering the context of two rare diseases and thus two small sample sizes; it has been decided since the fifth annual report from 2018 to focus on descriptive statistics and graphical representations of all biological parameters of interest in order to facilitate understanding of Orphacol® treatment's efficacy. Tests of comparisons per treatment period have been nonetheless kept for information, but the exact test procedure was changed to take into account patients' repeated measures (i.e. Wilcoxon's test instead of non-paired Student t-test).

Definition of treatment periods

All data (socio-demographic data, laboratory investigations, treatment intakes) were recorded into the eCRF. The following treatment periods were generated using start and end dates of treatment by UDCA and CA:

- No treatment (i.e., before initiation of any treatment [calculated from birth of patient until first treatment], when there are gaps between treatments, or after liver transplantation);
- UDCA;
- UDCA+CA, when CA has been added to UDCA treatment;
- CA first year, defined as the first year of intake of only CA treatment (i.e., without combination with UDCA);
- CA second year, defined as the second year of intake of only CA treatment;
- CA after second year, defined as the years after the second year of intake of only CA treatment;
- CA monotherapy (or CA pooled), defined as the pooled of CA first year, CA second year and CA after second year.

Thus, each laboratory investigation (identified by a date of investigation) could be classified in a treatment period.

Categorization of values for blood biochemistry parameters

For each treatment period, the best and the worst values of ALAT, ASAT, GGT, total bilirubin and ALP were classified as:

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- Normal;
- >1 to 2-fold the upper limit of normal (ULN);
- >2 to 3-fold ULN;
- >3-fold ULN.

For each treatment period, the best and the worst values of vitamin E were classified as:

- Below the lower limit of normal (LLN);
- Normal;
- Above ULN.

Additionally, because of the small sample size of each modality mentioned above, the following classification was also used for each parameter:

- Normal;
- Abnormal (combination of the following modalities: >1 to 2-fold ULN, >2 to 3-fold ULN and >3-fold ULN; or below the LLN and above the ULN for vitamin E).

Normal values that are used are those from the reference Centre 001 with some exceptions (patients 002001, 002002, 002003, 002004, 004002 and 007001; details are provided in Appendix 15.1.2) and are based on their experience in the disease area since no consensual criteria currently exist.

Statistical analysis

Demographic and other baseline characteristics were described according to the type of deficiency (3β-HSD / Δ^4-3-oxoR).

For each blood biochemistry parameter, the categorical values were described in each treatment period, according to the type of deficiency. Indeed, since last year report, the number of patients with Δ^4-3-oxoR deficiency has reached a total of 9, which was considered as sufficient to perform descriptive analyses.

Considering all values, the mean value per patient was calculated for each blood biochemistry parameter and for each treatment period. These mean values were described in each treatment period according to the type of deficiency.

Moreover, comparisons of the mean values between each treatment period (UDCA, UDCA+CA, CA first year, CA second year, CA after second year and CA pooled vs no treatment and UDCA) were performed using the Wilcoxon's test for paired data at level 0.05, only for patients with 3β-HSD deficiency.

In addition, boxplots and individual time-response graphs were generated for illustration purposes. Normal values are represented in each boxplot and graphs to facilitate their visual analysis.

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9.7.2 DETERMINATION OF SAMPLE SIZE

Due to the rarity of the indications, no formal sample size could actually be calculated. It is expected that the number of inclusions will not exceed 100 patients.

9.8 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

9.8.1 PROTOCOL AMENDMENTS

The Sponsor's name has been changed from Laboratoire CTRS to Theravia in the study protocol in 2024. No other amendments have been performed since 2024.

9.8.2 CRF AMENDMENTS

The eCRF that was deployed since 2010 for the capture of the patients' data became obsolete (i.e., updates are not supported any longer by the navigation system Internet Explorer) and since the General Data Protection Regulation (GDPR) in force since 25 May 2018 imposed more restrictive constraints in terms of data security, it was decided in 2023 to deploy a new eCRF.

This new eCRF is fully compliant with the GDPR, is compatible with the new navigation system and includes features that facilitate the centres' use and the Sponsor's monitoring of the data.

The data captured by the centres in the previous eCRF have been re-entered and quality controlled by ICTA between May and June 2024, before being released for centres' use on 01 July 2024. The data recaptured by ICTA as well as new data recorded by the centres after the new eCRF release, have been electronically signed by the investigators.

9.8.3 DATA ENTRY

Data entry has been performed directly by the physician, delegated person at the centre or a contractor, but should always be checked/validated by the principal investigator of the site. Data that could not be signed by the investigators in time for this analysis have not been used for this analysis.

9.8.4 DATA ANALYSES

The Statistical Analysis Plan has been slightly modified since the previous report. A version 8.0 has been issued in which conversion factor following the change of eCRF and the possibility to enter the data in different units have been introduced.

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10 STUDY POPULATION

10.1 DISPOSITION OF PATIENTS

As of 12 September 2025, 52 patients have been included in the study database and considered in the statistical analysis of this twelfth report (see Figure 1). Compared to last year's report, 4 new patients with 3β-HSD deficiency and 2 with Δ4-3-oxoR deficiency were included.

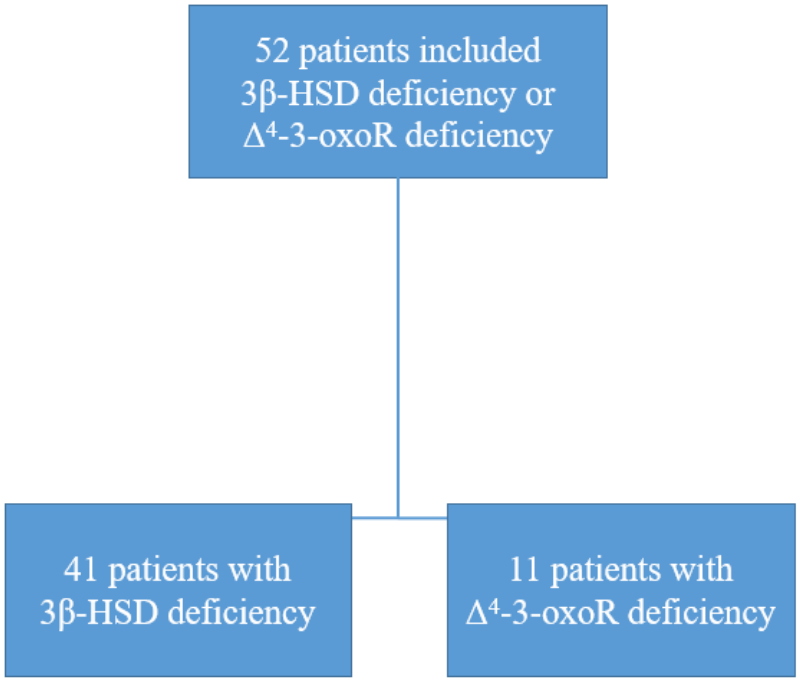


Figure 1: Orphacol® Patient Database – Patient Disposition for Evaluation

Notes: 3β-HSD: 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase; Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase.
Source: Figure 1.1 – Tables, Figures, Listings output (TFL) V1.0 24OCT2025.

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No patients have been lost to follow-up according to the treating physicians. However, the last available data points are variable, depending essentially on the visits of the patients captured in the hospital records (Table 2).

Table 2: Years of Last Data Point (N = 52)

Year of last data point	Number of Patients Report 1	Number of Patients Report 2	Number of Patients Report 3	Number of Patients Report 4	Number of Patients Report 5	Number of Patients Report 6	Number of Patients Report 7	Number of Patients Report 8	Number of Patients Report 9	Number of Patients Report 10	Number of Patients Report 11	Number of Patients Report 12
2008	4	–	–	–	–	–	–	–	–	–	–	-
2009	3	–	–	–	–	–	–	–	–	–	–	-
2010	1	–	–	–	–	–	–	–	–	–	–	-
2013	3	1	1	–	–	–	–	–	–	–	–	-
2014	11	10	7	2	–	–	–	–	–	–	–	-
2015	NA	12	8	6	1	1	1	–	–	–	–	-
2016	NA	NA	7	5	5	1	2	2	2	1	1	1
2017	NA	NA	NA	10	9	3	1	1	1	–	–	-
2018	NA	NA	NA	NA	7	4	2	1	1	1	–	-
2019	NA	NA	NA	NA	NA	18	10	5	3	2	1	1
2020	NA	NA	NA	NA	NA	NA	19	8	1	–	–	-
2021	NA	NA	NA	NA	NA	NA	NA	22	11	2	1	-
2022	NA	NA	NA	NA	NA	NA	NA	NA	25	19	6	6
2023	NA	NA	NA	NA	NA	NA	NA	NA	NA	21	11	6
2024	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	26	14
2025	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	24

Note: NA: Not applicable. Source: adapted from Table 1.2 - TFL V1.0 24OCT2025.

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10.2 TREATMENT DURATION

Durations of observation under the different treatment modalities are displayed in Table 4 in an individual fashion. Since the fifth report, patients are identified by the identification codes as used in the Orphacol® study database and not anymore as it was in the MAA dossier, where applicable. See Section 15.1.1, a table provides the correspondence between these two types of identification.

The treatment sequence was as follows:

Table 3: Treatment sequence

Treatment sequence	Number of patients
From no treatment to CA monotherapy	27 patients
From no treatment to UDCA monotherapy to CA monotherapy	11 patients
From no treatment to UCDA monotherapy, came back to no treatment, took UCDA monotherapy again and then started CA monotherapy	1 patient (004002)
From no treatment to UDCA monotherapy, then started UDCA+CA combination therapy, and went back to no treatment after liver transplantation	1 patient
From no treatment to UDCA+CA combination therapy to CA monotherapy	1 patient (001004)
From combination of chenodeoxycholic acid (CDCA) alone before being under CA	1 patient (004001)*
From CDCA and UDCA before being under CA alone	1 patient (008001)*
All treatment period (no treatment, UDCA monotherapy, UDCA+CA combination therapy, CA monotherapy)	9 patients

* These two patients are currently the only patients for whom CDCA/UDCA combination or CDCA alone are known, which present insufficient data for an analysis of CDCA/UDCA and CDCA alone treatment periods. For this year's report, it has been decided to not consider biochemistry parameters' results obtained during CDCA treatment periods.

The longest treatment durations were generally with CA monotherapy. Patient 001005 with Δ^4-3-oxoR deficiency returned to UDCA+CA dual therapy after increases of transaminases on CA monotherapy. As the level of unusual metabolites in urine was pretty high, the treating physician decided to decrease the dose of CA and add UDCA. In this way the decrease of urinary metabolites has been achieved successfully, and the patient stayed on this combination therapy.

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The longest CA treatment duration (including a period of combined UDCA+CA therapy), reported for 6 patients, was from 27, 28, 29 or 30 October 1993 to 12 September 2025, which corresponded to approximately 31 years and 11 months. However, for patient 001001, we did not have any new information entered since October 2016 (this patient is currently treated outside France) but we assumed that (s)he is still on treatment. Finally, patient 018001 with Δ^4-3-oxoR was not on treatment anymore since May 7th, 2023, because he underwent a liver transplant. The transplantation was not related to treatment but was performed because the patient experienced a sepsis that had led to deteriorating liver function.

Table 4: Durations of Treatment Period per Patient (Years) (N = 52)

Patient identifier	No treatment	UDCA only	UDCA + CA	CDCA only or UDCA + CDCA	CA only
Patients with 3ß-HSD deficiency					
001001	4.2	3.6	0.5	.	31.3
001002	2.3	0.2	4.4	.	24.8
001003	2.2	0.2	.	.	29.7
001006	0.2	0.5	0.5	.	27.5
001007	3.2	1.1	0.6	.	31.3
001008	0.5	0.2	.	.	22.4
001009	0.2	.	.	.	23.3
001010	4.7	0.5	.	.	24.0
001013	1.1	0.1	.	.	2.1
002001	4.3	.	.	.	19.7
002002	0.3	.	.	.	18.5
002003	15.8	.	.	.	17.4
002004	0.4	0.2	.	.	17.7
002005	1.3	.	.	.	7.6
002006	1.2	.	.	.	5.6
003001	11.5	0.2	.	.	29.2
003002	2.5	1.5	0.5	.	31.4
003003	10.3	2.8	0.5	.	31.4
003004	4.1	1.1	0.5	.	31.4
003005	3.6	0.8	0.7	.	31.2
004001 (a)	5.3	.	.	11.0	8.0
004002	1.9	0.9	.	.	12.8
004003	1.3	.	.	.	1.9
007001	8.7	.	.	.	5.2
008001 (b)	8.4	.	.	10.9	9.9
009001	9.5	.	.	.	5.7
011001	6.2	.	.	.	9.7

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Patient identifier	No treatment	UDCA only	UDCA + CA	CDCA only or UDCA + CDCA	CA only
012001	5.0	.	.	.	9.8
012002	0.5	.	.	.	9.9
015001	11.5	.	.	.	7.8
015002	12.1	.	.	.	7.1
016001	11.7	.	.	.	4.9
016002	6.3	.	.	.	5.7
017002	17.7	.	.	.	7.0
017003	22.0	.	.	.	7.0
017004	19.9	.	.	.	7.0
017005	16.5	.	.	.	6.9
017006	11.7	.	.	.	6.9
018002	2.9	.	.	.	2.1
020002	0.8	0.3	.	.	4.9
022001	0.9	.	.	.	2.6
Patients with Δ^4-3-oxoR deficiency					
001004	0.7	.	1.2	.	26.7
001005	0.4	0.3	26.9	.	1.0
001011	0.3	0.2	.	.	12.6
001012	0.6	.	.	.	6.2
010001	0.2	0.3	.	.	8.4
010002	0.2	2.4	.	.	6.2
014001	0.2	.	.	.	8.6
017001	0.7	.	.	.	8.4
018001	0.2	0.1	0.2	.	.
020001	11.6	1.5	7.8	.	.
021001	0.2	0.0	.	.	2.6

Notes: 3β-HSD:3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase; Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase; CA: Cholic acid; UDCA: Ursodeoxycholic acid. Source: Listing 1.1 - TFL V1.0 24OCT2025.

Patient 018-001 has been transplanted, so he is not on treatment anymore. The time post-transplantation was not taken into account in the 'No treatment' period.

For patient 021-001, the duration of “UDCA only” period is 0.04 year (i.e. 2 weeks). Because of rounding, the duration is put to 0.0 in the listing.

(a) and (b): These patients received CDCA only (004001) or CDCA + UDCA (008001):

(a): 11/09/2006-01/09/2017 = CDCA; 01/09/2017- on going = CA only.

(b): 06/12/2004-11/09/2010 = CDCA + UDCA; 25/09/2010-29/10/2015 = CDCA + UDCA; 30/10/2015-on going = CA only.

Since they are the only patients in this situation, it has been decided to not consider biochemistry parameters’ results obtained during CDCA treatment periods. If upcoming new patient(s) present with the same pathway, a new category will be created.

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10.3 TREATMENT DOSAGE

A total of 39.0 % of patients with 3β-HSD deficiency received a CA dose between more than 250 mg/day and up to 450 mg/day (Table 5). Only 29.3 % received a CA dose up to 250 mg/day and 31.8 % received a CA dose of more than 450 mg/day. In average, patients with 3β-HSD deficiency and aged between 2 and 11 years old received a mean CA dose of 165.0 (± 89.9) mg/day, those aged between 12 and 17 years, a CA dose of 358.3 (± 86.1) mg/day, and patients of at least 18 years old, a CA dose of 425.9 (± 143.0) mg/day (Table 6).

Table 5. Current doses (in classes) according to the type of deficiency on the 12SEP2025 - Analysis population (N=52)

		3ß-HSD (n = 41)	Δ^4-3-oxoR (n =11)	Total (N = 52)
Current doses (mg/day) (in classes)	[50 – 100]	2 (4.9%)	2 (18.2%)	4 (7.7%)
	]100 – 250]	10 (24.4%)	5 (45.5%)	15 (28.8%)
	]250 – 450]	16 (39.0%)	1 (9.1%)	17 (32.7%)
	]450 – 500]	9 (22.0%)	2 (18.2%)	11 (21.2%)
	] 500	4 (9.8%)	1 (9.1%)	5 (9.6%)

Notes: Patients with current doses > 500 mg/day on the 12SEP2025 are the following: 002-001, 002-002, 002-004, 003-005 and 020-001.
Source: Table 3.3 - TFL V1.0 24OCT2025.

Table 6. Current doses according to the age on the 12SEP2025 in patients with 3ß-HSD deficiency - Analysis population (N=41)

		2 - 11 years (N = 8)	12 - 17 years (N = 6)	≥ 18 years (N = 27)	Total (N = 41)
Current doses (mg/day)	Mean ± SD	165.0 ± 89.9	358.3 ± 86.1	425.9 ± 143.0	365.1 ± 161.7
	Median	150.0	350.0	450.0	350.0
	Q1 ; Q3	110.0 ; 225.0	300.0 ; 400.0	300.0 ; 500.0	250.0 ; 500.0
	Min ; Max	50 ; 300	250 ; 500	150 ; 750	50 ; 750

Source: Table 3.1 - TFL V1.0 24OCT2025.

For patients with Δ^4-3-oxoR deficiency, more than half (63.7 %) received a CA dose up to 250 mg/day (Table 5). One patient (020-001) received more than 500 mg/day. In average, the patient aged between 2 and 11 years old received a CA dose of 171.4.0 (± 103.5) mg/day, the patient aged between 12 and 17 years, a CA dose of 250 mg/day and finally the three patients of at least 18 years old, a CA dose of 583.3 (± 144.3) mg/day (Table 7).

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Table 7. Current doses according to the age on the 12SEP2025 in patients with Δ4-3-oxoR deficiency - Analysis population (N=11)

		2 – 11 years (N = 7)	12 - 17 years (N = 1)	≥ 18 years (N = 3)	Total (N = 11)
Current doses (mg/day)	Mean ± SD	171.4 ± 103.5	250.0	583.3 ± 144.3	290.9 ± 215.4
	Median	200.0	250.0	500.0	200.0
	Q1 ; Q3	50.0 ; 200.0	250.0 ; 250.0	500.0 ; 750.0	150.0 ; 500.0
	Min ; Max	50 ; 350	250 ; 250	500 ; 750	50 ; 750

Source: Table 3.2 - TFL V1.0 24OCT2025.

In addition, it has to be noted that four patients with 3β-HSD deficiency and one patient with Δ^4-3-oxoR deficiency received a CA dose of more than 500 mg/day (Table 5). In fact, in the sixth report, it has been reported a dose of 600 mg/day of CA for two patients at site 002, since December 2015 (patient 002001) and September 2018 (patient 002002). From July 2021 for the patient 002002 the daily CA dose has been increased to 700 mg/day. In 2022, one patient at site 003 (patient 003005) received a dose of 550 mg/day (still ongoing). Since July 2023 and Octobre 2024, two patients at sites 020 and 002 respectively, received a dose of 750 mg/day (patients 020001 and 002004). These are not in line with recommended dosing instructions presented in Orphacol® SmPC. No adverse effects have been reported for those five patients, so far. Besides these cases discussed above, to the Sponsor knowledge, CA capsules were administered following instructions in the SmPC.

10.4 Protocol Deviations

During the reporting period, there were no protocol deviations.

11 RESULTS

This report focuses on the data obtained in blood biochemistry, in particular regarding liver function and nutritional status (Vitamin E), in relation to each treatment period.

11.1 Data Sets Analysed

Data from 52 patients were included in the analyses, 41 of whom had 3β-HSD deficiency and 11 had Δ^4-3-oxoR deficiency as shown in Figure 1. This patient population represents almost 61 % of the known population in European countries (EU) and UK with hereditary defects of primary bile acid synthesis due to 3β-HSD or Δ^4-3-oxoR deficiency.

Compared to the previous report, the data set in the present report differs as follows: in total, data from 85 additional visits were integrated into the database (i.e. 34 more visits than during the previous period), raising the total number of visits to 999. New additional data points include 46 ‘old’ visits (i.e., which happened before 12 September 2024) and 39 ‘new’ visits (i.e., which happened between 12 September 2024 and 12 September 2025). In total, 40 laboratory measurements were added between 12 September 2024 and 12 September 2025.

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11.2 Demographic and Other Baseline Characteristics

Demographic details of all analysed patients are shown in Table 8.

For the 41 patients with 3β-HSD deficiency:

- The population was almost equally distributed between genders (46.3 % male vs 53.7 % female).
- A total of 46.3 % of the patients were born after 2005 and the remaining between the years 1980 and 2000.
- Country repartition was as follows:
  - 21 patients (51.2 %) were treated in French centres (centres 001, 002, 003 and 020),
  - 10 patients (24.4 %) in Italy (centres 007, 011, 016, 017 and 018),
  - 2 patients (4.9 %) in Germany (centres 008 and 009),
  - 3 patients in Switzerland (centre 004),
  - 2 patients in Spain (centre 015),
  - 3 patients in UK (centres 012 and 022).
- Diagnosis was made:
  - 29.0% of patients in the first year of life;
  - 26.3 % between 1 and 3 years of old;
  - 34.2 % between 3 and 10 years old;
  - 10.5 % between 10 and 15 years old.
- At the time of data cut-off, 35 patients (85.4 %) had been treated with CA (alone or in combination with UDCA) for more than 5 years, of whom 17 (41.5 %) had been treated for more than 15 years.

For the 11 patients with Δ^4-3-oxoR deficiency:

- There were more males than females (72.7 % vs 27.3 %).
- Most patients (63.7 %) were born after 2016 and the remaining between the years 1996 and 2015.
- Country repartition was as follows:
  - 5 patients (45.5 %) were treated in France (centres 001 and 020),
  - 3 patients (27.3 %) in Germany (centres 010 and 014),
  - 3 patients (27.3 %) in Italy (centres 017, 018 and 021).
- Diagnosis was made in the first 6 months of life for all patients except for one who was diagnosed between 10 and 15 years.
- At the time of data cut-off, 2 patients had been treated with CA (alone or in combination with UDCA) for less than 5 years, 6 patients for 5 to 10 years, 1 patient for 10 to 15 years and 2 patients for more than 20 years.

These baseline characteristics are identical to what was reported last year.

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Table 8: Demographic Details of all Included Patients in Total and by type of deficiency (N = 52).

		3ß-HSD (n = 41)	Δ^4-3-oxoR (n = 11)	Total (N = 52)
Year of birth	1980-1985	2 (4.9)	-	2 (3.8)
	1986-1990	5 (12.2)	-	5 (9.6)
	1991-1995	3 (7.3)	-	3 (5.8)
	1996-2000	5 (12.2)	2 (18.2)	7 (13.5)
	2001-2005	7 (17.1)	1 (9.1)	8 (15.4)
	2006-2010	9 (22.0)	-	9 (17.3)
	2011-2015	3 (7.3)	1 (9.1)	4 (7.7)
	2016-2020	4 (9.8)	5 (45.5)	9 (17.3)
	≥ 2021	3 (7.3)	2 (18.2)	5 (9.6)
Gender	Male	19 (46.3%)	8 (72.7%)	27 (51.9%)
	Female	22 (53.7%)	3 (27.3%)	25 (48.1%)
Treatment centre*	Centre 001	9 (22.0)	4 (36.4)	13 (25.0)
	Centre 002	6 (14.6)	-	6 (11.5)
	Centre 003	5 (12.2)	-	5 (9.6)
	Centre 004	3 (7.3)	-	3 (5.8)
	Centre 007	1 (2.4)	-	1 (1.9)
	Centre 008	1 (2.4)	-	1 (1.9)
	Centre 009	1 (2.4)	-	1 (1.9)
	Centre 010	-	2 (18.2)	2 (3.8)
	Centre 011	1 (2.4)	-	1 (1.9)
	Centre 012	2 (4.9)	-	2 (3.8)
	Centre 014	-	1 (9.1)	1 (1.9)
	Centre 015	2 (4.9)	-	2 (3.8)
	Centre 016	2 (4.9)	-	2 (3.8)
	Centre 017	5 (12.2)	1 (9.1)	6 (11.5)
	Centre 018	1 (2.4)	1 (9.1)	2 (3.8)
	Centre 020	1 (2.4)	1 (9.1)	2 (3.8)
	Centre 021	-	1 (9.1)	1 (1.9)
	Centre 022	1 (2.4)	-	1 (1.9)
Age at diagnosis (classes)	Missing values	3**	-	3
	<3 months	4 (10.5)	3 (27.3)	7 (14.3)
	[3 months;6 months]	2 (5.3)	7 (63.6)	9 (18.4)
	]6 months;12 months]	5 (13.2)	-	5 (10.2)
	]12 months;36 months]	10 (26.3)	-	10 (20.4)
	]3 years;10 years]	13 (34.2)	-	13 (26.5)
	]10 years;15 years]	4 (10.5)	1 (9.1)	5 (10.2)

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		3ß-HSD (n = 41)	Δ⁴-3-oxoR (n = 11)	Total (N = 52)
CA treatment duration (classes)	≤1 year	-	1 (9.1)	1 (1.9)
	]1 year;2 years]	1 (2.4)	-	1 (1.9)
	]2 years;3 years]	3 (7.3)	1 (9.1)	4 (7.7)
	]3 years;5 years]	2 (4.9)	-	2 (3.8)
	]5 years;10 years]	17 (41.5)	6 (54.5)	23 (44.2)
	]10 years;15 years]	1 (2.4)	1 (9.1)	2 (3.8)
	]15 years;20 years]	4 (9.8)	-	4 (7.7)
	>20 years	13 (31.7)	2 (18.2)	15 (28.8)

Notes: Data are presented as n(%). 3β-HSD: 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase; Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase. CA: Cholic acid.
* List of initiated sites but no recruitment performed: sites 006, 013 and 019. In the site 023, 3 new patients signed ICF but data were not entered yet.
** For patients 002001, 002003 and 017002, the date of diagnosis was missing in their medical records and confirmed as missing by the investigator.
Source: Table 2.1 - TFL V1.0 24OCT2025.

11.3 Measurements of Treatment Compliance

Measurement of treatment compliance from CA consumption was not possible. Poor intermittent compliance has been only reported for 2 patients (001002 and 003002).

11.4 Blood Biochemistry

11.4.1 Handling of Dropouts or Missing Data

One patient (009001) refused to sign the new ICF version in January 2021. Therefore, his/her data stopped being collected after 01JAN2021. Data collected before that date remain in the database. There have been no dropouts.

Data that were missing have not been replaced since we are in the context of routine care visits. However, with the new eCRF, the investigators can indicate if some investigations have not been performed. This should thus increase data accuracy, even if some data will be still missing due to the context of routine care follow-up.

11.4.2 Patients with 3β-HSD Deficiency

11.4.2.1 Descriptive Analyses on Categorical Parameters

Patients generally underwent treatment in a progression based on historical clinical observations (Gonzales et al., 2009). Following no treatment, patients were generally treated with UDCA, followed by UDCA+CA dual therapy, then CA monotherapy. By contrast newly diagnosed patients are more likely to move from no treatment to treatment by CA as a monotherapy.

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The number of visits and blood biochemistry parameters measures were depending on the development of the clinical condition of the patient, the duration of the treatment, the need for dose adjustments as well as on other factors. Therefore, patients could have had multiple clinic visits during any treatment period, and potentially multiple measures of blood biochemistry parameters per period. In these categorical analyses of liver parameters, the best and worst values per treatment period were considered. These values are first described as a four-modality categorical variable (normal, > 1 to 2-fold ULN, > 2 to 3-fold ULN, > 3-fold ULN) and then as a binary variable (normal vs abnormal).

As previously mentioned, four new patients with 3β-HSD deficiency have been included since last year's report. Patients already included in the database had 28 new blood measurements collected for this year's report.

11.4.2.1.1 Aspartate Aminotransferase

Table 9 and Table 10 present the numbers and percentages of each best and worst values of the ASAT parameter per treatment period.

When compared to the reference (worst value 'no treatment' or UDCA alone), these tables show a normalisation of the best ASAT values during UDCA, UDCA+CA or CA alone treatments, whichever the variable classification used (categorical vs binary). But the best results are obtained when patients are under CA as a monotherapy especially after the second year of treatment.

However, this trend toward normalisation of ASAT values is less noticeable when comparing the worst ASAT values during UDCA+CA or CA alone treatment to the reference (worst value 'no treatment' or UDCA alone). Compared to previous study reports, these results on ASAT parameter remain similar demonstrating a parameter normalization under treatment.

Table 9: Serum ASAT Values as a Categorical Variable (Patients with 3β-HSD deficiency, N = 41).

		N	Missing	Normal	>1 to 2-fold ULN	>2 to 3-fold ULN	>3-fold ULN
References (worst values)	No treatment	31	10	9 (29.0)	10 (32.3)	4 (12.9)	8 (25.8)
	UDCA	16	25	6 (37.5)	6 (37.5)	2 (12.5)	2 (12.5)
Best values	UDCA	16	25	12 (75.0)	2 (12.5)	1 (6.3)	1 (6.3)
	UDCA + CA	7	34	6 (85.7)	1 (14.3)	-	-
	CA first year	36	5	32 (88.9)	4 (11.1)	-	-
	CA second year	36	5	33 (91.7)	3 (8.3)	-	-
	CA after second year	35	6	35 (100.0)	-	-	-
	CA pooled	40	1	40 (100.0)	-	-	-

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		N	Missing	Normal	>1 to 2-fold ULN	>2 to 3-fold ULN	>3-fold ULN
Worst values	UDCA + CA	7	34	6 (85.7)	1 (14.3)	-	-
	CA first year	36	5	24 (66.7)	9 (25.0)	1 (2.8)	2 (5.6)
	CA second year	36	5	30 (83.3)	6 (16.7)	-	-
	CA after second year	35	6	23 (65.7)	10 (28.6)	1 (2.9)	1 (2.9)
	CA pooled	40	1	23 (57.5)	12 (30.0)	2 (5.0)	3 (7.5)

Notes: data are presented as n(%). ASAT: Aspartate Aminotransferase; CA: Cholic Acid; UDCA: Ursodeoxycholic acid; ULN: Upper Limit of Normal. Source: adapted from Table 4.1.1 - TFL V1.0 24OCT2025.

Table 10: Serum ASAT Values as a Binary Variable (Patients with 3β-HSD deficiency, N = 41).

		N	Missing	Normal	Abnormal
References (worst values)	No treatment	31	10	9 (29.0)	22 (71.0)
	UDCA	16	25	6 (37.5)	10 (62.5)
Best values	UDCA	16	25	12 (75.0)	4 (25.0)
	UDCA + CA	7	34	6 (85.7)	1 (14.3)
	CA first year	36	5	32 (88.9)	4 (11.1)
	CA second year	36	5	33 (91.7)	3 (8.3)
	CA after second year	35	6	35 (100.0)	-
	CA pooled	40	1	40 (100.0)	-
Worst values	UDCA + CA	7	34	6 (85.7)	1 (14.3)
	CA first year	36	5	24 (66.7)	12 (33.3)
	CA second year	36	5	30 (83.3)	6 (16.7)
	CA after second year	35	6	23 (65.7)	12 (34.3)
	CA pooled	40	1	23 (57.5)	17 (42.5)

Notes: data are presented as n(%). ASAT: Aspartate Aminotransferase; CA: Cholic Acid; UDCA: Ursodeoxycholic acid. Source: adapted from Table 4.1.2 - TFL V1.0 24OCT2025.

11.4.2.1.2 ALANINE AMINOTRANSFERASE

Table 11 and Table 12 present the numbers and percentages of each best and worst values of the ALAT parameter per treatment period.

As for ASAT parameter, when compared to the reference (worst value ‘no treatment’ or UDCA alone), these tables show a normalisation of the best ALAT values during UDCA, UDCA+CA and CA alone treatments, whichever the variable classification used (categorical vs binary). But the best results are obtained when patients are under CA as a monotherapy, especially after the second year of treatment.

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This trend toward normalisation of ALAT values is less noticeable when comparing the worst ALAT values during UDCA+CA or CA alone to the reference (worst value 'no treatment' or UDCA alone). Compared to previous study reports, these results on ALAT parameter remain also similar.

Table 11: Serum ALAT Values as a Categorical Variable (Patients with 3β-HSD deficiency, N = 41).

		N	Missing	Normal	>1 to 2-fold ULN	>2 to 3-fold ULN	>3-fold ULN
References (worst values)	No treatment	31	10	8 (25.8)	11 (35.5)	3 (9.7)	9 (29.0)
	UDCA	16	25	6 (37.5)	4 (25.0)	2 (12.5)	4 (25.0)
Best values	UDCA	16	25	11 (68.8)	2 (12.5)	1 (6.3)	2 (12.5)
	UDCA + CA	7	34	7 (100.0)	-	-	-
	CA first year	37	4	30 (81.1)	7 (18.9)	-	-
	CA second year	36	5	33 (91.7)	3 (8.3)	-	-
	CA after second year	35	6	33 (94.3)	2 (5.7)	-	-
	CA pooled	40	1	38 (95.0)	2 (5.0)	-	-
Worst values	UDCA + CA	7	34	6 (85.7)	1 (14.3)	-	-
	CA first year	37	4	22 (59.5)	8 (21.6)	3 (8.1)	4 (10.8)
	CA second year	36	5	31 (86.1)	4 (11.1)	1 (2.8)	-
	CA after second year	35	6	20 (57.1)	8 (22.9)	2 (5.7)	5 (14.3)
	CA pooled	40	1	16 (40.0)	12 (30.0)	4 (10.0)	8 (20.0)

Notes: data are presented as n(%). ALAT: Alanine Aminotransferase; CA: Cholic Acid; UDCA: Ursodeoxycholic acid; ULN: Upper Limit of Normal. Source: adapted from Table 4.2.1 - TFL V1.0 24OCT2025.

Table 12: Serum ALAT Values as a Binary Variable (Patients with 3β-HSD deficiency, N = 41).

		N	Missing	Normal	Abnormal
References (worst values)	No treatment	31	10	8 (25.8)	23 (74.2)
	UDCA	16	25	6 (37.5)	10 (62.5)
Best values	UDCA	16	25	11 (68.8)	5 (31.3)
	UDCA + CA	7	34	7 (100.0)	-
	CA first year	37	4	30 (81.1)	7 (18.9)
	CA second year	36	5	33 (91.7)	3 (8.3)
	CA after second year	35	6	33 (94.3)	2 (5.7)
	CA pooled	40	1	38 (95.0)	2 (5.0)

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		N	Missing	Normal	Abnormal
Worst values	UDCA + CA	7	34	6 (85.7)	1 (14.3)
	CA first year	37	4	22 (59.5)	15 (40.5)
	CA second year	36	5	31 (86.1)	5 (13.9)
	CA after second year	35	6	20 (57.1)	15 (42.9)
	CA pooled	40	1	16 (40.0)	24 (60.0)

Notes: data are presented as n(%). ALAT: Alanine Aminotransferase; CA: Cholic Acid; UDCA: Ursodeoxycholic acid. Source: adapted from Table 4.2.2 - TFL V1.0 24OCT2025.

11.4.2.1.3 Gamma-Glutamyl Transferase

Table 13 and Table 14 present the numbers and percentages of each best and worst values of the GGT parameter per treatment period.

When compared to the reference (worst value 'no treatment' or UDCA alone), these tables show a fast normalisation of the best GGT values once a treatment is started, i.e. during UDCA alone, UDCA+CA and CA alone, whichever the variable classification used (categorical vs binary). But the best results are obtained when patients are under CA as a monotherapy, especially at the second year of treatment.

This trend toward normalisation of GGT values is also noticeable when comparing the worst GGT values during UDCA+CA or CA alone to the reference (worst value 'no treatment' or UDCA alone). These results on GGT parameter remain similar to previous reports.

Table 13: Serum GGT Values as a Categorical Variable (Patients with 3β-HSD deficiency, N = 41).

		N	Missing	Normal	>1 to 2-fold ULN	>2 to 3-fold ULN	>3-fold ULN
References (worst values)	No treatment	30	11	23 (76.7)	7 (23.3)	-	-
	UDCA	16	25	14 (87.5)	1 (6.3)	1 (6.3)	-
Best values	UDCA	16	25	15 (93.8)	1 (6.3)	-	-
	UDCA + CA	7	34	7 (100.0)	-	-	-
	CA first year	37	4	35 (94.6)	2 (5.4)	-	-
	CA second year	36	5	35 (97.2)	1 (2.8)	-	-
	CA after second year	35	6	34 (97.1)	1 (2.9)	-	-
	CA pooled	40	1	39 (97.5)	1 (2.5)	-	-

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		N	Missing	Normal	>1 to 2-fold ULN	>2 to 3-fold ULN	>3-fold ULN
Worst values	UDCA + CA	7	34	7 (100.0)	-	-	-
	CA first year	37	4	31 (83.8)	5 (13.5)	-	1 (2.7)
	CA second year	36	5	35 (97.2)	1 (2.8)	-	-
	CA after second year	35	6	29 (82.9)	4 (11.4)	1 (2.9)	1 (2.9)
	CA pooled	40	1	29 (72.5)	8 (20.0)	1 (2.5)	2 (5.0)

Notes: data are presented as n(%). CA: Cholic Acid; GGT: Gamma-Glutamyl Transferase; UDCA: Ursodeoxycholic acid; ULN: Upper Limit of Normal. Source: adapted from Table 4.3.1 - TFL V1.0 24OCT2025.

Table 14: Serum GGT Values as a Binary Variable (Patients with 3β-HSD deficiency, N = 41).

		N	Missing	Normal	Abnormal
References (worst values)	No treatment	30	11	23 (76.7)	7 (23.3)
	UDCA	16	25	14 (87.5)	2 (12.5)
Best values	UDCA	16	25	15 (93.8)	1 (6.3)
	UDCA + CA	7	34	7 (100.0)	-
	CA first year	37	4	35 (94.6)	2 (5.4)
	CA second year	36	5	35 (97.2)	1 (2.8)
	CA after second year	35	6	34 (97.1)	1 (2.9)
	CA pooled	40	1	39 (97.5)	1 (2.5)
Worst values	UDCA + CA	7	34	7 (100.0)	-
	CA first year	37	4	31 (83.8)	6 (16.2)
	CA second year	36	5	35 (97.2)	1 (2.8)
	CA after second year	35	6	29 (82.9)	6 (17.1)
	CA pooled	40	1	29 (72.5)	11 (27.5)

Notes: data are presented as n(%). CA: Cholic Acid; GGT: Gamma-Glutamyl Transferase; UDCA: Ursodeoxycholic acid. Source: adapted from Table 4.3.2 - TFL V1.0 24OCT2025.

11.4.2.1.4 TOTAL BILIRUBIN

Table 15 and Table 16 present the numbers and percentages of each best and worst values of the total bilirubin parameter per treatment period.

When compared to the reference (worst value ‘no treatment’ or UDCA), these tables show a normalisation of the best total bilirubin values during UDCA, UDCA+CA or CA alone treatments, whichever the variable classification used (categorical vs binary). Indeed, nearly all patients under UDCA normalise this parameter. But the best results are obtained when patients are under CA monotherapy, and this is already present during the first year of treatment. Nonetheless as indicated in previous reports, an exception has been reported for one patient on CA monotherapy after second year presenting a total bilirubin >1 to 2- fold ULN (patient 001007). The data from the last reporting period show normal bilirubin values for this patient. One patient (017005) presented a total bilirubin

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level >1 to 2-fold ULN during the first and second year of CA but level normalized after the second year of treatment.

This trend toward normalisation of total bilirubin values is less noticeable when comparing the worst total bilirubin values during UDCA+CA or CA alone to the reference (worst value 'no treatment' or UDCA alone).

The trend observed in previous reports is maintained in this twelfth report and confirms the parameter reduced variability (while already and mainly within the norms) under CA treatment.

Table 15: Serum Total Bilirubin Values as a Categorical Variable (Patients with 3β-HSD deficiency, N = 41).

		N	Missing	Normal	>1 to 2-fold ULN	>2 to 3-fold ULN	>3-fold ULN
References (worst values)	No treatment	30	11	8 (26.7)	9 (30.0)	3 (10.0)	10 (33.3)
	UDCA	16	25	6 (37.5)	4 (25.0)	2 (12.5)	4 (25.0)
Best values	UDCA	16	25	14 (87.5)	-	-	2 (12.5)
	UDCA + CA	7	34	7 (100.0)	-	-	-
	CA first year	36	5	35 (97.2)	1 (2.8)	-	-
	CA second year	34	7	33 (97.1)	1 (2.9)	-	-
	CA after second year	35	6	34 (97.1)	1 (2.9)	-	-
	CA pooled	40	1	39 (97.5)	1 (2.5)	-	-
Worst values	UDCA + CA	7	34	6 (85.7)	1 (14.3)	-	-
	CA first year	36	5	28 (77.8)	6 (16.7)	-	2 (5.6)
	CA second year	34	7	30 (88.2)	4 (11.8)	-	-
	CA after second year	35	6	22 (62.9)	8 (22.9)	2 (5.7)	3 (8.6)
	CA pooled	40	1	24 (60.0)	9 (22.5)	2 (5.0)	5 (12.5)

Notes: data are presented as n(%). CA: Cholic Acid; UDCA: Ursodeoxycholic acid; ULN: Upper Limit of Normal.
Source: adapted from Table 4.4.1 - TFL V1.0 24OCT2025.

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Table 16: Serum Total Bilirubin Values as a Binary Variable (Patients with 3β-HSD deficiency, N = 41).

		N	Missing	Normal	Abnormal
References (worst values)	No treatment	30	11	8 (26.7)	22 (73.3)
	UDCA	16	25	6 (37.5)	10 (62.5)
Best values	UDCA	16	25	14 (87.5)	2 (12.5)
	UDCA + CA	7	34	7 (100.0)	-
	CA first year	36	5	35 (97.2)	1 (2.8)
	CA second year	34	7	33 (97.1)	1 (2.9)
	CA after second year	35	6	34 (97.1)	1 (2.9)
	CA pooled	40	1	39 (97.5)	1 (2.5)
Worst values	UDCA + CA	7	34	6 (85.7)	1 (14.3)
	CA first year	36	5	28 (77.8)	8 (22.2)
	CA second year	34	7	30 (88.2)	4 (11.8)
	CA after second year	35	6	22 (62.9)	13 (37.1)
	CA pooled	40	1	24 (60.0)	16 (40.0)

Notes: data are presented as n(%). CA: Cholic Acid; UDCA: Ursodeoxycholic acid. Source: adapted from Table 4.4.2 - TFL V1.0 24OCT2025.

11.4.2.1.5 ALKALINE PHOSPHATASE

Table 17 and Table 18 present the numbers and percentages of each best and worst values of the ALP parameter per treatment period.

When compared to the reference (worst value 'no treatment' or UDCA alone), these tables show better values of ALP once a treatment is started, i.e. during UDCA alone, UDCA+CA and CA alone, whichever the variable classification used (categorical vs binary). The best results are obtained when patients are under CA as a monotherapy during the second year of treatment and entirely after the second year of CA treatment. However, there remain some patients for whom their ALP value remains between >1 to 2- fold ULN even under treatment, and whichever the type of treatment and/or its duration.

This trend toward a close normalisation of ALP values is less noticeable when comparing the worst ALP values during UDCA+CA or CA alone to the reference (worst value 'no treatment' or UDCA alone).

These results on ALP parameter remain in line with the previously reported results and show the parameter normalisation under treatment.

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Table 17: Serum ALP Values as a Categorical Variable (Patients with 3β-HSD deficiency, N = 41).

		N	Missing	Normal	>1 to 2-fold ULN	>2 to 3-fold ULN	>3-fold ULN
References (worst values)	No treatment	27	14	15 (55.6)	6 (22.2)	4 (14.8)	2 (7.4)
	UDCA	15	26	6 (40.0)	6 (40.0)	2 (13.3)	1 (6.7)
Best values	UDCA	15	26	12 (80.0)	3 (20.0)	-	-
	UDCA + CA	7	34	5 (71.4)	2 (28.6)	-	-
	CA first year	31	10	28 (90.3)	3 (9.7)	-	-
	CA second year	30	11	29 (96.7)	1 (3.3)	-	-
	CA after second year	30	11	30 (100.0)	-	-	-
	CA pooled	35	6	35 (100.0)	-	-	-
Worst values	UDCA + CA	7	34	3 (42.9)	3 (42.9)	1 (14.3)	-
	CA first year	31	10	20 (64.5)	10 (32.3)	1 (3.2)	-
	CA second year	30	11	26 (86.7)	4 (13.3)	-	-
	CA after second year	30	11	17 (56.7)	12 (40.0)	-	1 (3.3)
	CA pooled	35	6	16 (45.7)	17 (48.6)	1 (2.9)	1 (2.9)

Notes: data are presented as n(%). ALP: Alkaline Phosphatase; CA: Cholic Acid; UDCA: Ursodeoxycholic acid; ULN: Upper Limit of Normal. Source: adapted from Table 4.5.1 - TFL V1.0 24OCT2025.

Table 18: Serum ALP Values as a Binary Variable (Patients with 3β-HSD deficiency, N = 41).

		N	Missing	Normal	Abnormal
References (worst values)	No treatment	27	14	15 (55.6)	12 (44.4)
	UDCA	15	26	6 (40.0)	9 (60.0)
Best values	UDCA	15	26	12 (80.0)	3 (20.0)
	UDCA + CA	7	34	5 (71.4)	2 (28.6)
	CA first year	31	10	28 (90.3)	3 (9.7)
	CA second year	30	11	29 (96.7)	1 (3.3)
	CA after second year	30	11	30 (100.0)	-
	CA pooled	35	6	35 (100.0)	-
Worst values	UDCA + CA	7	34	3 (42.9)	4 (57.1)
	CA first year	31	10	20 (64.5)	11 (35.5)
	CA second year	30	11	26 (86.7)	4 (13.3)
	CA after second year	30	11	17 (56.7)	13 (43.3)
	CA pooled	35	6	16 (45.7)	19 (54.3)

Notes: data are presented as n(%).ALP: Alkaline Phosphatase; CA: Cholic Acid; UDCA: Ursodeoxycholic acid. Source: adapted from Table 4.5.2 - TFL V1.0 24OCT2025.

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11.4.2.1.6 VITAMIN E

Table 19 and Table 20 present the numbers and percentages of each best and worst values of the vitamin E parameter per treatment period.

When compared to the worst value of 'no treatment', these tables show a normalisation of the best vitamin E values for any treatment periods, i.e. during UDCA alone, UDCA+CA and CA alone, and whichever the variable classification used (categorical vs binary). The best results are obtained when patients are under CA as a monotherapy especially after the second year of treatment.

This trend toward normalisation of vitamin E values is less noticeable when comparing the worst vitamin E values during UDCA+CA or CA alone to the worst value of 'no treatment'.

These results on vitamin E remain in line with the previously reported results and show normalisation under treatment.

Table 19: Serum Vitamin E Values as a Categorical Variable (Patients with 3β-HSD deficiency, N = 41).

		N	Missing	Normal	Above ULN	Below LLN
References (worst values)	No treatment	23	18	3 (13.0)	4 (17.4)	16 (69.6)
	UDCA	14	27	7 (50.0)	4 (28.6)	3 (21.4)
Best values	UDCA	14	27	11 (78.6)	2 (14.3)	1 (7.1)
	UDCA + CA	8	33	7 (87.5)	1 (12.5)	-
	CA first year	29	12	23 (79.3)	6 (20.7)	-
	CA second year	26	15	24 (92.3)	2 (7.7)	-
	CA after second year	32	9	31 (96.9)	1 (3.1)	-
	CA pooled	38	3	36 (94.7)	2 (5.3)	-
Worst values	UDCA + CA	8	33	5 (62.5)	2 (25.0)	1 (12.5)
	CA first year	29	12	16 (55.2)	9 (31.0)	4 (13.8)
	CA second year	26	15	23 (88.5)	2 (7.7)	1 (3.8)
	CA after second year	32	9	20 (62.5)	9 (28.1)	3 (9.4)
	CA pooled	38	3	17 (44.7)	13 (34.2)	8 (21.1)

Notes: data are presented as n(%). CA: Cholic Acid; LLN: Lower Limit of Normal; UDCA: Ursodeoxycholic acid; ULN: Upper Limit of Normal. Source: adapted from Table 4.6.1 - TFL V1.0 24OCT2025.

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Table 20: Serum Vitamin E Values as a Binary Variable (Patients with 3β-HSD deficiency, N = 41).

		N	Missing	Normal	Abnormal
References (worst values)	No treatment	23	18	3 (13.0)	20 (87.0)
	UDCA	14	27	7 (50.0)	7 (50.0)
Best values	UDCA	14	27	11 (78.6)	3 (21.4)
	UDCA + CA	8	33	7 (87.5)	1 (12.5)
	CA first year	29	12	23 (79.3)	6 (20.7)
	CA second year	26	15	24 (92.3)	2 (7.7)
	CA after second year	32	9	31 (96.9)	1 (3.1)
	CA pooled	38	3	36 (94.7)	2 (5.3)
Worst values	UDCA + CA	8	33	5 (62.5)	3 (37.5)
	CA first year	29	12	16 (55.2)	13 (44.8)
	CA second year	26	15	23 (88.5)	3 (11.5)
	CA after second year	32	9	20 (62.5)	12 (37.5)
	CA pooled	38	3	17 (44.7)	21 (55.3)

Notes: data are presented as n(%). CA: Cholic Acid; UDCA: Ursodeoxycholic acid. Source: adapted from Table 4.6.2 - TFL V1.0 24OCT2025.

11.4.2.2 Descriptive Analyses on Quantitative Parameters

Table 21 and Table 22 present descriptive and comparative data for liver biochemistry parameters and for Vitamin E respectively when considered as quantitative (or continuous) variables, per treatment period and for patients with 3β-HSD deficiency. Patients could have had multiple clinic visits during any treatment period, and therefore could potentially have had multiple measures of blood biochemistry parameters. In the categorical and binary analyses of liver parameters in Section 11.4.2.1, only the best and worst value of each treatment period was considered. Here, every sample providing data on liver parameters and Vitamin E per patient was pooled for each treatment period to obtain a mean per patient per parameter. Then a general mean standard deviation (SD), median, Q1-Q3, minimum and maximum per parameter were calculated to be presented in these two tables.
Data are also presented graphically using boxplots for each liver parameter in Figure 2 to Figure 6 and for Vitamin E in Figure 7. Each parameter is discussed in the context of the defined norms issued from Centre 001 norms (with some exceptions, as described in Appendix 15.1.2), which are represented in each boxplot by a red line to have an easy-to-read visual view on the observed data.

11.4.2.2.1 Liver Parameters

Compared to the no treatment period (181.1 ± 300.5 IU/L), mean ASAT levels decreased with UDCA monotherapy (75.9 ± 81.6 IU/L) and then decreased more importantly to be below the ULN during the UDCA+CA dual therapy period (29.2 ± 13.6 IU/L). Mean ASAT levels remained under the ULN throughout subsequent CA treatment periods (CA pooled, 31.8 ± 17.3 IU/L). The reduction in ASAT

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was statistically significant for all treatments, and both versus no treatment and versus UDCA (Figure 2 and Table 21).

Mean ALAT levels decreased with UDCA monotherapy (82.3 ± 117.9 IU/L) and then decreased notably during the UDCA+CA dual therapy period (22.2 ± 14.4 IU/L) to be within the normal range, compared to the no treatment period (174.2 ± 288.2 IU/L). All mean ALAT levels remained in the normal range when under CA treatment after the first year of treatment (35.0 ± 25.5 IU/L) and thereafter (CA pooled, 27.2 ± 18.8 IU/L). The reduction of ALAT levels was statistically significant for any treatments versus the references (Figure 3 and Table 21).

Mean GGT levels were below the ULN – either untreated (24.6 ± 14.1 IU/L) or under UDCA alone (19.4 ± 13.4 IU/L), with only very few patients having an individual level above the normal threshold. Mean GGT levels then decreased during the UDCA+CA dual therapy period (11.0 ± 4.4 IU/L) to be completely within the normal range. During the first year of CA monotherapy, mean GGT levels (19.9 ± 20.0 IU/L) returned to that seen with UDCA or no treatment due to atypically high GGT levels in 1 patient (001010) that started in the UDCA treatment period and continued in the first year of CA monotherapy. Given close clinical monitoring, more GGT values towards the mean were gathered from this patient than from the others, therefore skewing the average. CA dose adjustment led to reduction of GGT levels for this patient. Mean GGT levels decreased again during subsequent years of treatment (CA pooled, 17.8 ± 10.9 IU/L). The mean reduction in GGT was statistically significant for treatments containing CA at second year versus no treatment and UDCA. These results are not surprising considering that mean GGT levels were in the normal limit for any analysed period including the period without any treatment (Figure 4 and Table 21).

Compared to the no treatment period (52.4 ± 62.7 µmol/L), mean total bilirubin levels decreased slightly with UDCA monotherapy (33.0 ± 42.2 µmol/L) and then decreased notably to below the ULN during the UDCA+CA dual therapy period (8.0 ± 3.2 µmol/L). Mean total bilirubin levels stayed below the ULN throughout subsequent treatment phases (CA pooled, 11.9 ± 11.2 µmol/L). The reduction was statistically significant for all treatments versus not treatment period and during the first and second years of CA treatment when compared to UDCA (Figure 5 and Table 21).

Mean ALP levels were above the ULN without treatment (380.3 ± 168.3 IU/L), with UDCA monotherapy (396.0 ± 194.0 IU/L) and UDCA+CA dual therapy (348.7 ± 191.7 IU/L). Mean ALP levels then decreased below the ULN during the first year of CA treatment (271.9 ± 129.9 IU/L) and throughout subsequent treatments with CA (CA pooled, 213.9 ± 90.2 IU/L). The reduction was statistically significant for CA treatments alone versus no treatment, and then for CA treatment after the first year versus UDCA (Figure 6 and Table 21).

As mentioned before, the addition of the data from the follow-up visits included during this reporting period only had little impact on the trends seen in previous study reports on these liver parameters. Besides, the significant differences found are globally consistent with the ones observed in previous reports, even though these results must be interpreted with caution considering the high number of performed statistical tests.

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Table 21: Continuous Serum Liver Parameters: Description and Comparison to References ('no treatment' or UDCA alone) – in patients with 3β-HSD Deficiency (N = 41).

		No treatment	UDCA	UDCA + CA	CA first year	CA second year	CA after second year	CA pooled
ASAT (IU/L)	N	31	16	7	36	36	35	40
	Missing values	10	25	34	5	5	6	1
	Mean ± SD	181.1 ± 300.5	75.9 ± 81.6	29.2 ± 13.6	39.1 ± 25.1	30.7 ± 9.9	27.6 ± 11.9	31.8 ± 17.3
	Median	60.8	47.5	25.0	32.5	31.7	26.0	27.9
	Q1 ; Q3	44.6 ; 182.0	38.8 ; 77.6	21.0 ; 32.3	24.5 ; 44.2	24.3 ; 36.0	21.7 ; 27.1	22.6 ; 34.8
	Min ; Max	26.4 ; 1 431.7	23.0 ; 359.1	15.1 ; 57.3	15.0 ; 153.6	15.0 ; 65.0	13.7 ; 72.0	14.2 ; 115.3
	p vs. no treatment		**0.0166**	**0.0156**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	p vs. UDCA			**0.0156**	**0.0023**	**0.0002**	**0.0003**	**<0.0001**
ALAT (IU/L)	N	31	16	7	37	36	35	40
	Missing values	10	25	34	4	5	6	1
	Mean ± SD	174.2 ± 288.2	82.3 ± 117.9	22.2 ± 14.4	35.0 ± 25.5	24.6 ± 14.3	23.7 ± 17.6	27.2 ± 18.8
	Median	62.5	36.1	17.7	25.4	20.6	17.8	21.9
	Q1 ; Q3	26.8 ; 132.0	23.0 ; 82.4	12.3 ; 26.0	19.0 ; 44.0	15.0 ; 29.5	15.4 ; 26.6	17.2 ; 30.1
	Min ; Max	13.0 ; 1 239.0	11.0 ; 481.7	9.8 ; 52.5	11.5 ; 124.8	7.0 ; 79.0	11.3 ; 113.5	11.9 ; 100.5
	p vs. no treatment		**0.0256**	**0.0156**	**0.0003**	**<0.0001**	**<0.0001**	**<0.0001**
	p vs. UDCA			**0.0313**	**0.0052**	**0.0024**	**0.0006**	**0.0006**

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		No treatment	UDCA	UDCA + CA	CA first year	CA second year	CA after second year	CA pooled
GGT (IU/L)	N	30	16	7	37	36	35	40
	Missing values	11	25	34	4	5	6	1
	Mean ± SD	24.6 ± 14.1	19.4 ± 13.4	11.0 ± 4.4	19.9 ± 20.0	14.2 ± 8.9	18.0 ± 11.3	17.8 ± 10.9
	Median	22.0	15.2	10.0	13.0	11.3	14.4	13.9
	Q1 ; Q3	14.8 ; 33.0	10.0 ; 24.5	7.5 ; 12.9	10.0 ; 21.5	9.3 ; 14.8	12.6 ; 20.0	12.5 ; 18.5
	Min ; Max	5.0 ; 64.0	7.0 ; 51.0	6.8 ; 19.3	6.0 ; 111.1	5.0 ; 51.5	8.3 ; 73.8	7.7 ; 66.1
	p vs. no treatment		0.2478	0.1094	**0.0150**	**0.0007**	0.1023	0.0633
	p vs. UDCA			0.4688	0.2958	**0.0205**	0.8361	0.8999
Total bilirubin (µmol/L)	N	30	16	7	36	34	35	40
	Missing values	11	25	34	5	7	6	1
	Mean ± SD	52.4 ± 62.7	33.0 ± 42.2	8.0 ± 3.2	10.8 ± 14.1	7.8 ± 4.3	11.9 ± 7.4	11.9 ± 11.2
	Median	25.2	18.1	6.7	7.4	7.8	9.6	8.7
	Q1 ; Q3	13.5 ; 71.2	7.0 ; 30.4	5.6 ; 10.8	5.1 ; 11.6	5.0 ; 9.4	6.9 ; 13.2	6.5 ; 11.5
	Min ; Max	0.2 ; 247.0	3.0 ; 150.0	3.8 ; 12.2	0.3 ; 86.6	1.0 ; 19.0	5.0 ; 36.5	2.7 ; 66.5
	p vs. no treatment		**0.0107**	**0.0156**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	p vs. UDCA			**0.0313**	**0.0032**	**0.0068**	0.1688	0.1754

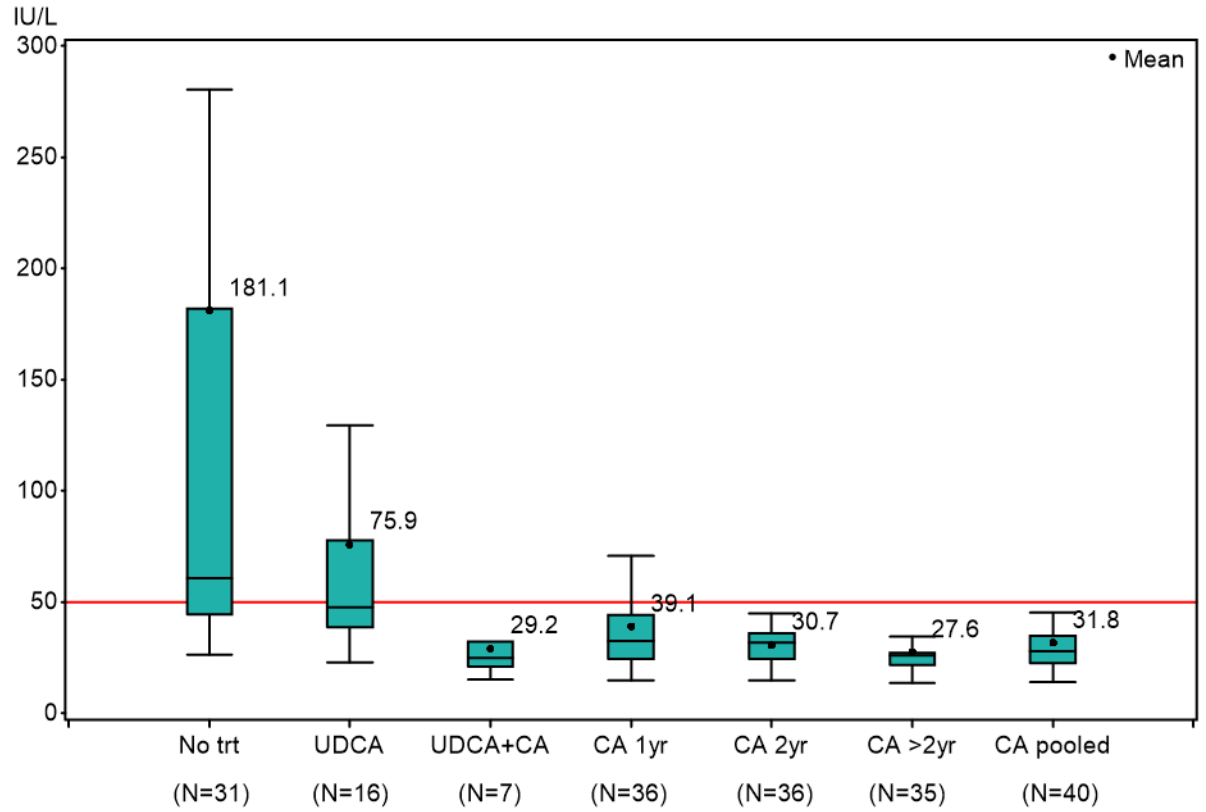
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		No treatment	UDCA	UDCA + CA	CA first year	CA second year	CA after second year	CA pooled
ALP (IU/L)	N	27	15	7	31	30	30	35
	Missing values	14	26	34	10	11	11	6
	Mean ± SD	380.3 ± 168.3	396.0 ± 194.0	348.7 ± 191.7	271.9 ± 129.9	230.0 ± 89.5	185.5 ± 96.2	213.9 ± 90.2
	Median	331.5	349.0	311.8	259.3	251.0	170.2	226.7
	Q1 ; Q3	284.5 ; 449.8	285.3 ; 475.3	267.3 ; 580.8	187.0 ; 330.5	179.0 ; 276.0	132.0 ; 229.7	153.8 ; 266.4
	Min ; Max	178.5 ; 861.3	180.0 ; 985.0	52.7 ; 603.5	60.0 ; 622.0	56.0 ; 484.0	46.4 ; 448.9	50.7 ; 472.8
	p vs. no treatment		0.4631	0.9375	**0.0014**	**0.0010**	**<0.0001**	**<0.0001**
	p vs. UDCA			0.9375	0.0681	**0.0273**	**0.0002**	**0.0003**

Notes: CA: Cholic Acid; UDCA: Ursodeoxycholic acid. ASAT: Aspartate aminotransferase; ALAT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; ALP: Alkaline phosphatase; N: number of samples; SD: Standard Deviation. Pairwise comparisons with Wilcoxon test.
Sources: issued from all Tables of Section 7 - TFL V1.0 24OCT2025.

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Figure 2: Mean Value of Serum ASAT According to Treatment Period in Patients with 3ß-HSD Deficiency (N = 41).

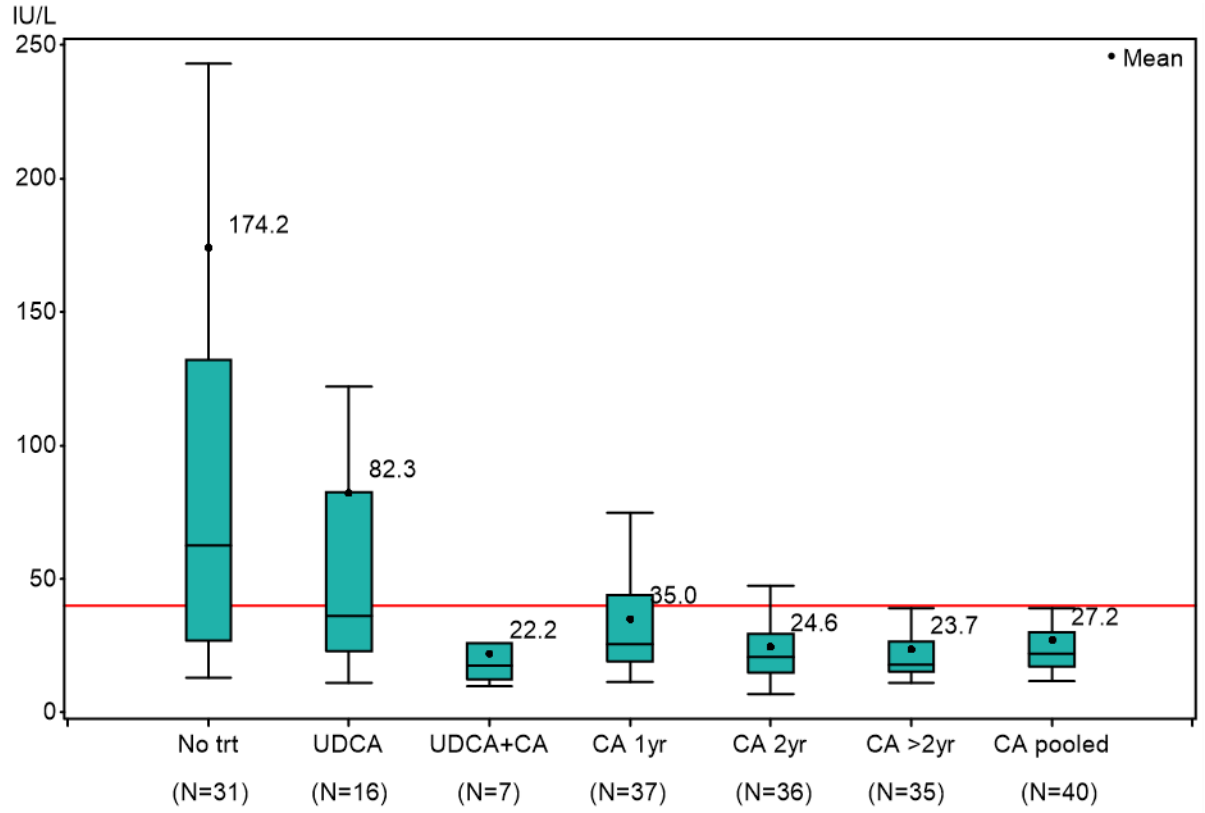


No trt: No Treatment, UDCA:Ursodeoxycholic acid, CA: Cholic Acid, yr:year

Notes: The box represents the inter-quartile range (Q3-Q1); the black dot the mean, the — the median; and the upper & lower bars the observed maximum and minimum average values. The upper limit of the normal ranges is issued from Centre 001 (except for some patients) and is indicated by a red line. ASAT: Aspartate aminotransferase; CA: Cholic Acid; trt: treatment; UDCA: Ursodeoxycholic acid; 1yr: first year; 2yr: second year. Source: Figure 8.1.1 - TFL V1.0 24OCT2025.

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Figure 3: Mean Value of Serum ALAT According to Treatment Period in Patients with 3ß-HSD Deficiency (N = 41).

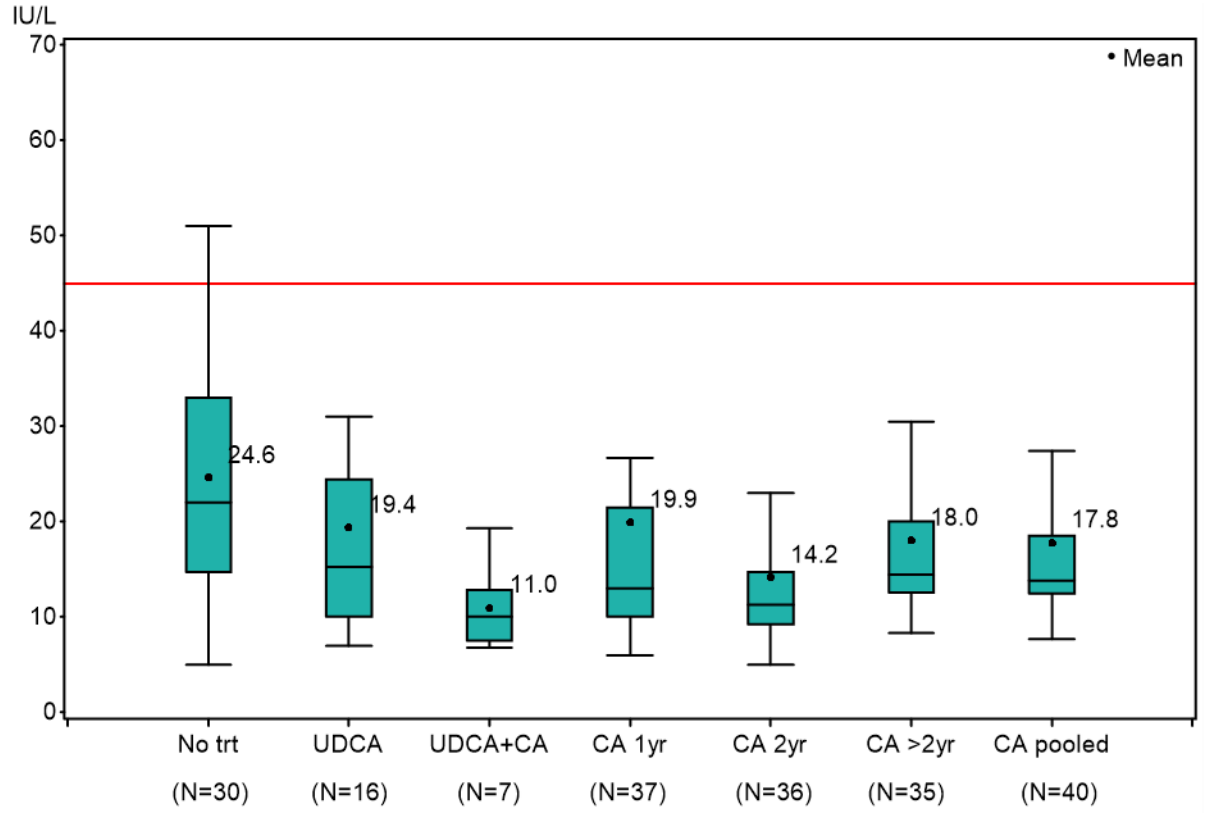


No trt: No Treatment, UDCA:Ursodeoxycholic acid, CA: Cholic Acid, yr:year

Notes: The box represents the inter-quartile range (Q3-Q1); the black dot the mean, the — the median; and the upper & lower bars the observed maximum and minimum average values. The upper limit of the normal ranges is issued from Centre 001 (except for some patients) and is indicated by a red line. ALAT: Alanine aminotransferase; CA: Cholic Acid; trt: treatment; UDCA: Ursodeoxycholic acid; 1yr: first year; 2yr: second year. Source: Figure 8.1.2 - TFL V1.0 24OCT2025.

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Figure 4: Mean Value of Serum GGT According to Treatment Period in Patients with 3ß-HSD Deficiency (N = 41).

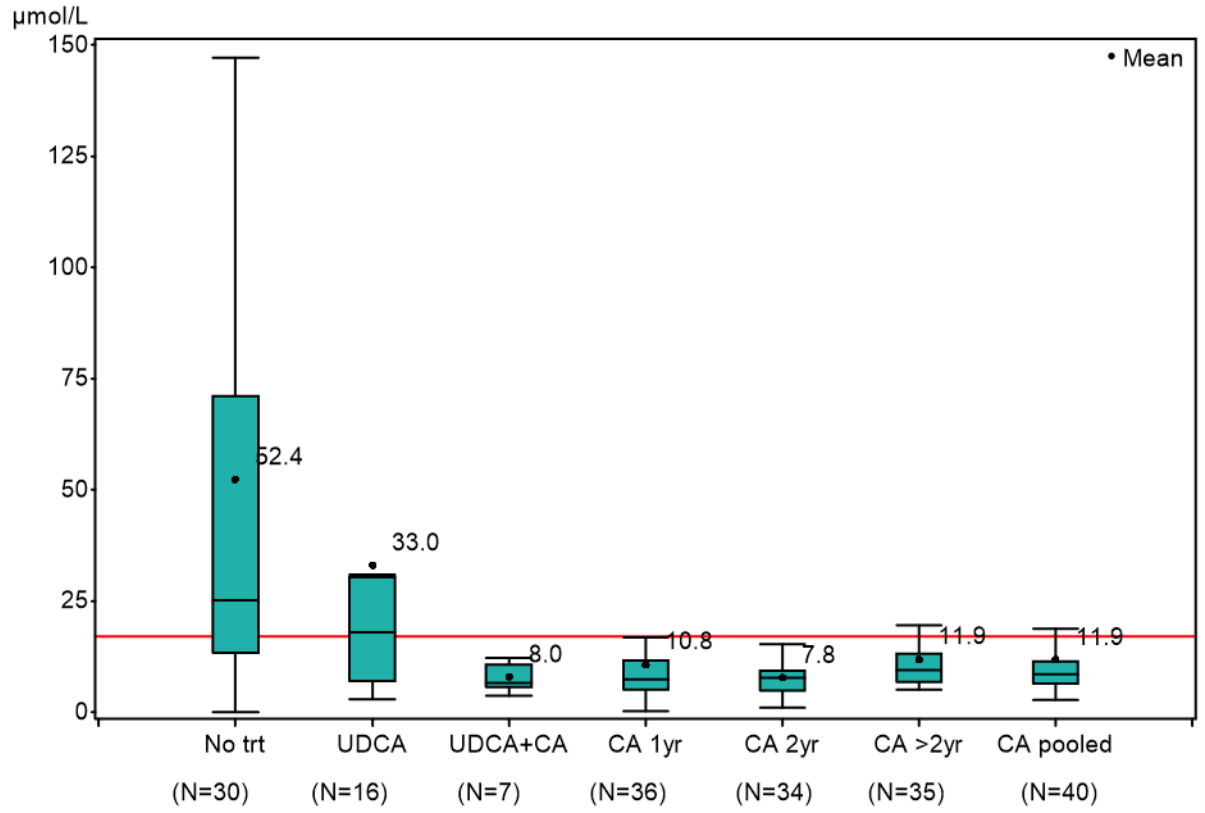


No trt: No Treatment, UDCA:Ursodeoxycholic acid, CA: Cholic Acid, yr:year

Notes: The box represents the inter-quartile range (Q3-Q1); the black dot the mean, the — the median; and the upper & lower bars the observed maximum and minimum average values. The upper limit of the normal ranges is issued from Centre 001 (except for some patients) and is indicated by a red line. GGT: Gamma-glutamyl transferase; CA: Cholic Acid; trt: treatment; UDCA: Ursodeoxycholic acid; 1yr: first year; 2yr: second year. Source: Figure 8.1.3 - TFL V1.0 24OCT2025.

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Figure 5: Mean Value of Serum Total Bilirubin According to Treatment Period in Patients with 3ß-HSD Deficiency (N = 41).

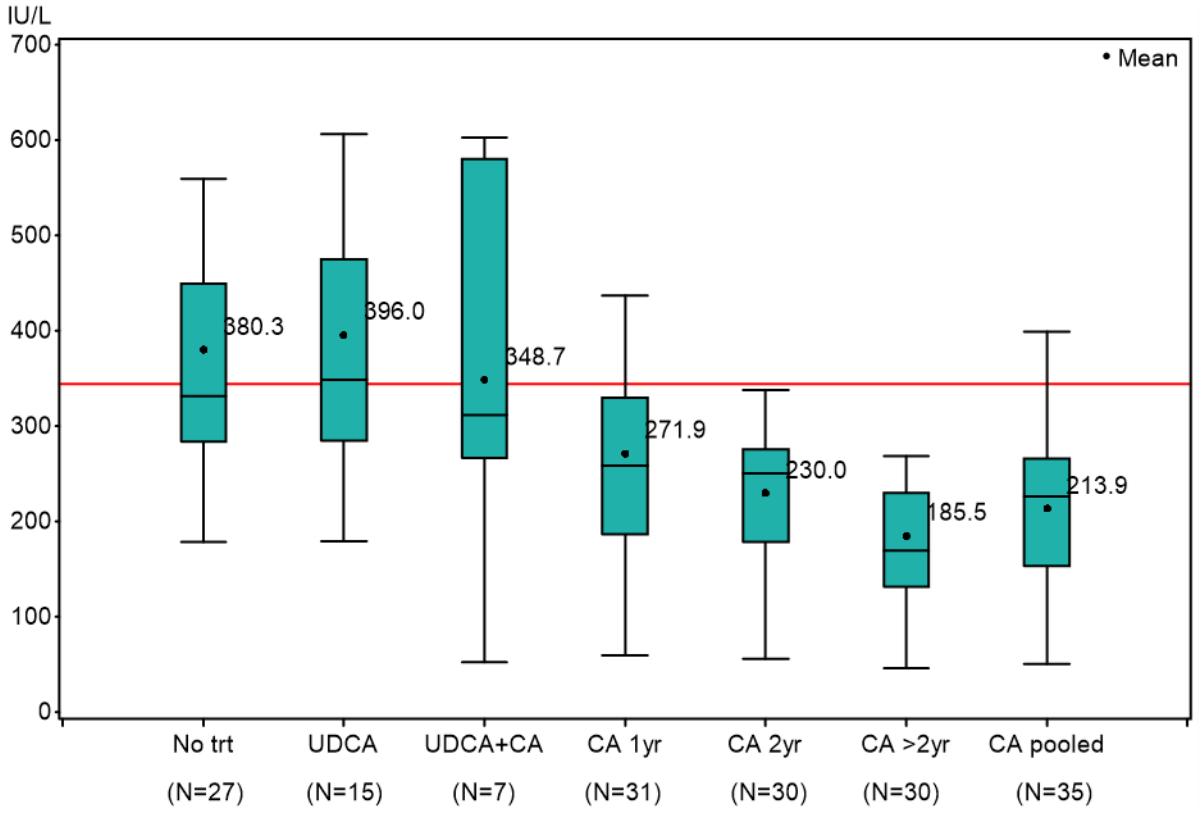


No trt: No Treatment, UDCA:Ursodeoxycholic acid, CA: Cholic Acid, yr:year

Notes: The box represents the inter-quartile range (Q3-Q1); the black dot the mean, the — the median; and the upper & lower bars the observed maximum and minimum average values. The upper limit of the normal ranges is issued from Centre 001 (except for some patients) and is indicated by a red line. CA: Cholic acid; trt: treatment; UDCA: Ursodeoxycholic acid; 1yr: first year; 2yr: second year. Source: Figure 8.1.4 - TFL V1.0 24OCT2025.

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Figure 6: Mean Value of Serum ALP According to Treatment Period in Patients with 3ß-HSD Deficiency (N = 41).



No trt: No Treatment, UDCA:Ursodeoxycholic acid, CA: Cholic Acid, yr:year

Notes: The box represents the inter-quartile range (Q3-Q1); the black dot the mean, the — the median; and the upper & lower bars the observed maximum and minimum average values. The upper limit of the normal ranges is issued from Centre 001 (except for some patients) and is indicated by a red line. ALP: Alkaline phosphatase; CA: Cholic acid; trt: treatment; UDCA: Ursodeoxycholic acid; 1yr: first year; 2yr: second year. Source: Figure 8.1.5 - TFL V1.0 24OCT2025.

11.4.2.2.2 VITAMIN E

Mean Vitamin E levels, which were already slightly above the lower limit of the normal range without treatment (6.3 ± 6.6 µg/L), increased with UDCA monotherapy (9.0 ± 5.9 µg/L) and the subsequent treatment phases, getting closer to the middle of the normal range, especially in the second year of CA treatment (10.2 ± 3.2 µg/L) (Table 22 and Figure 7). Thus, it lasted up to two years to reach normal vitamin E levels. Vitamin E values exceeded slightly the upper limit of the normal range during UDCA + CA treatment and all CA treatment periods, except during CA treatment after 2 years. These results are comparable with the ones presented in the previous report.

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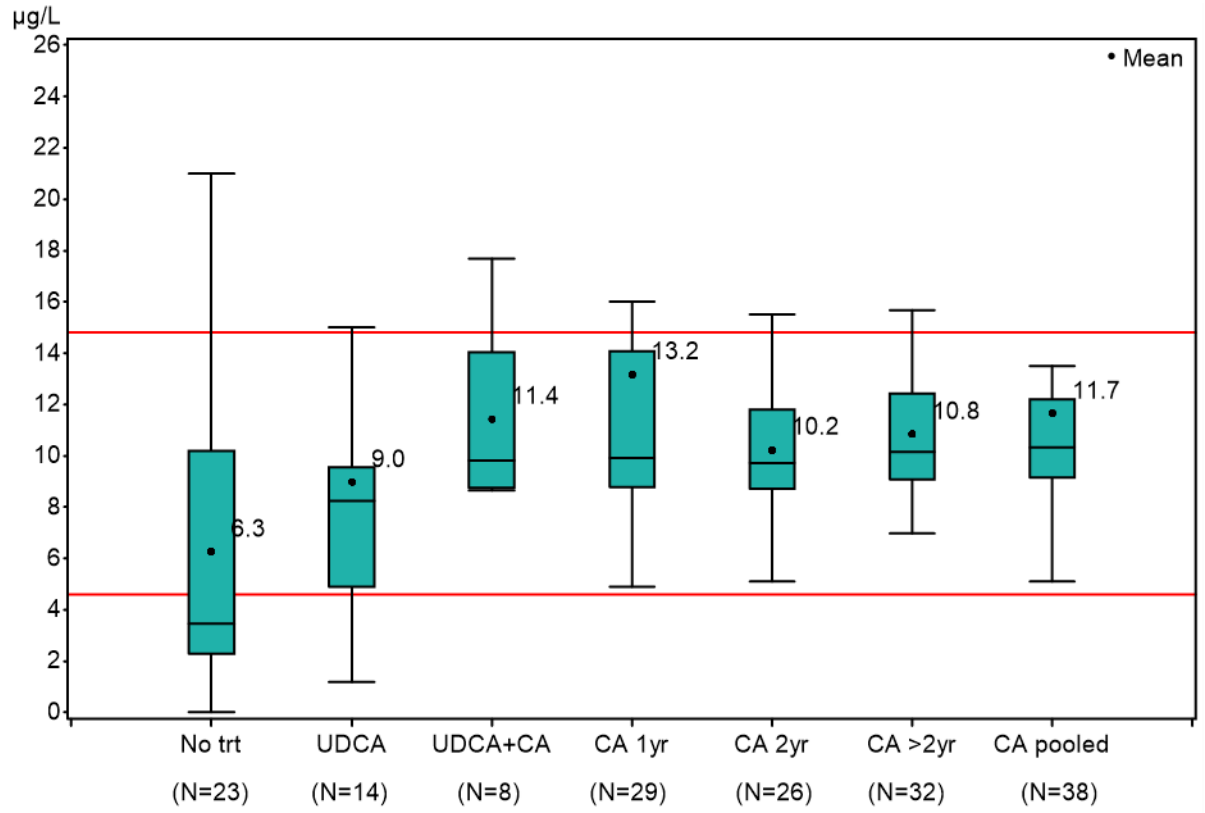
Table 22: Continuous Vitamin E Parameter: Description and Comparison to References ('no treatment' or UDCA alone) in Patients with 3β-HSD Deficiency (N = 41).

		No treatment	UDCA	UDCA + CA	CA first year	CA second year	CA after second year	CA pooled
Vitamin E (µg/L)	N	23	14	8	29	26	32	38
	Missing values	18	27	33	12	15	9	3
	Mean ± SD	6.3 ± 6.6	9.0 ± 5.9	11.4 ± 3.5	13.2 ± 8.7	10.2 ± 3.2	10.8 ± 2.7	11.7 ± 5.3
	Median	3.5	8.2	9.8	9.9	9.7	10.1	10.3
	Q1 ; Q3	2.3 ; 10.2	4.9 ; 9.6	8.7 ; 14.0	8.8 ; 14.1	8.7 ; 11.8	9.1 ; 12.4	9.2 ; 12.2
	Min ; Max	0.0 ; 23.0	1.2 ; 25.6	8.7 ; 17.7	4.9 ; 47.3	5.1 ; 20.4	7.0 ; 20.0	5.1 ; 33.6
	p vs. no treatment		0.0186	0.0313	0.0006	0.0008	**0.0001**	**<0.0001**
	p vs. UDCA			**0.0156**	0.2402	0.6953	0.0342	0.0681

Notes: CA: Cholic Acid; UDCA: Ursodeoxycholic acid. N: number of samples; SD: standard deviation.
Pairwise comparisons with Wilcoxon test. Source: Tables 7.6.1 and 7.6.2 - TFL V1.0 24OCT2025.

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Figure 7: Mean Value of Serum Vitamin E According to Treatment Period in Patients with 3ß-HSD Deficiency (N = 41).



No trt: No Treatment, UDCA:Ursodeoxycholic acid, CA: Cholic Acid, yr:year

Notes: The box represents the inter-quartile range (Q3-Q1); the black dot the mean, the — the median; and the upper & lower bars the observed maximum and minimum average values. The upper and lower limits of the normal ranges are issued from Centre 001 and are indicated by red lines. CA: Cholic Acid; trt: treatment; UDCA: Ursodeoxycholic acid; 1yr: first year; 2yr: second year. Source: Figure 8.1.6 - TFL V1.0 24OCT2025.

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11.4.2.3 BY-PATIENT DISPLAYS

For this twelfth report, all figures presented below have been updated and include graphical representations of transaminases and bilirubin over time. They are presented for the same 5 selected patients with 3β-HSD deficiency than in last year's report.

When comparing the observation periods of these patients:

- 3 patients had very long periods of observation, including ≥ 25 years of treatment for patients 003002 and 001006 and > 20 years of treatment for patient 001010;
- 1 patient (002001) had a > 15-year observation period, including > 15 years of treatment;
- 1 patient (004002) had an observation period > 10 years, including 13 years of treatment.

11.4.2.3.1 PATIENT 003002: TREATMENT DURATION > 25 YEARS

The treatment sequence for this patient (indicated on the graphs by green dots) was: no treatment, UDCA monotherapy, UDCA+CA dual therapy, first year of CA monotherapy, second year of CA monotherapy, after second year of CA monotherapy.

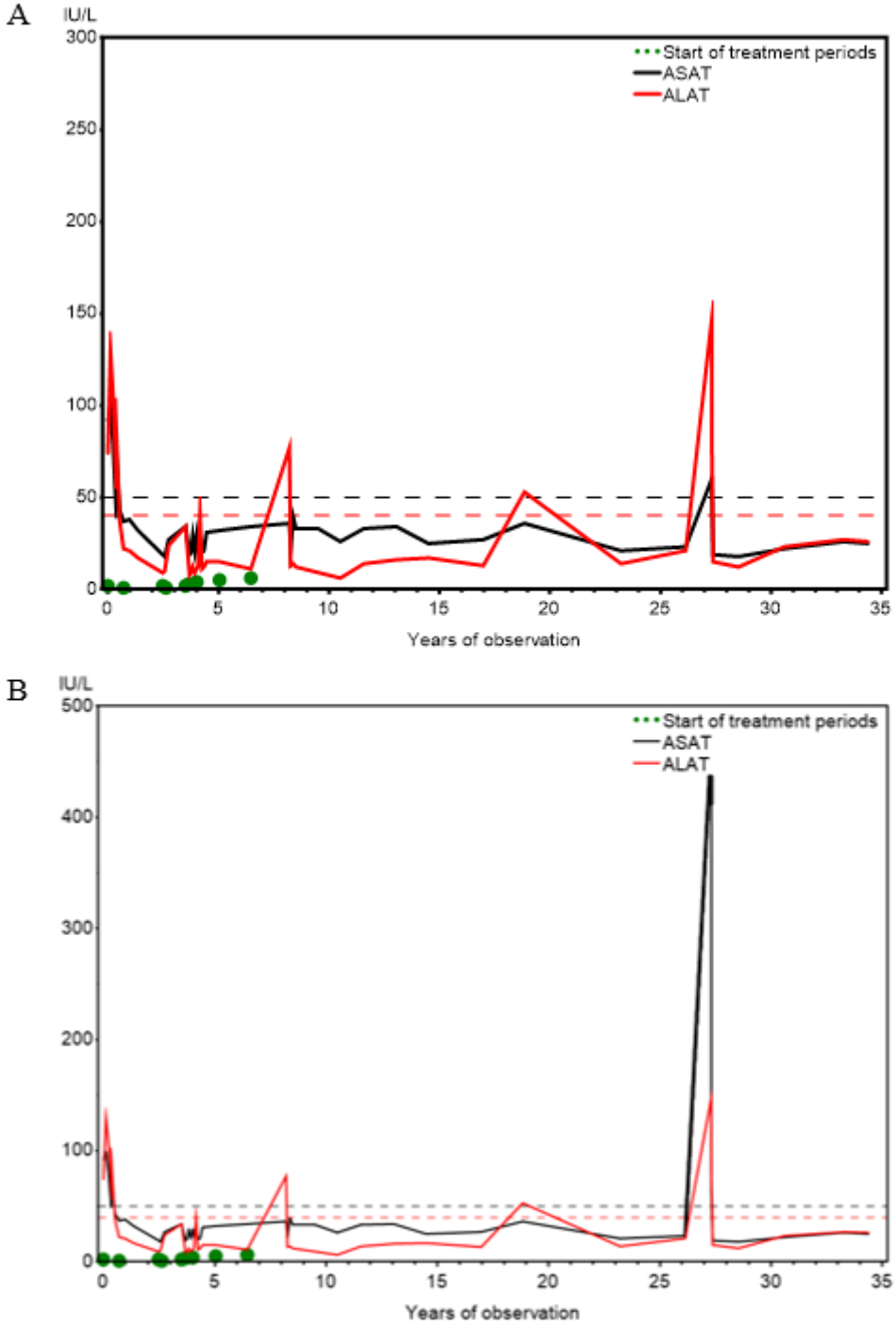
This patient was treated for 33.4 years overall and for 31.4 years with CA only (Table 4). One new visit and one laboratory measurement were performed since last year's report.

In general, once treated, patient's transaminases levels reduced to normal ranges, although the ALAT levels were above the normal limit after 8 and 18 years of observation and spiked at about 27 years of observation before normalising rapidly (Figure 8). As it was reported in the 7th Orphabase report, based on the information received from the treating physician, this increase could have been related to a poor treatment adherence. Moreover, this patient stayed without any treatment for two periods of almost 2 years and 11 months respectively, during the 2nd and the 3rd years of observation. The no treatment period during the 3rd year of observation explained the transient peaks observed in transaminases levels. The levels normalised below the ULN when the patient returned to UCDA monotherapy.

Total bilirubin levels were below the ULN from the time of UDCA monotherapy and thereafter, except one peak after 3 and 27 years of observation (Figure 9). As for transaminases, the peak observed after 3 years of observation is explained by a transient period without any treatment.

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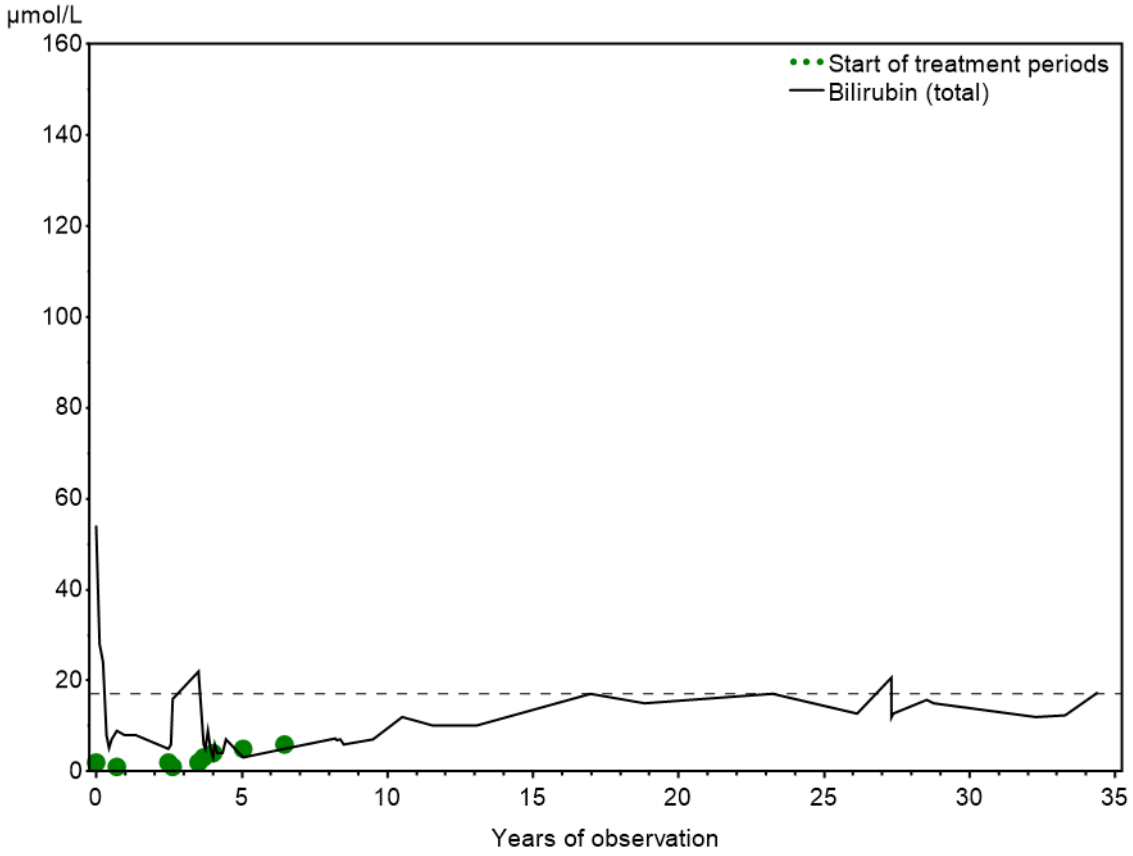
Figure 8: Evolution of Transaminases Over Time – Patient 003002, 3β-HSD Deficiency.



A. Graph displaying transaminases levels from 0 to 300 UI/L (same scale used for all patients and in previous reports).
B. Graph displaying transaminases levels from 0 to 500 UI/L as values > 300 UI/L were collected for this patient.
Notes: The black and red dotted lines represent the upper limits of the normal range of respectively ASAT and ALAT. 3β-HSD: 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase, ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase. Source: Figures 8.7.1 and 8.7.2 - TFL V1.0 24OCT2025.

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Figure 9: Evolution of Total Bilirubin Over Time – Patient 003002, 3β-HSD Deficiency.



Notes: The dotted line represents the upper limit of the normal range. 3β-HSD: 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase, Source: Figure 8.7.4 - TFL V1.0 24OCT2025.

11.4.2.3.2 PATIENT 001006: TREATMENT DURATION > 25 YEARS

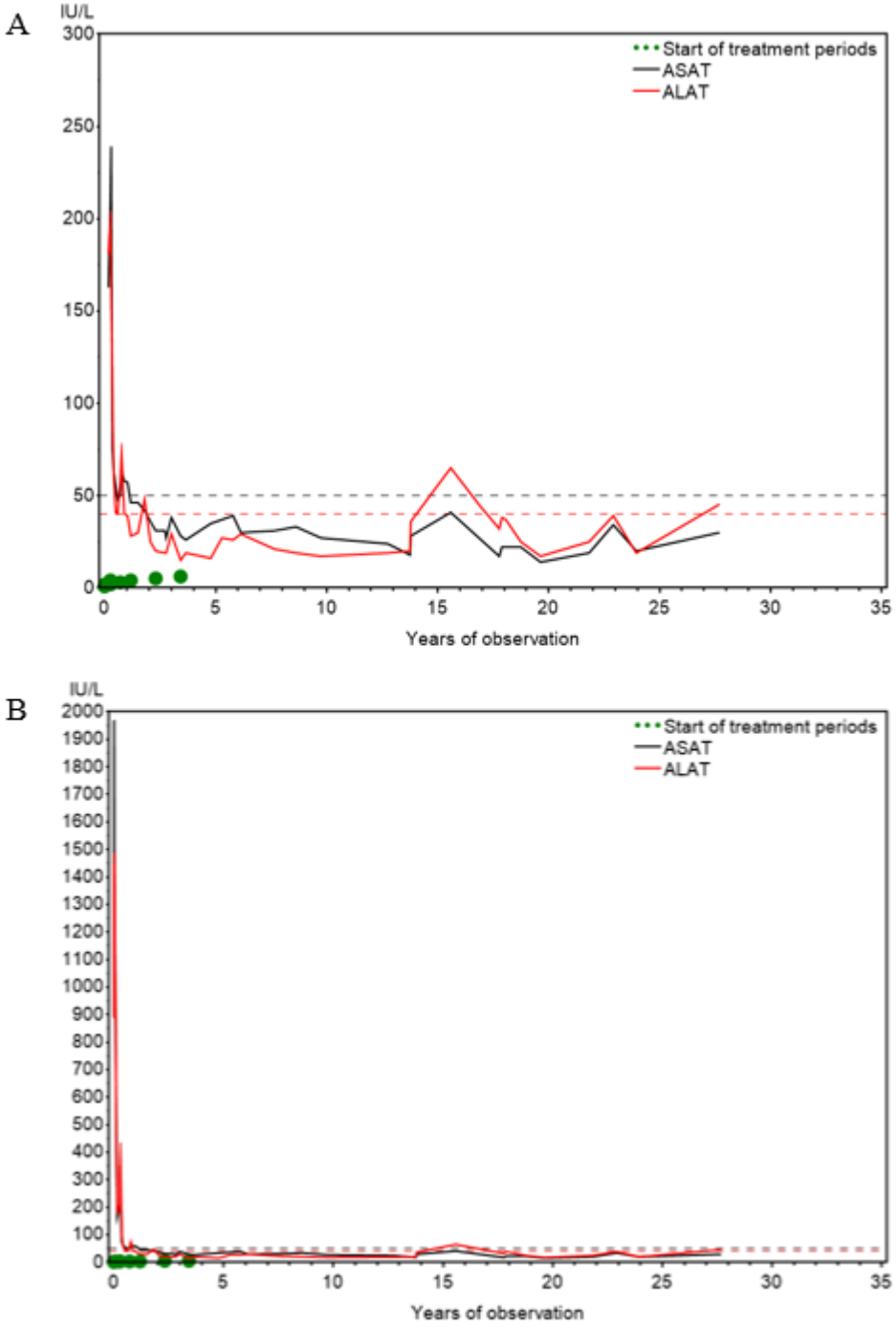
The treatment sequence for this patient (indicated on the graphs by green dots) was: no treatment, UDCA monotherapy, UDCA+CA dual therapy, first year of CA monotherapy, second year of CA monotherapy, after second year of CA monotherapy. It is noteworthy that this patient was under CA monotherapy for a short period of time (one month) during the first year of observation but had to stop the treatment because suffering from severe pruritus. Patient then returned to UDCA monotherapy.

This patient was treated for 28.5 years overall and for 27.5 years with CA only (Table 4). One new visit and one laboratory measurement were performed since last year's report.

In general, transaminases were below ULN once treated, except a peak of ALAT observed after 15 years of observation. In addition, since the past 3 years, ALAT level increased to reach 45 UI/L, just above ULN. Total bilirubin was below or around the ULN from the time of UDCA monotherapy onwards (Figure 10 and Figure 11).

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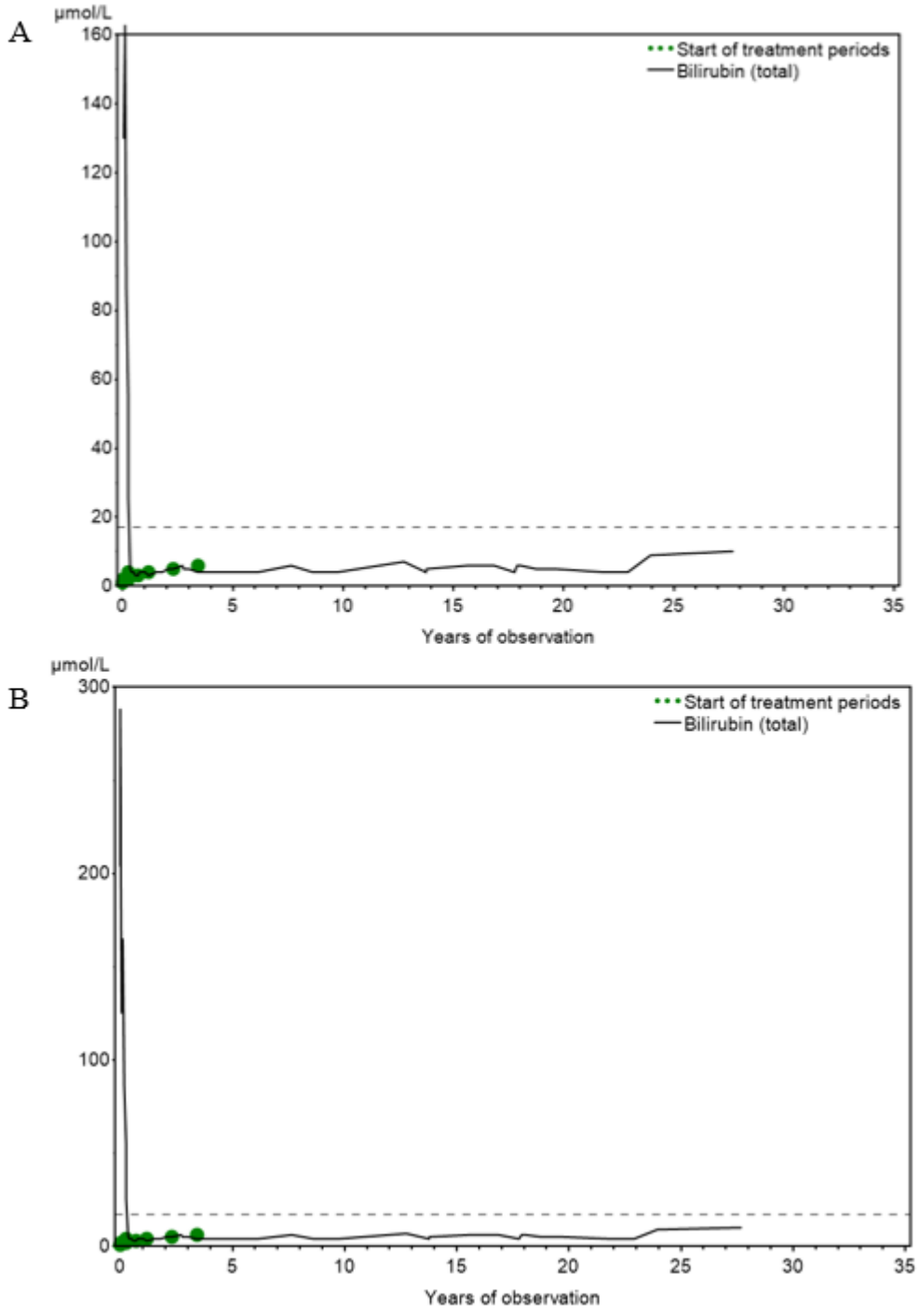
Figure 10. Evolution of Transaminases Over Time – Patient 001006, 3β-HSD Deficiency.



A. Graph displaying transaminases levels from 0 to 300 UI/L (same scale used for all patients and in previous reports).
B. Graph displaying transaminases levels from 0 to 2 000 UI/L as values > 300 UI/L were collected for this patient.
Notes: The black and red dotted lines represent the upper limits of the normal range for respectively ASAT and ALAT.
3β-HSD: 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase, ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase. Source: Figures 8.4.1 and 8.4.2 - TFL V1.0 24OCT2025.

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Figure 11. Evolution of Total Bilirubin Over Time – Patient 001006, 3β-HSD Deficiency.



A. Graph displaying bilirubin levels from 0 to 160 µmol/L (same scale used for all patients and in previous reports).
B. Graph displaying bilirubin levels from 0 to 300 µmol/L as values > 160 µmol/L were collected for this patient.
Notes: The dotted line represents the upper limit of the normal range. 3β-HSD: 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase. Source: Figures 7.4.3 and 7.4.4 - TFL V1.0 24OCT2025.

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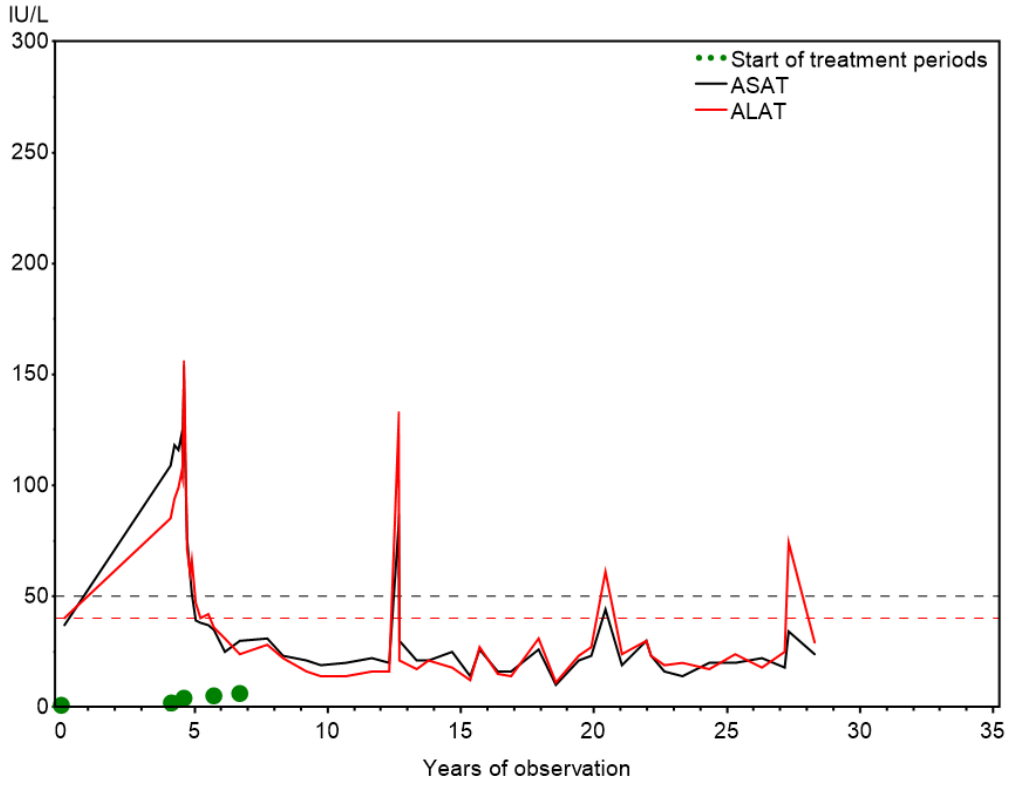
11.4.2.3.3 PATIENT 001010: TREATMENT DURATION > 20 YEARS

The treatment sequence for this patient (indicated on the graphs by green dots) was: no treatment, UDCA monotherapy, first year of CA monotherapy, second year of CA monotherapy, after second year of CA monotherapy.

This patient was treated for 24.5 years including 24 years with CA only (Table 4). One new visit and one biochemical analysis result were added compared to last year's report.

Transaminases increased during the no treatment period and UDCA monotherapy but rapidly normalised after CA monotherapy. There were three short and isolated peaks in transaminases levels above ULN at approximately years 4, 12 and 20 of observation, after which values returned below ULN. In addition, a peak above ULN (for ALAT) was also noticed last year, at approximately 27 years of observation but the level normalised below the ULN this year. This patient was treated with a dose of 450 mg/day between 27 June 2023 and 17 June 2024. A laboratory assessment performed on 18 June 2024 identified an increase in ALAT while all other investigations were in a normal range. The daily dose has been adjusted to 500 mg. Total bilirubin levels also normalised in the first year of CA monotherapy and remained below ULN throughout the observation period (Figure 12 and Figure 13).

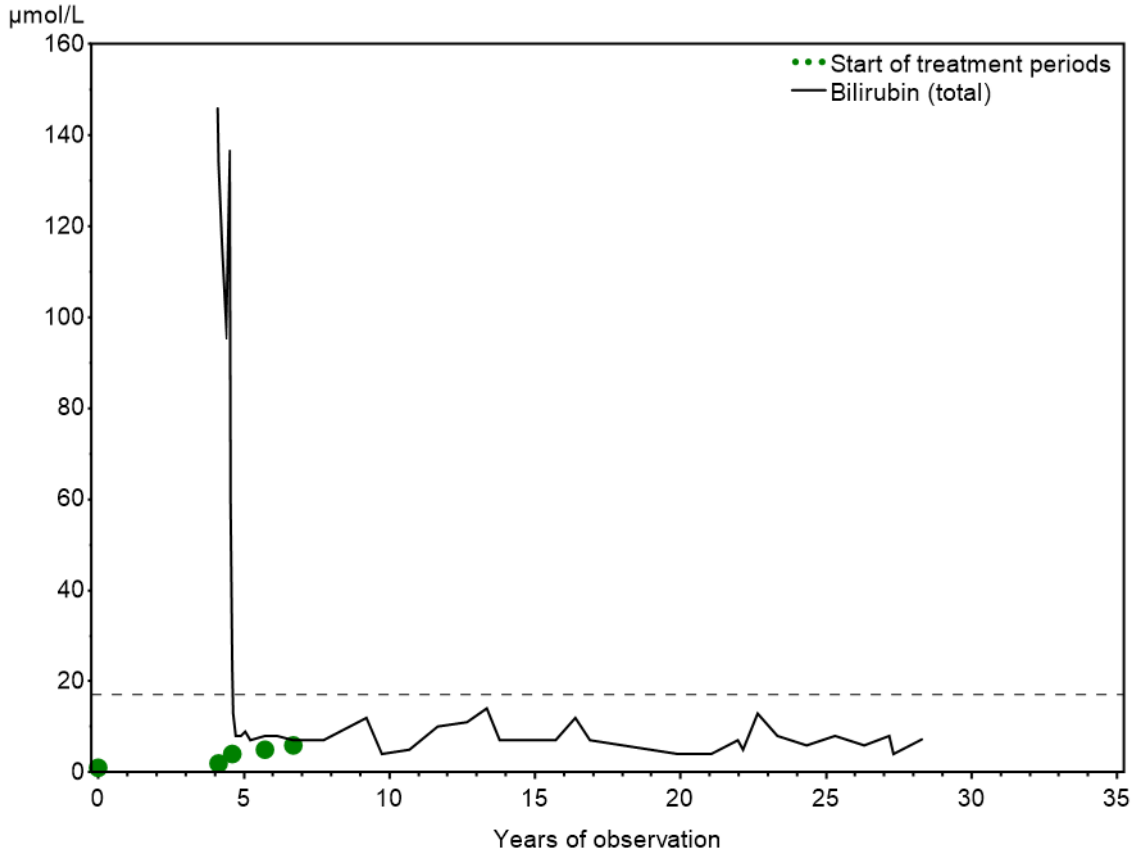
Figure 12: Evolution of Transaminases Over Time – Patient 001010, 3β-HSD Deficiency.



Notes: The black and red dotted lines represent the upper limits of the normal range for respectively ASAT and ALAT. 3β-HSD: 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase, ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase. Source: Figure 8.5.2 - TFL V1.0 24OCT2025.

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Figure 13: Evolution of Total Bilirubin Over Time – Patient 001010, 3β-HSD Deficiency.



Notes: The dotted line represents the upper limit of the normal range. 3β-HSD: 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase. Source: Figure 8.5.4 - TFL V1.0 24OCT2025.

11.4.2.3.4 PATIENT 002001: TREATMENT DURATION > 15 YEARS

The treatment sequence for this patient (indicated on the graphs by green dots) was: no treatment, first year of CA monotherapy, second year of CA monotherapy, after second year of CA monotherapy.

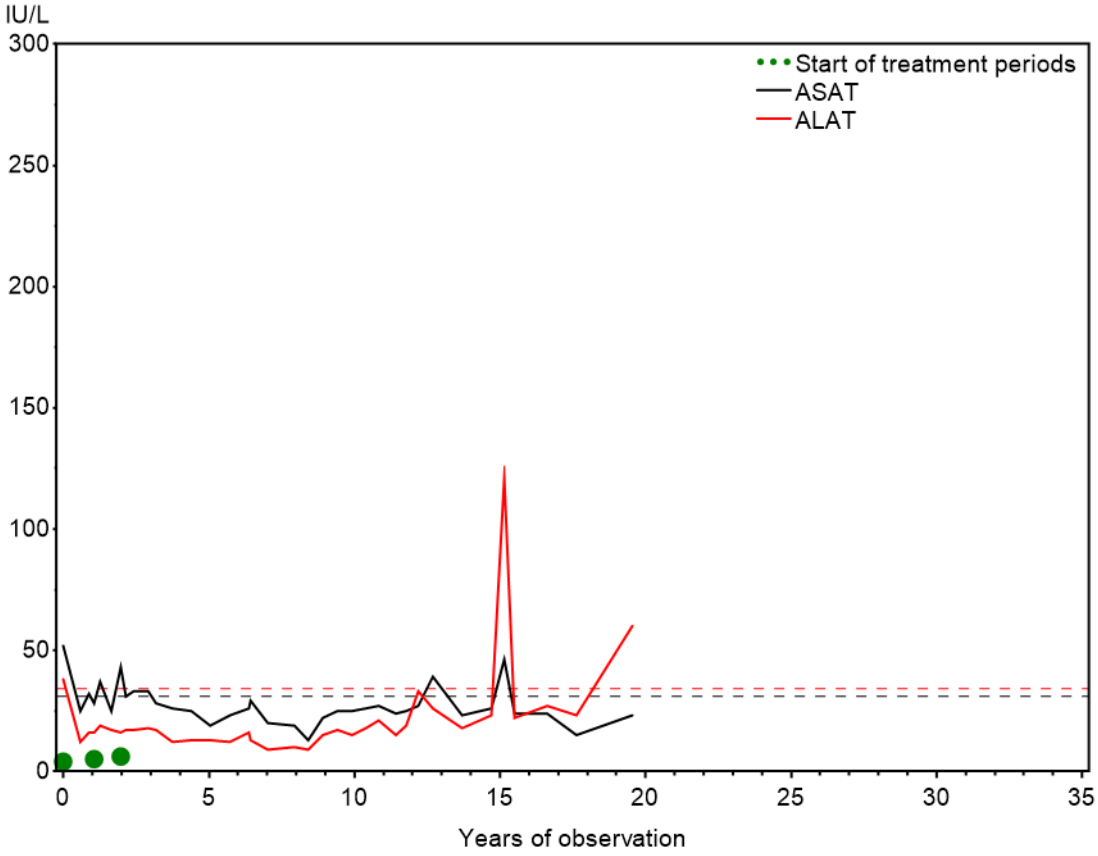
This patient was treated only with CA for 19.7 years (Table 4). No blood parameters results were recorded during the no treatment period for this patient. The comparative effect of CA treatment cannot therefore be evaluated. One new visit and one biochemical analysis result were added in the database since last year's report.

Transaminases were generally below or around the ULN during CA monotherapy (Figure 14). There were four short and isolated peaks in ASAT levels after 2, 3, 13 and 15 years of observation after which values returned below ULN. ALAT levels transiently peaked to 121 UI/L in February 2021 and were reported as normal 4 months later. No other biochemical abnormalities or adverse events were reported at the time of the peak. The centre was thus required to verify this peak data. They

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confirmed that it was correct. Another peak in ALAT above ULN occurred this year to reach 60 UI/L. Total bilirubin levels were below the ULN during CA monotherapy (Figure 15).

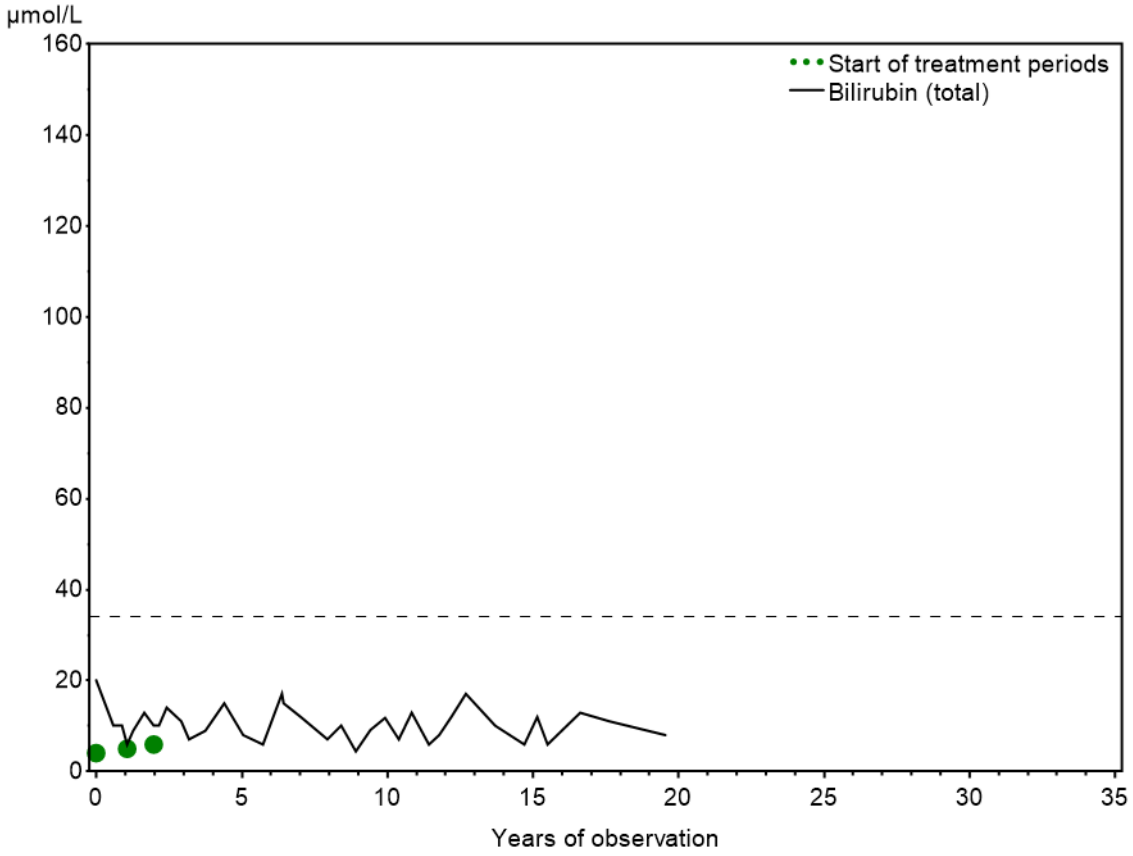
Figure 14: Evolution of Transaminases Over Time – Patient 002001, 3β-HSD Deficiency.



Notes: The black and red dotted lines represent the upper limits of the normal range for respectively ASAT and ALAT. 3β-HSD: 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase, ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase. Source: Figure 8.6.2 - TFL V1.0 24OCT2025.

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Figure 15: Evolution of Bilirubin Over Time – Patient 002001, 3β-HSD Deficiency.



Notes: The dotted line represents the upper limit of the normal range. 3β-HSD: 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase. Source: Figure 8.6.4 - TFL V1.0 24OCT2025.

11.4.2.3.5 PATIENT 004002: TREATMENT DURATION OF >12 YEARS

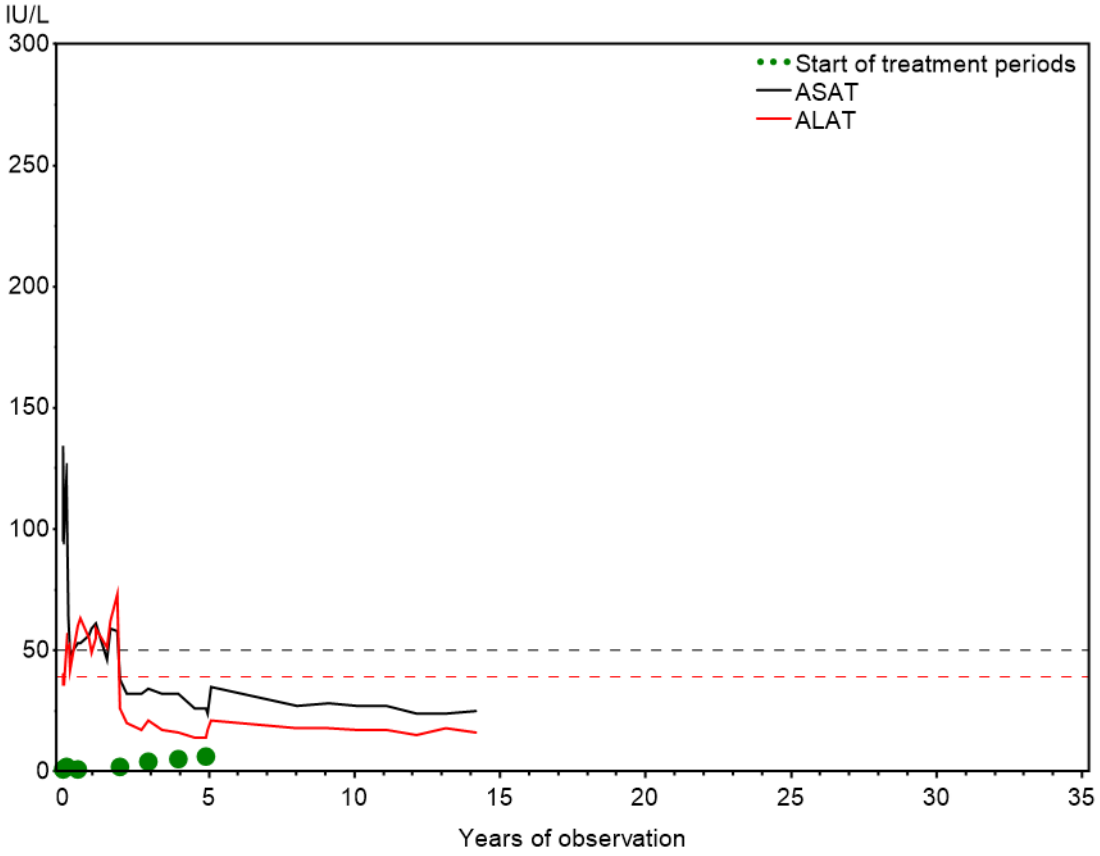
The treatment sequence for this patient (indicated on the graphs by green dots) was: no treatment, UDCA monotherapy, no treatment, UCDA monotherapy, first year of CA monotherapy, second year of CA monotherapy, after second year of CA monotherapy.

This patient was treated for 13.7 years overall and for 12.8 years with CA only (Table 4). One new visit was added since last year's report, but no biochemical analysis results.

Transaminases and bilirubin levels were above the ULN without treatment and normalised during the first period of UDCA monotherapy (Figure 16 and Figure 17). Then, levels increased above the ULN or stayed close to the ULN during the no treatment period started at the end of the first year of observation. Levels returned below the ULN when patient restarted UDCA monotherapy at the end of the second year of observation. Since then, transaminases and bilirubin levels stayed below the ULN.

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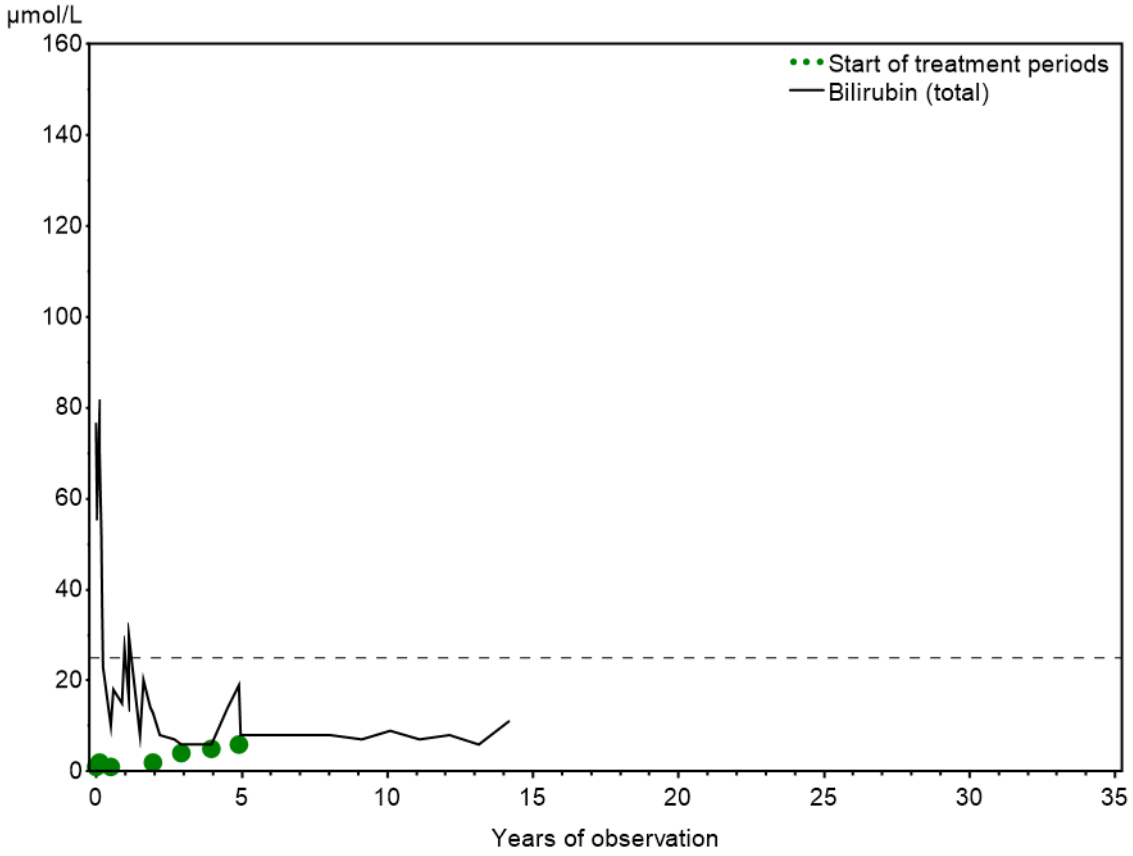
Figure 16: Evolution of Transaminases Over Time – Patient 004002, 3β-HSD Deficiency.



Notes: The black and red dotted lines represent the upper limits of the normal range for respectively ASAT and ALAT. 3β-HSD: 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase, ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase. Source: Figure 8.8.2 - TFL V1.0 24OCT2025.

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Figure 17: Evolution of Bilirubin Over Time – Patient 004002, 3β-HSD Deficiency.



Notes: The dotted line represents the upper limit of the normal range. 3β-HSD: 3β-hydroxy-Δ^5-C_{27}-steroid oxidoreductase. Source: Figure 8.8.4 - TFL V1.0 24OCT2025.

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11.4.3 PATIENTS WITH Δ4-3-OXOR DEFICIENCY

As previously mentioned, two new patients with Δ4-3-oxoR deficiency have been included since last year's report. Patients already included in the database had 12 new blood measurements collected for this year's report.

11.4.3.1 DESCRIPTIVE ANALYSES ON CATEGORICAL PARAMETERS

11.4.3.1.1 ASPARTATE AMINOTRANSFERASE

Table 23 and Table 24 present the numbers and percentages of each best and worst values of the ASAT parameter per treatment period.

When compared to the reference (worst value 'no treatment' or UDCA alone), these tables show a fast normalisation of the best ASAT values once a treatment is started (UDCA, UDCA+CA or CA alone), whichever the variable classification used (categorical vs binary). But the best results are obtained when patients are under CA as a monotherapy with all patients displaying normal values under CA treatment on the first year and after.

This normalisation of ASAT values is less noticeable for certain patients when comparing the worst ASAT values to the reference (worst value 'no treatment' or UDCA alone).

Table 23: Serum ASAT Values as a Categorical Variable (Patients with Δ4-3-oxoR deficiency, N = 11).

		N	Missing	Normal	>1 to 2-fold ULN	>2 to 3-fold ULN	>3-fold ULN
References (worst values)	No treatment	9	2	1 (11.1)	-	-	8 (88.9)
	UDCA	6	5	1 (16.7)	1 (16.7)	-	4 (66.7)
Best values	UDCA	6	5	3 (50.0)	-	-	3 (50.0)
	UDCA + CA	4	7	3 (75.0)	-	-	1 (25.0)
	CA first year	8	3	8 (100.0)	-	-	-
	CA second year	5	6	5 (100.0)	-	-	-
	CA after second year	8	3	8 (100.0)	-	-	-
	CA pooled	9	2	9 (100.0)	-	-	-
Worst values	UDCA + CA	4	7	1 (25.0)	-	-	3 (75.0)
	CA first year	8	3	3 (37.5)	2 (25.0)	-	3 (37.5)
	CA second year	5	6	5 (100.0)	-	-	-
	CA after second year	8	3	7 (87.5)	1 (12.5)	-	-
	CA pooled	9	2	3 (33.3)	3 (33.3)	-	3 (33.3)

Notes: data are presented as n(%). ASAT: Aspartate Aminotransferase; CA: Cholic Acid; UDCA: Ursodeoxycholic acid; ULN: Upper Limit of Normal. Source: adapted from Table 4.1.1 - TFL V1.0 24OCT2025.

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Table 24: Serum ASAT Values as a Binary Variable (Patients with Δ4-3-oxoR deficiency, N = 11).

		N	Missing	Normal	Abnormal
References (worst values)	No treatment	9	2	1 (11.1)	8 (88.9)
	UDCA	6	5	1 (16.7)	5 (83.3)
Best values	UDCA	6	5	3 (50.0)	3 (50.0)
	UDCA + CA	4	7	3 (75.0)	1 (25.0)
	CA first year	8	3	8 (100.0)	-
	CA second year	5	6	5 (100.0)	-
	CA after second year	8	3	8 (100.0)	-
	CA pooled	9	2	9 (100.0)	-
Worst values	UDCA + CA	4	7	1 (25.0)	3 (75.0)
	CA first year	8	3	3 (37.5)	5 (62.5)
	CA second year	5	6	5 (100.0)	-
	CA after second year	8	3	7 (87.5)	1 (12.5)
	CA pooled	9	2	3 (33.3)	6 (66.7)

Notes: data are presented as n(%). ASAT: Aspartate Aminotransferase; CA: Cholic Acid; UDCA: Ursodeoxycholic acid. Source: adapted from Table 4.1.2 - TFL V1.0 24OCT2025.

11.4.3.1.2 ALANINE AMINOTRANSFERASE

Table 25 and Table 26 present the numbers and percentages of each best and worst values of the ALAT parameter per treatment period.

As for ASAT parameter, when compared to the reference, these tables show a fast normalisation of the best ALAT values once a treatment is started, whichever the variable classification used (categorical vs binary). The best results are obtained when patients are under CA treatment: as a monotherapy, especially as from the second year of treatment.

This normalisation of ALAT values is less noticeable for certain patients when comparing the worst ALAT values to the reference (worst value ‘no treatment’ or UDCA alone).

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Table 25: Serum ALAT Values as a Categorical Variable (Patients with Δ4-3-oxoR deficiency, N = 11).

		N	Missing	Normal	>1 to 2-fold ULN	>2 to 3-fold ULN	>3-fold ULN
References (worst values)	No treatment	9	2	1 (11.1)	-	-	8 (88.9)
	UDCA	6	5	1 (16.7)	-	-	5 (83.3)
Best values	UDCA	6	5	3 (50.0)	-	-	3 (50.0)
	UDCA + CA	4	7	3 (75.0)	-	1 (25.0)	-
	CA first year	8	3	6 (75.0)	2 (25.0)	-	-
	CA second year	5	6	5 (100.0)	-	-	-
	CA after second year	8	3	8 (100.0)	-	-	-
	CA pooled	9	2	8 (88.9)	1 (11.1)	-	-
Worst values	UDCA + CA	4	7	1 (25.0)	-	1 (25.0)	2 (50.0)
	CA first year	8	3	3 (37.5)	1 (12.5)	1 (12.5)	3 (37.5)
	CA second year	5	6	5 (100.0)	-	-	-
	CA after second year	8	3	7 (87.5)	1 (12.5)	-	-
	CA pooled	9	2	3 (33.3)	2 (22.2)	1 (11.1)	3 (33.3)

Notes: data are presented as n(%). ALAT: Alanine Aminotransferase; CA: Cholic Acid; UDCA: Ursodeoxycholic acid; ULN: Upper Limit of Normal. Source: adapted from Table 4.2.1 - TFL V1.0 24OCT2025.

Table 26: Serum ALAT Values as a Binary Variable (Patients with Δ4-3-oxoR deficiency, N = 11).

		N	Missing	Normal	Abnormal
References (worst values)	No treatment	9	2	1 (11.1)	8 (88.9)
	UDCA	6	5	1 (16.7)	5 (83.3)
Best values	UDCA	6	5	3 (50.0)	3 (50.0)
	UDCA + CA	4	7	3 (75.0)	1 (25.0)
	CA first year	8	3	6 (75.0)	2 (25.0)
	CA second year	5	6	5 (100.0)	-
	CA after second year	8	3	8 (100.0)	-
	CA pooled	9	2	8 (88.9)	1 (11.1)
Worst values	UDCA + CA	4	7	1 (25.0)	3 (75.0)
	CA first year	8	3	3 (37.5)	5 (62.5)
	CA second year	5	6	5 (100.0)	-
	CA after second year	8	3	7 (87.5)	1 (12.5)
	CA pooled	9	2	3 (33.3)	6 (66.7)

Notes: data are presented as n(%). ALAT: Alanine Aminotransferase; CA: Cholic Acid; UDCA: Ursodeoxycholic acid. Source: adapted from Table 4.2.2 - TFL V1.0 24OCT2025.

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11.4.3.1.3 GAMMA-GLUTAMYL TRANSFERASE

Table 27 and Table 28 present the numbers and percentages of each best and worst values of the GGT parameter per treatment period.

When compared to the reference, these tables show a fast normalisation of the best GGT values once a treatment is started, whichever the variable classification used. The best results are obtained when patients are under CA as a monotherapy, especially as from the second year of treatment.

This trend toward normalisation of GGT values is also noticeable when comparing the worst GGT values during CA alone to the reference, especially as from the second year of treatment.

Table 27: Serum GGT Values as a Categorical Variable (Patients with Δ4-3-oxoR deficiency, N = 11).

		N	Missing	Normal	>1 to 2-fold ULN	>2 to 3-fold ULN	>3-fold ULN
References (worst values)	No treatment	9	2	2 (22.2)	6 (66.7)	-	1 (11.1)
	UDCA	6	5	2 (33.3)	3 (50.0)	1 (16.7)	-
Best values	UDCA	6	5	5 (83.3)	1 (16.7)	-	-
	UDCA + CA	4	7	4 (100.0)	-	-	-
	CA first year	8	3	7 (87.5)	-	1 (12.5)	-
	CA second year	5	6	5 (100.0)	-	-	-
	CA after second year	8	3	8 (100.0)	-	-	-
	CA pooled	9	2	8 (88.9)	-	1 (11.1)	-
Worst values	UDCA + CA	4	7	2 (50.0)	-	1 (25.0)	1 (25.0)
	CA first year	8	3	5 (62.5)	-	1 (12.5)	2 (25.0)
	CA second year	5	6	5 (100.0)	-	-	-
	CA after second year	8	3	8 (100.0)	-	-	-
	CA pooled	9	2	6 (66.7)	-	1 (11.1)	2 (22.2)

Notes: data are presented as n(%). CA: Cholic Acid; GGT: Gamma-Glutamyl Transferase; UDCA: Ursodeoxycholic acid; ULN: Upper Limit of Normal. Source: adapted from Table 4.3.1 - TFL V1.0 24OCT2025.

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Table 28: Serum GGT Values as a Binary Variable (Patients with Δ4-3-oxoR deficiency, N = 11).

		N	Missing	Normal	Abnormal
References (worst values)	No treatment	9	2	2 (22.2)	7 (77.8)
	UDCA	6	5	2 (33.3)	4 (66.7)
Best values	UDCA	6	5	5 (83.3)	1 (16.7)
	UDCA + CA	4	7	4 (100.0)	-
	CA first year	8	3	7 (87.5)	1 (12.5)
	CA second year	5	6	5 (100.0)	-
	CA after second year	8	3	8 (100.0)	-
	CA pooled	9	2	8 (88.9)	1 (11.1)
Worst values	UDCA + CA	4	7	2 (50.0)	2 (50.0)
	CA first year	8	3	5 (62.5)	3 (37.5)
	CA second year	5	6	5 (100.0)	-
	CA after second year	8	3	8 (100.0)	-
	CA pooled	9	2	6 (66.7)	3 (33.3)

Notes: data are presented as n(%). CA: Cholic Acid; GGT: Gamma-Glutamyl Transferase; UDCA: Ursodeoxycholic acid. Source: adapted from Table 4.3.2 - TFL V1.0 24OCT2025.

11.4.3.1.4 TOTAL BILIRUBIN

Table 29 and Table 30 present the numbers and percentages of each best and worst values of the total bilirubin parameter per treatment period.

When compared to the reference, these tables show a fast normalisation of the best total bilirubin values since a treatment is started, with all patients displaying normal values under CA treatment on the first year and after.

This normalisation of total bilirubin values is also observed when comparing the worst total bilirubin values during CA alone to the reference, especially as from the second year of treatment.

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Table 29: Serum Total Bilirubin Values as a Categorical Variable (Patients with Δ4-3-oxoR deficiency, N = 11).

		N	Missing	Normal	>1 to 2-fold ULN	>2 to 3-fold ULN	>3-fold ULN
References (worst values)	No treatment	9	2	3 (33.3)	-	-	6 (66.7)
	UDCA	6	5	3 (50.0)	1 (16.7)	-	2 (33.3)
Best values	UDCA	6	5	4 (66.7)	1 (16.7)	-	1 (16.7)
	UDCA + CA	4	7	3 (75.0)	-	-	1 (25.0)
	CA first year	7	4	7 (100.0)	-	-	-
	CA second year	5	6	5 (100.0)	-	-	-
	CA after second year	8	3	8 (100.0)	-	-	-
	CA pooled	9	2	9 (100.0)	-	-	-
Worst values	UDCA + CA	4	7	1 (25.0)	-	1 (25.0)	2 (50.0)
	CA first year	7	4	6 (85.7)	-	-	1 (14.3)
	CA second year	5	6	5 (100.0)	-	-	-
	CA after second year	8	3	8 (100.0)	-	-	-
	CA pooled	9	2	8 (88.9)	-	-	1 (11.1)

Notes: data are presented as n(%). CA: Cholic Acid; UDCA: Ursodeoxycholic acid; ULN: Upper Limit of Normal. Source: adapted from Table 4.4.1 - TFL V1.0 24OCT2025.

Table 30: Serum Total Bilirubin Values as a Binary Variable (Patients with Δ4-3-oxoR deficiency, N = 11).

		N	Missing	Normal	Abnormal
References (worst values)	No treatment	9	2	3 (33.3)	6 (66.7)
	UDCA	6	5	3 (50.0)	3 (50.0)
Best values	UDCA	6	5	4 (66.7)	2 (33.3)
	UDCA + CA	4	7	3 (75.0)	1 (25.0)
	CA first year	7	4	7 (100.0)	-
	CA second year	5	6	5 (100.0)	-
	CA after second year	8	3	8 (100.0)	-
	CA pooled	9	2	9 (100.0)	-
Worst values	UDCA + CA	4	7	1 (25.0)	3 (75.0)
	CA first year	7	4	6 (85.7)	1 (14.3)
	CA second year	5	6	5 (100.0)	-
	CA after second year	8	3	8 (100.0)	-
	CA pooled	9	2	8 (88.9)	1 (11.1)

Notes: data are presented as n(%). CA: Cholic Acid; UDCA: Ursodeoxycholic acid. Source: adapted from Table 4.4.2 - TFL V1.0 24OCT2025.

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11.4.3.1.5 ALKALINE PHOSPHATASE

Table 31 and Table 32 present the numbers and percentages of each best and worst values of the ALP parameter per treatment period.

When compared to the reference, these tables show a fast normalisation of best ALP values once a treatment is started, especially CA treatment alone. After the second year of CA treatment alone, all patients display normal ALP values.

This normalisation of ALP values is less noticeable when comparing the worst ALP values to the reference except during CA for the second year of CA treatment.

Table 31: Serum ALP Values as a Categorical Variable (Patients with Δ4-3-oxoR deficiency, N = 11).

		N	Missing	Normal	>1 to 2-fold ULN	>2 to 3-fold ULN	>3-fold ULN
References (worst values)	No treatment	5	6	3 (60.0)	1 (20.0)	-	1 (20.0)
	UDCA	5	6	2 (40.0)	2 (40.0)	-	1 (20.0)
Best values	UDCA	5	6	4 (80.0)	1 (20.0)	-	-
	UDCA + CA	4	7	3 (75.0)	1 (25.0)	-	-
	CA first year	6	5	5 (83.3)	1 (16.7)	-	-
	CA second year	5	6	4 (80.0)	1 (20.0)	-	-
	CA after second year	6	5	6 (100.0)	-	-	-
	CA pooled	7	4	7 (100.0)	-	-	-
Worst values	UDCA + CA	4	7	1 (25.0)	1 (25.0)	2 (50.0)	-
	CA first year	6	5	3 (50.0)	2 (33.3)	1 (16.7)	-
	CA second year	5	6	4 (80.0)	1 (20.0)	-	-
	CA after second year	6	5	2 (33.3)	4 (66.7)	-	-
	CA pooled	7	4	1 (14.3)	5 (71.4)	1 (14.3)	-

Notes: data are presented as n(%). ALP: Alkaline Phosphatase; CA: Cholic Acid; UDCA: Ursodeoxycholic acid; ULN: Upper Limit of Normal. Source: adapted from Table 4.5.1 - TFL V1.0 24OCT2025.

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Table 32: Serum ALP Values as a Binary Variable (Patients with Δ4-3-oxoR deficiency, N = 11).

		N	Missing	Normal	Abnormal
References (worst values)	No treatment	5	6	3 (60.0)	2 (40.0)
	UDCA	5	6	2 (40.0)	3 (60.0)
Best values	UDCA	5	6	4 (80.0)	1 (20.0)
	UDCA + CA	4	7	3 (75.0)	1 (25.0)
	CA first year	6	5	5 (83.3)	1 (16.7)
	CA second year	5	6	4 (80.0)	1 (20.0)
	CA after second year	6	5	6 (100.0)	-
	CA pooled	7	4	7 (100.0)	-
Worst values	UDCA + CA	4	7	1 (25.0)	3 (75.0)
	CA first year	6	5	3 (50.0)	3 (50.0)
	CA second year	5	6	4 (80.0)	1 (20.0)
	CA after second year	6	5	2 (33.3)	4 (66.7)
	CA pooled	7	4	1 (14.3)	6 (85.7)

Notes: data are presented as n(%).ALP: Alkaline Phosphatase; CA: Cholic Acid; UDCA: Ursodeoxycholic acid. Source: adapted from Table 4.5.2 - TFL V1.0 24OCT2025.

11.4.3.1.6 VITAMIN E

Table 33 and Table 34 present the numbers and percentages of each best and worst values of the vitamin E parameter per treatment period.

When compared to the references, these tables show a normalisation of the best vitamin E values for any treatment periods, especially as from the second year of CA treatment.

This trend toward normalisation of vitamin E values is less noticeable when comparing the worst vitamin E values to the worst value of ‘no treatment’ except during UDCA + CA and CA after the second year of CA treatment.

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Table 33: Serum Vitamin E Values as a Categorical Variable (Patients with Δ4-3-oxoR deficiency, N = 11).

		N	Missing	Normal	Above ULN	Below LLN
References (worst values)	No treatment	7	4	3 (42.9)	-	4 (57.1)
	UDCA	4	7	2 (50.0)	-	2 (50.0)
Best values	UDCA	4	7	3 (75.0)	-	1 (25.0)
	UDCA + CA	4	7	4 (100.0)	-	-
	CA first year	7	4	5 (71.4)	2 (28.6)	-
	CA second year	4	7	4 (100.0)	-	-
	CA after second year	7	4	7 (100.0)	-	-
	CA pooled	9	2	9 (100.0)	-	-
Worst values	UDCA + CA	4	7	4 (100.0)	-	-
	CA first year	7	4	3 (42.9)	4 (57.1)	-
	CA second year	4	7	3 (75.0)	1 (25.0)	-
	CA after second year	7	4	7 (100.0)	-	-
	CA pooled	9	2	5 (55.6)	4 (44.4)	-

Notes: data are presented as n(%). CA: Cholic Acid; LLN: Lower Limit of Normal; UDCA: Ursodeoxycholic acid; ULN: Upper Limit of Normal. Source: adapted from Table 4.6.1 - TFL V1.0 24OCT2025.

Table 34: Serum Vitamin E Values as a Binary Variable (Patients with Δ4-3-oxoR deficiency, N = 11).

		N	Missing	Normal	Abnormal
References (worst values)	No treatment	7	4	3 (42.9)	4 (57.1)
	UDCA	4	7	2 (50.0)	2 (50.0)
Best values	UDCA	4	7	3 (75.0)	1 (25.0)
	UDCA + CA	4	7	4 (100.0)	-
	CA first year	7	4	5 (71.4)	2 (28.6)
	CA second year	4	7	4 (100.0)	-
	CA after second year	7	4	7 (100.0)	-
	CA pooled	9	2	9 (100.0)	-
Worst values	UDCA + CA	4	7	4 (100.0)	-
	CA first year	7	4	3 (42.9)	4 (57.1)
	CA second year	4	7	3 (75.0)	1 (25.0)
	CA after second year	7	4	7 (100.0)	-
	CA pooled	9	2	5 (55.6)	4 (44.4)

Notes: data are presented as n(%). CA: Cholic Acid; UDCA: Ursodeoxycholic acid. Source: adapted from Table 4.6.2 - TFL V1.0 24OCT2025.

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11.4.3.2 Descriptive Analyses on Quantitative Parameters

As presented in Section 11.4.2.2 for patients with 3β-HSD deficiency, Table 35 and Table 36 present descriptive data for liver biochemistry parameters and for Vitamin E when considered as quantitative variables for patients with Δ4-3-oxoR deficiency.

Data are also presented graphically using boxplots for each liver parameter in Figure 18 to Figure 22 and for Vitamin E in Figure 23.

11.4.3.2.1 Liver Parameters

Mean ASAT levels increased from no treatment (304.8 ± 197.1 IU/L) to UDCA monotherapy (399.2 ± 367.3 IU/L) and showed a high variability between patients (large interquartile range) (Table 35 and Figure 18)Figure 18: Mean Value of Serum ASAT According to Treatment Period in patients with Δ4-3-oxoR deficiency (N = 11).. Mean levels then importantly decreased during the UDCA+CA dual therapy period (124.5 ± 116.7 IU/L) and the first year of CA alone (114.7 ± 158.6 IU/L) but remained above the ULN. They decreased below the ULN during the second year of CA treatment (37.4 ± 1.9 IU/L) and remained low after 2 years of CA treatment (32.0 ± 4.9 IU/L).

The same pattern was observed for mean ALAT levels except for the first year of CA alone when the mean ALAT level increased to 128.7 (± 221.9) IU/L before decreasing again (Table 35 and Figure 19).

Mean GGT levels were above the ULN without treatment (52.4 ± 35.7 IU/L) and then decreased below the ULN when under UDCA (36.9 ± 17.0 IU/L) (Table 35 and Figure 20). Mean GGT levels increased then during UDCA+CA (49.4 ± 26.3 IU/L), above the ULN, but this increase should be considered with caution as only 4 patients were treated with UDCA+CA. Mean GGT levels then decreased during CA monotherapy (41.8 ± 39.3 IU/L) and remained below the ULN during all CA treatment periods (CA after two years, 11.8 ± 2.7 IU/L).

Mean total bilirubin levels were above the ULN without treatment (75.4 ± 86.9 µmol/L), with UDCA monotherapy (60.0 ± 118.7 µmol/L) and during UDCA+CA period (54.6 ± 65.3 µmol/L) (Table 35 and Figure 21). Mean total bilirubin levels then dropped below the ULN during the first year of CA treatment (12.0 ± 15.4 µmol/L) and remained low after two years of treatment (5.6 ± 1.3 µmol/L).

Mean ALP levels were above the ULN without treatment (472.9 ± 318.1 IU/L), with UDCA monotherapy (377.6 ± 136.6 IU/L), during UDCA+CA period (346.5 ± 190.0 IU/L) and the first year of CA treatment (377.0 ± 166.6 IU/L) (Table 35 and Figure 22). Mean ALP levels dropped below the ULN during the second year of CA treatment (276.3 ± 73.1 IU/L) and remained low after two years of treatment (271.3 ± 77.8 IU/L).

Although these data concern only 11 patients, effect of CA treatment on liver parameters normalisation are evident. The inclusion of new patients in the next years will allow to reduce the variability of the mean for the analysed parameters.

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Table 35: Continuous Serum Liver Parameters: Description and Comparison to References ('no treatment' or UDCA alone) – in patients with Δ4-3-oxoR deficiency (N = 11).

		No treatment	UDCA	UDCA + CA	CA first year	CA second year	CA after second year	CA pooled
ASAT (IU/L)	N	9	6	4	8	5	8	9
	Missing values	2	5	7	3	6	3	2
	Mean ± SD	304.8 ± 197.1	399.2 ± 367.3	124.5 ± 116.7	114.7 ± 158.6	37.4 ± 1.9	32.0 ± 4.9	72.2 ± 81.5
	Median	286.0	381.1	87.1	56.8	37.5	31.8	36.9
	Q1 ; Q3	176.3 ; 345.0	46.3 ; 696.0	52.1 ; 196.8	35.8 ; 104.7	35.5 ; 39.3	28.6 ; 35.6	31.1 ; 66.0
	Min ; Max	35.0 ; 634.7	33.0 ; 857.9	29.6 ; 294.0	24.0 ; 499.5	35.3 ; 39.3	24.5 ; 39.5	26.0 ; 281.8
ALAT (IU/L)	N	9	6	4	8	5	8	9
	Missing values	2	5	7	3	6	3	2
	Mean ± SD	337.0 ± 208.4	425.7 ± 468.2	71.9 ± 41.6	128.7 ± 221.9	19.0 ± 3.8	16.7 ± 1.9	74.0 ± 120.2
	Median	257.0	312.2	76.8	59.1	19.3	16.2	21.1
	Q1 ; Q3	205.0 ; 452.0	89.7 ; 517.4	38.5 ; 105.3	24.9 ; 86.3	17.5 ; 20.9	15.7 ; 16.8	17.9 ; 61.6
	Min ; Max	16.0 ; 658.0	22.7 ; 1 300.3	21.1 ; 113.0	16.0 ; 673.1	13.5 ; 23.8	15.1 ; 21.1	16.0 ; 387.8
GGT (IU/L)	N	9	6	4	8	5	8	9
	Missing values	2	5	7	3	6	3	2
	Mean ± SD	52.4 ± 35.7	36.9 ± 17.0	49.4 ± 26.3	41.8 ± 39.3	10.9 ± 3.2	11.8 ± 2.7	32.0 ± 33.2
	Median	47.7	34.7	45.4	19.6	9.7	11.3	13.5
	Q1 ; Q3	43.0 ; 50.0	26.7 ; 45.5	30.3 ; 68.6	12.5 ; 78.7	8.5 ; 12.0	9.5 ; 13.2	11.3 ; 38.3
	Min ; Max	13.0 ; 138.0	15.7 ; 64.0	22.6 ; 84.4	9.0 ; 103.8	8.5 ; 16.0	9.0 ; 17.2	9.4 ; 103.8

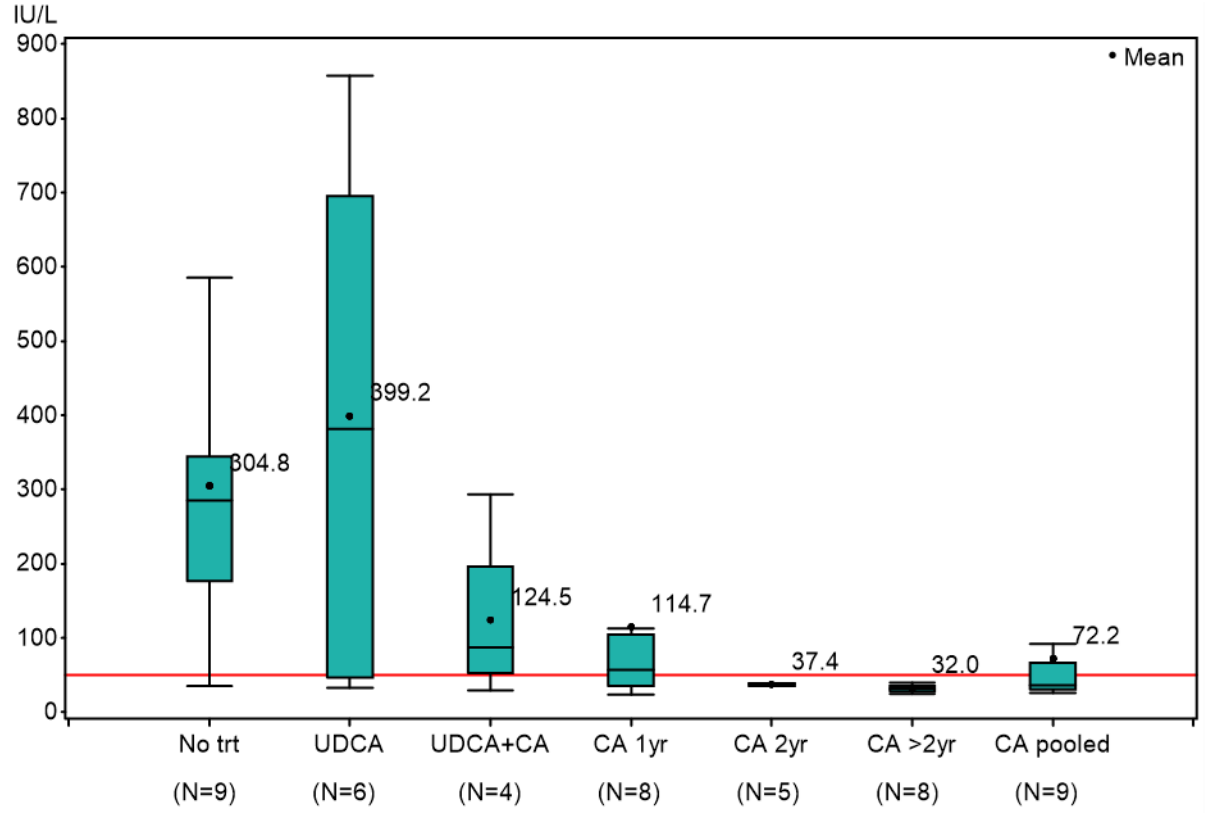
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		No treatment	UDCA	UDCA + CA	CA first year	CA second year	CA after second year	CA pooled
Total bilirubin (µmol/L)	N	9	6	4	7	5	8	9
	Missing values	2	5	7	4	6	3	2
	Mean ± SD	75.4 ± 86.9	60.0 ± 118.7	54.6 ± 65.3	12.0 ± 15.4	4.0 ± 2.6	5.6 ± 1.3	9.5 ± 10.8
	Median	43.9	10.2	32.1	6.3	4.0	5.9	6.4
	Q1 ; Q3	8.1 ; 79.5	7.7 ; 24.2	8.4 ; 100.8	5.0 ; 9.6	3.2 ; 5.5	4.6 ; 6.5	4.8 ; 7.0
	Min ; Max	7.1 ; 243.3	6.0 ; 302.0	7.2 ; 147.0	4.0 ; 46.7	0.2 ; 7.3	3.5 ; 7.0	4.3 ; 38.1
ALP (IU/L)	N	5	5	4	6	5	6	7
	Missing values	6	6	7	5	6	5	4
	Mean ± SD	472.9 ± 318.1	377.6 ± 136.6	346.5 ± 190.0	377.0 ± 166.6	276.3 ± 73.1	271.3 ± 77.8	338.6 ± 148.5
	Median	274.0	360.7	304.5	360.3	286.0	301.0	318.0
	Q1 ; Q3	266.0 ; 575.0	255.3 ; 508.0	216.2 ; 476.7	235.2 ; 477.5	213.5 ; 292.0	227.2 ; 324.3	215.4 ; 382.8
	Min ; Max	260.0 ; 989.3	236.5 ; 527.5	166.9 ; 610.0	193.4 ; 635.5	204.5 ; 385.7	135.1 ; 339.5	179.4 ; 635.5

Notes: CA: Cholic Acid; UDCA: Ursodeoxycholic acid. ASAT: Aspartate aminotransferase; ALAT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; ALP: Alkaline phosphatase; N: number of samples; SD: Standard Deviation.
Sources: issued from all Tables of Section 7 - TFL V1.0 24OCT2025.

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Figure 18: Mean Value of Serum ASAT According to Treatment Period in patients with Δ4-3-oxoR deficiency (N = 11).

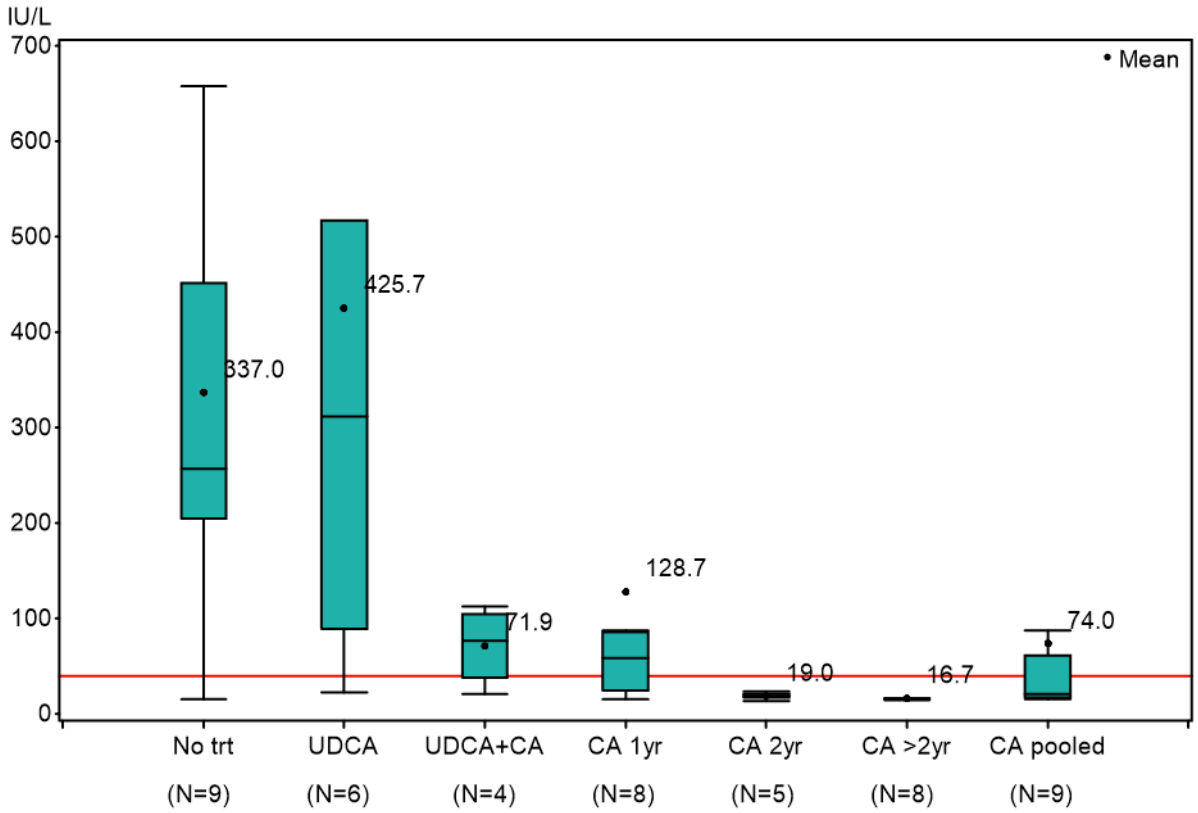


No trt: No Treatment, UDCA:Ursodeoxycholic acid, CA: Cholic Acid, yr:year

Notes: The box represents the inter-quartile range (Q3-Q1); the black dot the mean, the — the median; and the upper & lower bars the observed maximum and minimum average values. The upper limit of the normal ranges is issued from Centre 001 (except for some patients) and is indicated by a red line. ASAT: Aspartate aminotransferase; CA: Cholic Acid; trt: treatment; UDCA: Ursodeoxycholic acid; 1yr: first year; 2yr: second year. Source: Figure 8.2.1 - TFL V1.0 24OCT2025.

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Figure 19: Mean Value of Serum ALAT According to Treatment Period in patients with Δ4-3-oxoR deficiency (N = 11).

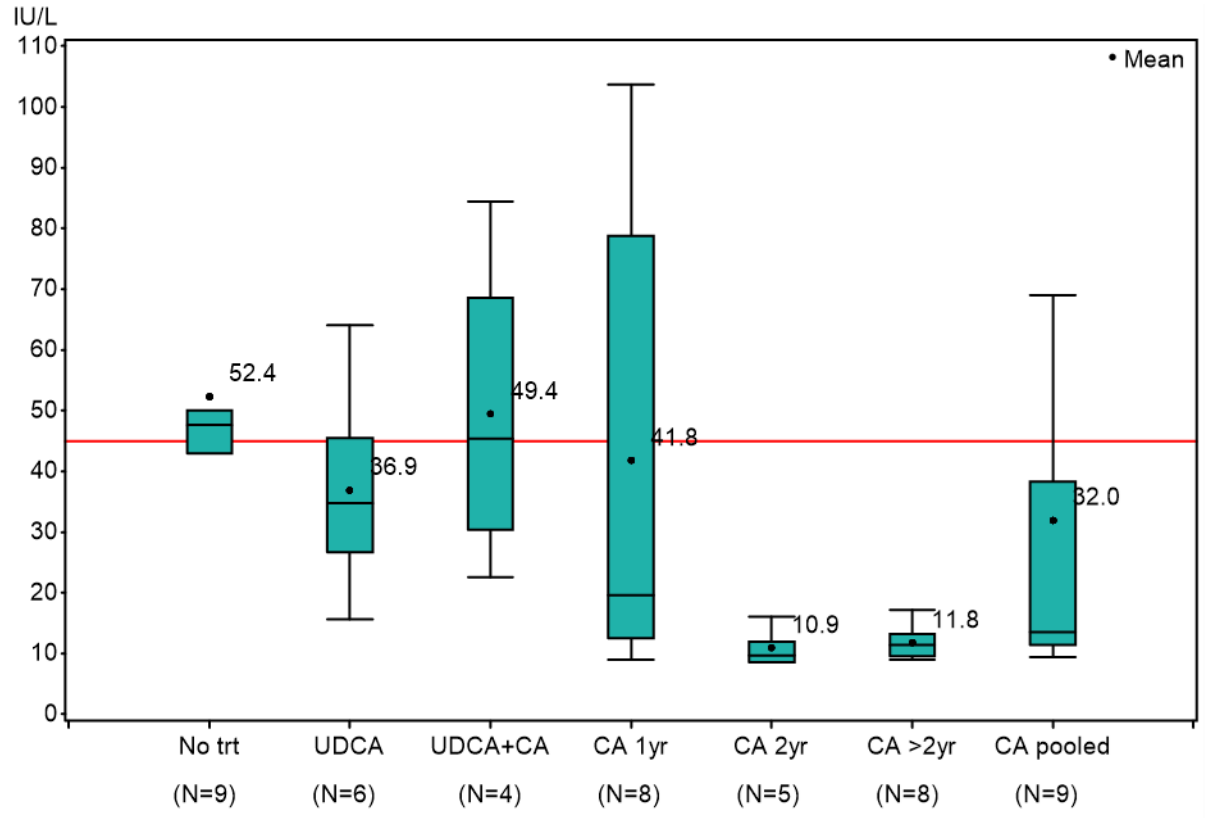


No trt: No Treatment, UDCA:Ursodeoxycholic acid, CA: Cholic Acid, yr:year

Notes: The box represents the inter-quartile range (Q3-Q1); the black dot the mean, the — the median; and the upper & lower bars the observed maximum and minimum average values. The upper limit of the normal ranges is issued from Centre 001 (except for some patients) and is indicated by a red line. ALAT: Alanine aminotransferase; CA: Cholic Acid; trt: treatment; UDCA: Ursodeoxycholic acid; 1yr: first year; 2yr: second year. Source: Figure 8.2.2 - TFL V1.0 24OCT2025.

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Figure 20: Mean Value of Serum GGT According to Treatment Period in patients with Δ4-3-oxoR deficiency (N = 11).

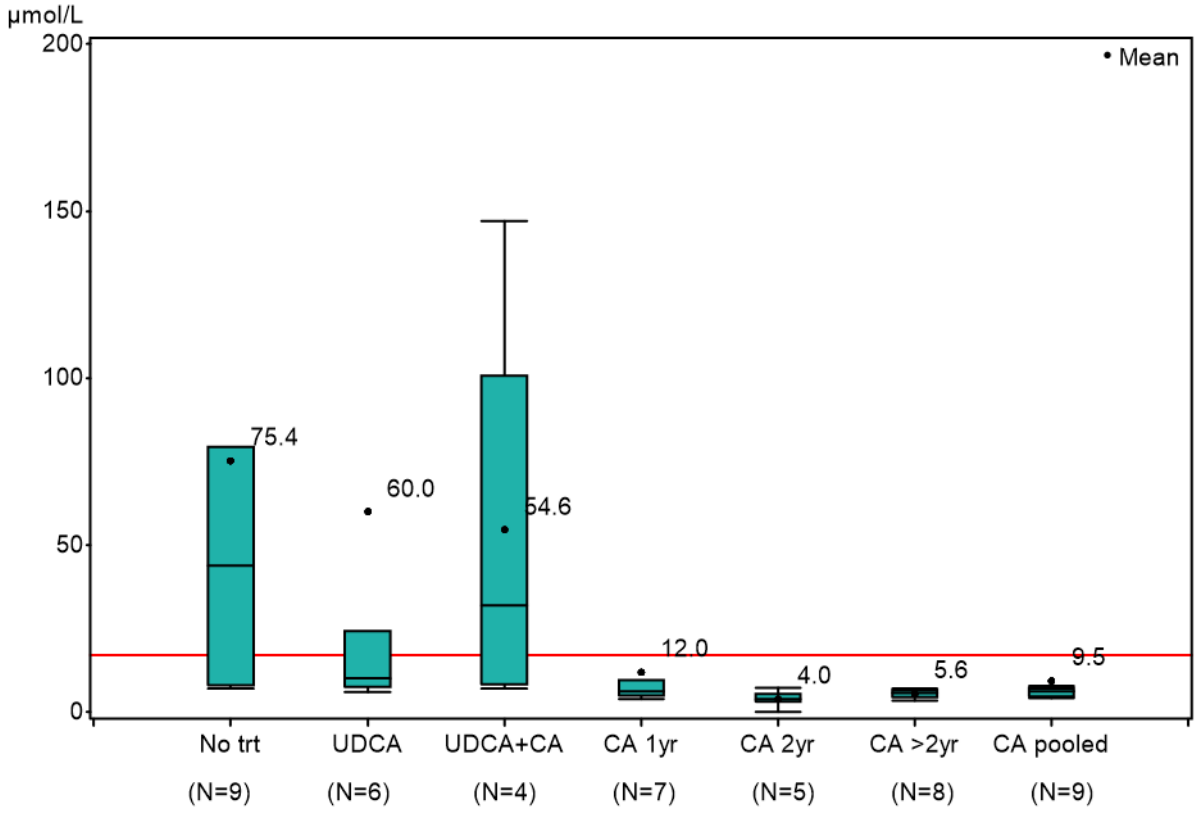


No trt: No Treatment, UDCA:Ursodeoxycholic acid, CA: Cholic Acid, yr:year

Notes: The box represents the inter-quartile range (Q3-Q1); the black dot the mean, the — the median; and the upper & lower bars the observed maximum and minimum average values. The upper limit of the normal ranges is issued from Centre 001 (except for some patients) and is indicated by a red line. GGT: Gamma-glutamyl transferase; CA: Cholic Acid; trt: treatment; UDCA: Ursodeoxycholic acid; 1yr: first year; 2yr: second year. Source: Figure 8.2.3 - TFL V1.0 24OCT2025.

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Figure 21: Mean Value of Serum Total Bilirubin According to Treatment Period in patients with Δ4-3-oxoR deficiency (N = 11).

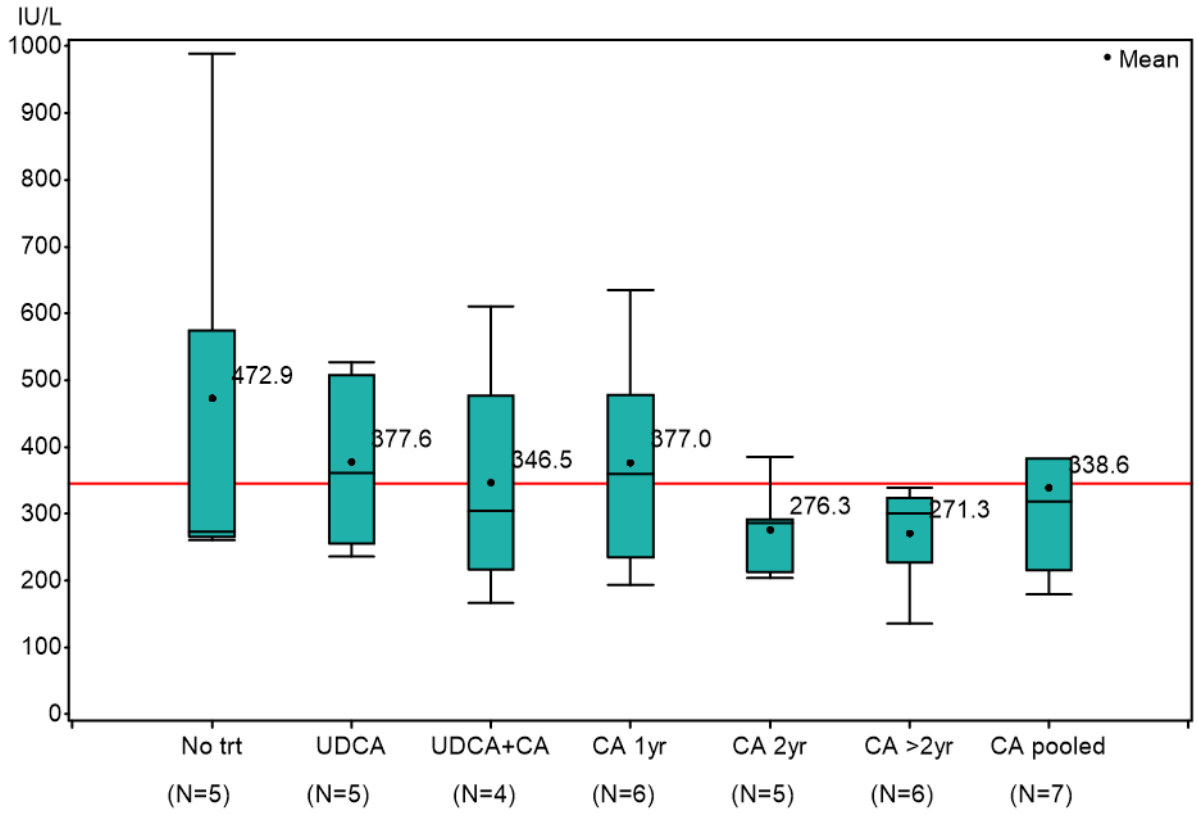


No trt: No Treatment, UDCA:Ursodeoxycholic acid, CA: Cholic Acid, yr:year

Notes: The box represents the inter-quartile range (Q3-Q1); the black dot the mean, the — the median; and the upper & lower bars the observed maximum and minimum average values. The upper limit of the normal ranges is issued from Centre 001 (except for some patients) and is indicated by a red line. CA: Cholic acid; trt: treatment; UDCA: Ursodeoxycholic acid; 1yr: first year; 2yr: second year. Source: Figure 8.2.4 - TFL V1.0 24OCT2025.

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Figure 22: Mean Value of Serum ALP According to Treatment Period in patients with Δ4-3-oxoR deficiency (N = 11).



No trt: No Treatment, UDCA:Ursodeoxycholic acid, CA: Cholic Acid, yr:year

Notes: The box represents the inter-quartile range (Q3-Q1); the black dot the mean, the — the median; and the upper & lower bars the observed maximum and minimum average values. The upper limit of the normal ranges is issued from Centre 001 (except for some patients) and is indicated by a red line. ALP: Alkaline phosphatase; CA: Cholic acid; trt: treatment; UDCA: Ursodeoxycholic acid; 1yr: first year; 2yr: second year. Source: Figure 8.2.5 - TFL V1.0 24OCT2025.

11.4.3.2.2 VITAMIN E

Mean Vitamin E levels, which were at the lower limit of the normal range without treatment (5.0 ± 3.2 µg/L), increased with UDCA monotherapy (7.9 ± 3.2 µg/L) and the subsequent treatment phases, getting closer to the middle of the normal range, especially during the second year of CA treatment (9.6 ± 3.4 µg/L) (Table 36 and Figure 23). During the first year of CA treatment (14.9 ± 4.9 µg/L), vitamin E levels were on the ULN.

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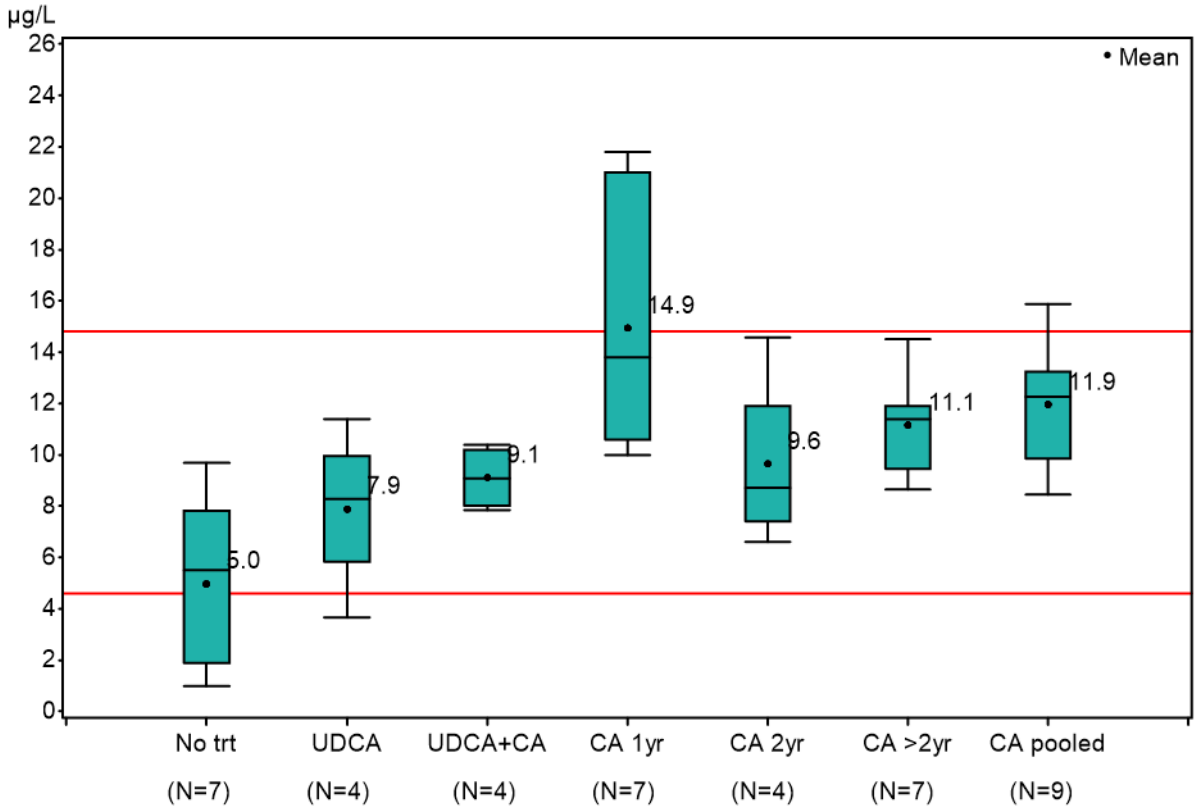
Table 36: Continuous Vitamin E Parameter: Description and Comparison to References ('no treatment' or UDCA alone) in patients with Δ4-3-oxoR deficiency (N = 11).

		No treatment	UDCA	UDCA + CA	CA first year	CA second year	CA after second year	CA pooled
Vitamin E (µg/L)	N	7	4	4	7	4	7	9
	Missing values	4	7	7	4	7	4	2
	Mean ± SD	5.0 ± 3.2	7.9 ± 3.2	9.1 ± 1.3	14.9 ± 4.9	9.6 ± 3.4	11.1 ± 1.9	11.9 ± 2.3
	Median	5.5	8.3	9.1	13.8	8.7	11.4	12.3
	Q1 ; Q3	1.9 ; 7.8	5.8 ; 10.0	8.0 ; 10.2	10.6 ; 21.0	7.4 ; 11.9	9.4 ; 11.9	9.9 ; 13.2
	Min ; Max	1.0 ; 9.7	3.7 ; 11.4	7.9 ; 10.4	10.0 ; 21.8	6.6 ; 14.6	8.7 ; 14.5	8.4 ; 15.9

Notes: CA: Cholic Acid; UDCA: Ursodeoxycholic acid. N: number of samples; SD: standard deviation.
Source: Table 7.6.1 - TFL V1.0 24OCT2025.

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Figure 23: Mean Value of Serum Vitamin E According to Treatment Period in patients with Δ4-3-oxoR deficiency (N = 11).



No trt: No Treatment, UDCA:Ursodeoxycholic acid, CA: Cholic Acid, yr:year

Notes: The box represents the inter-quartile range (Q3-Q1); the black dot the mean, the — the median; and the upper & lower bars the observed maximum and minimum average values. The upper limit of the normal ranges is issued from Centre 001 (except for some patients) and is indicated by a red line. CA: Cholic Acid; trt: treatment; UDCA: Ursodeoxycholic acid; 1yr: first year; 2yr: second year. Source: Figure 8.2.6 - TFL V1.0 24OCT2025.

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11.4.3.3 BY-PATIENT DISPLAYS

For this twelfth report, all figures presented below have been updated and include graphical representations of transaminases, bilirubin, GGT and ALP over time. They are presented for the same patients with Δ^4-3-oxoR deficiency as in previous reports.

When comparing the observation periods of these 7 patients with Δ^4-3-oxoR deficiency:

- The two first patients (001004 and 001005) had more than 25 years of observation and treatment,
- 1 patient (001011) had more than10 years of observation and almost 13 years of treatment,
- 3 patients (010001, 010002 and 017001) had more than 8 years of observation and treatment,
- The last patient (001012) had less than 7 years of observation, with a treatment period of 6.2 years.

11.4.3.3.1 PATIENT 001005: TREATMENT DURATION > 25 YEARS

The treatment sequence for this patient (indicated on the graphs by green dots) was: no treatment, UDCA, UDCA+CA dual therapy, first year of CA monotherapy, back to UDCA+CA dual therapy for the remaining duration.

This patient was treated for 28.2 years overall and with UDCA+CA for 26.9 years (Table 4). CA treatment alone was only temporary (1 year) before returning to the dual therapy UDCA+CA. One new visit and two biochemistry analysis results were added since last year's report.

Transaminases and bilirubin were notably above the ULN under no treatment and decreased below or close to the ULN under UDCA monotherapy and UDCA+CA combination therapy. Bilirubin levels stayed below the ULN during the subsequent treatment period but transaminases spiked with CA monotherapy starting during the 3rd year of observation, before normalising with UDCA+CA dual therapy (Figure 24 and Figure 25). Transient pikes of ALAT and ALAT were still observed during UDCA+CA dual therapy, after 8, 17, 22, 25 and 28 years of observation.

GGT and ALP were elevated under no treatment, UDCA, UDCA+CA dual therapy, spiked again under CA monotherapy, and normalised subsequently under UDCA+CA dual therapy (Figure 26 and Figure 27). Similar to her twin sister – patient 001004 –, this patient had a transient elevation of ALP around at around 10 years of observation, probably related to rapid bone growth in puberty (see Section 11.4.3.3.2).

As noted in the previous report, an increase in GGT levels was observed during the follow up visit done in February 2018: GGT activity values were in the normal range during many previous years of observation. Clinical situation of the patient has been evaluated by the treating physician as "stable and favourable". Indeed, other liver function tests did not show any deterioration of the condition. According to the physician, observed increase of GGT value (61 UI/L) in 2018 could be possibly explained by a general viral infection which the patient had at the time of blood analysis. However, GGT levels were still above the ULN few months later (May 2018), so that the physician decided to

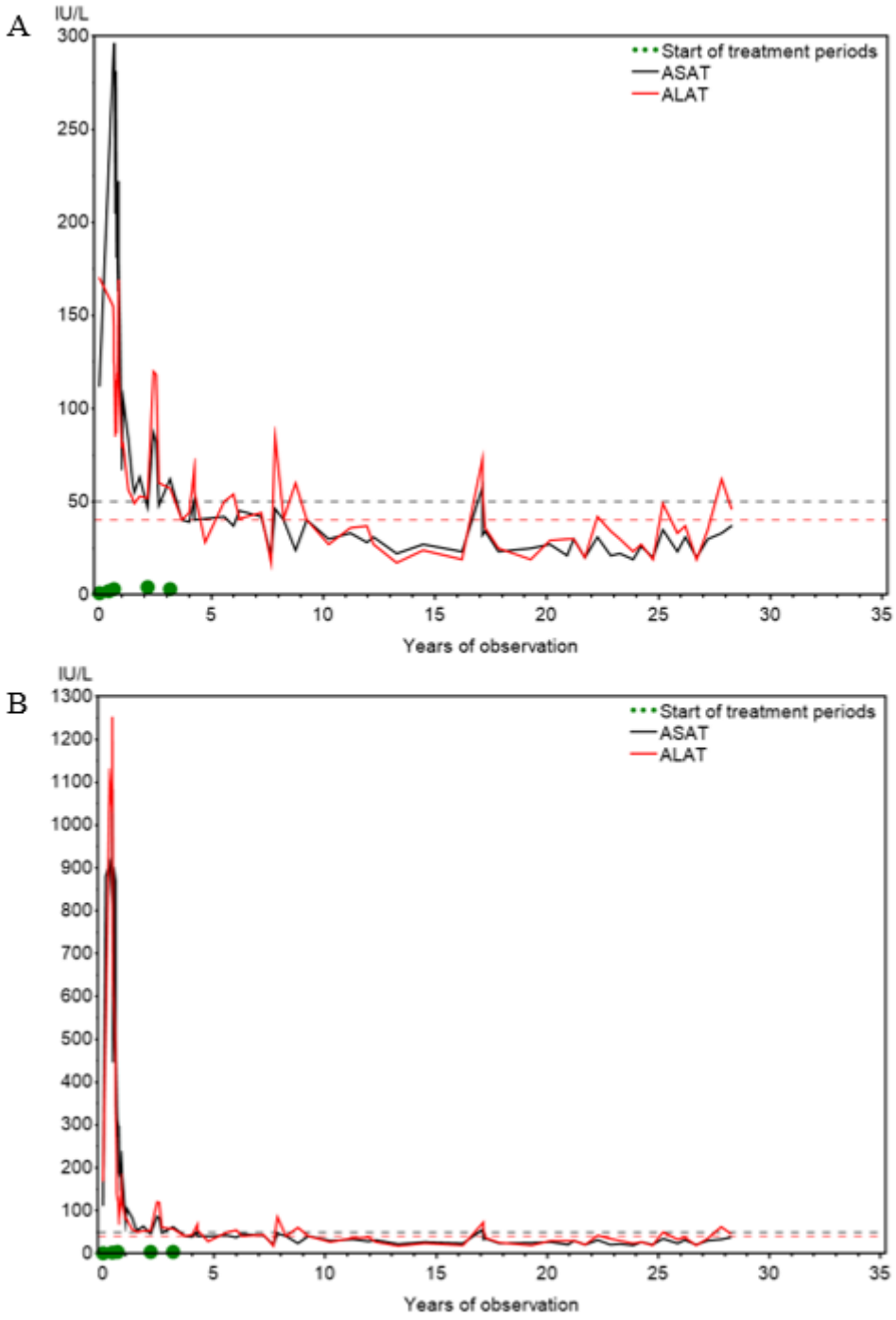
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increase the treatment daily dose to 450 mg. Since this dose modification, GGT levels decreased, but remained slightly above the ULN (May 2023). During the previous reporting period, GGT has been detected elevated. However, other liver parameters remained in the normal range. Since 31 January 2023 the daily dose has been increased from 450 to 500 mg/day. During this year, GGT levels decreased to 61 UI/L. No additional comments were provided by the investigator.

Too few data points for a meaningful display of serum Vitamin E levels were available. Briefly, levels were below the normal range at UDCA treatment and normalised under subsequent treatment; data for no treatment were not available.

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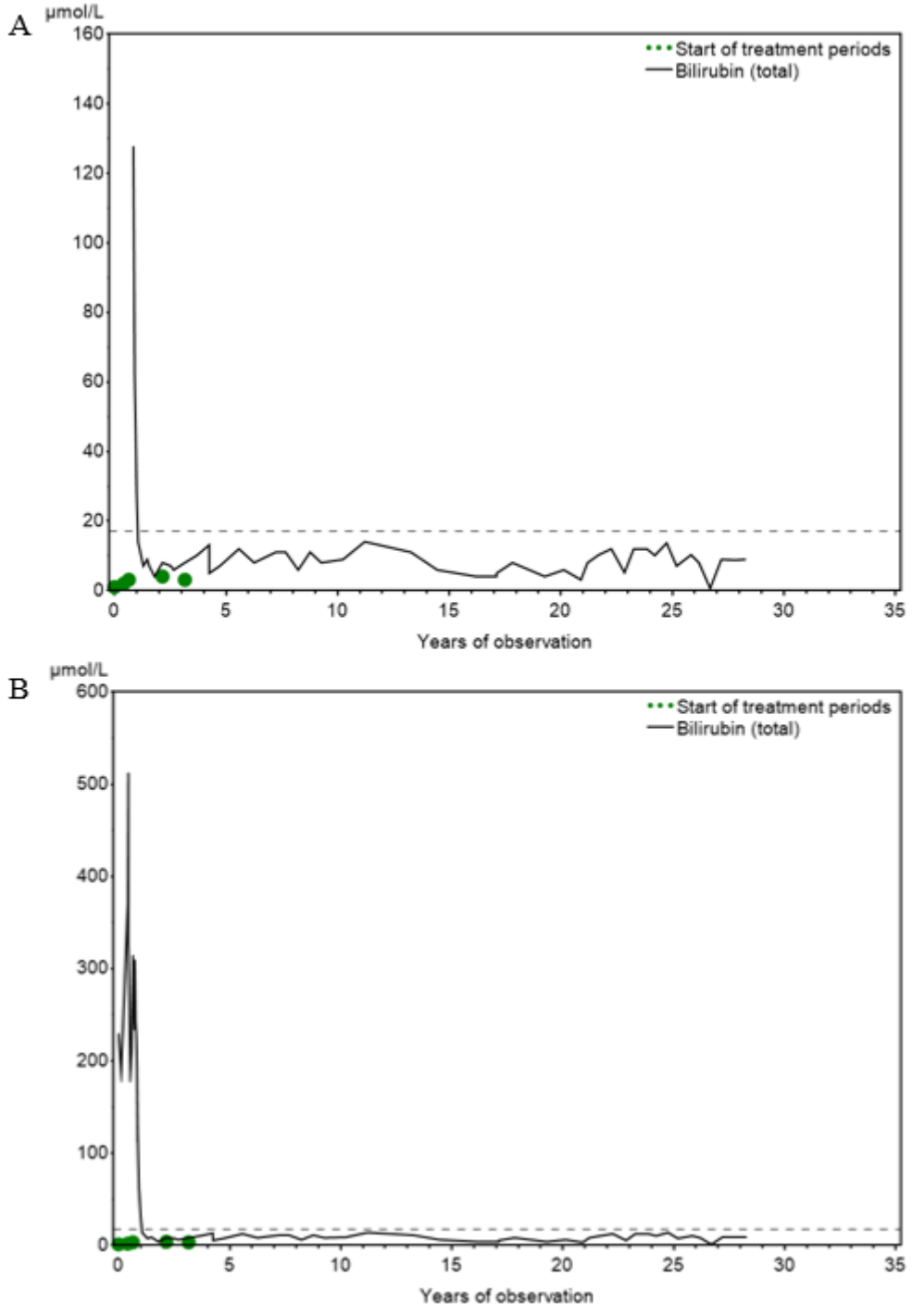
Figure 24: Evolution of Transaminases Over Time – Patient 001005, Δ^4-3-oxoR Deficiency.



A, Graph displaying transaminases levels from 0 to 300 UI/L (same scale used for all patients and in previous reports). B, Graph displaying transaminases levels from 0 to 1 300 UI/L as values > 300 UI/L were collected for this patient. Notes: The black and red dotted lines represent the upper limits of the normal range for respectively ASAT and ALAT. Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase, ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase. Source: Figures 8.10.1 and 8.10.2 - TFL V1.0 24OCT2025.

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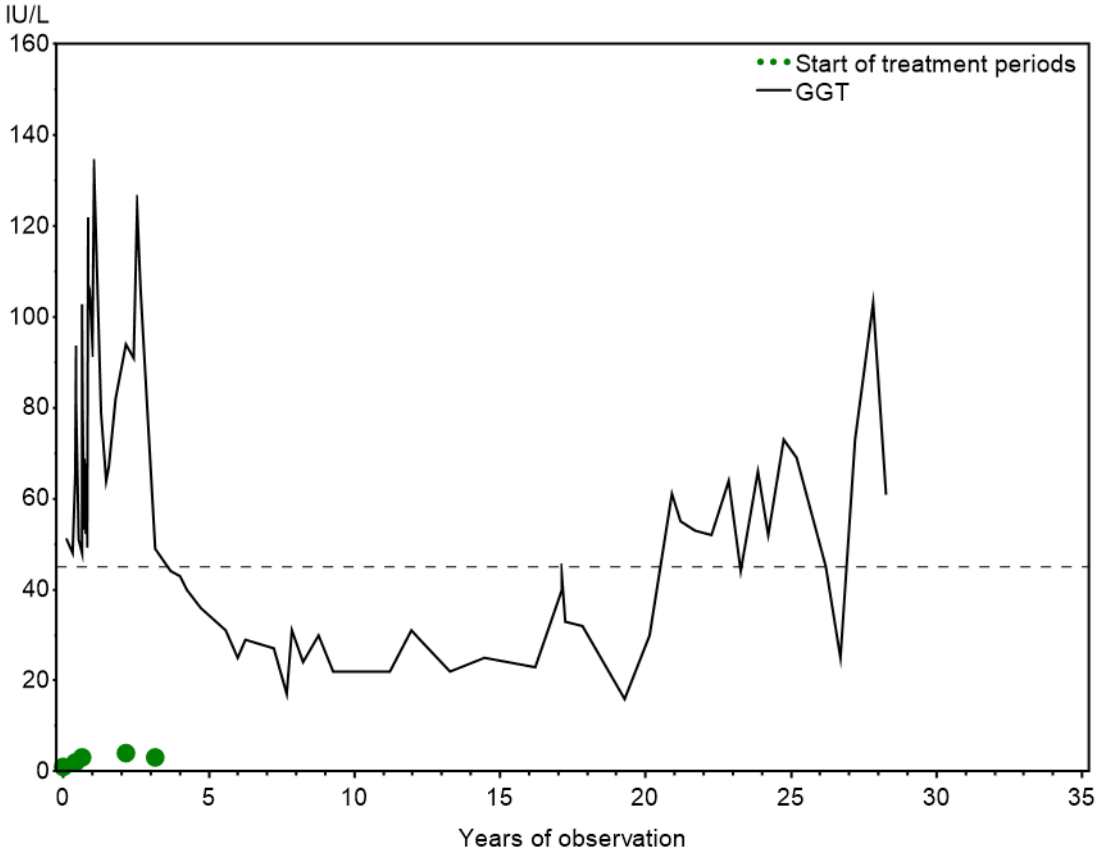
Figure 25: Evolution of Total Bilirubin Over Time – Patient 001005, Δ^4-3-oxoR Deficiency.



A, Graph displaying bilirubin levels from 0 to 160 µmol/L (same scale used for all patients and in previous reports). B, Graph displaying bilirubin levels from 0 to 600 µmol/L as values > 160 µmol/L were collected for this patient. Notes: The dotted line represent the upper limit of the normal range. Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase. Source: Figures 8.10.3 and 8.10.4 - TFL V1.0 24OCT2025.

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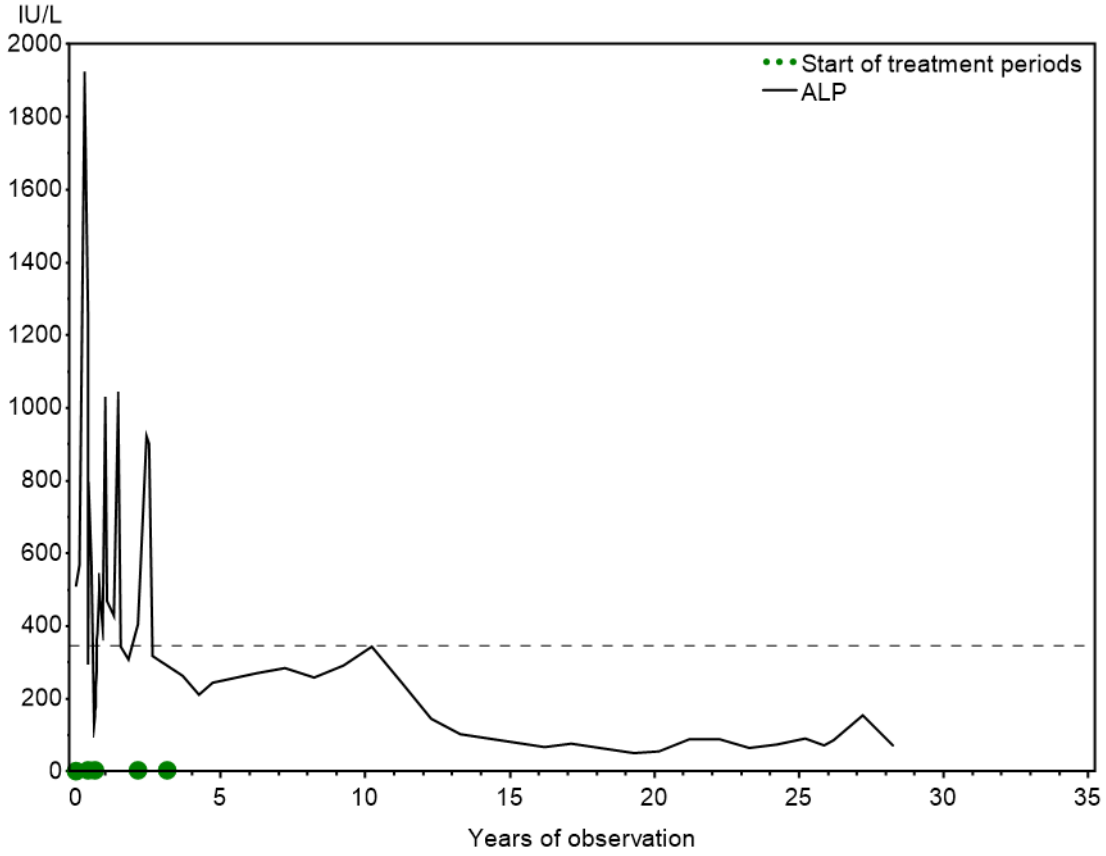
Figure 26: Evolution of GGT Over Time – Patient 001005, Δ^4-3-oxoR Deficiency.



Notes: The dotted lines represent the upper limit of the normal range. Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase, GGT: Gamma-glutamyl transferase. Source: Figure 8.10.6 - TFL V1.0 24OCT2025.

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Figure 27: Evolution of ALP Over Time – Patient 001005, Δ^4-3-oxoR Deficiency.



Notes: The dotted line represents the upper limit of the normal range. Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase, ALP: Alkaline phosphatase. Source: Figure 8.10.8 - TFL V1.0 24OCT2025.

11.4.3.3.2 PATIENT 001004: TREATMENT DURATION > 25 YEARS

The treatment sequence for this patient (indicated on the graphs by green dots) was: no treatment, UDCA+CA dual therapy, first year of CA monotherapy, second year of CA monotherapy, after second year of CA monotherapy.

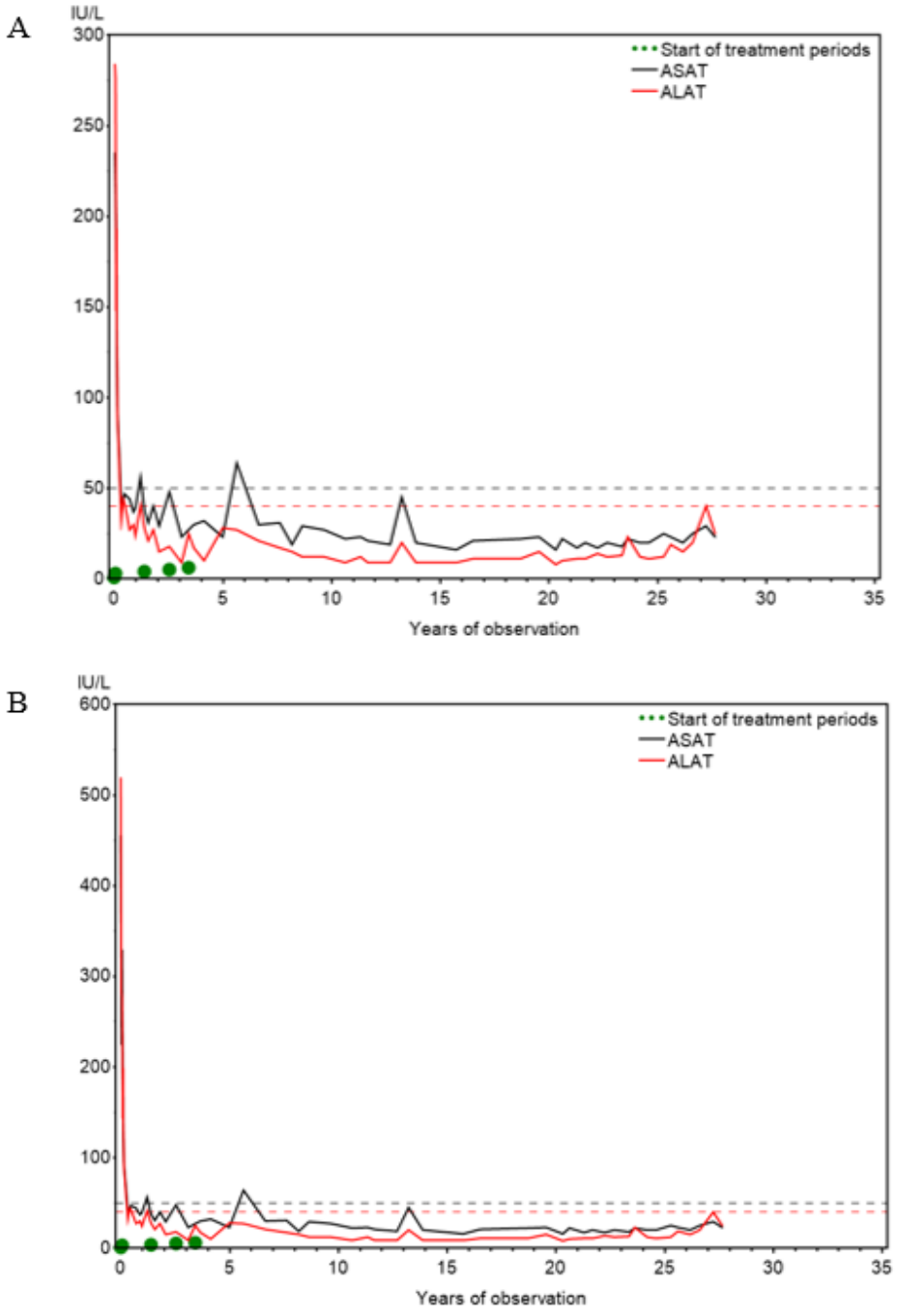
This patient was treated for 27.9 years overall and for 26.7 years with CA only (Table 4). One new visit and two biochemistry analysis results were added since last year's report.

In general, transaminases, GGT and total bilirubin were below or around the ULN from the time of UDCA+CA dual therapy onwards, except once where the ASAT level was above ULN after 6 years of observation (Figure 28, Figure 29 and Figure 30). ALP only normalised in the second year of CA and, similarly to her twin sister – patient 001005 (see Section 11.4.3.3.1) – this patient had a transient elevation of ALP levels after around 10 years of observation (Figure 31). This was probably related to rapid bone growth in puberty.

Too few data points were available for a meaningful display of serum Vitamin E levels. Briefly, levels were in the normal range at all observations, not including the no treatment period.

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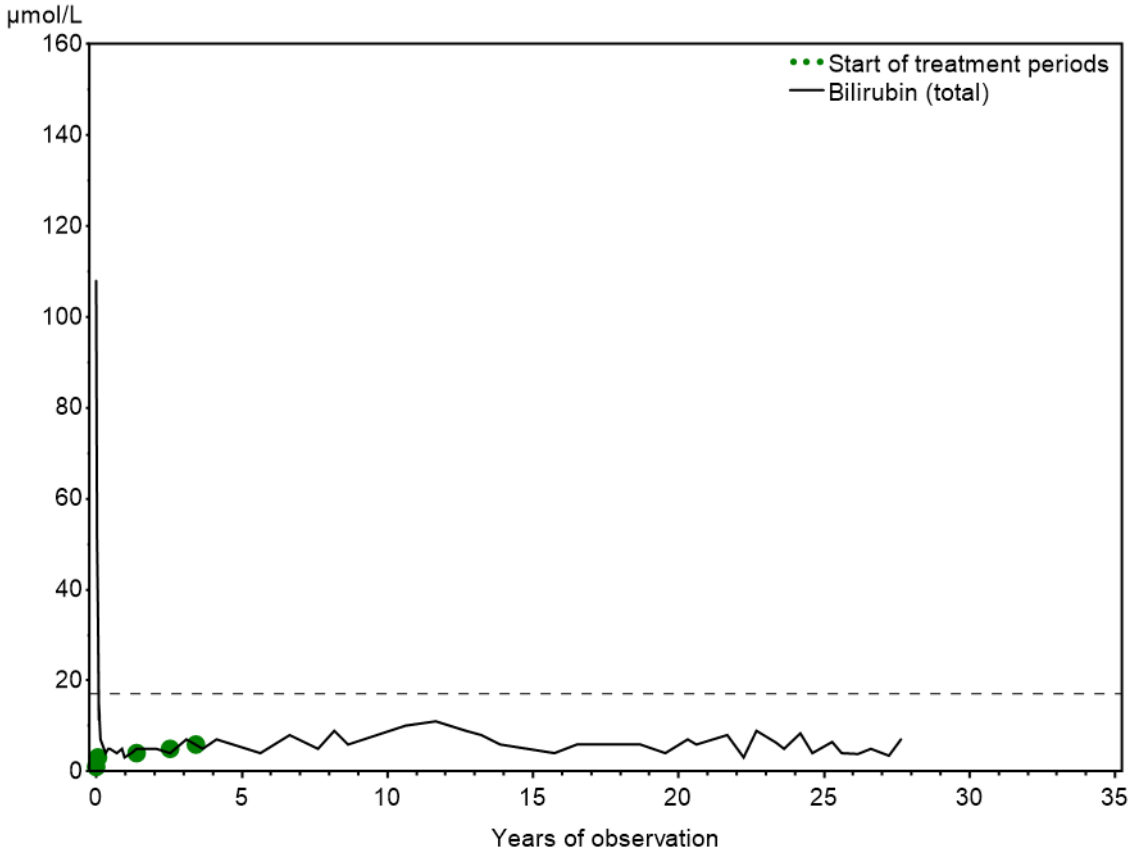
Figure 28: Evolution of Transaminases Over Time – Patient 001004, Δ^4-3-oxoR Deficiency.



A, Graph displaying transaminases levels from 0 to 300 UI/L (same scale used for all patients and in previous reports). B, Graph displaying transaminases levels from 0 to 600 UI/L as values > 300 UI/L were collected for this patient. Notes: The black and red dotted lines represent the upper limits of the normal range of respectively ASAT and ALAT. Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase, ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase. Source: Figures 8.9.1 and 8.9.2 - TFL V1.0 24OCT2025.

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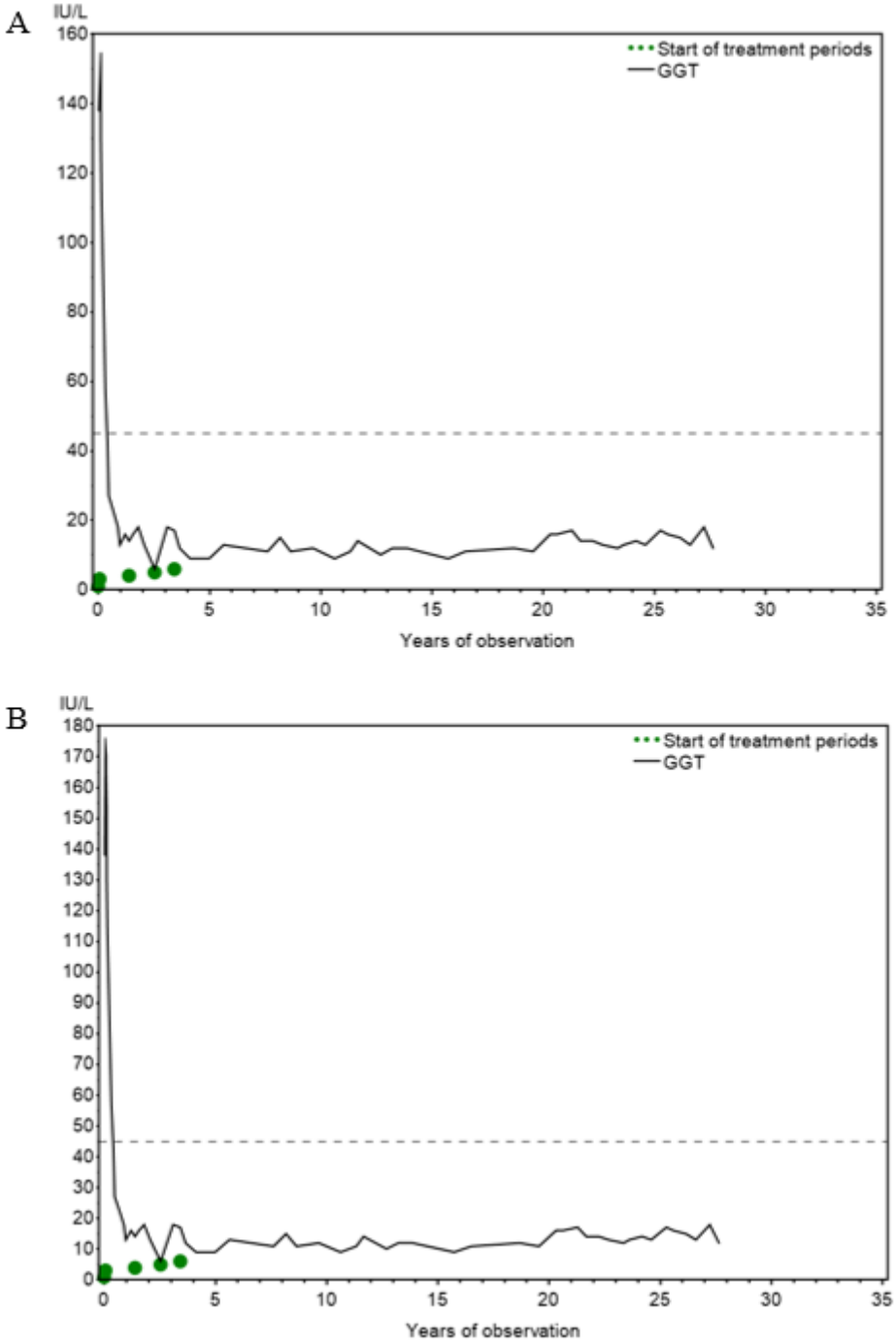
Figure 29: Evolution of Total Bilirubin Over Time – Patient 001004, Δ^4-3-oxoR Deficiency.



Notes: The dotted line represents the upper limit of the normal range. Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase. Source: Figure 8.9.4 - TFL V1.0 24OCT2025.

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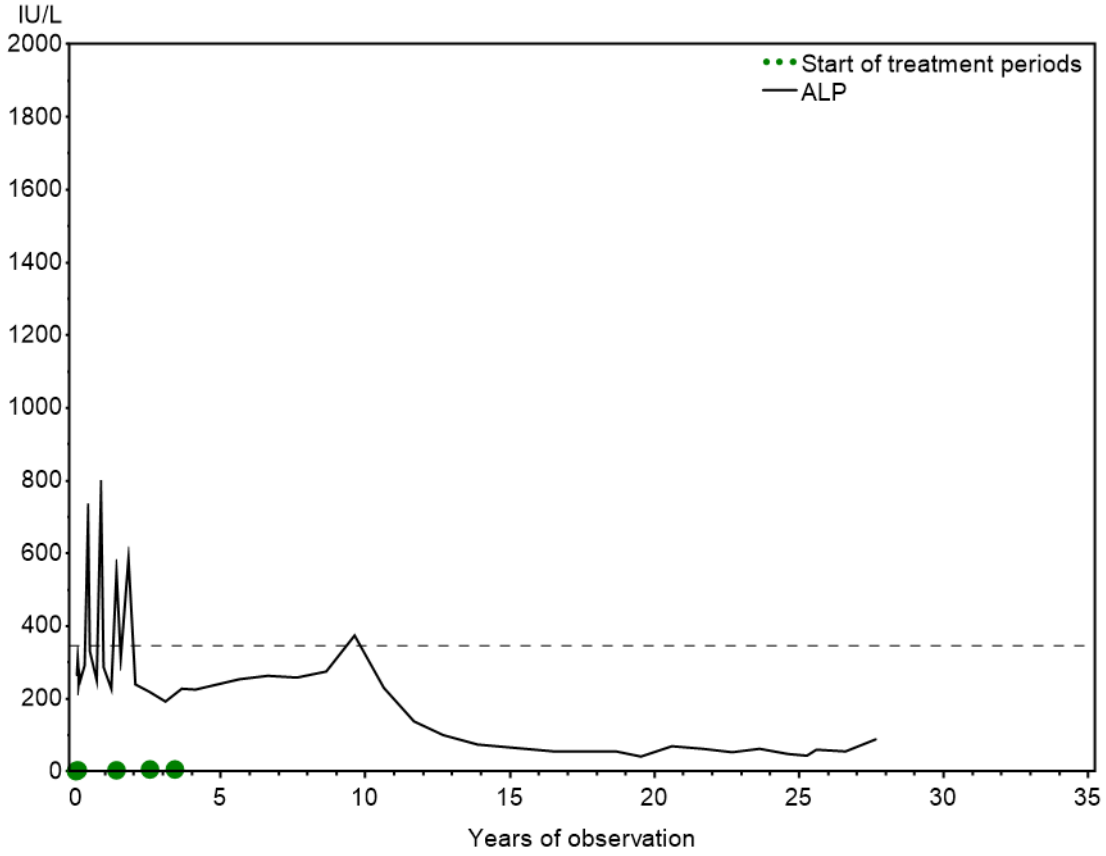
Figure 30: Evolution of GGT Over Time – Patient 001004, Δ⁴-3-oxoR Deficiency.



A, Graph displaying GGT levels from 0 to 160 UI/L (same scale used for all patients and in previous reports). B, Graph displaying GGT levels from 0 to 180 UI/L as values > 160 UI/L were collected for this patient. Notes: The dotted line represents the upper limit of the normal range. Δ⁴-3-oxoR: Δ⁴-3-oxosteroid 5β-reductase, GGT: Gamma-glutamyl transferase. Source: Figures 8.9.5 and 8.9.6 - TFL V1.0 24OCT2025.

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Figure 31: Evolution of ALP Over Time – Patient 001004, Δ^4-3-oxoR Deficiency.



Notes: The dotted line represents the upper limit of the normal range. Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase, ALP: Alkaline phosphatase. Source: Figure 8.9.8 - TFL V1.0 24OCT2025.

11.4.3.3.3 PATIENT 001011: TREATMENT OF ALMOST 13 YEARS

The treatment sequence for this patient (indicated on the graphs by green dots) was: no treatment, UCDA monotherapy, first year of CA monotherapy, second year of CA monotherapy, after second year of CA monotherapy.

This patient was treated for 12.8 years overall including 12.6 years with CA only (0.2 years with UDCA only) (Table 4). Two visits and two biochemistry analysis results were added since last year's report.

Transaminases and bilirubin were notably above the ULN under no treatment (Figure 32 and Figure 33). Levels started decreasing under UDCA monotherapy. Transaminases were below or around the ULN from CA monotherapy onwards. Bilirubin normalised with UDCA monotherapy and remained stable throughout subsequent treatments. GGT was slightly above the ULN under no treatment and normalised rapidly under UDCA monotherapy and subsequent therapies. ALP was in the normal range throughout all treatment sequences but peaked to 440 UI/L in September 2021. Following this peak, CA daily dose was increased from 150 to 250 mg, and the decrease of ALP under the ULN was observed few months later until just before year 11 when it slightly increased above the ULN before

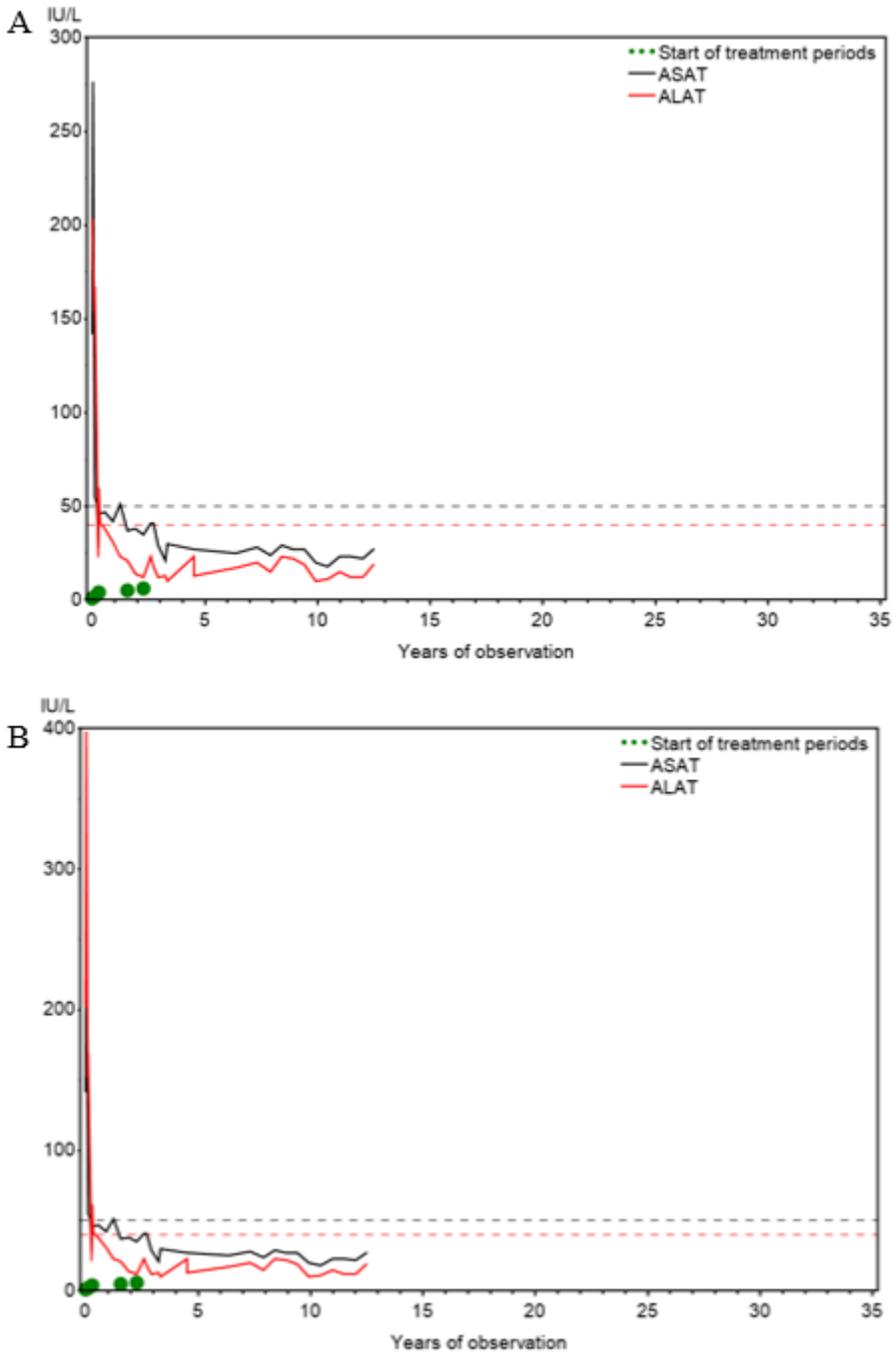
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decreasing again (Figure 34 and Figure 35). During this reporting period, ALP peaked again to 544 UI/L (April 2025). The dose was still 250 mg. As per comments of the treating physician, the increase in ALP was probably due to growth and vitamin D deficiency (although there was no rickets at this stage). It didn't seem to be due to liver disease, as all liver blood tests were normal, as were serum bile acid levels. Furthermore, there was no worsening of fibrosis: liver elastometry values were within the normal range.

Vitamin E levels were below the normal range under no treatment and normalised with UDCA and subsequent therapies.

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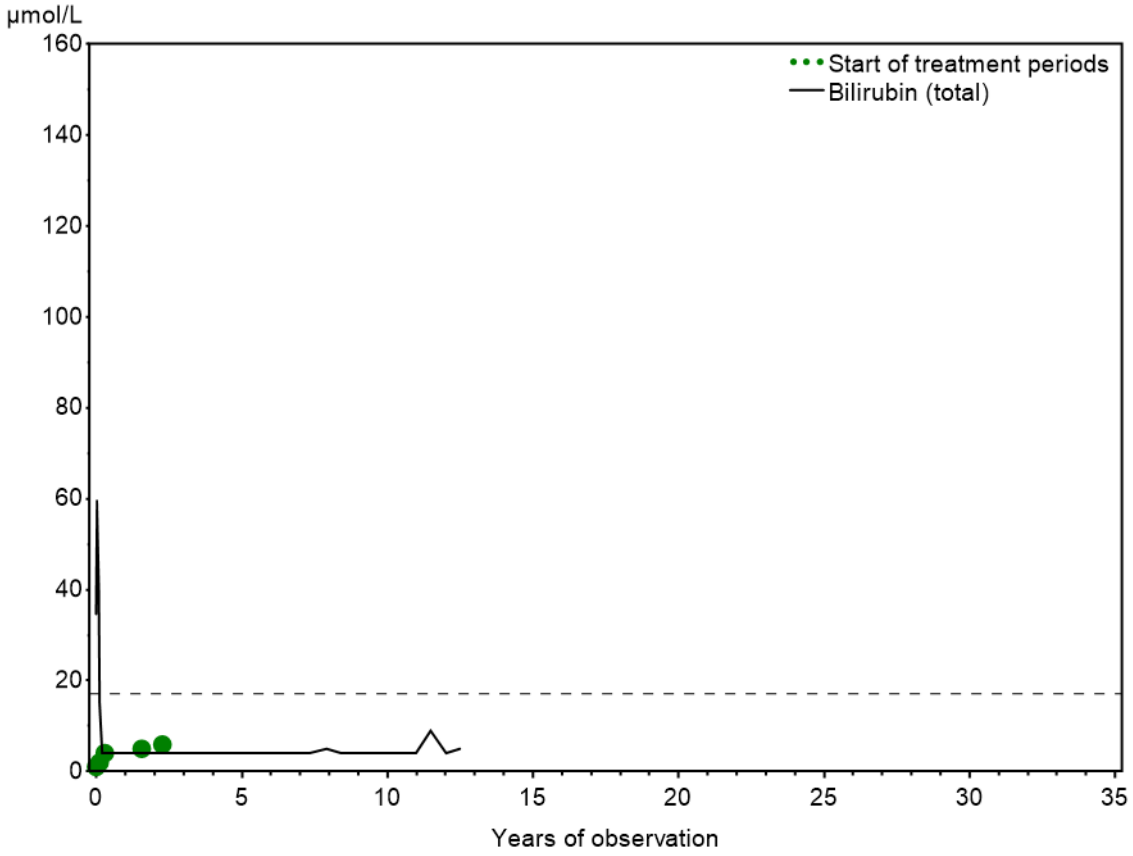
Figure 32: Evolution of Transaminases Over Time – Patient 001011, Δ^4-3-oxoR Deficiency.



A, Graph displaying transaminases levels from 0 to 300 UI/L (same scale used for all patients and in previous reports). B, Graph displaying transaminases levels from 0 to 400 UI/L as values > 300 UI/L were collected for this patient. Notes: The black and red dotted lines represent the upper limits of the normal range of respectively ASAT and ALAT. Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase, ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase. Source: Figures 8.11.1 and 8.11.2 - TFL V1.0 24OCT2025.

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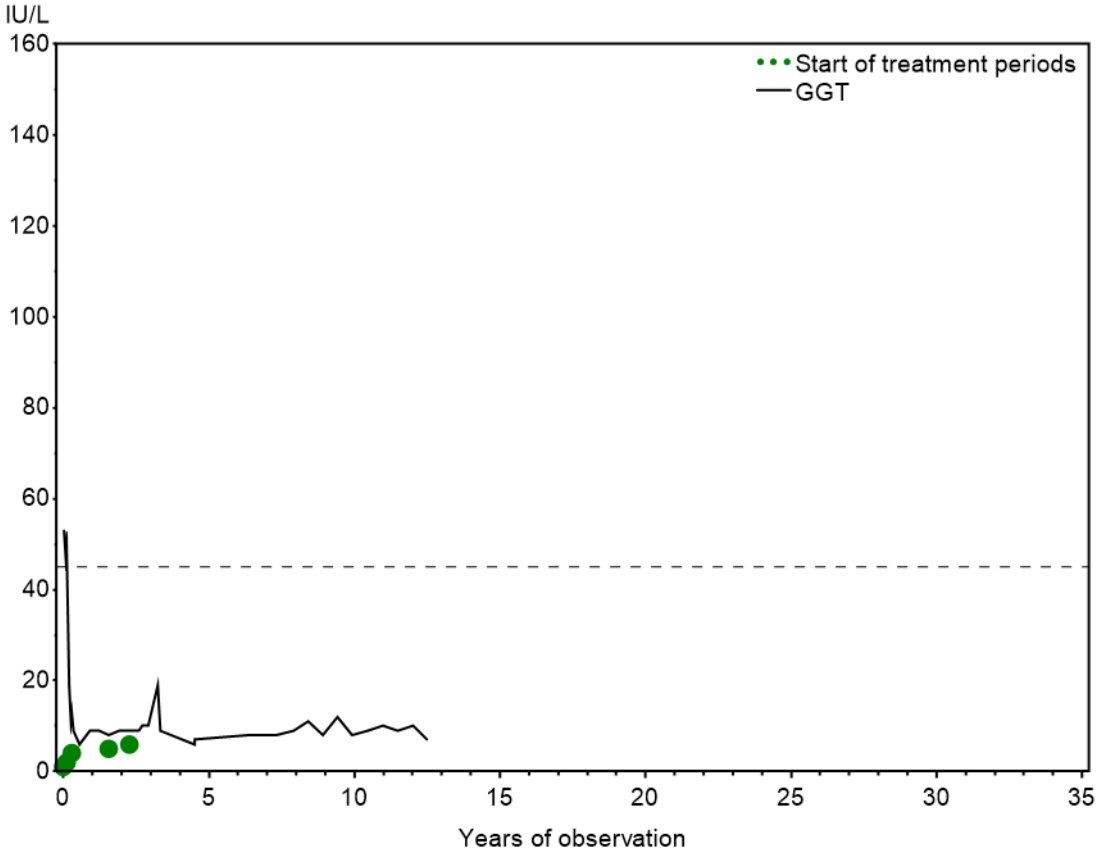
Figure 33: Evolution of Total Bilirubin Over Time – Patient 001011, Δ^4-3-oxoR Deficiency.



Notes: The dotted line represents the upper limits of the normal range. Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase. Source: Figure 8.11.4 - TFL V1.0 24OCT2025.

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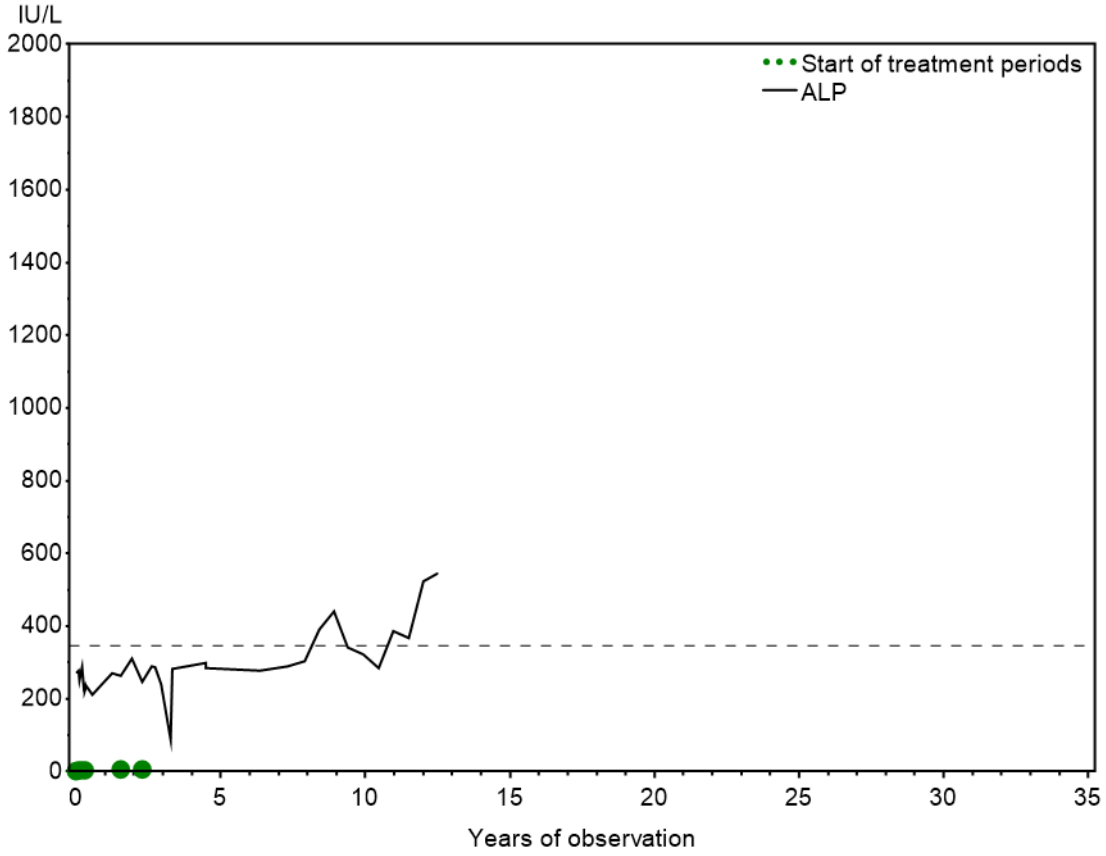
Figure 34: Evolution of GGT Over Time – Patient 001011, Δ^4-3-oxoR Deficiency.



Notes: The dotted line represents the upper limit of the normal range. GGT: Gamma-glutamyl transferase. Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase. Source: Figure 8.11.6 - TFL V1.0 24OCT2025.

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Figure 35: Evolution of ALP Over Time – Patient 001011, Δ^4-3-oxoR Deficiency.



Notes: The dotted line represents the upper limit of the normal range. Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase, ALP: Alkaline phosphatase. Source: Figure 8.11.8 - TFL V1.0 24OCT2025.

11.4.3.3.4 Patient 001012: Treatment duration 6.2 years

The treatment sequence for this patient (indicated on the graphs by green dots) was: no treatment, first year of CA monotherapy, second year of CA monotherapy, after second year of CA monotherapy.

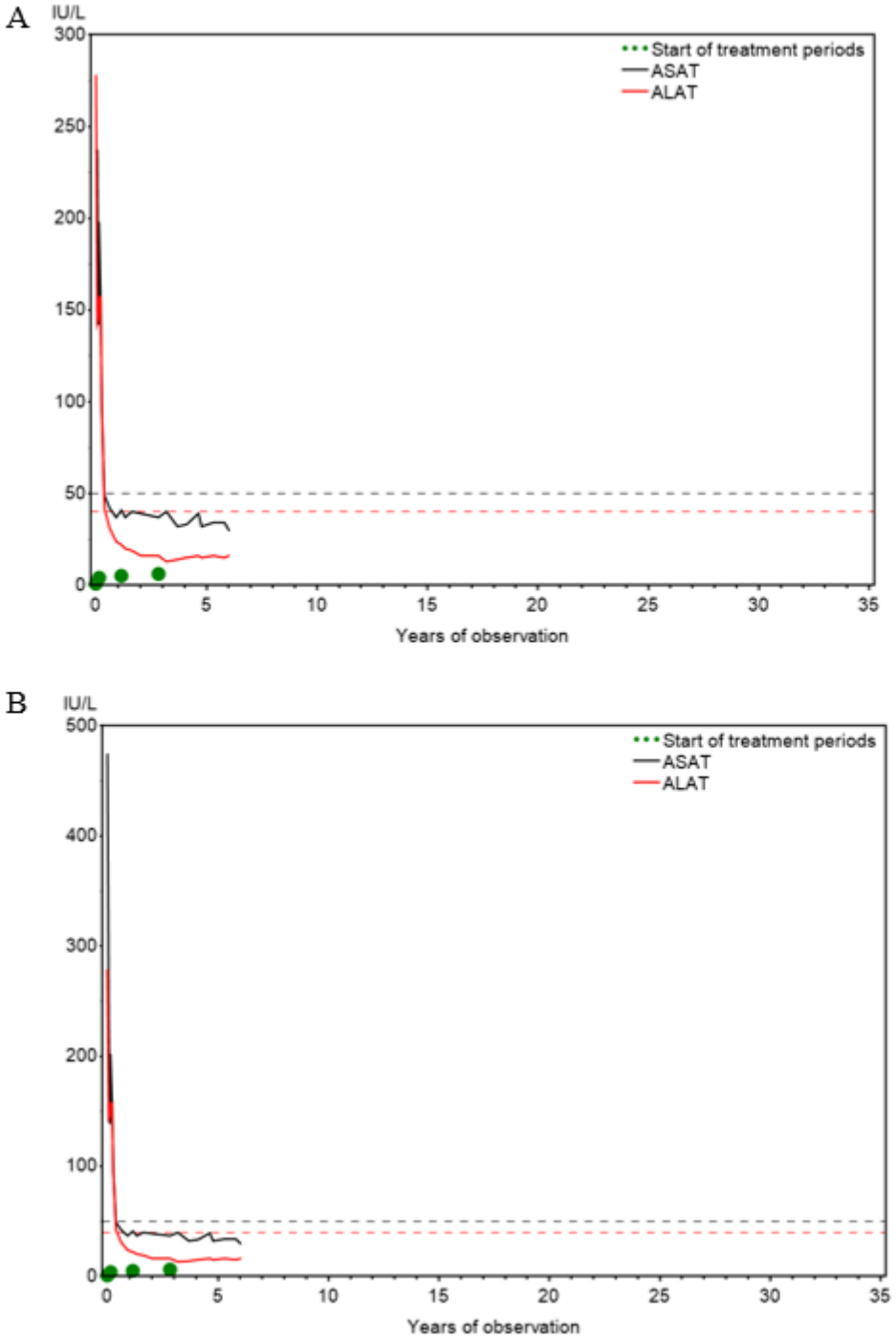
This patient was treated with CA monotherapy for 6.2 years (Table 4). One new visit was reported since last year's report and two biochemistry analysis results were performed.

Transaminases, GGT and total bilirubin levels were notably above the ULN under no treatment (Figure 36, Figure 37 and Figure 38) and decreased rapidly to reach normal levels when under CA monotherapy. ALP levels were in the normal range without treatment and during CA treatment (and Figure 39).

Too few data points for a meaningful display of serum Vitamin E levels were available.

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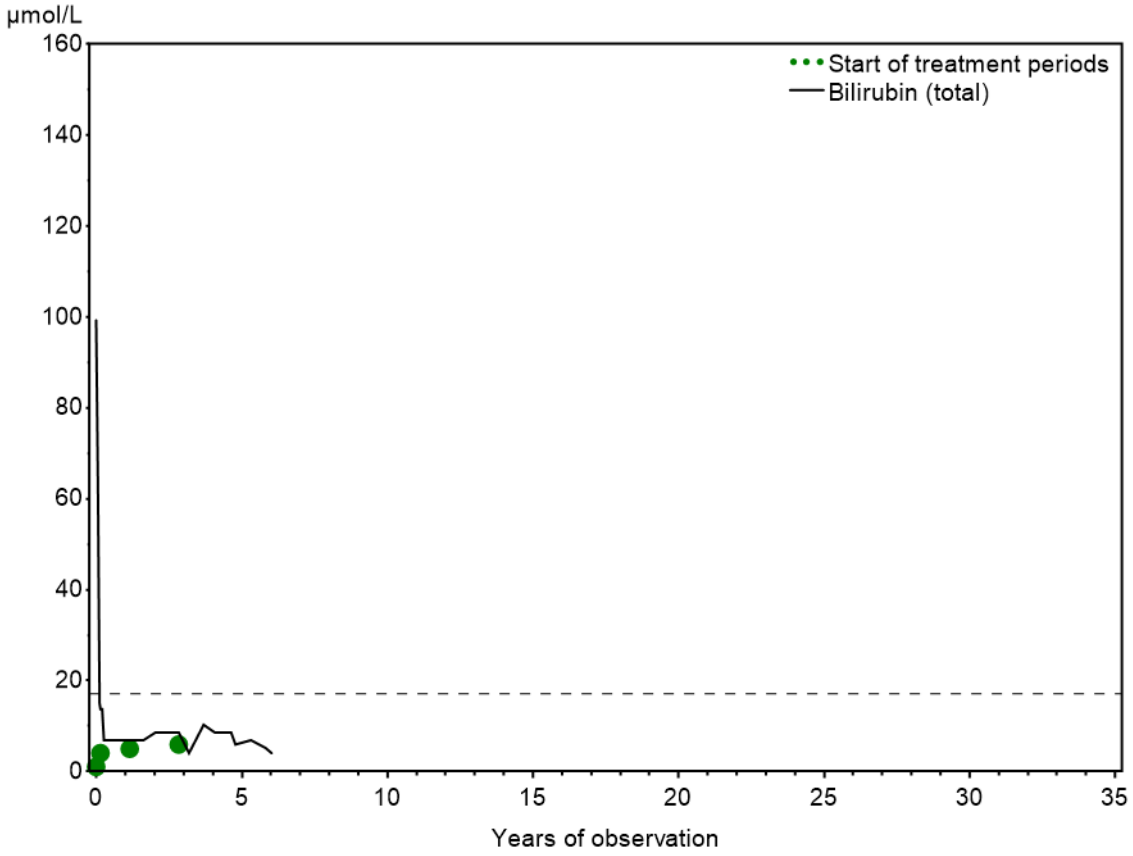
Figure 36: Evolution of Transaminases Over Time – Patient 001012, Δ^4-3-oxoR Deficiency.



A, Graph displaying transaminases levels from 0 to 300 UI/L (same scale used for all patients and in previous reports). B, Graph displaying transaminases levels from 0 to 500 UI/L as values > 300 UI/L were collected for this patient. Notes: The black and red dotted lines represent the upper limits of the normal range for respectively ASAT and ALAT. Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase, ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase. Source: Figures 8.12.1 and 8.12.2 - TFL V1.0 24OCT2025.

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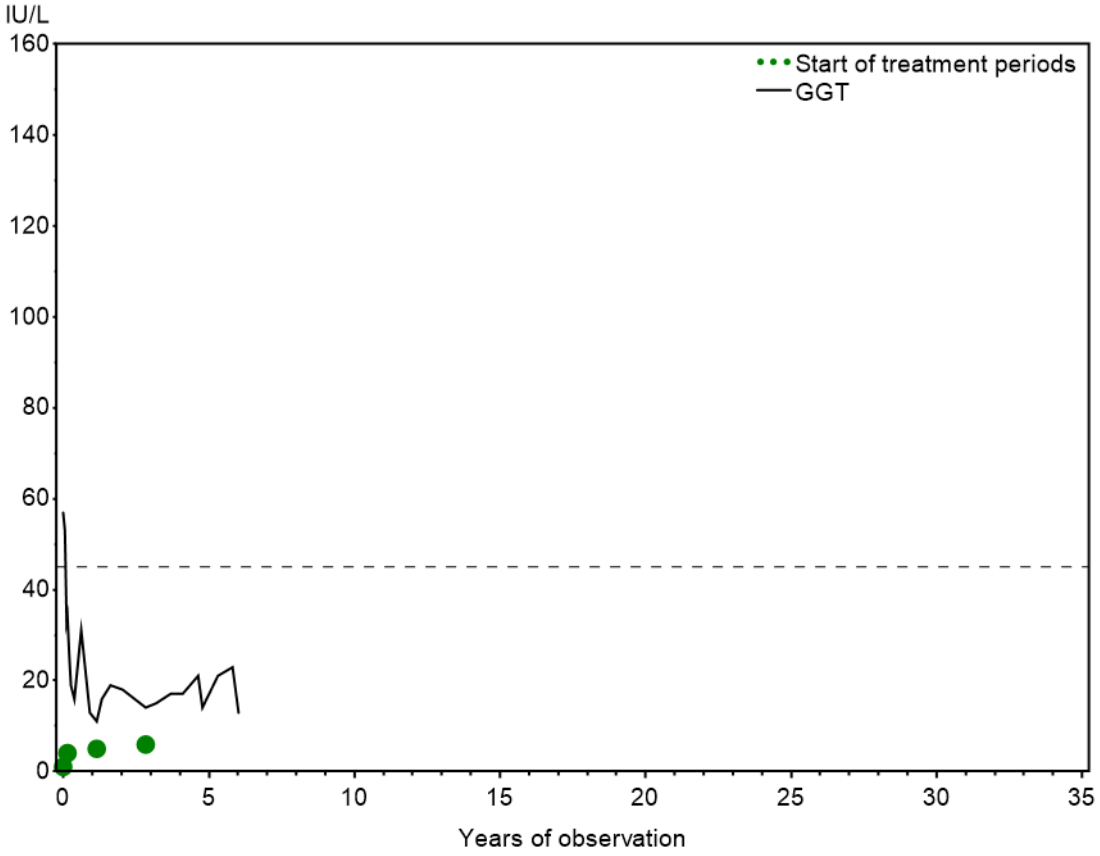
Figure 37: Evolution of Total Bilirubin Over Time – Patient 001012, Δ^4-3-oxoR Deficiency.



Notes: The dotted line represent the upper limit of the normal range. Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase. Source: Figure 8.12.4 - TFL V1.0 24OCT2025.

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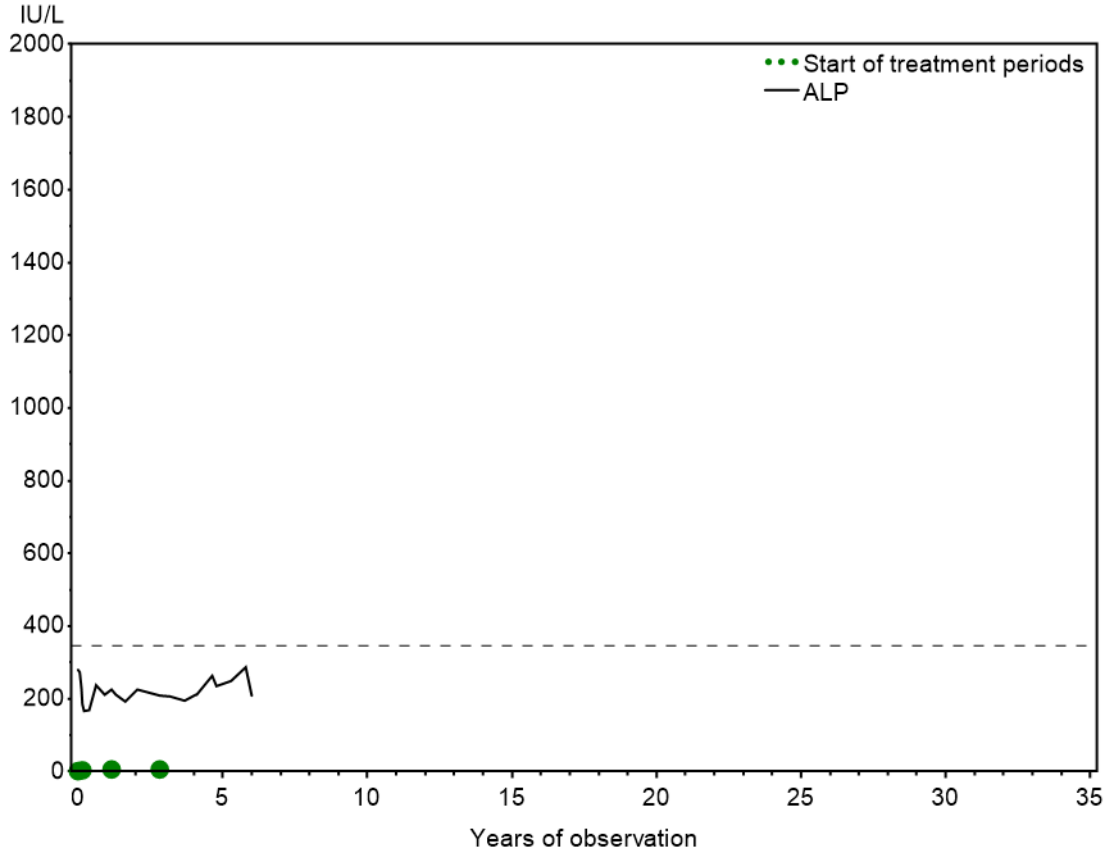
Figure 38: Evolution of GGT Over Time – Patient 001012, Δ^4-3-oxoR Deficiency.



Notes: The dotted lines represent the upper limit of the normal range. Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase, GGT: Gamma-glutamyl transferase. Source: Figure 8.12.6 - TFL V1.0 24OCT2025.

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Figure 39: Evolution of ALP Over Time – Patient 001012, Δ^4-3-oxoR Deficiency.



Notes: The dotted line represents the upper limit of the normal range. Δ^4-3-oxoR: Δ^4-3-oxosteroid 5β-reductase, ALP: Alkaline phosphatase. Source: Figure 8.12.8 - TFL V1.0 24OCT2025.

11.4.3.3.5 PATIENTS 010001 AND 010002: TREATMENT DURATION > 8 YEARS

The data for these two patients are identical to those of the tenth report (2023) because no new visit nor laboratory measurement were performed. Therefore, the individual data for these two patients were not presented in this report.

11.4.3.3.6 PATIENT 017001: TREATMENT DURATION > 8 YEARS

The data for this patient are identical to those of last year's report because no new visit nor laboratory measurement were performed. Therefore, the individual data for this patient were not presented in this report.

11.5 TREATMENT DISCONTINUATION

During the period between 13 September 2024 and 12 September 2025, the Sponsor did not receive any information regarding discontinuation of treatment with Orphacol®.

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12 SAFETY EVALUATION

12.1 ADVERSE EVENTS

As of 12th September 2025, 29 adverse events (AEs) in 17 patients were entered into the database including five new AEs entered in the database since last year's report (details are provided below).

Among AEs reported during this reporting period:

- 5 non-serious AEs (3 not related to the study disease and treatment and 2 not assessable) in 3 patients:
  - **1 renal insufficiency** (not related),
  - **1 gastroenteritis** (not related);
  - **1 otitis** (not related);
  - **1 macular damage** (not assessable);
  - **1 polyp** (not assessable).

Among AEs reported in previous reports:

- 6 AEs in 3 patients among which **four were an ADR related to another cholic acid**, before Orphacol® launch in these countries:
  - **1 increased itching** (related to another cholic acid);
  - **1 body heat** (related to another cholic acid);
  - **1 increased pruritus** (related to another cholic acid);
  - **1 pruritus** (related to another cholic acid – occurred in 1997);
  - **1 pruritus and diarrhoea** (not-related – occurred in 1998);
  - **1 pruritus** (not related – occurred in 1999).

- 2 ADRs in 1 patient:
  - **1 swelling of upper eye;**
  - **1 slight nausea in the morning.**

- 14 non-related AEs in 9 patients:
  - **4 Covid infections**;
  - **1 eczema in the popliteal fossa**;
  - **1 wart in the right hand**;
  - **1 gestational diabetes**:
  - **1 cholelithiasis**;
  - **1 hypothyroidism**;
  - **1 eczema (fold of elbow and neck)**;
  - **1 scalp keratosis**;
  - **1 gastroesophageal reflux disease**;
  - **1 depressive syndrome**;

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- **1 throat infection**.

- 2 AEs not assessable in 2 patients:
  - **1 GGT elevation**;
  - **1 swelling of upper eye lids**.

Of note, all events were considered as non-serious except for 3 for which the seriousness could not be assessed. However, "gestational diabetes" which was considered as non-serious by the investigator has been reclassified as serious AE by the marketing authorisation holder.

Two non-serious events of swelling of upper eye lids were related to the same patient. The first AE was reported as an ADR in 2016, and the second AE was in 2018 (relationship to treatment unknown). Both AEs were closely monitored by the PV service provider.

No ADRs were reported during this reporting period via Orphabase. However, 7 special situations were reported to GRA, PV/MV department within the period and during the preparation of this report: 4 maternal exposures and 3 medically prescribed overdose cases.

No new safety concerns have been entered in the database following these reports. Finally, no new and significant information on previously identified important risk, off-label use, drug interactions, overdose, drug abuse, misuse, pregnancy and lactation, effects of long-term treatment was identified during the reporting interval.

The detailed analysis and follow up of ADRs is performed by the GRA, PV/MV department and will be summarized in the PSUR.

12.2 Serious Adverse Events and Other Significant Adverse Events

No serious AEs have been reported during the whole period captured in the database. However, as previously mentioned, "gestational diabetes" which was considered as non-serious has been reclassified as serious.

12.3 Off-label doses

As mentioned in Section 10.3, three patients had an off-label dose reported during a previous period:

- One patient at site 002 had a dose of 600 mg/day of CA since December 2015 (patient 002001);
- One patient at site 002 (patient 002002) had a dose of 600 mg/day of CA since September 2018. Since July 2021, the daily CA dose has been increased to 700 mg/day;
- One patient at site 003 (patient 003005) had a dose of 550 mg/day since 2022;

During this period, two patients (002004 and 020001) with off-label doses were reported:

- On patient at site 020 (patient 020001) had a dose of 750 mg/day since July 2023;
- On patient at site 002 (patient 002004) had a dose of 750 mg/day since October 2024.

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These doses are not in line with recommended dosing instructions presented in Orphacol® SmPC. No adverse effects have been reported for those five patients, so far.

12.4 Deaths

No patient deaths occurred during the whole period captured in the database.

12.5 Vital Signs, Physical Findings and Other Observations Related To Safety

While vital signs and other data relevant to safety, including exposure during pregnancy, are captured in the database, they are not the focus of this report. They have been reported in publications (Gonzales, 2006; Gonzales et al., 2009; Gonzales et al., 2018; Gardin et al., 2023) and continue to be reported in PSUR.

12.6 Safety Conclusions

Twenty-five non-serious AEs, one AE reclassified as serious and three AEs for which the seriousness is unknown have been reported during the whole period captured in the database, including five more since the previous report. Among all AEs, two were considered related to Orphacol®, 23 were not related to Orphacol® and four were not assessable. No serious AEs have been entered in the database since last report, indicating good tolerance of the treatment. The detailed analysis and follow up of all ADRs/AEs are performed by the GRA, PV/MV department and will be summarized in the PSUR.

13 DISCUSSION AND OVERALL CONCLUSIONS

Data from patients treated in France have previously been summarised in a doctoral thesis (Gonzales, 2006) and in two publications (Gonzales et al., 2009; Gonzales et al., 2018). In this report, we focused on monitoring liver parameters and Vitamin E including a larger number of sampling points which confirm the fact that Orphacol® treatment is effective and safe. In addition, we provided data on the combined treatment with UDCA+CA.

Results from this open-label observation study, which included data on 52 patients (41 patients with 3β-HSD deficiency and 11 patients with Δ^4-3-oxoR deficiency) in this reporting period, showed that real life treatment with CA monotherapy effectively reduces serum levels of the liver enzymes ALAT, ASAT, ALP, GGT and of total bilirubin, indicating a reduction in liver damage as shown by each parameter boxplot. CA treatment also increased serum Vitamin E to normal levels, despite a bit later (after the second year of CA treatment) as observed in previous reports, but nonetheless indicating restoration of the absorption of dietary fat-soluble vitamins.

The results presented in this twelfth report confirm those provided by earlier observations of parts of this population (Gonzales, 2006; Gonzales et al., 2009; Gonzales et al., 2018; Gardin et al., 2023), while adding additional details on the development of liver parameters and Vitamin E levels in the intermediate term. Previous publications on the efficacy of bile acid therapy for 3β-HSD deficiency have usually reported short-term results (weeks or months (Fischler et al., 2007; Kobayashi et al., 2000; K.D.R. Setchell, Balistreri, Piccoli, & Clerici, 1991; Vanderpas et al., 1987)) or long-term

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results (Gonzales et al., 2009; Gonzales et al., 2018; Heubi, Bove, & Setchell, 2017; Heubi & Setchell, 2020; Subramaniam et al., 2010; Gardin et al., 2023). Patients included in this report generally went through several treatment periods of various durations, with the longest treatment durations observed with CA monotherapy (more than 25 years).

Regarding **3β-HSD deficiency**, all descriptive analyses performed on categorical (using best-to-worst values) and quantitative variables (using patient's mean values) per treatment period showed a general normalisation of values in serum liver functions and Vitamin E, well shown by each parameter boxplot (see Figure 2 to Figure 7). ASAT, ALAT, total bilirubin and Vitamin E normalised with UDCA+CA combination therapy and all parameters tested (ASAT, ALAT, total bilirubin, GGT, ALP and Vitamin E) normalised with CA monotherapy (for the first and second years of treatment and after the second year of treatment).

In this cohort, liver parameters and Vitamin E parameters had generally a significant shift towards normal values versus no treatment and versus UDCA, the latest indicating that CA monotherapy was more effective than UDCA monotherapy, usually after two years of CA. Indeed, biological parameters levels were usually lower during UDCA+CA period (n±7) than CA first year period (n±36). This difference could be explained by a different treatment sequence and different patients evaluated in both groups. All patients with UDCA+CA have been under UDCA before, what contributed to the decrease of abnormal parameters before CA introduction. Since most of patients in the CA first year period had no treatment before, it resulted that levels of some biological parameters were high in the first weeks of CA treatment, before normalisation.

The descriptive analyses for worst-to-worst data supported the results seen for CA treatment (individual treatment periods and pooled) for ASAT, ALAT, total bilirubin and Vitamin E (but not for GGT or ALP) although less clearly than the best-to-worst analysis.

Most parameters had stable results between the different durations of CA monotherapy, that is, biochemistry parameters did not significantly change with increased duration of CA monotherapy, although being more stable after 2 years of CA treatment. Few patients displayed outliers (mainly for transaminases) with levels > 2 ULN. These values were isolated and transient and could be explained by a lack of treatment adherence.

In patients with 3β-HSD deficiency, the analyses on continuous parameters of treatment response showed that all serum transaminases, GGT, total bilirubin and Vitamin E levels remained normal under long-term CA monotherapy, especially after 1 year of treatment. Serum ALP levels continued to fall.

In this twelfth report, descriptive analyses were also performed in patients with **Δ^4-3-oxoR deficiency**. Quantitative analyses showed comparable results to those observed in patients with 3β-HSD deficiency, namely a normalisation of all parameters tested under CA monotherapy, especially after the first year. These parameters remained stable throughout CA monotherapy. Normal serum liver tests were also confirmed by Gardin et al., 2023 for patients with Δ^4-3-oxoR deficiency and showed that CA allowed a 12-fold decrease of 3-oxo-Δ^4 derivatives in urine.

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Categorical analyses, using best-to-worst data, showed similar results except for total bilirubin and ASAT which normalized under UDCA+CA.

The descriptive analyses for worst-to-worst data supported the results seen for CA treatment although usually less clearly than the best-to-worst analysis.

Due to the small sample size, no statistical comparison was performed in this population. The inclusion of new patients in the next years could allow such analysis and will reduce the variability of the mean observed for some parameters.

Overall, this twelfth report shows that CA monotherapy led to significant improvements in most markers of hepatic impairment, with normalisation or near normalisation of most liver parameters. Statistical comparisons were performed in patients with 3β-HSD deficiency (as the sample size allowed such analysis) and showed that CA monotherapy was generally more effective than UDCA monotherapy: all biochemistry parameter outcomes were statistically significantly different with CA monotherapy versus UDCA, except for GGT. Normal GGT is one of the diagnostic features for primary bile acid synthesis disorders (Gonzales et al., 2009), and mean GGT levels were indeed already below the ULN with no treatment. With CA monotherapy, serum GGT levels were maintained normal. Addition of CA to UDCA (UDCA+CA combination therapy) led to significant normalisation versus no treatment in 3 keys liver cholestasis parameters (ASAT, ALAT, total bilirubin).

The favourable outcome observed in patients with 3β-HSD or Δ^4-3-oxoR deficiencies undergoing oral CA therapy is consistent with what is expected with the supplementation of low bile acid pool with external primary bile acid (CA), such as:

- Normal fat and fat-soluble vitamin absorption;
- Normal bile flow;
- Down-regulation of bile acid synthesis by negative feedback regulation. This, in turn, results in the inhibition of the synthesis and decreased accumulation of toxic and cholestatic bile acid metabolites).

Correction of the malabsorption syndrome was shown by normalisation of steatorrhea and serum vitamin E concentration during CA therapy. Restoration of bile acid–dependent bile flow ameliorated cholestasis, leading to improved liver function test results, reduction in the urinary excretion of total bile acids, and maintenance of normal serum bile acids and GGT levels (Gonzales et al., 2009; Gonzales et al., 2018; Heubi et al., 2017; Heubi & Setchell, 2020). It should also be noted that UDCA is a poor agonist of farnesoid X receptor and does not reduce the production of atypical hepatotoxic bile acids' precursors (Jahnel et al., 2017). Therefore, its effect on clinical and biochemical parameters is less pronounced in patients with bile acid synthesis disorders.

Regarding nutrition, the data presented here confirm that exogenous CA monotherapy can functionally replace primary bile acids in terms of absorption of the fat-soluble Vitamin E (and by extension, fat and other fat-soluble vitamins), as its levels normalise on CA monotherapy, in both populations.

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Overall, the results presented in this twelfth study report were in accordance with previous study reports. The decision made in the fifth report to change the statistical analyses and focus on more descriptive representations using boxplots, continues to strengthen the benefits of CA treatment over other treatments and no treatment, on all the liver parameters of interest and the vitamin E levels.

Major limitations of this study are still its limited sample size (in total and per subtypes) preventing any complex data analyses; and its observational and voluntary participation nature further impacting the data quality and risk of missing data. However, these two drawbacks are quite common in rare diseases context. So far, 52 patients (41 patients with 3β-HSD deficiency and 11 with Δ4-3-oxoR deficiency) have been included in 18 centres in 6 countries (i.e. France, Germany, Italy, Spain, Switzerland and UK). In addition, a total of 85 follow-up visits have been performed during this reporting period. Compared to last year's report, these represents the inclusion of 4 new patients with 3β-HSD deficiency and 2 with Δ4-3-oxoR deficiency, the opening of four new centres (two in France, one in Italy and one in the UK) and the addition of 34 more follow-up visits. Furthermore, one more centre has been opened in France this year, but the patients (N=3) were included at the date of the database lock, and therefore no data were yet entered for this period. In addition, the data for 2 other new patients in one new site in Italy started to be entered just after the database lock. Therefore, at least 5 new patients will be included in the next reporting period.

Although important efforts have been made by the Sponsor the last few years, it still could remain challenging to open new centres, especially in the UK. Discussions are ongoing with four new centres in the UK, representing the potential inclusion of 8 patients. In fact, the process remains lengthy due to site-specific regulatory requirements. Currently, financial agreement is expected for these four centres, including Leeds General Infirmary and the Royal Manchester Children's Hospital. The Data Protection Impact Agreement is awaiting approval for Birmingham Adults / Queen Elizabeth Hospital, and for the Great Ormond Street Hospital – GOSH, financial agreement approval is expected from the site governance team. More details are provided in Section 15.1.6. To address these challenges and speed up the process, reinforced follow-up measures such as regular phone and email reminders have been implemented in order to open these centres during the next period.

Nevertheless, the Sponsor continues to involve as many sites as possible and collect as many data as possible to increase data quality and obtain valid and reliable results on Orphacol® treatment's effectiveness on a representative sample of patients. Efforts have thus been rewarded with the opening of new centres and the inclusion of new patients during this reporting year. Finally, in addition to the discussions with the four centres in the UK, discussions are also in place for two more countries (Belgium and Singapore) in which two centres with potentially 3 patients are planned to be opened next year.

Furthermore, as the records are the results of the examinations and laboratory exams that were made on an as-needed and as-scheduled basis, not all of the analysed data were generated at every examination. Despite continuous reminders to the investigators on the established annual data lock point, they may not have had the possibility to enter the data before this date, or for some patients, the scheduled annual follow-up visits were not falling in the period of the yearly data capture. In these cases, the data will be entered and included in the next annual report. Finally, the number of examinations is different for each patient, as it depends on the development of the clinical condition

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of the patient, the duration of the treatment, the need for dose adjustments as well as on other factors. As a result, the data set for every included patient is different.

Safety

Twenty-five non-serious AEs, one AE reclassified as serious and three AEs for which the seriousness is unknown have been reported cumulatively in the database, including five more since the previous report. Among all AEs, two were considered related to Orphacol®, 23 were not related to Orphacol® and four were not assessable. No serious AEs have been entered in the database since last report, indicating good tolerance of the treatment. The detailed analysis and follow up of ADRs/AEs reported in the Orphabase are performed by the GRA, PV/MV department and will be summarized in the PSUR.

Conclusions

These results confirm previous observations that CA monotherapy is the treatment for patients with 3β-HSD deficiency with an extremely favourable benefit/risk ratio for patients. Although the number of patients with Δ^4-3-oxoR deficiency increases each year, the number of such patients included in the database remains insufficient for statistical analysis. Nevertheless, descriptive analyses showed a fast normalisation of liver parameters and Vitamin E while under CA monotherapy showing that this treatment is effective and safe in this patient population.

The results presented in this twelfth report support the value of the data contained in Orphabase for the detailed evaluation of the effectiveness and safety of Orphacol® therapy in 3β-HSD and Δ^4-3-oxoR deficiencies.

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15 APPENDICES

15.1 STUDY INFORMATION

15.1.1 PATIENT IDENTIFICATION CORRESPONDENCE

Correspondence between previously used identifiers in Annual Reports n°1 to 4 and new identifiers used since Report n°5.

Patient identifier currently used	Patient identifier previously used
001001	3 (C)
001002	9 (G1)
001003	8 (F)
001006	11 (H)
001007	4 (D1)
001008	5 (D2)
001009	13 (I2)
001010	12 (I1)
002001	17 (J)
002002	18
002003	19
002004	20
003001	10 (G2)
003002	1 (A)
003003	7 (E2)
003004	6 (E1)
003005	2 (B)
008001	-
001004	15 (K2)
001005	14 (K1)
001011	16

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15.1.2 EXCEPTIONS IN THE NORMAL VALUES USED FOR THE ANALYSIS

Normal values of Centre 001 have been used for the analysis of all patients included in the database, with some exceptions, as indicated in Table 37.

Table 37: Normal values used for the analysis, other than Centre 001.

Parameter	ULN	Exceptions (site + patient code)	ULN for exceptions
ALAT	<40 IU/l	002001, 002002, 002003, 002004	<34 IU/l
		004002	<39 IU/l
		007001	<35 IU/L
ASAT	<50 IU/l	002001, 002002, 002003, 002004	<31 IU/l
		007001	<40 IU/L
GGT	<45 IU/L	002001, 002002, 002003, 002004	<38 IU/l
		004002	<40 IU/l
		007001	<25 IU/L
Total bilirubin	<17 µmol/L	002001, 002002, 002003, 002004	<34 IU/l
		004002	<25 µmol/L
ALP	<345 IU/l	002001, 002002, 002003, 002004	<321 IU/l
		004002	<300 IU/l
		007001	<390 IU/L
Vitamin E	4.6-14.8 µg/L	-	-

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15.1.3 PROTOCOL AND PROTOCOL AMENDMENTS

See attached for:

- Protocol version 1.1 dated 10 January 2018,
- Protocol version 2.0 dated 04 February 2020,
- Protocol version 2.1 dated 23 April 2024.

15.1.4 CASE REPORT FORM

See relevant attachment. One amendment was made to the CRF during the reporting period: CRF version 2.0 dated 25 March 2024.

15.1.5 PATIENT AND/OR LEGAL REPRESENTATIVE CONSENT FORM

See attached for:

- Consent form version 1.1 dated 27 June 2018,
- Consent form version 2.0 dated 25 June 2020,
- Consent form version 2.1 dated 30 November 2023.

Docusign Envelope ID: 2A49FC02-DE41-469C-83B2-1286A7B0B800

15.1.6 SITES AND PATIENTS' INCLUSION IN UK

ORPHABASE: SITES AND PATIENTS' INCLUSION IN UK
12SEP2024 -> 12SEP2025

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	Center included in Orphabase ?	Number of treated patients	Number of included patients	Action performed during the repporting période (including number of interaction) And Reason for the lengthy process
KING'S COLLEGE HOSPITAL - LONDON	Yes active site since 24JUL2019	3	2	3rd patient participation pending. Patient's visit has been postponed 3 times since September 2024. Recruitment still followed up.
BIRMINGHAM WOMEN'S AND CHILDREN'S NHS FOUNDATION TRUST - BIRMINGHAM	Yes active site since 03JUL2025	1	1	CDA signed on 15JAN2024 Financial agreement signed by the site on 02JUN2025 Initiation performed on 03JUN2025 Inclusion of patient on 04JUL 2025
LEEDS GENERAL INFIRMARY	No	3		CDA signed on 10OCT2022 Financial agreement phase ongoing On 2024-2025, 17 exchanges were performed with the site. Long process of review by the site of the study file, especially the Data privacy questionnaires, and expecting now the approval of the financial agreement. Last reminder sent on 06OCT2025
BIRMINGHAM ADULTS /QUEEN ELIZABETH HOSPITAL	No	2		CDA signed on 20JUN2022 DPI / Financial agreement phase ongoing On 2024-2025: 17 exchanges were performed Long review process by the site governance team and expecting now the completion of Data Protection Impact Assessment Last reminder sent on 13OCT2025
GREAT ORMOND STREET HOSPITAL – GOSH	No	1		CDA signed on 06MAY2025 Financial agreement phase ongoing On 2024-2025 12 exchanges related to CDA signature and financial agreement review and signature. On 10OCT2025: pending feedback from the site governance team.
ROYAL MANCHESTER CHILDREN'S HOSPITAL	No	2		CDA signed on 03JUN2025 Financial agreement phase ongoing On 2024-2025: 12 exchanges related to CDA signature and financial agreement review and signature Last reminder sent on 10OCT2025