REGISTRY PROTOCOL A4250-019

Study title:	Prospective Registry-Based Study of the Long-Term Safety of Odevixibat in Patients with Progressive Familial Intrahepatic Cholestasis (PFIC)
Version of the protocol:	1.0
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Medicinal product:	Bylvay
Procedure no.:	EU/1/21/1566 – EMEA/H/C/004691
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Research question and objectives:	The objective of this registry-based study is to assess the long-term, real-world safety profile of odevixibat treatment in patients with PFIC compared to patients not receiving odevixibat (untreated control cohort).
Countries of study:	Global investigation
Marketing authorisation holder:	Albireo AB
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PROTOCOL TITLE: Prospective Registry-Based Study of the Long-Term Safety of Odevixibat in Patients with Progressive Familial Intrahepatic Cholestasis (PFIC)

PROTOCOL NUMBER: A4250-019

Albireo AB

PPD

PPD , Clinical Development

Date (day/month/year)

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List of Abbreviations and Definitions of Terms

Abbreviation	Definition
ABC	ATP-binding cassette
AE(s)	adverse event(s)
ALT	alanine aminotransferase
AP	alkaline phosphatase
AST	aspartate aminotransferase
BMI	body mass index
BSEP	bile salt export pump
CERC	Critical Events Review Committee
eCRF(s)	electronic case report form(s)
EDC	electronic data capture
EMA	European Medicines Agency
ENcePP	European Network of Centres for Pharmacoepidemiology and Pharmacovigilance
FDA	Food and Drug Administration
GGT	gamma-glutamyl transferase
IBAT-I	ileal bile acid transporter inhibitor
INR	international normalised ratio
MAH	marketing authorisation holder (Albireo AB)
MDR3	multidrug resistance protein 3
NAPPED	Natural Course and Prognosis of PFIC and Effect of Biliary Diversion
PASS	post-authorisation safety study
PBRER	Periodic Benefit Risk Evaluation Report
PFIC	progressive familial intrahepatic cholestasis
P-gp	P-glycoprotein
REDCap	Research Electronic Data Capture web application
SAP	statistical analysis plan
StdDev	standard deviation
TreatFIC	Treating patients with Familial Intrahepatic Cholestasis
UDCA	ursodeoxycholic acid

UMCG	University Medical Center Groningen
US	United States
WMA	World Medical Association

1. Responsible Parties

Marketing authorisation holder:

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Sweden

Principal investigator:

Not applicable.

Coordinating investigator for each country in which the study is to be performed:

Not applicable.

2. Abstract/Protocol Synopsis

Title: Prospective Registry-Based Study of the Long-Term Safety of Odevixibat in Patients with Progressive Familial Intrahepatic Cholestasis (PFIC)

Rationale and Background: Odevixibat (Bylvay) is an authorised medical treatment for PFIC, a group of rare genetic disorders that results from defects in bile secretion and presents with intrahepatic cholestasis. Odevixibat acts as a potent, selective inhibitor of the human ileal bile acid transporter (IBAT), an integral brush border membrane glycoprotein that co-transports sodium and bile acids and appears to be a major regulator of the bile acids pool in animals and humans.

This will be a long-term non-interventional, observational, and voluntary participation registry-based study designed to examine the safety of odevixibat in the treatment of PFIC using data prospectively collected in the **Treat**ing patients with **F**amilial **I**ntrahepatic **C**holestasis (TreatFIC) registry.

Research Question and Objectives: The aim of this study is to assess the long-term, real-world safety profile of odevixibat treatment in patients with PFIC compared to patients not receiving odevixibat (untreated control cohort). The overall objectives of this registry-based study are to evaluate the long-term safety of odevixibat and:

- To evaluate the impact of odevixibat treatment on occurrence of severe diarrhoea.
- To evaluate the impact of odevixibat treatment on the clinical manifestations of fat-soluble vitamin deficiency.
- To evaluate the impact of odevixibat treatment on the effectiveness of fat-soluble drugs.
- To evaluate the impact of odevixibat treatment on nutritional status.
- To evaluate the impact of odevixibat treatment on hepatic function and signs of hepatoxicity.

Study Design: This post-authorisation safety study (PASS) represents a registry-based study analysing secondary data. This will be a long-term non-interventional, observational, and voluntary participation registry-based safety study designed to examine the real-world usage of odevixibat for the treatment of PFIC using data prospectively collected in the TreatFIC registry.

The TreatFIC registry is a disease registry that is open to enrolment for all patients with PFIC, including patients treated with odevixibat as well as untreated patients. There is no cut-off date for enrolment or a maximum number of patients to be enrolled.

This registry-based safety study will continue until a minimum of 50 patients with PFIC treated with odevixibat have been followed for a minimum of 3 years. It is estimated that 50 patients with PFIC will enrol in a 1- to 2-year period. The length of time for duration of follow-up will be adjusted as needed to ensure the study provides meaningful results.

Population: The study population will comprise patients with PFIC (all types) enrolled into the TreatFIC registry. Patients who are homozygous for a known, disease-causing mutation of the PFIC family (any type), compound homozygous for two disease-causing mutations or heterozygous for one disease-causing mutation in combination with the clinical phenotype intrahepatic cholestasis will be eligible.

Variables: The TreatFIC registry captures individual patient data including demographics, odevixibat treatment information, other treatments (e.g. vitamin supplementation, ursodeoxycholic acid [UDCA], rifampicin), growth, monitoring of longitudinal serum biochemical parameters, clinical symptoms, clinical outcomes (e.g. alive with native liver, liver transplantation, death), and safety parameters including diarrhoea, bloody diarrhoea, dehydration, fat soluble vitamin levels, clinical manifestations of fat-soluble vitamin deficiency, and fat soluble vitamin deficiency refractory to dose increase.

Outcomes:

The safety of odevixibat treatment will be evaluated using the following outcomes:

- Incidence of diarrhoea lasting > 3 days, bloody diarrhoea, or diarrhoea leading to dehydration or electrolyte imbalance and any treatment.
- Incidence of clinical manifestations related to fat-soluble vitamin deficiency (e.g. bleeding, rickets, osteopenia) including symptoms and treatment.
- Change in fat-soluble vitamin levels.
- Incidence of reports of ineffectiveness of fat-soluble drugs that were previously effective.
- Incidence of reports of new nutritional interventions (special diets, nasogastric feeding, gastrostomy, parenteral nutrition).
- Incidence of clinical manifestations related to hepatoxicity.
- Changes in alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, international normalised ratio (INR)
- Changes in growth parameters (height, weight, body mass index [BMI]).
- Incidence of hospitalisations due to diarrhoea, fat-soluble vitamin deficiency, hepatoxicity.
- Discontinuation of treatment due to diarrhoea, fat-soluble vitamin deficiency, hepatoxicity.

Data Sources: Data for this study will be obtained from the TreatFIC registry.

Study Size: No formal sample size calculation was performed. Based upon the incidence of episodes of fat-soluble vitamin deficiencies, diarrhoea, and elevations in AST, ALT, and bilirubin observed in both the placebo and odevixibat-treated patients in the pivotal Phase 3 study (A4250-005), the data collected by following 50 patients for a minimum of 3 years is expected to be sufficient for meaningful analyses and interpretation of the results. Overall, this registry-based safety study will target a total of approximately 50 patients with PFIC treated with odevixibat and approximately 50 patients with PFIC in the untreated control cohort.

Data Analysis: Patients will be stratified based upon whether or not they received odevixibat treatment and PFIC type. When a minimum of 50 patients with PFIC treated with odevixibat have been followed for a minimum of 3 years, data will be collated, and descriptive statistics will be presented. Similar data from a control group of patients who did not receive odevixibat treatment will be presented for comparison. Interim analyses will be conducted for interim reporting.

Study Milestones

Milestone	Planned Date
Date of study registration in the EU PASS Register (estimated)	26 June 2023
Start of data collection (estimated)	26 June 2023
First interim study report	30 September 2024
Second interim study report	30 September 2025
Third interim study report	30 September 2026
Fourth interim study report	30 September 2027
End of data collection	30 June 2028
Final report of study results	31 December 2028

3. Amendments and Updates

Update No.	Date	Protocol Sections Affected	Update	Rationale for Change
1	04 Nov 2022	Synopsis Section 7.1 Study Design Section 7.2.4 Patient Follow-Up	Patients will be followed for a 2-year enrolment period and 3-year follow-up period.	The duration of enrolment and follow-up has been clarified throughout.
		Section 7.2.1 TreatFIC Registry	Revised the background information regarding the TreatFIC registry.	Updated text to increase accuracy and consistency with the TreatFIC registry protocol.
		Section 7.2.4 Patient Follow-Up	Added that clinic visits with patients are to occur every 6 months and that before declaring a patient is lost to follow-up, physicians may contact patient or the patient's family to exclude death as a reason for study termination.	Clarifies the frequency of clinic visits and procedure to exclude death as a reason for study termination.
		Section 7.3 Variables	Added that for patients who discontinue odevixibat treatment, endpoint events occurring within 180 days of discontinuation are to be recorded.	Clarifies the timeframe for recording of events following discontinuation of odevixibat treatment.
		Section 7.6 Data Management	Added text outlining data management, including storage and sharing through REDCap and SPSS.	Updated text to increase accuracy and consistency with the TreatFIC registry protocol.
		Section 7.7 Data Analysis	Revised text to highlight that results will be assessed yearly as part of interim analyses as part of annual re-assessments.	Updated text to reflect that the length of time for duration of follow-up will be adjusted to ensure the study provides meaningful results.

Update No.	Date	Protocol Sections Affected	Update	Rationale for Change
		Section 7.8 Quality Control and Quality Assurance	Added text outlining data monitoring, validation, and associated measures.	Text reflects measures taken by the TreatFIC registry to improve the validity, quality, and reliability of the data.
2	24 March 2023	Section 4. Study Milestones	Milestones added	Milestones added to allow for a 1-2 year period of patients' enrolment and a minimum of 3 years of follow-up.

REDCap=Research Electronic Data Capture web application; TreatFIC= Treating patients with Familial Intrahepatic Cholestasis.

4. Study Milestones

MILESTONE	PLANNED DATE
Date of study registration in the EU PASS Register (estimated)	26 June 2023
Start of data collection (estimated)	26 June 2023
First interim study report	30 September 2024
Second interim study report	30 September 2025
Third interim study report	30 September 2026
Fourth interim study report	30 September 2027
End of data collection	30 June 2028
Final report of study results	31 December 2028

The **Treat**ing patients with **F**amilial **I**ntrahepatic **C**holestasis (TreatFIC) registry is planning to launch their patient registry in January 2023. The collaboration with the TreatFIC registry and accrual of data is therefore estimated to start in January 2023.

5. Background and Rationale

5.1 Background

Progressive familial intrahepatic cholestasis (PFIC) is a group of rare genetic disorders that results from defects in bile secretion and presents with intrahepatic cholestasis. Onset is usually in infancy or childhood [Srivastava 2014]. Transmission is autosomal recessive, and the estimated incidence is about 1 per 50,000 to 1 per 100,000 births. The disorders affect both males and females equally and have been reported in all geographical regions [Srivastava 2014]. PFIC is generally categorised into 3 types, PFIC 1-3, although at least 2 other types have been described in literature [Srivastava 2014; Jacquemin 2000; Mehl 2016; Gunaydin 2018]. PFIC1, also known as Byler disease, is associated with defects in *ATP8B1* gene, which encodes for familial intrahepatic cholestasis 1 protein, a flippase located on the canalicular membrane of hepatocytes. PFIC2, previously known as Byler's syndrome, results from mutations in the ATP-binding cassette (ABC) subfamily B member 4 *B11* gene which encodes for bile salt export pump (BSEP), a transporter protein expressed at the canalicular membrane of hepatocytes, and which is the main exporter of bile acids from hepatocytes to canaliculi against a concentration gradient. PFIC3 is associated with defects in the *ABCB4* gene encoding multidrug resistance protein 3 (MDR3), a P-glycoprotein (P-gp), which is expressed in the canalicular membrane of hepatocytes and is responsible for biliary secretion of phospholipids, predominantly phosphatidylcholine. The underlying mutations for PFIC1 and PFIC2 both either directly or indirectly affect BSEP expression and function in the canalicular membrane of the hepatocyte leading to impaired bile acid secretion [Hori 2010]. Whereas in PFIC3, the basic defect results in reduced secretion of biliary phospholipids, resulting in injury to the biliary epithelia and canaliculi, as the concentration of free bile acids is increased in the extracellular space. Furthermore, the cholestasis in PFIC3 is associated with high gamma-glutamyl transferase (GGT) levels, while in PFIC1 and 2 GGT levels are normal or low [Srivastava 2014].

Patients with PFIC often present with worsening jaundice and severe pruritus within the first few years of life [Mehl 2016]. Median age at onset of symptoms is approximately 3 months; 78% of patients develop jaundice before 12 months of age [Pawlikowska 2010]. Examination reveals icterus, hepatomegaly, scratch marks with excoriation, and hyperpigmentation of skin and shiny nails. Other features include fat

malabsorption resulting in weight and height below normal centiles, and fat-soluble vitamin (A, D, E, and K) deficiency. Portal hypertension and decompensation may be evident earlier in the first year of life in PFIC2 and in early childhood in PFIC1 [Davit Spraul 2009, Srivastava 2014]. Liver biopsy reveals canalicular cholestasis and, later, the appearance of portal fibrosis. Liver biochemistry shows cholestasis with hyperbilirubinemia and elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The concentrations of bile acids in serum are typically very high, while serum GGT is normal or low (except for PFIC3), and cholesterol is typically normal [Hori 2010]. Many of these patients will progress to end-stage liver disease and require liver transplantation [Mehl 2016].

Odevixibat is currently the only approved pharmaceutical treatment for PFIC, with the exception of ursodeoxycholic acid (UDCA), which has national approval for the treatment of PFIC3 in France. The therapeutic choices are restricted to non-specific symptomatic treatment, nutritional support, vitamin supplementation, and treatment of complications. Other medical treatment options include off-label use of UDCA, rifampicin, antihistamines, and naltrexone to treat pruritus. A minority of patients (approximately 30%) respond to these medications, some only transiently.

Surgical options include biliary diversion and liver transplant. Treatment-resistant pruritus is the leading indication for surgical biliary diversion, particularly in patients with PFIC2 [Melter 2000; Yang 2009; Arnell 2010; Schukfeh 2012]. Liver transplantation is currently the only definitive treatment for PFIC. Survival in patients with PFIC not undergoing surgical diversion or liver transplant is 50% at 10 years of age and almost none at 20 years of age, highlighting the rapid rate of progression of this life-threatening disease [Pawlikowska 2010].

Odevixibat is a small molecule that acts as a potent, selective inhibitor of the human ileal bile acid transporter, an integral brush border membrane glycoprotein that co-transports sodium and bile acids and appears to be a major regulator of the bile acids pool in animals and humans. Odevixibat, minimally absorbed following oral administration, represents a novel, non-invasive medical therapy for infants and children with PFIC. The safety of odevixibat in patients with PFIC was demonstrated in two Phase 3 studies. In the pivotal Phase 3 study A4250-005, a randomised, double-blind, placebo-controlled

study, the most frequently reported adverse events (AEs) included diarrhoea, abdominal pain, soft faeces, hepatomegaly, liver test abnormalities, vomiting, and fat-soluble vitamin deficiency. In the long-term, open-label Phase 3 extension study A4250-008, the AEs profile has been similar to that in the pivotal study.

5.2 Rationale

Odevixibat (Bylvay) was authorised in July 2021 for the treatment of PFIC in patients ≥6 months of age (European Medicines Agency [EMA]) and for the treatment of pruritus in patients ≥3 months of age with PFIC (United States Food and Drug Administration [US FDA]). Long-term follow-up information is needed to provide comprehensive safety data, including clinically significant or severe diarrhoea leading to dehydration and/or electrolyte imbalance, fat-soluble vitamin deficiencies, interaction with fat-soluble drugs, and hepatotoxicity.

This registry-based safety study aims to evaluate the long-term safety profile in patients with PFIC receiving odevixibat treatment relative to untreated patients.

6. Research Questions and Objectives

The aim of this study is to assess the long-term, real-world safety profile of odevixibat treatment in patients with PFIC compared to patients not receiving odevixibat (untreated control cohort).

The overall objectives of this registry-based study are:

- To evaluate the impact of odevixibat treatment on the occurrence of severe diarrhoea.
- To evaluate the impact of odevixibat treatment on clinical manifestations of fat-soluble vitamin deficiency.
- To evaluate the impact of odevixibat treatment on the effectiveness of fat-soluble drugs.
- To evaluate the impact of odevixibat treatment on nutritional status.
- To evaluate the impact of odevixibat treatment on hepatic function and signs of hepatoxicity.

7. Research Methods

7.1 Study Design

This post-authorisation safety study (PASS) represents a registry-based study analysing secondary data. This will be a long-term non-interventional, observational, and voluntary participation registry-based safety study designed to examine the real-world usage of odevixibat for the treatment of PFIC using data prospectively collected in the TreatFIC registry.

The TreatFIC registry is a disease registry that is open to enrolment for all patients with PFIC, including patients treated with odevixibat as well as untreated patients. There is no cut-off date for enrolment or cap on the number of patients to be enrolled.

This study will continue until a minimum of 50 patients with PFIC treated with odevixibat have been followed for a minimum of 3 years. It is estimated that 50 patients with PFIC will enrol in a 1- to 2-year period. The length of time for duration of follow-up will be adjusted as needed to ensure the study provides meaningful results.

7.2 Study Population and Setting

7.2.1 TreatFIC Registry

TreatFIC is a prospective registry managed by a Steering Committee of global PFIC experts based in Europe. PFIC expert centres world-wide are being invited to form a global study group. Clinicians who previously published or presented data on patients with PFIC and clinicians who are actively treating these patients will be identified and approached for participation in TreatFIC. Upon entering the prospective registry, the clinical (including medical history, concomitant medications, and odevixibat treatment information), biochemical, genetic and, if applicable, surgical data will be retrospectively collected from patients diagnosed with the indicated forms of PFIC disease, followed by prospective collection from that moment onwards.

The retrospective data collection will be aligned with that of the retrospective **NA**tural Course and **P**rognosis of **P**FIC and **E**ffect of Biliary **D**iversion (NAPPED) registry. The NAPPED registry has the largest genetically defined cohort of PFIC patients to date, providing retrospective analysis of approximately 130 PFIC1 and 264 PFIC2 patients in

68 centres globally as of 2021 (van Wessel 2021a; van Wessel 2021b). However, the aim of NAPPED is to characterize the natural course of disease in PFIC1 and PFIC2, determine associations between genotype and phenotype, assess effects of surgical biliary diversion on native liver survival, and identify an early surrogate marker for long-term native liver survival. No prospective data has been or is being collected in NAPPED.

In addition to clinicians who currently have patients enrolled in the NAPPED registry, clinicians who previously published or presented data on patients with PFIC and clinicians who are actively treating these patients will be identified and approached for participation in TreatFIC. A prerequisite for participation will be local regulatory and/or review board and ethics approval and consent to use the patient data in aggregated form for regulatory purposes, such as submissions to the EMA, the FDA, and comparable international agencies.

7.2.2 Inclusion Criteria

Patients who are homozygous for a known, disease-causing mutation of the PFIC family (any type), compound homozygous for two disease-causing mutations or heterozygous for one disease-causing mutation in combination with the clinical phenotype intrahepatic cholestasis will be eligible. Patients treated with odevixibat as well as those not treated with odevixibat will be eligible for this study.

7.2.3 Exclusion Criteria

There are no exclusion criteria.

7.2.4 Patient Follow-Up

Patients will be followed for a minimum of 3 years, or until withdrawal, transfer out of the registry, or loss to follow-up, whichever comes first. It is anticipated that physicians will have clinic visits with patients every 6 months; however, if clinic visits are delayed, the physician may contact the patient to update information. Before declaring that a patient is lost to follow-up, the physician may contact the patient or the patient's family to exclude death as a reason for study termination.

7.3 Variables

Data of included patients will be regularly updated at 6-month intervals. For patients who discontinue odevixibat treatment, endpoint events occurring within 180 days of discontinuation are to be recorded with the date of discontinuation and the date of the event.

The TreatFIC registry captures individual patient data including:

- Demographics: date of birth, gender, diagnosis, age at diagnosis, specific mutation, extrahepatic manifestations, other diagnoses, genetic analysis not (yet) performed, and growth parameters.
- Odevixibat treatment: start date, dosage, treatment end date, and reasons for dose modification/treatment discontinuation.
- Other treatments: vitamin supplementation (type, dosage, response to supplementation, and route of administration), UDCA, rifampicin, cholestyramine, surgical interruption of the enterohepatic circulation (partial external biliary diversion and other variants), medical interruption of the enterohepatic circulation (ileal bile acid transporter inhibitor [IBAT-I], odevixibat, maralixibat).
- Monitoring: longitudinal serum biochemical parameters, including pre- and post-odevixibat treatment changes as far as available: alkaline phosphatase (AP), total bilirubin, albumin, GGT, AST, ALT, bile acids, thrombocytes, vitamins A, D, and E, triglycerides, cholesterol, and international normalized ratio (INR).
- Clinical symptoms: pruritus and sleep disturbance, if (semi)-objective scoring data are available.
- Clinical outcomes: alive with native liver, with or without biliary diversion (internal or external) and with or without IBAT-I therapy, imaging indications of liver cirrhosis and portal hypertension, liver transplantation, indication for transplantation (pruritus, end-stage liver disease, hepatocellular carcinoma, other), death, cause of death, variceal haemorrhage, diagnosis of hepatocellular carcinoma.

- Growth: height, weight, and body mass index (BMI), over time. Nutritional interventions (e.g. special diets, nasogastric feeding, gastrostomy, parenteral nutrition).
- Safety: diarrhoea (>3 days), bloody diarrhoea, dehydration, hepatotoxicity, fat soluble vitamin levels, clinical manifestations of fat-soluble vitamin deficiency (e.g. fractures, bleeding, rickets), fat soluble vitamin deficiency refractory to dose increase.

7.3.1 Exposure

The main exposure of interest is treatment with odevixibat. Dosing information will be collected as outlined in Section 7.3.

7.3.2 Outcomes

Patients will be stratified based upon whether or not they received odevixibat treatment and PFIC type. The safety of odevixibat treatment will be evaluated using the following outcomes:

- Incidence of diarrhoea lasting > 3 days, bloody diarrhoea, or diarrhoea leading to dehydration or electrolyte imbalance and any treatment.
- Incidence of clinical manifestations related to fat-soluble vitamin deficiency (e.g., bleeding, rickets, osteopenia) including symptoms and treatment.
- Change in fat-soluble vitamin levels.
- Incidence of reports of ineffectiveness of fat-soluble drugs that were previously effective.
- Incidence of reports of new nutritional interventions (special diets, nasogastric feeding, gastrostomy, parenteral nutrition).
- Incidence of clinical manifestations related to hepatoxicity.
- Changes in ALT, AST, bilirubin, INR.
- Changes in growth parameters (height, weight, BMI).

- Incidence of hospitalisations due to diarrhoea, fat-soluble vitamin deficiency, hepatoxicity.
- Discontinuation of treatment due to diarrhoea, fat-soluble vitamin deficiency, hepatoxicity.

7.4 Data Sources

Data for this study will be obtained from the TreatFIC registry. A TreatFIC registry specific electronic case report form (eCRF) will be designed in Research Electronic Data Capture web application (REDCap). The eCRF will also provide a data dictionary or codebook containing descriptive information on all data variables and, if applicable, its units of measurement. Data will be stored in REDCap and in a study specific folder within the University Medical Center Groningen (UMCG) network.

Upon entering the registry, the clinical (including medical history, concomitant medications, and odevixibat treatment information), biochemical, genetic and, if applicable, surgical data will be retrospectively collected from patients diagnosed with the indicated forms of PFIC disease. The retrospective data collected in the TreatFIC registry will be aligned with that of the data collected in the NAPPED registry. The investigators within each centre will identify all patients with a genetically confirmed form of the indicated PFIC diseases that are currently still in treatment/follow up. There is no age limit. Follow-up data on these patients will be obtained from databases, patient files, and digital hospital information systems.

7.5 Study Size

No formal sample size calculation was performed. Based upon the incidence of episodes of fat-soluble vitamin deficiencies, diarrhoea, and elevations in AST, ALT, and bilirubin observed in both the placebo and odevixibat-treated patients in the pivotal Phase 3 study (A4250-005), the data collected by following 50 patients for a minimum of 3 years is expected to be sufficient for meaningful analyses and interpretation of the results. Overall, this registry-based safety study will target a total of approximately 50 patients with PFIC treated with odevixibat and approximately 50 patients with PFIC in the untreated control cohort.

7.6 Data Management

Data for this study will be obtained from the TreatFIC registry, which obtains data from databases, patient files and digital hospital information systems used by physicians. Data of included patients will be regularly updated by physicians at 6-month intervals following clinic visits (Section 7.2.4). If delayed, physicians will be contacted by the TreatFIC registry. Data on patients will be obtained from databases, patient files, and digital hospital information systems and entered into the registry database. The registry database will be managed by a team in Groningen, the Netherlands.

Data will be managed in REDCap and in a study specific folder by a team at theUMCG. The infrastructure of REDCap has access management, an audit trail and automated back-up in place. The use of this infrastructure is in line with current UMCG policy. All data will be thoroughly reviewed and shared through the REDCap and statistical software (SPSS). Data shared through REDCap is stored centrally in a secure MySQL database. The statistical analysis will be conducted by a statistical group that is independent to the marketing authorisation holder (MAH) and the final outputs will be provided to the MAH.

Individual patient data will not be shared with the MAH in the regular course of the registry; however, based on research questions approved by the Steering Committee and the contributing centres opting in, pseudonymised data can be made available and analysed for further use.

7.7 Data Analysis

Detailed methodology and comprehensive information relating to this registry-based safety study will be provided in a statistical analysis plan (SAP). An outline of the planned statistical analyses is provided below.

When a minimum of 50 patients with PFIC treated with odevixibat have been followed for a minimum of 3 years, data will be collated, and descriptive statistics will be presented. Similar data from a control group with patients who did not receive odevixibat treatment will be presented for comparison. Interim analyses will be conducted for interim reporting. The length of time for duration of follow-up will be adjusted to ensure the study provides meaningful results.

Descriptive analysis will be conducted for all patients enrolled in the registry, including the following cohorts:

- Odevixibat cohort: Patients with PFIC who received odevixibat at any time before or during the study.
- Control cohort: Patients with PFIC who did not receive odevixibat.

To minimize immortal time bias, the historical data collected in the registry will not be included in the analysis. For the odevixibat cohort, Day 1 is the date of enrolment for prevalent users (patients who received odevixibat before enrolment) and date of first dose of odevixibat for incident users (patients who received odevixibat when enrolled in the registry). For the control cohort, Day 1 is the date of enrolment.

Baseline is defined as the last available value before Day 1.

Enrolment and disposition will be summarised by cohort. Demographic and baseline characteristics of all patients will be described by cohort using mean, standard deviation (StdDev), median, minimum, and maximum for continuous variables and count and percentages for discrete variables. Baseline data will be presented for both cohorts.

The expected intercurrent event in this study will be liver transplant and death. The hypothetical strategy will be implemented by excluding data after an intercurrent event. For the safety endpoints outlined below, the number of events and incident rates will be calculated. Patients receiving odevixibat after enrolling in the study will be counted in both cohorts. Two time periods will be defined. Period 1 is from enrolment to 1 day before the first dose of odevixibat. Period 2 is after the first dose of odevixibat. If an event occurs in Period 1, it will be counted in the control cohort. If an event occurs in Period 2, it will be counted in the odevixibat cohort. Descriptive statistics will be presented by odevixibat cohort and control cohort. Within the odevixibat cohort, data for incident and prevalent users will be report separately.

The incidence rate per 100 patient years for AE or hospitalisation will be calculated as the number of total events/total cumulative time (years) * 100, where total cumulative time is the sum of individual time at risk. For patients in the control cohort or the odevixibat cohort receiving odevixibat before enrolling in the study, the time at

risk = date of last follow-up - date of enrolment. Patients receiving odevixibat after enrolment will be counted in both cohorts. The time at risk from enrolment to the date before the first dose of odevixibat will be included in the control cohort (Period 1) and from the first dose of odevixibat to the date of last follow-up will be included in the odevixibat cohort (Period 2).

The occurrence of AEs with an early onset after treatment initiation (e.g. diarrhoea) may differ between the incident users and prevalent users. AEs will also be analysed by incident users and prevalent users separately as a part of a subgroup analysis.

For clinical laboratory variables, descriptive statistics for results and change from baseline at each follow-up visit (year) will be presented for each cohort. Patients receiving odevixibat during the study will be counted in both cohorts. The data from enrolment to 1 day before the first dose of odevixibat will be included in the control cohort (Period 1) and data on and after Day 1 will be included in the odevixibat cohort (Period 2). The baseline for Period 1 will be the last available assessment before enrolment. The baseline for Period 2 will be the last available assessment before Day 1.

Descriptive statistics will be used to analyse the outcomes as listed in Section 7.3.2.

7.8 Quality Control and Quality Assurance

In the TreatFIC registry, physicians will enter all data directly into eCRFs. Guidelines for completion of the eCRFs will be developed. In the electronic data capture (EDC), all patient data will be pseudonymised by using a study specific case number. Upon registration of new patients, the registry will subsequently require data entry for each patient at a minimum of every 6 months. All data will be monitored for verification. A data validation plan will be developed. Data validation will be performed with the EDC data quality tools for standard data quality checks and with manual checks. The standard data quality checks will include missing data checks, range checks, and date checks. A data manager will review the data entered into the registry every 6 months and contact the physician regarding missing data (data that has been collected for a patient but not yet reported; as this is a registry based on real-world experience, not all data will be collected for each patient as per standard of care) or to verify data entered. The timely data review and follow-up will minimize the amount of the missing data. In addition, for patients who

discontinue odevixibat treatment, clinical events (surgical biliary diversion, liver transplant, or death) occurring within 180 days of discontinuation are to be recorded with the date and reason of discontinuation and the date of the event. Thus, the missing data on these clinical events is expected to be low.

To ensure high quality data and analyses, the following measures will be installed, which will improve the validity, quality, and reliability of the data:

- Data of included patients will be regularly updated at 6-month intervals.
- All data will be thoroughly reviewed and, through the REDCap SPSS, automatically queried.
- Quality audits will be conducted, as well as monitoring of a subset of sites performed based on risk assessment.
- Training on data entry will be conducted.
- A data review plan will be prepared and executed, containing procedures for retrieving missing information and checking spurious data.
- A Critical Events Review Committee (CERC) will be established to perform a blinded adjudication of all clinical outcomes used to define the primary endpoints and to evaluate data quality issues that may arise.

7.9 Limitations of Research Methods

The long-term safety of patients with PFIC that are chronically treated with odevixibat will be assessed using data collected in the TreatFIC registry. This is a non-interventional observational study without randomisation. Limitations of this study are consistent with those of most observational studies. A potential limitation is patient enrolment. The TreatFIC registry is new. At this time, the proportion of patients who will agree to participate in the registry-based studies is unknown. Clinicians who previously published or presented data on patients with PFIC and clinicians who are actively treating these patients will be identified and approached for participation in the TreatFIC Study Group. This will increase the likelihood of patient participation since these experts have long-standing established relationships with PFIC patients.

8. Protection of Human Participants

8.1 Informed Consent

Appropriate informed consent will be obtained from participants who agree to participate in this registry-based study based on the requirements at participating centres.

8.2 Ethical Conduct of Study

This study will be performed in accordance with the protocol, the principles of the Declaration of Helsinki 1964 as modified by the 52nd World Medical Association (WMA) General Assembly, Edinburgh, Scotland, October 2000 with notes of clarification on paragraph 29 & 30 added by the WMA General Assembly, Washington 2002 & Tokyo 2004 and the local national laws governing the conduct of clinical research studies.

9. Management and Reporting of Safety Information

This is a non-interventional study based on the secondary use of data collected in the TreatFIC registry. No administration of any therapeutic or prophylactic agent is required in this protocol, and there are no procedures required as part of this protocol. AEs/adverse reactions will be summarised in periodic safety reports (e.g., Periodic Benefit Risk Evaluation Report [PBRER]). The reporting of adverse reactions in the form of individual case safety reports is not required.

The MAH is responsible for analysing reports of collected safety information and reporting to regulatory agencies as determined by country-specific legislation or regulations. Participating healthcare providers are encouraged to report all suspected adverse drug reactions to the national pharmacovigilance systems.

Collected outcomes will be summarised in the final study report. Safety information will be summarised in periodic safety update reports and other periodic regulatory reports, as outlined in EMA *Good Pharmacovigilance Practices*, Module V and VII.

9.1 Adverse Events

An AE is defined as any untoward medical occurrence in an enrolled patient regardless of causal relationship with study drug. An AE can therefore be any clinically significant unfavourable and unintended sign, symptom, or disease that occurs once a patient is enrolled in the registry and until the end of follow-up whether or not the AE is related to odevixibat. An AE can be a new condition or worsening of a pre-existing condition.

The information on AEs will be collected by the TreatFIC registry and may not include event characteristics such as seriousness, causality (suspected or non-suspected), severity, start and end dates, actions taken, and outcome.

10. Plans for Disseminating and Communicating Study Results

A final study report will be submitted to appropriate authorities, according to the schedule located in Section 4. A summary of the final report will also be published on the EU electronic Register of Post-Authorisation Studies. The report will include information about the number of patients included in the study, cumulative follow-up time accrued, descriptive analyses of baseline demographic and clinical characteristics, and summary safety information in accordance with EMA Module VIII of *Good Pharmacovigilance Practices*.

Results of this study may be submitted to a peer-reviewed journal for publication and submitted for presentations on national and international conferences. The core publication will be authored by the investigators, who contribute significantly to the implementation and conduct of the TreatFIC registry and personnel who contribute substantially to the design, interpretation, or analysis of the study. The MAH is entitled to review the results and interpretations included in the manuscript and provide comment prior to submission of the manuscript for publication.

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Appendix 1: ENcePP Checklist for Study Protocols (Revision 4)

(Adopted by the ENCePP Steering Group on 15/10/2018)

The European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) welcomes innovative designs and new methods of research. This checklist has been developed by ENCePP to stimulate consideration of important principles when designing and writing a pharmacoepidemiological or pharmacovigilance study protocol. The checklist is intended to promote the quality of such studies, not their uniformity. The user is also referred to the *ENCePP Guide on Methodological Standards in Pharmacoepidemiology*, which reviews and gives direct electronic access to guidance for research in pharmacoepidemiology and pharmacovigilance.

For each question of the checklist, the investigator should indicate whether or not it has been addressed in the study protocol. If the answer is "yes," the section number of the protocol where this issue has been discussed should be specified. It is possible that some questions do not apply to a particular study (for example, in the case of an innovative study design). In this case, the answer 'N/A' (not applicable) should be checked and the "Comments" field included for each section should be used to explain why. The "Comments" field can also be used to elaborate on a "no" answer.

This checklist should be included as an Appendix by marketing authorisation holders when submitting the protocol of a non-interventional PASS to a regulatory authority (see Guidance on the format and content of the protocol of non-interventional PASS). The checklist is a supporting document and does not replace the format of the protocol for PASS presented in the guidance and Module VIII of the Good Pharmacovigilance Practices.

Study title: Prospective Registry-Based Study of the Long-Term Safety of Odevixibat in Patients with Progressive Familial Intrahepatic Cholestasis (PFIC)

EU PAS Register® number: TBD
Study reference number (if applicable): A4250-019

Section 1: Milestones	**Yes**	**No**	**N/A**	**Section Number**
1.1 Does the protocol specify timelines for				
1.1.1 Start of data collection[1]	☒	☐	☐	Section 4
1.1.2 End of data collection[2]	☒	☐	☐	Section 4
1.1.3 Progress report(s)	☐	☐	☒	
1.1.4 Interim report(s)	☒	☐	☐	Section 4
1.1.5 Registration in the EU PAS Register®	☒	☐	☐	Section 4
1.1.6 Final report of study results.	☒	☐	☐	Section 4

Comments:

Section 2: Research question	**Yes**	**No**	**N/A**	**Section Number**
2.1 Does the formulation of the research question and objectives clearly explain:				
2.1.1 Why the study is conducted? (e.g. to address an important public health concern, a risk identified in the risk management plan, an emerging safety issue)	☒	☐	☐	Section 5.2
2.1.2 The objective(s) of the study?	☒	☐	☐	Section 6
2.1.3 The target population? (i.e. population or subgroup to whom the study results are intended to be generalised)	☒	☐	☐	Section 7.2
2.1.4 Which hypothesis(-es) is (are) to be tested?	☐	☐	☒	
2.1.5 If applicable, that there is no *a priori* hypothesis?	☒	☐	☐	Section 7.7

Comments:

Descriptive statistics will be used; no hypothesis will be tested.

[1] Date from which information on the first study is first recorded in the study dataset or, in the case of secondary use of data, the date from which data extraction starts.
[2] Date from which the analytical dataset is completely available.

Section 3: Study design		**Yes**	**No**	**N/A**	**Section Number**
3.1	Is the study design described? (e.g. cohort, case-control, cross-sectional, other design)	☒	☐	☐	Section 7.1
3.2	Does the protocol specify whether the study is based on primary, secondary or combined data collection?	☒	☐	☐	Section 7.1 Section 9
3.3	Does the protocol specify measures of occurrence? (e.g., rate, risk, prevalence)	☐	☐	☒	
3.4	Does the protocol specify measure(s) of association? (e.g. risk, odds ratio, excess risk, rate ratio, hazard ratio, risk/rate difference, number needed to harm (NNH))	☐	☐	☒	
3.5	Does the protocol describe the approach for the collection and reporting of adverse events/adverse reactions? (e.g. adverse events that will not be collected in case of primary data collection)	☒	☐	☐	Section 9.1

Comments:

Descriptive statistics will be used; there is no plan for measures of association.

Section 4: Source and study populations		**Yes**	**No**	**N/A**	**Section Number**
4.1	Is the source population described?	☒	☐	☐	Section 7.2
4.2	Is the planned study population defined in terms of:				
	4.2.1 Study time period	☒	☐	☐	Section 4
	4.2.2 Age and sex	☐	☐	☒	
	4.2.3 Country of origin	☐	☐	☒	
	4.2.4 Disease/indication	☒	☐	☐	Section 7
	4.2.5 Duration of follow-up	☒	☐	☐	Section 7.2.4
4.3	Does the protocol define how the study population will be sampled from the source population? (e.g. event or inclusion/exclusion criteria)	☒	☐	☐	Section 7.2

Comments:

Section 5: Exposure definition and measurement	**Yes**	**No**	**N/A**	**Section Number**
5.1 Does the protocol describe how the study exposure is defined and measured? (e.g. operational details for defining and categorising exposure, measurement of dose and duration of drug exposure)	☒	☐	☐	Section 7
5.2 Does the protocol address the validity of the exposure measurement? (e.g. precision, accuracy, use of validation sub-study)	☐	☐	☒	
5.3 Is exposure categorised according to time windows?	☒	☐	☐	Section 7
5.4 Is intensity of exposure addressed? (e.g. dose, duration)	☒	☐	☐	Section 7
5.5 Is exposure categorised based on biological mechanism of action and taking into account the pharmacokinetics and pharmacodynamics of the drug?	☒	☐	☐	Section 7
5.6 Is (are) (an) appropriate comparator(s) identified?	☒	☐	☐	Section 7

Comments:

Section 6: Outcome definition and measurement	**Yes**	**No**	**N/A**	**Section Number**
6.1 Does the protocol specify the primary and secondary (if applicable) outcome(s) to be investigated?	☒	☐	☐	Section 7.3
6.2 Does the protocol describe how the outcomes are defined and measured?	☒	☐	☐	Section 7.3
6.3 Does the protocol address the validity of outcome measurement? (e.g. precision, accuracy, sensitivity, specificity, positive predictive value, use of validation sub-study)	☐	☐	☒	
6.4 Does the protocol describe specific outcomes relevant for Health Technology Assessment? (e.g. HRQoL, QALYs, DALYS, health care services utilisation, burden of disease or treatment, compliance, disease management)	☐	☐	☒	

Comments:

Section 7: Bias	**Yes**	**No**	**N/A**	**Section Number**
7.1 Does the protocol address ways to measure confounding? (e.g. confounding by indication)	☐	☐	☒	
7.2 Does the protocol address selection bias? (e.g. healthy user/adherer bias)	☒	☐	☐	Section 7.7
7.3 Does the protocol address information bias? (e.g. misclassification of exposure and outcomes, time-related bias)	☒	☐	☐	Section 7.7

Comments:

Section 8: Effect measure modification	**Yes**	**No**	**N/A**	**Section Number**
8.1 Does the protocol address effect modifiers? (e.g. collection of data on known effect modifiers, sub-group analyses, anticipated direction of effect)	☐	☐	☒	

Comments:

Section 9: Data sources	**Yes**	**No**	**N/A**	**Section Number**
9.1 Does the protocol describe the data source(s) used in the study for the ascertainment of:				
9.1.1 Exposure? (e.g. pharmacy dispensing, general practice prescribing, claims data, self-report, face-to-face interview)	☒	☐	☐	Section 7.3
9.1.2 Outcomes? (e.g. clinical records, laboratory markers or values, claims data, self-report, patient interview including scales and questionnaires, vital statistics)	☒	☐	☐	Section 7.3
9.1.3 Covariates and other characteristics?	☐	☐	☒	
9.2 Does the protocol describe the information available from the data source(s) on:				
9.2.1 Exposure? (e.g. date of dispensing, drug quantity, dose, number of days of supply prescription, daily dosage, prescriber)	☒	☐	☐	Section 7.3
9.2.2 Outcomes? (e.g. date of occurrence, multiple event, severity measures related to event)	☒	☐	☐	Section 7.3
9.2.3 Covariates and other characteristics? (e.g. age, sex, clinical and drug use history, co-morbidity, co-medications, lifestyle)	☒	☐	☐	Section 7.3

Section 9: Data sources	**Yes**	**No**	**N/A**	**Section Number**
9.3 Is a coding system described for:				
9.3.1 Exposure? (e.g. WHO Drug Dictionary, Anatomical Therapeutic Chemical (ATC) Classification System)	☐	☐	☒	
9.3.2 Outcomes? (e.g. International Classification of Diseases (ICD), Medical Dictionary for Regulatory Activities (MedDRA))	☐	☐	☒	
9.3.3 Covariates and other characteristics?	☐	☐	☒	
9.4 Is a linkage method between data sources described? (e.g. based on a unique identifier or other)	☐	☐	☒	

Comments:

Section 10: Analysis plan	**Yes**	**No**	**N/A**	**Section Number**
10.1 Are the statistical methods and the reason for their choice described?	☒	☐	☐	Section 7.7
10.2 Is study size and/or statistical precision estimated?	☒	☐	☐	Section 7.5
10.3 Are descriptive analyses included?	☒	☐	☐	Section 7.5
10.4 Are stratified analyses included?	☒	☐	☐	Section 7.3
10.5 Does the plan describe methods for analytic control of confounding?	☐	☐	☒	
10.6 Does the plan describe methods for analytic control of outcome misclassification?	☐	☐	☒	
10.7 Does the plan describe methods for handling missing data?	☐	☐	☒	
10.8 Are relevant sensitivity analyses described?	☐	☐	☒	

Comments:

Section 11: Data management and quality control	**Yes**	**No**	**N/A**	**Section Number**
11.1 Does the protocol provide information on data storage? (e.g. software and IT environment, database maintenance and anti-fraud protection, archiving)	☒	☐	☐	Section 7.4
11.2 Are methods of quality assurance described?	☒	☐	☐	Section 7.8

Section 11: Data management and quality control	**Yes**	**No**	**N/A**	**Section Number**
11.3 Is there a system in place for independent review of study results?	☒	☐	☐	Section 9

Comments:

Section 12: Limitations	**Yes**	**No**	**N/A**	**Section Number**
12.1 Does the protocol discuss the impact on the study results of:				
12.1.1 Selection bias?	☒	☐	☐	Section 7.7
12.1.2 Information bias?	☒	☐	☐	Section 7.7
12.1.3 Residual/unmeasured confounding? (e.g. anticipated direction and magnitude of such biases, validation sub-study, use of validation and external data, analytical methods).	☐	☐	☒	
12.2 Does the protocol discuss study feasibility? (e.g. study size, anticipated exposure uptake, duration of follow-up in a cohort study, patient recruitment, precision of the estimates)	☒	☐	☐	Section 7.5

Comments:

Section 13: Ethical/data protection issues	**Yes**	**No**	**N/A**	**Section Number**
13.1 Have requirements of Ethics Committee/ Institutional Review Board been described?	☒	☐	☐	Section 8.2
13.2 Has any outcome of an ethical review procedure been addressed?	☐	☐	☒	
13.3 Have data protection requirements been described?	☒	☐	☐	Section 8

Comments:

Section 14: Amendments and deviations	**Yes**	**No**	**N/A**	**Section Number**
14.1 Does the protocol include a section to document amendments and deviations?	☒	☐	☐	Section 3

Comments:

Section 15: Plans for communication of study results	Yes	No	N/A	Section Number
15.1 Are plans described for communicating study results (e.g. to regulatory authorities)?	☒	☐	☐	Section 10
15.2 Are plans described for disseminating study results externally, including publication?	☒	☐	☐	Section 10

Comments:

Name of the main author of the protocol: PPD Clinical Development

Date: dd/Month/year 24 March 2023 | 12:52 EDT

Signature: PPD

Qualified P

Date: dd/M

Signature: PPD