CLINICAL STUDY PROTOCOL A4250-005

A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

IND Number:	130591
EudraCT Number	2017-002338-21
Test Product:	A4250
Indication:	Progressive Familial Intrahepatic Cholestasis (PFIC)
Sponsor:	Albireo AB
Development Phase:	Phase 3
Sponsor Signatory:	[redacted]
Sponsor Medical Monitor:	[redacted]
Principal Investigator:	[redacted]
Date of the Protocol:	24 June 2019
Version of the Protocol:	Final Amendment 06

Confidential

The information in this document contains trade secrets and commercial information that are privileged or confidential and may not be used, divulged, published, or otherwise disclosed without the written consent of Albireo AB. These restrictions on disclosure apply equally to all future information supplied to you or generated by you in connection with the study.

SPONSOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

Albireo AB

[redacted] Date (day/month/year)

INVESTIGATOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

I have read this protocol and agree that it contains all necessary details for performing this study. I will conduct the study as outlined herein and will complete the study within the time designated, in accordance with all stipulations of the protocol and in accordance with Good Clinical Practice (GCP), local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study drug and the conduct of the study.

I will use only the informed consent approved by the Independent Ethics Committee (IEC) and will fulfil all responsibilities for submitting pertinent information to the IEC responsible for this study.

I agree that the sponsor (Albireo AB) shall have access to any source documents from which case report form information may have been generated.

I further agree not to originate or use the name of Albireo AB, A4250 in any publicity, news release, or other public announcement, written or oral, whether to the public, press, or otherwise, relating to this protocol, to any amendment to the protocol, or to the performance of this protocol without the prior written consent of Albireo AB.

[redacted]

Signature

Date (day/month/year)

1 ADMINISTRATIVE INFORMATION

A Double-blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Protocol No.:	A4250-005
Date of the initial Protocol:	20 September 2017
Date and Number of Amendment(s):	Amendment 01, 06 December 2017 Amendment 02, 10 May 2018 Amendment 03, 22 November 2018 Amendment 04, 01 March 2019 Amendment 05, 29 April 2019 Amendment 06, 24 June 2019
Sponsor:	Albireo AB Arvid Wallgrens backe 20 413 46 Göteborg Sweden
Clinical Research Organization:	[redacted]
Sponsor Signatory:	
Sponsor Medical Monitor:	
Principal Investigator:	

2 STUDY SYNOPSIS

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250

Title of Study:
A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Principal Investigator:
[redacted]

Study Centers:
Up to 50 sites will be initiated for this study in the United States (US), Canada, European Union (EU), Middle East, and Australia.

Publication(s):
None.

Planned Study Period:	**Development Phase:**
Q2 2018 to Q4 2019	Phase 3

Objectives:

Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with progressive familial intrahepatic cholestasis (PFIC) Types 1 and 2, as determined by the following:

- Proportion of patients experiencing at least a 70% reduction in fasting serum bile acid (s-BA) concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- The proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period

Secondary Objectives

- To evaluate the effect of A4250 on serum alanine aminotransferase (ALT) concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

Methodology:

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of familial intrahepatic cholestasis-1 or bile salt export pump).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-up Period. After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo. Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008) in which all patients will receive active treatment.

There will be up to 10 clinic visits during the study and one scheduled telephone contact.

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Number of Patients:

Approximately 60 patients diagnosed with PFIC-1 or PFIC-2 will be randomized. The study will target 15% of enrolled patients to be PFIC-1 patients. If enrollment of all PFIC-2 patients is completed and the 15% enrollment of PFIC-1 patients has not yet been achieved, then enrollment of PFIC-2 patients will continue in order to reach the total study target enrollment.

Diagnosis and Main Criteria for Inclusion:

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have clinical genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples at least 7 days apart (Visits 1 and 2) prior to randomization
4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the electronic diary (eDiary) average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients are expected to have a consistent caregiver for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

Test Product, Dose and Mode of Administration:

A4250, 40 µg/kg/day or 120 µg/kg/day, orally administered

Reference Therapy, Dose and Duration of Administration:

Matching placebo, orally administered

Duration of Treatment:

24 weeks

Variables

Efficacy:

Primary Efficacy Endpoints

The primary efficacy endpoints are:

- **EU and rest of world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.
- **US**: Proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period.

Secondary Efficacy Endpoints

Secondary efficacy endpoints include the following:

- **EU and RoW**: Proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period.
- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

All Regions:

All secondary endpoints will be compared to placebo.

- Change from baseline to Week 12 and to Week 24 in fasting s-BA
- Change from baseline to Week 12 and to Week 24 in serum ALT concentration

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Albireo	A4250	A4250

- Change in growth from baseline to Week 12 and Week 24
- Proportion of responders for pruritus scores at Weeks 12 and 24 based on the Albireo PRO and ObsRO instruments
- Change in sleep parameters measured with the Albireo PRO and ObsRO instruments from baseline over the 24-week Treatment Period
- Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level over the 24-week Treatment Period. A positive pruritus assessment includes an itch score ≤1, or at least a one-point drop from baseline based on the Albireo PRO instrument; only patients ≥8 years of age will complete the Albireo PRO instrument
- Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Proportion of individual AM assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Proportion of individual PM assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Number of patients undergoing biliary diversion surgery or liver transplantation

Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the patient global impression of change (PGIC) items
- Change from baseline in fasting s-BA at Week 4
- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline by each 4-week interval of the Treatment Period as measured by the Albireo ObsRO instrument
- Change from baseline by each 4-week interval of the Treatment Period in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline by each 4-week interval of the Treatment Period in patient-reported and observer-reported morning time itching and scratching severity scores, respectively
- Change from baseline by each 4-week interval of the Treatment Period in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline by each 4-week interval of the Treatment Period in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in Pediatric Quality of Life Inventory (PedsQL)
- Change from baseline in serum ALT concentration at Week 4
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST and gamma-glutamyl transferase at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, plasma 7α-hydroxy-4-cholesten-3-one concentration [p-C4]) at Week 24
- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events.
- All-cause mortality

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Safety:

Safety criteria are as follows:

- Occurrence of treatment-emergent adverse events including severity and relatedness to study drug at all visits
- The incidence of treatment-emergent serious adverse events (SAEs), based upon information from patient reports, including the description, incidence, and severity of an SAE
- Trends evaluated for the following assessments: physical examinations, concomitant medications, vital signs, laboratory test results (including clinical chemistry, hematology, urinalysis, alfa-fetoprotein, vitamins A and E, 25-hydroxy vitamin D and INR), and abdominal ultrasound

Statistical Methods:

The primary objective of this study is to demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with PFIC Types 1 and 2.

The Cochran Mantel Haenzel (CMH) test stratified by PFIC class and age class will be performed to compare the proportion in fasting bile acid responders at the end of treatment (Week 22 and 24) in the two A4250 dose groups to the placebo group. To ascertain that all data are used in the CMH analysis, neighboring strata will be pooled if all subjects in a stratum have the same response. The proportion together with the corresponding 95% CI, odds ratio and corresponding 95% Clopper-Pearson exact CI and p-value for the CMH test will be presented.

For the primary efficacy variable of the proportion of positive pruritus assessments at subject level over the 24-week treatment period, an ANCOVA model will be used to analyse the comparisons between the treatment groups. The model will include treatment arm, AM baseline pruritus score, PM baseline pruritus score, and randomization stratification factors, i.e. PFIC class and age class. LS mean (SE) by treatment arm and LS mean difference (SE), 95% confidence intervals, and p-values (where applicable) between treatments (120 µg/kg/day and 40 µg/kg/day, respectively) vs. placebo will be provided. If there are concerns on model assumptions, i.e. normality and homogeneity of variances, a non-parametric ANCOVA based on the rank scores can be used as a sensitivity analysis. LS mean/SE on the outcome by treatment arm and LS mean difference/SE between active dose and placebo will be provided.

For each primary endpoint by region (EU & RoW and US), a pooled analysis for the closed testing procedure will be applied to control the 1-sided overall type I error rate for two treatment comparisons vs. the placebo at the 0.025 level, as specified below:

In the closed testing procedure, the low and high dose groups will be pooled to compare with the placebo group first. If the 1-sided p-value is ≤0.025, the 1-sided p-values for low dose vs. placebo and high dose vs. placebo will be calculated respectively. If both individual p-values are ≤0.025, a significant treatment effect will be declared on both dose groups. If only one of them is ≤0.025, a significant treatment effect will be declared on the corresponding dose group.

Other secondary and exploratory outcome variables will provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple comparisons when testing secondary and exploratory efficacy variables.

Date of the Protocol: 24 June 2019

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3.1 List of Tables

3.2 List of Figures

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4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADME	absorption, distribution, metabolism, and excretion
AE(s)	adverse event(s)
AFP	alfa-fetoprotein
ALT	alanine aminotransferase
APRI	AST to platelet ratio index
ASBT	apical sodium-dependent bile transporter (also known as IBAT)
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	body mass index
BSEP	bile salt export pump
CPK	creatine phosphokinase
CRA	clinical research associate
CYP	cytochrome P450
DSMB	Data and Safety Monitoring Board
eCRF	electronic case report form
eDiary	electronic diary
EOT	End of Treatment
EU	European Union
EudraCT	European Union drug regulatory agency clinical trial
FAS	full analysis set
FDA	United States Food and Drug Administration
FIC1	familial intrahepatic cholestasis-1
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
GI	gastrointestinal
GLMM	generalized linear mixed modelling
IB	Investigator's Brochure
IBAT	ileal bile acid transporter (also known as ASBT)
ICF	informed consent form
ICH	International Council for Harmonisation
[redacted]	[redacted] Clinical Research Organization
IEC	Independent Ethics Committee
INR	international normalized ratio
IRB	Institutional Review Board

IWRS	Interactive Web Response System
LDH	lactate dehydrogenase
LFT	liver function test
LPLV	last patient last visit
MDR3	multidrug-resistance protein 3
MedDRA	Medical Dictionary for Regulatory Activities
MELD	model for end-stage liver disease
MMRM	mixed model repeated measures
ObsRO	observer-reported outcome
PBC	primary biliary cholangitis
p-C4	plasma 7α-hydroxy-4-cholesten-3-one concentration
PCS	potentially clinically significant
PedsQL	Pediatric Quality of Life Inventory
PELD	pediatric end-stage liver disease
PFIC	progressive familial intrahepatic cholestasis
PGIC	patient global impression of change
PGIS	patient global impression of symptoms
PK	pharmacokinetic(s)
PP	per protocol
PRO	patient-reported outcome
PT	prothrombin time
PVM	pharmacovigilance manager
QoL	quality of life
RoW	rest of world
RR	respiratory rate
SAE(s)	serious adverse event(s)
SAP	statistical analysis plan
s-BA	serum bile acid
SD	standard deviation
s-FGF19	serum fibroblast growth factor 19 concentration
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction
TEAE(s)	treatment-emergent adverse event(s)
ULN	upper limit of normal
US	United States
WHO	World Health Organization

5 INTRODUCTION

5.1 Investigational Medicinal Product

A4250 is a small molecule and a potent selective inhibitor of the ileal bile acid transporter (IBAT), also known as the apical sodium-dependent bile acid transporter (ASBT). IBAT is an integral brush border membrane glycoprotein that co-transports sodium and bile acids and appears to be a critical component in the regulation of the bile acid pool size in animals and man. This transporter, expressed in the distal ileum, is a key element in the enterohepatic circulation of bile acids since it facilitates the high-affinity, high-capacity reabsorption of bile acids. Indeed, 95% of secreted bile acids are reabsorbed via IBAT [Hofmann 2009; Miethke 2016]. A4250 is orally administered and acts locally in the gut where it binds reversibly to IBAT to decrease the reuptake of bile acids. A4250 has minimal systemic exposure at the predicted therapeutic dose ranges.

No safety signals were found with A4250 in preclinical safety and toxicity studies. In the first clinical study of A4250 in healthy volunteers, A4250 was given as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. A4250 was well tolerated and the only findings were gastrointestinal (GI) related effects, such as diarrhea, at higher doses.

In an exploratory Phase 2 study in children with cholestatic pruritus, A4250 was given as oral doses of 10 to 200 µg/kg/day for 4 weeks. A4250 reduced mean levels of serum bile acids (s-BA) in all cohorts. In 11 of 13 progressive familial intrahepatic cholestasis (PFIC) patients, substantial reductions (ranging from 43% to 98%) were observed. Patient-reported diary data documented improvement of pruritus during treatment. A4250 was well tolerated. No serious adverse events (SAEs) were deemed to be related to study drug, and most adverse events (AEs), including some increased transaminases, were considered unrelated to study drug and transient in nature.

5.2 Background

5.2.1 Progressive Familial Intrahepatic Cholestasis

PFIC is a rare autosomal recessive cholestatic liver disease estimated to affect between 1 in every 50,000 to 100,000 children born worldwide. PFIC represents 10% to 15% of causes of cholestasis in children and 10% to 15% of liver transplantation indications in children. All types of PFIC exist worldwide and both sexes appear to be equally affected.

The common underlying pathogenesis of PFIC is disruption of bile formation and bile transport through the liver [Jacquemin 2000]. The classification of PFIC has evolved over the years. The most commonly used sub-classifications are PFIC Types 1, 2, and 3, which are based on the associated affected gene and described in more detail below.

- PFIC, Type 1: also referred to as “Byler disease” or “familial intrahepatic cholestasis-1 (FIC1) protein deficiency.” FIC1 protein is located on the canalicular

membrane of hepatocytes and facilitates movement of aminophospholipids from the outer to inner leaflet of the plasma membrane of the hepatocyte. The *ATP8B1* gene encodes FIC1 protein. Biallelic pathologic variants in the *ATP8B1* gene are associated with FIC1 dysfunction and classified clinically as PFIC Type 1 disease.

- PFIC, Type 2: also referred to as "Byler syndrome" or "bile salt export pump (BSEP) deficiency." BSEP is a transporter protein that is expressed at the canalicular membrane of hepatocytes and is the primary exporter of bile acids. The *ABCB11* gene encodes the BSEP protein. Biallelic pathologic variations in the *ABCB11* gene are associated with BSEP dysfunction and classified clinically as PFIC Type 2 disease.
- PFIC, Type 3: is caused by a deficiency of the multidrug-resistance protein 3 (MDR3) due to mutations in the *ABCB4* gene. MDR3 is a phospholipid translocase critical for phospholipid secretion.

Severe pruritus is common in children diagnosed with PFIC. Itching (and subsequent scratching) is a significant morbidity for these patients and their families [Suchy 2007]. A more severe degree of pruritus is experienced compared to patients with other forms of liver disease and to other pruritic conditions such as atopic dermatitis [Murray 2011]. In patients with PFIC, liver biopsy reveals canalicular cholestasis and, later, the appearance of portal fibrosis. Serum biochemistry indicates cholestasis with hyperbilirubinemia, elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The concentrations of bile acids in serum are very high, while serum gamma-glutamyl transferase (GGT) activity (exception being MDR3 variants) and cholesterol are normal [Hori 2010]. Symptoms of portal hypertension and liver failure will develop during the course of the disease [Davit-Spraul 2009; Alissa 2008]. Symptoms develop early; median age at onset of symptoms is 2 months, and 78% of PFIC patients present with jaundice [Pawlikowska 2010]. The life-threatening and debilitating nature of PFIC is reflected by the fact that survival in those not resorting to surgery is 50% at 10 years of age and almost zero at 20 years of age. Approximately half of PFIC patients undergo liver transplantation [Davit-Spraul 2009] and treatment-resistant pruritus is the leading indication for the surgical procedure partial external biliary diversion, mostly in PFIC Type 1 and 2 patients.

PFIC is life-threatening and debilitating. Currently, the treatment for PFIC is palliative and there is no pharmaceutical treatment approved for use in PFIC. The therapeutic choices are restricted to non-specific therapy of the symptoms and signs of the disease such as nutritional support, preventing vitamin deficiencies, and treatment of extrahepatic features. Medical treatment options include off-label use of ursodeoxycholic acid, rifampin, antihistamines, and naltrexone. A minority of patients respond nominally and transiently to these interventions. Biliary diversion is used to decrease systemic bile acids through interruption of the enterohepatic circulation and so avoid transplantation. Liver transplantation is typically only viewed as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

5.2.2 Summary of Clinical and Nonclinical Studies

This is a summary of nonclinical and clinical studies. More detail is provided in the Investigator's Brochure ([IB] see A4250 IB).

A4250 is a potent, selective IBAT inhibitor with no safety signals seen in the safety pharmacological studies. In addition, no safety signals were seen in repeat-dose oral toxicity studies in rats with up to 26 weeks of daily dosing and in dogs with up to 39 weeks of daily dosing. No safety signals were identified when A4250 was administered daily to juvenile rats from age 14 days to 63 days.

A4250 is minimally absorbed with very low systemic exposure in both humans and other species. In blood, protein binding is high (>99.7%). Systemically absorbed A4250 is slowly metabolized in humans and in preclinical studies by hydroxylation only, a metabolite that retains IBAT activity. A4250 did not show any significant inhibition of cytochrome P450 (CYP) enzymes in the rat. A4250 inhibited human CYP3A4 and CYP2D6 (IC_{50}: 16 µmol/L for both enzymes), and CYP2C9 (IC_{50}:1.2 µmol/L).

To date, A4250 has been studied in 3 Albireo-sponsored clinical studies: a study in healthy volunteers evaluating single and multiple administration of A4250; a single-dose absorption, distribution, metabolism, and excretion (ADME) study; and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with primary biliary cholangitis (PBC).

In the first clinical study (A4250-001), A4250 was given to healthy volunteers as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. Following a single oral dose of 10 mg, the median maximum drug concentration was 0.2 nmol/L and the area under the curve was 0.8 nmol × h/L (n=6). Pharmacodynamic evaluation of bile acid synthesis biomarkers (serum fibroblast growth factor 19 [s-FGF19] and plasma 7α Hydroxy 4-cholesten-3-one [p-C4]) identified the expected decrease in s-FGF19 and an increase in p-C4. In addition, total plasma bile acids decreased and total fecal bile acids increased in A4250-treated patients compared to placebo. A4250 was generally well tolerated. The only findings were GI-related effects, such as diarrhea at higher doses.

In the ADME study (A4250-007), healthy subjects were administered a single oral dose of 3 mg [^{14}C]-A4250 capsule. The main excretion route was determined to be fecal. There were no quantifiable concentrations of A4250 in plasma or total radioactivity in plasma or whole blood. A4250 was well tolerated. AEs were reported and most were mild and not related to A4250. The 3 AEs experienced by 2 subjects reported as related to study drug were GI in nature, including diarrhea (2 AEs; 2 subjects) and abdominal pain (1 AE; 1 subject).

In an exploratory Phase 2 study in children with cholestatic pruritus (A4250-003), A4250 was administered as oral daily doses of 10 to 200 µg/kg/day for 4 weeks. Twenty patients (8 females and 12 males) aged 1 to 17 years were enrolled in this open-label dose-escalation study. Four patients were re-enrolled into a second dose level. No

treatment-related SAEs were reported, and most AEs, including some increased transaminases, were transient. Four-week, once-daily treatment with A4250 reduced mean levels of s-BA in all cohorts. Substantial s-BA reductions in 11 of 13 PFIC patients (ranging from 43% to 98%) were observed. A4250 also reduced s-BA in the 11 non-PFIC patients, but to a lesser extent than in PFIC patients. Patient-reported diaries documented improvement of the visual analogue scale itch score in 17 of 24 patients during treatment. The dose with the greatest improvement showed a mean decrease (± standard error of the mean) of 2.9 ±1.1 from baseline. The results from this study form the basis of the current (A4250-005) study design.

5.3 Rationale

In this investigational study, A4250 is under development for treatment of PFIC and is also being considered for other orphan cholestatic liver diseases.

There is currently no medical treatment approved for PFIC. In PFIC patients with severe pruritus, biliary diversion surgery is used to decrease systemic bile acids through interruption of the enterohepatic circulation and thereby reducing pruritus [Whitington 1988]. A portion of patients who have biliary diversion surgery continue to have elevated serum bile acids and pruritus. Liver transplantation is typically used as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

By inhibiting IBAT with high selectivity and potency, A4250 has the potential to relieve cholestasis and improve liver function without surgical intervention in patients with PFIC. Since A4250 is minimally absorbed and achieves a substantial reduction of s-BA, it has the potential to be a safer and less invasive alternative to surgical biliary drainage. The rationale for using A4250 is to decrease s-BA levels, and to reduce the major morbidity of pruritus, improving the health and wellbeing of patients affected with PFIC. Ultimately, this noninvasive therapeutic approach has the potential to not only improve quality of life (QoL), but also reduce the need for biliary diversion and subsequent liver failure and liver transplantation. For those patients who have undergone biliary diversion surgery and continue to have elevated serum bile acids and symptomatic pruritus, IBAT inhibition has the potential to reduce both the serum bile acid levels and the pruritus.

The doses selected, 40 and 120 µg/kg/day, were based on the efficacy and safety data generated from patients with PFIC (n=10 + 3 re-entered) and supporting data from all patients (n=20 + 4 re-entered) in the five dose groups dosed in the A4250-003 study. Improvements in s-BA and pruritus were observed with doses from 30 µg/kg to 100 µg/kg, with a dose-related trend. The best dose response in all patients was at the 60 µg/kg/day for s-BA and 100 µg/kg/day for pruritus. The best dose response in the PFIC subgroup was at 30 µg/kg/day for both s-BA and pruritus. Patients with PFIC responded well to the doses given from 30 µg/kg to 200 µg/kg/day; however, a clear dose relationship could not be

established in order to select a single dose for this Phase 3 study. As efficacy was observed at both low and high doses, 40 µg/kg and 120 µg/kg/day are considered to be the most optimal for this study.

This study will be conducted in compliance with the protocol and with the International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP).

Patients completing 24 weeks of double-blind treatment will be offered to enter Study A4250-008 and receive open-label treatment with A4250.

5.3.1 Risk/Benefit

A4250 has been evaluated in three Albireo-sponsored clinical studies: a double-blind placebo-controlled study in healthy volunteers, a single-dose ADME study, and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with PBC.

Healthy subjects have been exposed to up to 10 mg as a single dose and up to 3 mg daily as part of a multiple-dose evaluation. Children with cholestatic liver disease have been treated with up to 0.2 mg/kg/day (200 µg/kg/day) for 4 weeks. In total, 2 SAEs have been reported; both were judged by the physician to be not related to the study drug.

Patients with cholestatic liver diseases suffer from excess bile acids in the liver resulting in tissue damage. A commonly used treatment in these patients is bile diversion surgery, whereby approximately 50% to 100% of the enterohepatic circulation of bile acids is interrupted. Inhibition of IBAT with A4250, thereby interrupting the enterohepatic circulation of bile acids, is therefore a potential medical alternative to surgery which could be of benefit to these patients if shown to be effective and safe. Data from the Phase 2 study showed efficacy of A4250 in reducing s-BA concentrations and pruritus in such patients.

Based on the mode of action of an IBAT inhibitor, loose stools or diarrhea could be expected. However, in the pediatric Phase 2 study with 4 weeks daily treatment, only one patient had mild transient diarrhea after a single dose that did not recur on multiple dosing.

6 STUDY OBJECTIVES

6.1 Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with PFIC Types 1 and 2, as determined by the following:

- The proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- The proportion of positive pruritus assessments at the subject level over the 24-week treatment period.

6.2 Secondary Objectives

- To evaluate the effect of A4250 on serum ALT concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

7 INVESTIGATIONAL PLAN

7.1 Overall Study Design and Plan

7.1.1 Description

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of FIC1 or BSEP).

Study data will be periodically (approximately on a quarterly basis) reviewed by an unblinded Data Safety and Monitoring Board (DSMB).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-Up Period.

After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo.

Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008; EudraCT Number 2017-002325-38, subject to a separate study approval) in which all patients will receive active treatment.

Visit windows applicable to each visit are presented in the schedule of assessments (Table 1). There will be up to 10 clinic visits during the study (Figure 1) and one scheduled telephone contact, as follows:

- Visit 1: Screening Period start. Patient is considered enrolled after signing the informed consent form (ICF) (Days -56 to -35)
- Visit 2: second Screening Visit (Days -28 to -7)
- Visit 3: Randomization Visit (Day 0)
- Telephone Contact 1: (Day 14)
- Visit 4: Clinic Visit (Day 28)
- Visit 5: Clinic Visit (Day 56)
- Visit 6: Clinic Visit (Day 84)
- Visit 7: Clinic Visit (Day 126)
- Visit 8: Clinic Visit (Day 154)
- Visit 9: Clinic Visit, End of Treatment ([EOT] Day 168)
- Visit 10: Follow-Up Visit, 28 days after last dose of study drug (Day 196). All patients continuing in Study A4250-008 will not have this Follow-up Visit.

Additional clinic visits may be required for patients who need direct site assistance, e.g., due to AE monitoring, to fulfil screening requirements, and/or for safety maintenance.

Screening Period

Informed consent must be obtained at Visit 1 prior to performing any study procedures. After signing the ICF, patients will be considered enrolled and evaluated for study eligibility.

Daily recording of pruritus using an electronic diary (eDiary) will be started to determine confirmation of the magnitude and the baseline pruritus symptoms before the study. After consultation with the Medical Monitor, patients failing eligibility criteria may be re-screened. Patients not fulfilling inclusion/exclusion criteria after 3 attempts are not allowed to re-screen.

Caregivers/patients will be instructed on the daily use of the eDiary. The eDiary will include patient-reported outcome (PRO) and observer-reported outcome (ObsRO) items for evaluation of itching (PRO), scratching (ObsRO), and sleep disturbance (PRO and ObsRO) during the Screening Period (study Days -56 to 0), throughout the 24-week Treatment Period (study Days 0 to 168), and Follow-up (Day 196).

The 14 consecutive days prior to randomization will be used for eligibility calculation of the diary entries. If a patient does not meet the minimum number of observations (see Section 9.2.2 for scoring and missing data definitions) to determine eligibility, the patient may be re-screened. Observer-reported outcomes in patients of all ages will be recorded by a caregiver. If possible, the same caregiver will complete the Albireo ObsRO items throughout the study.

At a second Screening Visit (Visit 2) additional laboratory samples will be taken for eligibility assessments.

Treatment Period

Eligibility for randomization will be determined at Visit 3 using the pruritus (scratching reports from the Albireo ObsRO) data in the 2 weeks before Visit 3, clinical genetic confirmation of diagnosis, and the liver biochemistry evaluations from the previous screening visits. Two weeks after Visit 3, there will be a telephone contact with the caregiver/patient to evaluate any AEs.

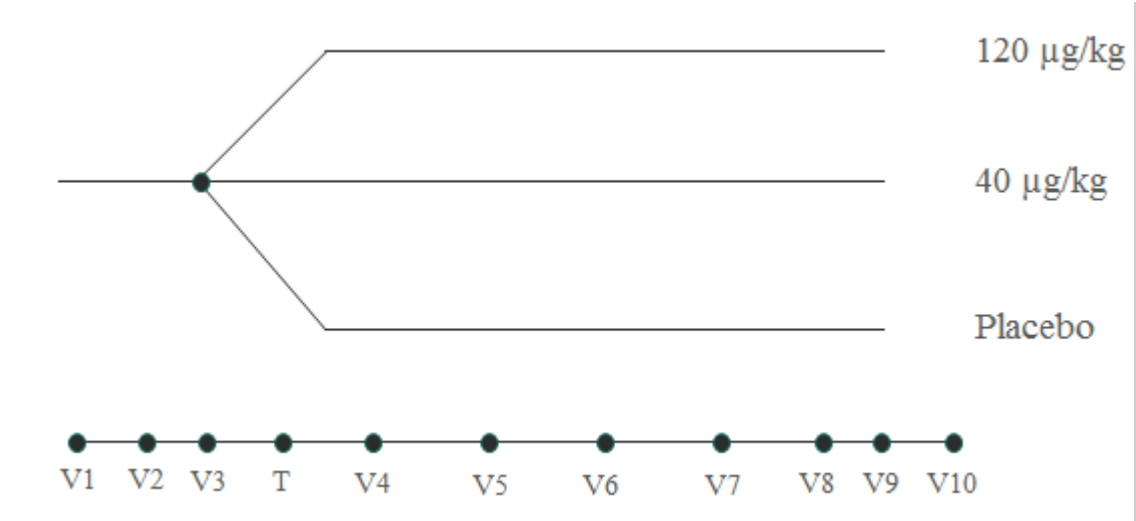
The patient will return to the clinic at Visit 4, Visit 5, Visit 6, Visit 7, Visit 8, and Visit 9 for assessments. If a new caregiver will begin to complete the Albireo ObsRO during the course of the trial, they will be provided with training during a clinic visit before they begin to use the eDiary. Additionally, caregivers will be requested to use the diary to report the time each dose of study drug is administered during the Treatment Period.

The last dose of study drug will be administered the day before Visit 9. At study Visit 9, the patient has the option to enroll in an open-label extension study (A4250-008) should they

meet the required entry criteria. If the patient is continuing into Study A4250-008 this will be the last visit in this study and the first visit in Study A4250-008. Visit 9/EOT should also be performed at the time patients are prematurely withdrawn from the study.

Clinic Visit 10 will take place 28 days after Visit 9 for all patients not continuing into Study A4250-008.

Figure 1 Study Design



T: telephone contact; V: study visit

End of Study

The end of the study is defined as follows:

a) End of study in one country: last patient last visit (LPLV) and sites are closed

b) End of study globally: LPLV globally and all sites closed

7.1.2 Schedule of Assessments

The schedule of assessments is presented in Table 1. For information on blood volumes, please see Appendix 7.

Table 1 Schedule of Assessments

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Informed consent [c]	X										
Inclusion/exclusion criteria	X		X								
Demographics	X										
Concomitant medication[d]	X	X	X	X	X	X	X	X	X	X	X
Medical and surgical history	X		X								
Physical examination including voluntary photography	X		X				X			X	
Skin examination	X		X		X		X			X	X
Vital signs[e]	X	X	X		X	X	X	X	X	X	X
eDiary: itching, scratching, and sleep scores[f]	Daily diary entry										
Clinical chemistry[g]	X		X		X	X	X	X		X	X
Hematology[g]			X		X	X	X	X		X	X
Urinalysis[g]		X					X			X	
Serum bile acids[h]	X	X	X		X	X	X	X	X	X	X

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
INR		X			X		X		X		X
Autotaxin[i]			X		X					X	
p-C4[i]			X		X					X	
AFP			X							X	
Vitamins A & E[h]		X					X			X	
25-hydroxy vitamin D			X				X			X	
Blood sample for A4250 PK[i]					X					X	
Abdominal Ultrasound			X							X	
Fibroscan® (where available)			X							X	
QoL questionnaire (PedsQL)			X							X	
Patient/Caregiver/Clinician Global Impression of Change					X		X			X	
Patient/Caregiver/Clinician Global Impression of Symptoms			X		X		X			X	
Pregnancy test[j]	X[j]	X	X		X	X	X	X	X	X[j]	X

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Study drug dispensed[k]			X		X	X	X	X	X		
Adverse events[l]	Continuous collection										
Study drug compliance					X	X	X	X	X	X	

AFP: alfa-fetoprotein; BMI: body mass index; eCRF: electronic case report form; eDiary: electronic diary; EOT: End of Treatment; ICF: informed consent form; INR: international normalized ratio; p-C4: plasma 7α Hydroxy 4-cholesten-3-one; PedsQL: Pediatric Quality of Life Inventory; PK: pharmacokinetic; QoL: quality of life; RR: respiratory rate; SAE: serious adverse event; s-BA: serum bile acid.

a This visit is the same as Visit 1 in the extension protocol (Study A4250-008). Must also be performed at the time a patient is prematurely withdrawn from the study.

b Assessments must be performed 28 days following the final dose of study drug. This visit is omitted if patients are entering Study A4250-008.

c The ICF (and assent) must be signed before any study procedures are performed.

d Past (within 1 month prior to Visit 1) and current medication will be documented in the eCRF.

e Includes blood pressure, pulse, RR, temperature, height (or length depending on age), and body weight (using a certified weight scale). BMI will be calculated.

f Includes training on use of the device at Screening (Visit 1) and review of patient daily entries during 14 days prior to Clinic Visit 3 to determine eligibility.

g See Table 3 for detailed parameters.

h Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA and vitamin A. Exceptions can be made for infants, less than 12 months of age, if unable to fast for the full 4 hours. All s-BA results during the treatment period and follow-up will be blinded.

i Samples will not be collected for patients ≤10 kg. In the event of an SAE, a PK sample should be collected as close to the onset of the event as possible.

j For girls who have reached menarche. Serum test at Visit 1; urine test at other visits. A serum pregnancy test will also be conducted at Visit 9 for patients entering Study A4250-008. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy.

k Study drug to be taken once daily on Day 1 through Day 167 as described in Section 8.2.

l Adverse event information will be collected from the time of signing of the ICF to study discontinuation.

7.1.3 Study Procedures and Assessments

7.1.3.1 Screening Period (Days -56 to 0)

Day -56 to Day -35/Clinic Visit 1

Patients will undergo a Screening Visit up to 56 days prior to the planned first day of study treatment. Screening procedures and assessments are as follows:

- Obtain written informed consent
- Assess inclusion/exclusion criteria (Section 7.2)
- Record demographics (age, full date of birth, gender, race, and ethnicity)
- Document concomitant medications
- Medical and surgical history (date of diagnosis of PFIC Types 1 or 2, prior investigational medications for PFIC, historical liver function test [LFT] values, any surgery performed including biliary diversion (if applicable), any other diagnosis, and historical liver biopsy data)
- Collect, if available, past confirmatory clinical genetic laboratory report for PFIC-1/PFIC-2 and send to central reader for review. If a historical report is equivocal, unavailable, or unobtainable, a blood sample for clinical genetic testing will be collected to determine eligibility
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- eDiary training and compliance requirements; caregivers and, if applicable, patients begin daily recording of pruritus using the eDiary (Appendix 2)
- Clinical chemistry (Section 10.2.1)
- s-BA (Section 9.2.1)
- Serum pregnancy test for girls who have reached menarche. Please see Appendix 6 for contraceptive requirements
- AE monitoring

Day -28 to Day -7/Clinic Visit 2

A second Screening Visit will be performed. Screening procedures and assessments are as follows:

- Review concomitant medications
- Vital signs (Section 10.2.6)

- Albireo PRO/ObsRO eDiary review for compliance
- Urinalysis (Section 10.2.1)
- s-BA (Section 9.2.1)
- International normalized ratio (INR)
- Vitamins A and E
- Urine pregnancy test for girls of who have reached menarche
- AE monitoring

7.1.3.2 Treatment Period (Day 0 to Day 168)

Study Day 0/Clinic Visit 3 (Randomization)

At the randomization visit, the following assessments will be conducted:

- Review inclusion/exclusion criteria (Section 7.2)
- Review concomitant medications, medical and surgical history
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance and entrance criteria requirement (see Inclusion Criteria 4, Section 7.2.1)
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin and p-C4 (samples will not be taken for patients ≤10 kg)
- 25-hydroxy vitamin D
- Alfa-fetoprotein (AFP)
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)
- Administer QoL (Pediatric Quality of Life Inventory [PedsQL]) questionnaire
- Patient/caregiver/clinician complete the patient global impression of symptoms (PGIS)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Study drug is dispensed and patients are instructed to administer daily from Day 1

Study Day 14 (Telephone Contact)

A study nurse will contact the patient/caregiver by telephone for monitoring of the following:

- Review concomitant medications
- Albireo PRO/ObsRO eDiary review for compliance
- AEs

Study Day 28 and Day 84/Clinic Visits 4 and 6

The following procedures and assessments will be performed:

- Review of concomitant medications
- Physical examination, including voluntary photography (at Clinic Visit 6 only; Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- Urinalysis (at Clinic Visit 6 only; Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- Autotaxin, p-C4, and A4250 pharmacokinetic (PK) assessment (at Clinic Visit 4 only; samples will not be taken for patients ≤10 kg)
- Vitamins A and E, and 25-hydroxy vitamin D (at Clinic Visit 6 only)
- Patient/caregiver/clinician complete the patient global impression of change (PGIC) and PGIS
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 56 and Day 126/Clinic Visits 5 and 7

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance

- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 154/Clinic Visit 8

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- s-BA (Section 9.2.1)
- INR
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 168/Clinic Visit 9/EOT

The last dose of study drug is taken in the morning the day before Clinic Visit 9, and the following procedures and assessments will be performed (also performed at the time a patient prematurely withdraws):

- Review of concomitant medications
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry, hematology, and urinalysis (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin, p-C4, and A4250 PK assessment (samples will not be taken for patients ≤10 kg)

- AFP
- Vitamins A, E, and 25-hydroxy vitamin D
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)
- Administer PedsQL questionnaire
- Patient/caregiver/clinician complete the PGIC and PGIS
- Urine pregnancy test for girls who have reached menarche, or serum pregnancy test for girls continuing to study A4250-008
- AE monitoring
- Evaluation of study drug compliance

7.1.3.3 Follow-Up Period

Study Day 196/Clinic Visit 10

All patients not continuing into Study A4250-008 will return to the study site 28 days after Clinic Visit 9 or the last dose of study drug for the following assessments:

- Review of concomitant medications
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- Urine pregnancy test for girls who have reached menarche
- AE monitoring

7.2 Study Population

A total of approximately 60 patients with a clinical diagnosis of PFIC Type 1 or 2 that have been genetically confirmed will be randomized. The study will target 15% of enrolled patients to be PFIC-1 patients. If enrollment of all PFIC-2 patients is completed and the 15% enrollment of PFIC-1 patients has not yet been achieved, then enrollment of PFIC-2 patients will continue in order to reach the total study target enrollment.

An attempt will be made to randomize a minimum of 10 patients from each age category (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years).

A maximum of 5 patients that were treated in Study A4250-003 will be allowed to participate in this study.

7.2.1 Inclusion Criteria

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have clinical genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples at least 7 days apart (Visits 1 and 2) prior to randomization
4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the eDiary average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients will be expected to have a consistent caregiver for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

7.2.2 Exclusion Criteria

1. Patient with pathologic variations of the *ABCB11* gene that predict complete absence of the BSEP protein
2. Patient with past medical history or ongoing presence of other types of liver disease including, but not limited to, the following:
   a) Biliary atresia of any kind
   b) Benign recurrent intrahepatic cholestasis, indicated by any history of normal s-BAs
   c) Suspected or proven liver cancer or metastasis to the liver on imaging studies
   d) Histopathology on liver biopsy is suggestive of alternate non-PFIC related etiology of cholestasis
3. Patient with a past medical history or ongoing presence of any other disease or condition known to interfere with the absorption, distribution, metabolism (specifically bile acid metabolism), or excretion of drugs in the intestine, including but not limited to, inflammatory bowel disease

4. Patient with past medical history or ongoing chronic (i.e., >3 months) diarrhea requiring intravenous fluid or nutritional intervention for treatment of the diarrhea and/or its sequelae
5. Patient has a confirmed past diagnosis of infection with human immunodeficiency virus or other present and active, clinically significant, acute, or chronic infection, or past medical history of any major episode of infection requiring hospitalization or treatment with parenteral anti-infective treatment within 4 weeks of treatment start (study Day 1) or completion of oral anti-infective treatment within 2 weeks prior to start of Screening Period
6. Any patient with suspected or confirmed cancers except for basal cell carcinoma, and non-liver cancers treated at least 5 years prior to Screening with no evidence of recurrence
7. Patient with a past medical history of chronic kidney disease with an impaired renal function and a glomerular filtration rate <70 mL/min/1.73 m^2
8. Patient with surgical history of disruption of the enterohepatic circulation (biliary diversion surgery) within 6 months prior to start of Screening Period.
9. Patient has had a liver transplant or a liver transplant is planned within 6 months of randomization
10. Decompensated liver disease, coagulopathy, history or presence of clinically significant ascites, variceal hemorrhage, and/or encephalopathy
11. INR >1.4 (the patient may be treated with Vitamin K intravenously, and if INR is ≤1.4 at resampling the patient may be randomized)
12. Serum ALT >10 × upper limit of normal (ULN) at Screening
13. Serum ALT >15 × ULN at any time point during the last 6 months unless an alternate etiology was confirmed for the elevation
14. Total bilirubin >10 × ULN at Screening
15. Patient suffers from uncontrolled, recalcitrant pruritic condition other than PFIC. Examples include, but not limited to, refractory atopic dermatitis or other primary pruritic skin diseases
16. Any patient who is pregnant or lactating or who is planning to become pregnant within 24 weeks of randomization
17. Sexually active males and females who are not using a reliable contraceptive method with ≤1% failure rate (such as hormonal contraception, intrauterine device, or complete abstinence) throughout the duration of the study and 90 days thereafter (from signed

informed consent through 90 days after last dose of study drug). See Appendix 6 for further details

18. Patient with a past medical history of alcohol or substance abuse will be excluded. Patient must agree to refrain from illicit drug and alcohol use during the study
19. Administration of bile acid or lipid binding resins and medications that slow GI motility (Refer to Appendix 1 – Concomitant Medications Guidelines)
20. Patient has had investigational exposure to a drug, biologic agent, or medical device within 30 days prior to Screening, or 5 half-lives of the study agent, whichever is longer
21. Patient who has been previously treated with an IBAT inhibitor whose pruritus has not responded to treatment
22. Any other conditions or abnormalities which, in the opinion of the investigator or Medical Monitor, may compromise the safety of the patient, or interfere with the patient participating in or completing the study

7.2.3 Withdrawal of Patients

Patients/caregivers will be informed that they have the right to withdraw from the study at any time, without prejudice to their medical care, and that they are not obliged to state their reasons.

Any withdrawal, and reasons for withdrawal, must be fully documented in the electronic case report form (eCRF) and source documents and the patient followed by the investigator/investigative staff. Even after the study is completed at the Follow-up Visit, the investigator/investigative staff will, regardless of reason for withdrawal, record any patient data they receive concerning SAEs, and all drug-related non-serious AEs, and subsequently report these according to Section 10.1. Withdrawn patients will not be replaced.

Patients will be withdrawn in the following circumstances:

- A patient's/caregiver's desire for withdrawal for any reason
- Lost to follow-up (every effort must be made to contact the patient/caregiver; a certified letter must be sent)
- An AE that, in the opinion of the investigator, necessitates withdrawal
- A worsening of the symptoms related to diarrhea occurs
- Death
- Patient/caregiver estimation of intolerable symptoms
- A patient's/caregiver's substantial noncompliance (eDiary and study drug compliance) or protocol violation

- An investigator's opinion that continuing the patient in the study is not appropriate. The investigator may withdraw a patient at any time, if it is considered to be in the patient's best interest

A patient who withdraws from treatment prematurely will have EOT (Visit 9) assessments at the time of withdrawal and a follow-up assessment (Visit 10) 28 days following the last dose of study drug, and the patient will be recorded as withdrawn.

7.2.4 Study Termination by Sponsor

This study may be terminated at any time by Albireo if serious side effects should occur, if the investigator does not adhere to the protocol; or if, in the sponsor's judgment, there are no further benefits to be achieved from the study. In this event, Albireo/designee will inform the study investigators, institutions, and all regulatory authorities.

Albireo may temporarily or permanently discontinue the study at an investigative site at any time for safety, ethical, compliance, or other reasons. If this is necessary, Albireo will endeavor to provide advance notification to the site. If a site or the study is suspended or discontinued, the investigator/investigative staff will be responsible for promptly informing the Independent Ethics Committee (IEC). If required by local regulations, Albireo/designee will be responsible for informing the IEC and the regulatory authority of study or site discontinuation. In such an event, all study data and unused study drug must be returned to Albireo.

8 TREATMENT OF PATIENTS

8.1 Identity of Study Drug

A4250 and placebo will be supplied as capsules for oral administration. White opaque capsules filled with pellets containing A4250 or placebo will be provided. Two different capsule sizes will be available, as follows:

- Capsule size 0 that can be opened
- Capsule size 3 that should be swallowed intact. These size 3 capsules may be opened only under exceptional circumstances, e.g., patient cannot swallow capsule intact

The content of the A4250 and placebo capsules will be identical in appearance. Capsule filling weight will also be identical for high dose, low dose, and placebo. Bottles with 34 capsules will be given to the patient at each visit. A patient who requires 2 or more capsules per day will be given multiple bottles. Refer to the investigational product manual.

8.2 Administration of Study Drug

Patients will be dosed with A4250 at a dose of 40 µg/kg/day, 120 µg/kg/day, or placebo for 24 weeks. Study drug will be dispensed to the patient at Visits 3, 4, 5, 6, 7, and 8, together with instructions on how to store and take the drug. Study drug administration data, including whether each patient took each dose or partial doses of study drug, whether there were any delayed or missed doses, and whether the capsule was opened or swallowed whole will be documented through the eDiary and transferred to the study database.

A4250 should be taken in the morning, together with food. On clinic visits days when laboratory assessments are conducted, study drug should be taken after the visit and after laboratory samples are taken. Patients should not crush or chew the capsule(s). When swallowing the capsule intact, the patient should administer the dose with a glass of water.

For a patient unable to swallow capsule intact, the capsule can be opened and the contents sprinkled and mixed in a small amount of soft room-temperature yogurt, apple sauce, or fruit purée. A list of food options can be found in the Pharmacy manual. The amount of food should be less than what the patient/infant is expected to eat and should be followed by water.

1. Hold the capsule over the prepared food, gently twist open and allow the content to fall into food
2. Mix the entire capsule content with 1 or 2 tablespoons (15 to 30 mL) of soft food (apple sauce, fruit purée, or yogurt)
3. Administer the mixture immediately after mixing.

The number of capsules provided to the patient will be based on body weight at Visit 3. At Visit 6, if a patient's weight has shifted to another weight category (identified in Table 2), the number of capsules per day may be adjusted.

Table 2 Dosing and Capsule Strength

Body Weight (kg)	Capsule Size	Number of Capsules per Day	Capsule Strength, Low Dose (µg)	Total Dose (µg)	Capsule Strength, High Dose (µg)	Total Dose (µg)	Capsule Size, Placebo (µg)
5.0 to <7.5	0	1	200	200	600	600	0
7.5 to <12.5	0	2	200	400	600	1200	0
12.5 to <17.5	0	3	200	600	600	1800	0
17.5 to <19.5	0	4	200	800	600	2400	0
19.5 to <25.5	3	2	400	800	1200	2400	3
25.5 to <35.5	3	3	400	1200	1200	3600	3
35.5 to <45.5	3	4	400	1600	1200	4800	3
45.5 to 55.5	3	5	400	2000	1200	6000	3
>55.5	3	6	400	2400	1200	7200	3

8.3 Study Treatment Packaging and Labeling

8.3.1 Packaging and Labeling

The capsules will be packed in high-density polyethylene containers, with child-proof polypropylene caps. Study drug capsules containing A4250 in the strength specified in Table 2 above will be manufactured.

Packaging and labeling will be prepared to comply with applicable regulatory requirements.

8.3.2 Storage

Treatment packs containing A4250 capsules should be stored and dispensed in accordance with regulations in their original containers. The storage facility at the investigative site should be locked and the storage temperature should be between 15°C and 25°C.

Patients/caregivers should be informed of appropriate storage conditions (i.e., room temperature, between 15°C and 25°C).

Any deviations from the recommended storage conditions should be immediately reported to Albireo and the study drug should not be used until authorization has been given by Albireo.

8.3.3 Blinding and Randomization of Study Drug

A double-blind design is employed so that both the investigators and the patients will be unaware of the treatment assignment during the study.

After written informed consent is obtained from an eligible patient, an 8-digit patient identification number will be assigned. The first 2 digits will denote the country, followed by a 3-digit site number and a 3-digit patient sequence number. This number will be created and

allocated by the Interactive Web Response System (IWRS), when the patient first enters the system at Screening. The randomization codes will be computer-generated by [redacted] Biostatistics and kept by a statistician independent from the project team. The randomization will be done in blocks and stratified according to PFIC class (Type 1 and 2) and age class (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) to ensure approximate balance between dose schemes (1:1:1). A separate randomization list will be prepared for the maximum 5 patients from Study A4250-003 regardless of stratification. Randomization codes will be assigned sequentially as patients become eligible for randomization.

Patients who are eligible for randomization will be assigned a unique 4-digit randomization number by IWRS. This randomization number identifies which treatment will be allocated to the patient. Patients who withdraw from the study after randomization visit are not to be replaced and their randomization number will not be reused.

To ensure blinding, the study drug and the matching placebo have the same shape and size. Patients will receive capsule(s) of A4250 according to their dose group or capsule(s) of matching placebo once a day during the double-blind Treatment Period. Labels on the study drug containers will not identify the treatment that a patient is randomized to. Traceability of the treatment is ensured by the study drug number.

The 5-digit study drug number will identify study drug packs and will be detailed on the study drug label. Dispensing of study drug will be coordinated by IWRS.

The system will assign a study drug number corresponding to the randomization arm. The randomization number will be used in the background only to ensure no unblinding and will not be displayed to end-users in the IWRS.

8.4 Procedure for Breaking the Randomization Code

Should a situation arise where unblinding is urgently required, i.e., only where knowledge of the study drug is required to adequately manage a life-threatening situation, the investigator at that study site may perform immediate unblinding through IWRS. The responsibility to break the treatment code in emergency situations resides solely with the investigator. The investigator should make every effort to discuss the rationale (status and outcome) for emergency unblinding with the sponsor Medical Monitor or the [redacted] Medical Monitor as soon as possible to review the individual patient details.

The study site investigator and appropriate [redacted] project team members will be authorized to access the emergency unblinding functionality within the IWRS. Emergency unblinding takes place in the IWRS via a dedicated emergency unblinding module. The system will require the user to enter their emergency unblinding, which is typically provided at the same time login credentials are distributed. The exact description of the treatment assigned to the individual patient then will be accessible to the user. Once the randomization code is broken for a patient, he/she must be withdrawn from the study and all assessments and procedures at EOT should be performed. As a follow-up, Visit 10, will be performed according to the time

schedule. Once a randomization code has been broken, the investigator must inform the [redacted] and sponsor Medical Monitor in writing within 24 hours. In case of technical issues accessing the system online, please see the IWRS site user manual for country-specific contact telephone numbers for the [redacted] HelpDesk 24/7 system support.

8.5 Patient Compliance

The study nurse will monitor eDiary compliance by routine review of the CRF health website. If both diary entries on a day are missing, the study nurse will call the patient/caregiver to remind them to complete all scheduled entries. Any noncompliance will be documented and explained in the source documents.

Study drug compliance will also be checked through diary responses. Additionally, patients will return all unused study drug at Visits 4, 5, 6, 7, 8, and 9. The study site staff will count all returned drug, assess compliance, and record details in the eCRF.

Treatment Compliance = 100 × ((Number of study drug dispensed – number of study drug returned)/number of study drug that should be taken).

Treatment compliance between 80% and 120% will be acceptable.

8.6 Study Drug Accountability

Records shall be maintained of the delivery of study treatment(s) to the study site(s), of the inventory at the study site(s), of each use of the study treatment(s) for each patient, and of the return of unused study treatment(s) to Albireo.

These records shall include dates, quantities, batch numbers, expiry dates, and the unique code numbers assigned to the study drug and to the study patients.

The investigator will be responsible for ensuring that the records adequately document that the patients were provided the quantities specified in the protocol and that all study drug received from Albireo is reconciled.

8.7 Concomitant Therapy and Prohibited Medications

The investigator will note all ongoing medication and any medication recently stopped (within 1 month prior to Visit 1) in the eCRF. At Visits 2 through 10, all changes in medication (stopping or starting new medication or changes in dose) will be recorded in the eCRF. All medications (prescribed or over the counter) for pruritus and sleep will be recorded.

All medications taken by a patient within 3 months prior to the first intake of study drug are regarded as prior medication.

All medications taken by a patient on or after the first intake of study drug, and which continue to be taken during the study, are regarded as concomitant medication.

Prohibited medications are listed in Appendix 1.

9 ASSESSMENT OF EFFICACY

9.1 Efficacy Endpoints

9.1.1 Primary Efficacy Endpoint

The primary efficacy endpoints are as follows:

- **European Union (EU) and rest of the world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  Fasting s-BA baseline will be calculated as the average of the value at randomization and the previous visit (Clinic Visit 2). The end value is the average of the values at Weeks 22 and 24 after the start of double-blind treatment.

- **United States (US)**: Proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period. A positive pruritus assessment is defined as a scratching score of ≤1 or at least a one-point drop from baseline on the Albireo ObsRO instrument. At each assessment, the AM score will be compared to the AM baseline, and the PM score will be compared to the PM baseline. Both AM baseline and PM baseline pruritus assessments will be included in the analysis of this endpoint. AM scores from the 14 days prior to the first dose of study medications will be averaged as AM baseline. PM scores from the 14 days prior to the first dose will be averaged as PM baseline.

9.1.2 Secondary Efficacy Endpoint

Secondary efficacy endpoints include the following:

- **EU and RoW**: Proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period. A positive pruritus assessment is defined as a scratching score of ≤1 or at least a one-point drop from baseline on the Albireo ObsRO instrument. At each assessment, the AM score will be compared to the AM baseline, and the PM score will be compared to the PM baseline. Both AM baseline and PM baseline pruritus assessments will be included in the analysis of this endpoint. AM scores from the 14 days prior to the first dose of study medications will be averaged as AM baseline. PM scores from the 14 days prior to the first dose will be averaged as PM baseline.
- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.
- **All Regions:**

All secondary endpoints are compared to placebo.

- Change from baseline to Week 12 and to Week 24 in fasting serum bile acids
- Change from baseline to Week 12 and to Week 24 in serum ALT concentration
- Change in growth from baseline to Week 12 and to Week 24
- Proportion of responders for pruritus scores at Weeks 12 and 24 based on the Albireo PRO and ObsRO instruments
- Change in sleep parameters measured with the Albireo PRO and ObsRO instruments from baseline over the 24-week Treatment Period
- Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level over the 24-week Treatment Period. A positive pruritus assessment includes an itch score ≤1, or at least a one-point drop from baseline based on the Albireo PRO instrument; only patients ≥8 years of age will complete the Albireo PRO instrument
- Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Proportion of individual AM assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Proportion of individual PM assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Number of patients undergoing biliary diversion surgery or liver transplantation

9.1.3 Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the PGIC items (Appendix 3)
- Change from baseline in fasting s-BA at Week 4

- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline by each 4 week-interval of the Treatment Period as measured by the Albireo ObsRO instrument
- Change from baseline by each 4-week interval of the Treatment Period in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline by each 4-week interval of the Treatment Period in patient-reported and observer-reported morning time itching and scratching severity scores, respectively
- Change from baseline by each 4-week interval of the Treatment Period in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline by each 4-week interval of the Treatment Period in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in PedsQL (Appendix 4)
- Change from baseline in serum ALT concentration at Week 4
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST and GGT at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, p-C4) at Week 24
- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events
- All-cause mortality

9.2 Efficacy Assessments

9.2.1 Serum Bile Acids

Blood samples for analysis of fasting total s-BA will be drawn at all visits. Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA. Exceptions can be made for infants, <12 months of age, if unable to fast for the full 4 hours. Any visit at which a bile acid sample result is unreportable, an additional unscheduled visit for a repeat sample collection may be scheduled. All s-BA results during

the treatment period and follow-up will be blinded. Samples will be processed and transported to a central laboratory per instructions in the laboratory manual.

9.2.2 Itching, Scratching and Sleep Score

Itching, observed scratching, and sleep disturbance will be recorded twice each day via eDiary. Patients and/or caregivers will be instructed to complete the eDiary every day in the morning after the patient wakes and in the evening just before the patient goes to sleep throughout the study. Patients and caregivers will receive a formal training session during Visit 1.

The eDiary includes Albireo ObsRO and PRO items. Patients <8 years of age will not be asked to complete the Albireo PRO items; only the Albireo ObsRO will be completed by caregivers of patients in this age group. Older patients, 8 to 18 years of age, will complete the Albireo PRO items and the caregiver will complete the Albireo ObsRO items. The Albireo PRO items assess severity of itch, aspects of sleep disturbance (morning diary only), and tiredness. For patients 8 to 12 years of age, the caregiver will read the Albireo PRO items along with the child and record the child's response. A guide will be provided to the caregivers that provides standardized explanations of the Albireo PRO items, in case the patient is confused or requires clarification about the meaning of a question. The Albireo ObsRO items assess severity of observed scratching, aspects of observed sleep disturbance (morning diary only), and observed signs of tiredness (evening diary only). The Albireo ObsRO and PRO scratching and itch severity items use 0 to 4 response scales, where each response is distinguished by a unique facial expression, verbal anchor, number, and color code (Appendix 2). To meet eligibility criteria, a scratch score will be calculated from daily diary entries (ObsRO items) the 14 consecutive days prior to randomization.

A daily AM & PM score for the Albireo ObsRO scratching item will be averaged from the 2 ratings for each day. A daily score will be considered missing if both of the daily assessments are missing. A weekly score will be calculated by averaging the daily scratching scores. A weekly score will be considered missing if ≥4 out of 7 days a week of data are missing. For AM & PM baseline, a 14-day daily score of AM & PM prior to the first dose of study medications will be averaged as the baseline score.

For AM baseline, a 14-day AM score prior to the first dose of study medications will be averaged as the AM baseline score. The same approach will be used for PM baseline and to calculate a patient-reported itch severity score. The handling of missing data is described in Section 11.2.2.2.

9.2.3 Growth

Growth will be measured as height (velocity) and weight (Z-score) using a certified weight scale. BMI will be calculated by weight (kg) / height $(m)^2$. Change will be defined as linear growth deficit (weight and BMI for age) compared to a standard growth curve (Z-score, SD from P50).

9.2.4 Quality of Life Questionnaire (PedsQL)

Patients and caregivers will be asked to complete a QoL questionnaire (PedsQL) at Visit 3 and Visit 9. Details of the questions included on the questionnaire are provided in Appendix 4.

9.2.5 Biomarkers and PK Samples

Blood samples for autotaxin and p-C4 will be drawn at Visits 3, 4, and 9 only for children with body weight >10 kg. Blood for A4250 PK assessment will only be drawn at Visits 4 and 9 (only for children with body weight >10 kg), and as close to the onset of an SAE as possible, should an SAE occur. Samples will be processed and transported to a laboratory per instructions in the laboratory manual.

9.2.6 PELD/MELD Score

At randomization and end of treatment, PELD score will be calculated for children up to 12 years of age. For children 13 years or older, the MELD score will be calculated.

PELD Score = 4.80 * ln(total bilirubin) + 18.57 * ln(INR) - 6.87 * ln(albumin) + 4.36 (if patient <1 year: scores for patients listed for liver transplantation before the patient's first birthday continue to include the value assigned for age (<1 year) until the patient reaches the age of 24 months) + 6.67 (if the patient has growth failure [≤2 SD])

Laboratory values less than 1.0 will be set to 1.0 for the calculation of the PELD score.

MELD Score = 9.57 * ln(creatinine) + 3.78 * ln(total bilirubin) + 11.2 * ln(INR) + 6.43

Laboratory values less than 1.0 will be set to 1.0 and serum creatinine values greater than 4.0 mg/dL (equivalent to 353.6 µmol/L) will be set to 4.0 for calculation of the MELD score.

9.2.7 Fibroscan®

Where available, Fibroscan® will be performed as per institution standard practice at Visits 3 and 9.

9.2.8 Markers of Fibrosis

APRI score and Fib-4 score will be calculated at randomization and at end of treatment.

APRI = [(AST in U/L) / (AST ULN in U/L)] / (Platelets in 10^9/L)

Fibrosis 4 Score = (Age*AST in U/L) / (Platelets in 10^9/L * √(ALT in U/L)).

9.2.9 Global Impression of Change and Global Impression of Symptom Measures

Patients (≥8 years of age), caregivers, and clinicians will complete the PGIC and the PGIS measures (Appendix 3) at randomization (Visit 3; PGIS only), Visits 4 and 6, and at EOT (Visit 9).

The PGIC items assess change in itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) since starting the study drug. The PGIS items assess itch

(patient version), scratching (caregiver and clinician versions), and sleep (all versions) in the past week. Caregivers and clinicians will complete the PGIC and PGIS for all patients; those patients ≥8 years of age will complete the patient version.

10 ASSESSMENT OF SAFETY

The timing and frequency of safety assessments are described in Section 7.1.3 and in Table 1 above.

The primary safety analysis for this study will include the incidence of total treatment-emergent adverse events (TEAE) and TEAEs categorized by causality, severity, and seriousness assessments made by the investigator by comparing study drug exposure to placebo.

Trends in safety will also be evaluated for the following assessments:

- Physical examinations
- Concomitant medications
- Vital signs
- Laboratory test results (including clinical chemistry, hematology, urinalysis, AFP, and vitamins A and E, 25-hydroxy vitamin D, and INR)
- Abdominal ultrasound
- Discontinuations due to AEs

10.1 Adverse Events

10.1.1 Definitions and Investigator Assessments

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 unfavorable and unintended sign, symptom, or disease that occurs once a patient is enrolled (ICF is signed) in the study until the patient is discharged from the study, whether or not related to the study drug.

10.1.1.1 Clinical Significance

Clinical significance is defined as any abnormal finding that results in further clinical investigation(s), treatment(s), or the diagnosis of new condition. Patient-reported events and protocol-mandated laboratory values, vital signs, and physical examination findings can be considered clinically significant (i.e., an AE) if there is a deterioration as compared to baseline. Examples of clinically significant worsening from baseline could include, but is not limited to, events causing withdrawal from the study and events requiring medical intervention outside of the study causing apparent clinical manifestations, or judged relevant by the investigator.

10.1.1.2 Serious Adverse Events

Serious criteria are applied by the investigator to each AE as specifically defined below. These criteria are used to determine whether an AE is serious or non-serious. The assessment

is made independently of severity assessment (Section 10.1.1.3). For example, the development of a severe rash that occurs after signing of informed consent may not meet serious criteria as defined below and therefore would be considered a severe, non-serious AE.

Any AE that meets any 1 of the following 6 criteria is considered an SAE:

- The outcome of the AE is **death**
- The AE is immediately **life-threatening**. Life-threatening means that the patient is, in the opinion of the investigator, at immediate risk of death from the reaction as it occurred. This does not include an AE that, if more severe, might have caused death
- The AE results in persistent or significant **disability/incapacity**. Disability means a substantial disruption of a person's ability to conduct normal life functions
- The AE requires or prolongs **hospitalization**
- The AE results in a **congenital anomaly/birth defect**
- **The AE is an important medical event**. Important medical events may meet serious criteria should the investigator assess that they may significantly jeopardize the patient, represent a significant hazard, or requires medical/surgical intervention to prevent one of the outcomes listed above. Examples of potential SAEs based on this criteria include, but are not limited to, allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions even if they do not result in inpatient hospitalization, or the development of drug dependency and drug abuse.

10.1.1.3 Severity Assessment

Severity assessments are based on the intensity of the event in relation to expectation. The investigator will assess the intensity of AEs based on the following definitions:

- Mild (awareness of sign or symptom, but easily tolerated)
- Moderate (discomfort sufficient to cause interference with normal activities)
- Severe (incapacitating, with inability to perform normal activities)

Severity is a measure of intensity where seriousness is defined by the criteria outlined in Section 10.1.1.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not meet serious criteria, and therefore would be assessed as a severe AE but not an SAE.

10.1.1.4 Causality Assessment

The investigator determines the causality of all AEs to the study drug using medical judgment and considering all relevant factors such as (but not limited to) the underlying study indication, coexisting disease, concomitant medication, relevant history, pattern of the AE, temporal relationship to the study drug, and de-challenge or re-challenge. The causality assessment of the AE/SAE is to be made as follows.

Related to study drug (possibly, probably, or definitely related)

Based on medical judgment, there is at least a reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event follows a reasonable temporal sequence from administration of study drug
- The event could not be reasonably attributed to the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient
- The event follows a known pattern of response to study drug
- The event disappears or decreases on cessation or reduction in dose of the study drug. (It should be noted that in some situations an AE will not disappear or decrease in intensity upon discontinuation of study drug despite other clear indications of relatedness)
- The event reappears or worsens when the study drug is re-administered

Unrelated to study drug (unlikely or unrelated)

Based on medical judgment there is no reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event does not follow a reasonable temporal sequence from administration of study drug
- The event could be reasonably attributed to the known characteristics of the patient's clinical state, concurrent illness, environment or toxic factors, or other modes of therapy administered to the patient
- The event does not follow a known pattern of response to study drug
- The event does not disappear or decrease on cessation or reduction in dose of the study drug, and it does not reappear or worsen when the study drug is re-administered

10.1.2 Recording of Adverse Events

It is the investigator's responsibility to assess whether each untoward event is a clinically significant worsening from baseline, thereby considered an AE. For all AEs, the severity, seriousness, and causality to study drug for each AE as outlined in Section 10.1.1 will be assessed and recorded in the eCRF.

All serious and non-serious AEs are collected once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10) or 28 days after the last dose of study drug.

Any AEs or SAEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator until resolution or stabilization up to the database lock and recorded in the eCRF. Albireo retains the right to request additional information for any patient with ongoing AE(s) or SAE(s) at the end of the study.

TEAEs are defined as any AE that occurs after randomization (Day 0). All AEs that occur in the Screening Period, i.e., after enrollment and prior to randomization, will be collected on the eCRF as non-treatment-emergent AEs.

If there is a clinically significant deterioration of a laboratory value/vital sign or other routine study assessment that is associated with a diagnosis, the clinical diagnosis will be reported as an AE and the associated signs and symptoms will be considered additional information unless the sign or symptom is more severe than expected given the diagnosis. For example, if an investigator diagnoses a study patient with hepatitis during the study period, hepatitis would be considered the AE and the concomitant signs and symptoms of abdominal pain, vomiting, and elevated ALT and AST would not be reported separately unless, in the opinion of the investigator, one of these signs or symptoms is more severe than expected and therefore a separate AE assessment is indicated.

10.1.3 Recording and Reporting of Serious Adverse Events

Every SAE (regardless of severity and causality) that occurs once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10), and 28 days after the final dose of study drug, should immediately and not later than within 24 hours of knowledge of the event, be reported by the investigator or delegate in the SAE report form.

Report of a SAE must include at least the following information:

- Patient identification information (study number, site number, initials, and date of birth [as per local country requirements for data protection])
- The last study drug administration date
- The diagnosis of the event with the description (or a brief description of signs/symptoms/clinical course if the diagnosis is not available) and the date of onset
- The action used to treat the event (i.e., treatment medications, temporary discontinuation)
- The reason(s) for considering the event serious
- The relationship of the event to the study drug or to the study procedure (e.g., the investigator's assessment of causality)
- A brief narrative of the SAE

Follow-up reports including all new information obtained of the subsequent course of the SAE must be prepared and the information collected in the SAE submitted to [redacted] (Pharmacovigilance and Safety Services Department) by e-mail within 24 hours of knowledge of the event.

The pharmacovigilance manager (PVM) may contact the investigator to obtain further information on a reported SAE. The investigator/investigative staff must respond to any

request for follow-up information or answers to questions regarding the SAE within the same timelines as for initial reports.

The PVM reports the occurrence of the SAE and follow-up to the Albireo medical monitor for medical assessment of the case.

Should an outcome of death occur within the study period or within 28 days after the last administration of study drug, an SAE form should be completed including the main and contributory causes of death.

All SAE reports must be e-mailed to the following e-mail address within **24 hours**:

[redacted]

If email is unavailable, SAEs may be transmitted via fax to the following number:

[redacted]

In a study-related medical emergency situation, when the assigned medical monitors for the study cannot be reached by a caller, an on call physician can be reached 24 hours per day, 7 days per week, via the [redacted] call center. The following number is a chargeable telephone number allowing a global reach from both landlines and mobile phones and the internet page indicated below contains a list of country-specific toll-free telephone numbers that is provided. It should be noted that not all countries globally have access to toll-free numbers as indicated on the "24/7 Medical Help desk" index. Countries without toll-free numbers need to dial the chargeable number as indicated below. Furthermore, toll-free numbers might not be available from mobile phones.

[redacted]

10.1.4 Reporting of Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reaction (SUSAR) is an SAE that occurs in a patient, the nature or severity of which is not expected per the applicable product information (e.g., the IB for an unauthorized investigational product or summary of product characteristics for an authorized product).

Reporting and tracking of SUSARs will be in accordance to all applicable competent authority regulations. The Ethics Committee and all investigators involved in this study will be informed according to national and international requirements.

10.2 Laboratory Values/Vital Signs/Physical Examinations and Other Safety Assessments

10.2.1 Laboratory Assessments

Samples will be collected for clinical chemistry, hematology, and urinalysis testing at the time points specified in Table 1. The parameters assessed are presented in Table 3.

Blood for AFP will be drawn at Visits 3 and 9. Fat-soluble vitamin levels including vitamins A and E, and 25-hydroxy vitamin D will be drawn at Visit 2 (vitamins A & E only), Visit 3 (25-hydroxy vitamin D only), Visit 6, and Visit 9. Blood for INR (surrogate for vitamin K) will be drawn at Visits 2, 4, 6, 8, and 10. Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for vitamin A. Exceptions can be made for infants, <12 months of age, if unable to fast for the full 4 hours. If a patient has any vitamin level(s) that are outside of the reference range, vitamin supplementation adjustments may be required. Target ranges and supplementation strategy guidelines can be found in Appendix 5.

A serum pregnancy test will be performed at Visit 1 and Visit 9 if the patient is entering the A4250-008 study. A urine pregnancy test will be collected at all other visits for girls who have reached menarche. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy. Study drug administration will be held pending the confirmatory results. If the serum pregnancy test is negative, the patient can resume dosing. If the serum pregnancy test is positive, the patient should be discontinued (see Section 10.2.9).

All samples will be processed and transported to a laboratory per instructions in the laboratory manual.

The observed values will be recorded and assessed as “normal” or “abnormal not clinically significant” or “abnormal clinically significant”.

Additional safety blood samples may be needed due to follow-up of an abnormal value or analysis failure. The blood samples collected for safety laboratory analysis will be destroyed after the analyses have been completed.

Table 3 Routine Laboratory Parameters

Clinical Chemistry	Hematology	Urinalysis
• Albumin • ALT • Alkaline phosphatase • AST • Bilirubin – total and direct • Calcium • Chloride • Creatinine • Creatine kinase • Gamma-glutamyl transferase • Potassium • Sodium	• Hematocrit • Hemoglobin • Platelet count • Red blood cell count • White blood cell count and differential (neutrophils, eosinophils, basophils, lymphocytes, and monocytes)	• Blood • Glucose • Ketones • Leukocytes • Nitrites • pH • Protein

10.2.2 Individual Patient Safety Monitoring

10.2.2.1 Liver Monitoring

Strategies to monitor markers of liver disease throughout the study are outlined below where the ULN will be based on central laboratory reference values for age and gender.

If isolated transaminase elevations are observed, defined as:

1. Normal bilirubin AND absence of clinical hepatitis symptoms AND
   - ALT or AST ≥5 × ULN (if normal at baseline) or an absolute threshold of 800 U/L, whichever comes first
   - OR ALT or AST ≥3 × baseline (if abnormal at baseline) or an absolute threshold of 800 U/L, whichever comes first

Then:

a) Repeat liver profile (AST, ALT, bilirubin, and prothrombin time [PT] or INR) within 2 to 3 days

b) Evaluate creatine phosphokinase (CPK) and lactate dehydrogenase (LDH)

c) As needed (for example, persistent ALT/total bilirubin elevation or worsening of liver function), consider evaluation for alternative etiologies

Monitor the patient using close observation found in Section 10.2.2.2

If any ONE of the following criteria are met:

1. Transaminases (ALT or AST ≥3 × baseline or 800 U/L, whichever comes first) AND bilirubin (total bilirubin > 2 × ULN) elevations

2. Transaminase elevations alone (ALT or AST >10 × ULN or 5 × baseline or absolute threshold of 800 U/L, whichever comes first) in presence of normal LDH and CPK
3. Total bilirubin increased, unrelated to hemolysis (elevated reticulocyte count) or established genetic diseases, such as Gilbert's syndrome
   a. Doubling if total bilirubin was <3 mg/dL (equivalent to 51.3 µmol/L) at baseline
   b. OR Increase by >3 mg/dL (equivalent to 51.3 µmol/L) if total bilirubin was ≥3 mg/dL (equivalent to 51.3 µmol/L) at baseline
4. INR increase refractory to vitamin K administration
   a. INR >1.5 if INR was normal at baseline
   b. OR Increase by >0.4 if INR was abnormal at baseline
5. Any increase in total bilirubin and transaminases if accompanied by EITHER a symptom of clinical hepatitis (e.g., vomiting, nausea, right upper quadrant pain) OR immunological reaction (rash or >5% eosinophilia)

Then:

a) Interrupt study medication
b) Initiate drug-induced liver injury work-up for alternative etiologies
c) Repeat liver profile (AST, ALT, total bilirubin, direct bilirubin) and PT or INR within 48 to 72 hours
d) Monitor the patient using close observation found in Section 10.2.2.2
e) If a patient lives in a remote area, they may be tested locally and the results communicated to the investigator site promptly

10.2.2.2 Close Observation

- Repeat liver enzyme and serum bilirubin tests two or three times weekly. Frequency of re-testing can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the subject is asymptomatic
- Obtain a more detailed history of symptoms and prior or concurrent diseases
- Obtain a history of concomitant drug use (including non-prescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets
- Consider ruling out alternate etiology including acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; non-alcoholic steatohepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease
- Obtain a history of exposure to environmental chemical agents.

- Obtain additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin)
- Consider gastroenterology or hepatology consultations.

10.2.2.3 De-challenge/Re-challenge for Liver and Clinical Hepatitis Monitoring

1. Re-challenge is not recommended:
   a. If a patient has had possible/probable drug-induced liver injury
   b. If a decompensation event has occurred (i.e., variceal hemorrhage, ascites, hepatic encephalopathy, etc.).
2. If due to underlying cholestatic liver disease variability or another alternative etiology is identified AND liver tests returned to baseline, re-challenge may be considered after consultation with the sponsor Medical Monitor.
3. If the ALT/total bilirubin elevations are observed after re-challenge, then repeat re-challenge is discouraged.

If patient is permanently discontinued, monitoring should be continued as outlined in Section 10.2.2.5.

10.2.2.4 Diarrhea

Study drug should be discontinued if a patient develops diarrhea with at least 1 of the following concomitant signs or symptoms: grossly bloody stools, vomiting, dehydration requiring treatment with oral or intravenous rehydration and/or electrolyte imbalances, fever (≥38 C) and/or the diarrhea persists for 7 or more days.

If there is gross blood in stool, INR and platelets should be measured.

Study drug will be reintroduced (re-challenge) when the symptoms have resolved. If the diarrhea re-occurs within 1 week after the re-challenge with no alternate etiology, the patient will be permanently discontinued and monitored as outlined in Section 10.2.2.5.

10.2.2.5 Monitoring after Permanent Discontinuation of Study Drug Due to Safety

Once study drug is permanently discontinued for liver, hepatitis, diarrhea, or other severe AE related to study drug, the patient will be monitored weekly until the laboratory and clinical parameters have normalized/stabilized.

10.2.3 Demographics/Medical and Surgical History

Demographic information per country regulations (age, full date of birth, gender, race, and ethnicity), along with medical and surgical history, will be obtained and recorded in the eCRF at Visit 1.

Medical and surgical history will be entered in the eCRF at Visit 1. This includes date of diagnosis of PFIC, prior investigational medications for PFIC, historical (3 months prior to

Screening) LFT values (AST, ALT, and total bilirubin), ongoing medication, any surgery performed, any other diagnosis, and historical biopsy data.

If a liver biopsy has been performed within 1 year prior to Screening, or during the study, the results will be recorded in the eCRF.

10.2.4 Clinical Genetic Testing

For previous confirmatory clinical genetic testing results for PFIC Type 1 or 2 performed prior to Screening Visit 1, the confirmatory clinical genetic testing laboratory report will be verified to determine eligibility.

If the historical clinical genetic result is equivocal, unavailable, or unobtainable, clinical genetic analysis to confirm pathologic biallelic variations in the *ATP8B1* or *ABCB11* genes will be performed and verified to determine eligibility. No other diagnostic genetic testing will be offered.

10.2.5 Physical Examination

A physician or suitably trained qualified assistant will perform a complete physical examination, including voluntary photography of skin lesions due to scratching, at Visits 1, 3, 6, and 9. A skin examination will be conducted at Visits 1, 3, 4, 6, 9, and 10 (see Table 1).

A complete physical examination will include assessment of general appearance, eyes, ears, nose, throat, head/neck/thyroid, lymph nodes, cardiovascular, lungs/chest, abdomen, genitourinary, extremities, skin, musculoskeletal, neurologic, and other.

Skin will be examined thoroughly and excoriations/scratch marks recorded.

10.2.6 Vital Signs

Evaluation of vital signs will be performed at all visits. This includes blood pressure (systolic and diastolic), pulse, respiratory rate, temperature, height (or length depending on age), and body weight (using a certified weight scale) at clinic visits. BMI will be calculated.

10.2.7 Abdominal Ultrasound

Ultrasound of the liver and spleen will be performed at randomization (Visit 3) and at EOT (Visit 9). Liver size, echogenicity, and presence of masses/nodules as well as spleen size will be recorded.

10.2.8 Overdose

A4250 is minimally absorbed and has a very low systemic availability. Based on toxicology data, for study purposes any dose exceeding a total of >3 mg/kg body weight of A4250 taken as a single dose or as a cumulative dose within 24 hours is defined as an overdose.

The no-adverse-effect dose level in the most sensitive species (defined as 20 mg/kg/day) in the rat 1 month toxicity study gives a human equivalent dose of approximately 194 mg/day in

a 60 kg person. This dose is approximately 10-fold higher than the maximum possible dose predicted in human studies (20 mg).

The investigator/investigative staff should immediately and not later than within 24 hours of knowledge of the overdose, be reported by the investigator or delegate on the SAE report form. In the event of an A4250 overdose, the patient should be monitored closely.

10.2.9 Pregnancy

If a pregnancy is discovered in a female patient enrolled in the study before the end of dosing, the patient will be immediately discontinued from the study and will attend the same visits as a prematurely withdrawn patient. If the pregnancy is discovered in a female patient enrolled in the study after the end of dosing, the patient will continue in the study per protocol. If a pregnancy occurs in a male patient's partner at any time during the study, the pregnancy should also be reported and followed.

Pregnancy is not considered to be an AE. However, if the patient has been dosed with the study drug, the pregnancy must be reported on the Paper Pregnancy Notification Form immediately within 24 hours after recognition to Safety Manager (please refer to Section 10.1.3). Date of exposure and as far as possible, details of the period of gestation at the time of exposure must be given.

The pregnancy should be followed to determine outcome, including spontaneous termination, details of birth, and presence of any birth defects, congenital anomalies or newborn or maternal complication. The infant who was exposed in-utero will be followed for up to 2 years after delivery. Individual cases with an abnormal outcome in association with the study drug should be reported on an expedited basis, i.e., reported rapidly to a competent authority.

11 STATISTICAL EVALUATION

All personnel involved with the analysis of the study will remain blinded until database lock for the double-blind Treatment Period and until major protocol violations have been identified.

All statistical analyses will be performed using SAS version 9.3 or higher.

11.1 Sample Size and Power

For the EU and RoW, the primary analysis will relate to the serum bile acid responder endpoint. For the US, the primary analysis will relate to the pruritus endpoint. For each primary endpoint, a pooled analysis for the closed testing procedure will be applied to control the 1-sided overall type I error rate for two treatment comparisons vs. the placebo at the 0.025 level (Kane et al, 2015, Lehmacher et al, 1991, and Marcus et al, 1976). The study will enroll 60 to 70 patients in order to obtain at least least 20 evaluable patients in each arm. For each primary endpoint, simulations with 5000 iterations using 20 patients per arm were conducted to estimate the power after multiplicity adjustment, resulting in a standard error of <0.7% for each estimated power.

Based on the Phase 2 A4250-003 study data both dose groups are predicted to have similar treatment effects for both serum bile acids and pruritus. For serum bile acids, simulated proportions were analyzed using the CMH test to generate the following 1-sided p-values: both A4250 arms pooled vs. placebo, low dose vs. placebo, and high dose vs. placebo. Assuming 60% responders in the A4250 arms and 10% responders in the placebo arm, the power to claim significance for a particular A4250 arm after multiplicity adjustment is approximately 94%. The power to claim significance for at least one arm or both arms are approximately 99% and 91%, respectively.

For the proportion of positive pruritus assessments at the subject level in pruritus scores, simulated proportions were analyzed using ANCOVA to generate the following 1-sided p-values: both A4250 arms pooled vs. placebo, low dose vs. placebo, and high dose vs. placebo. With an effect size of 1.0526, the simulated power to claim significance for a particular arm after multiplicity adjustment is approximately 89%. The probability to claim significance for at least one arm or both arms are approximately 95% and 83%, respectively.

11.2 Statistical Methods

11.2.1 Statistical Analysis Sets

Safety Analysis Set

The safety analysis set will consist of all patients who received at least 1 dose of study drug. Patients will be analyzed according to the treatment they actually received. The safety analysis set will be the primary analysis set for safety analyses.

Full Analysis Set

The full analysis set (FAS) will consist of all randomized patients who received at least 1 dose of assigned treatment. Patients will be analyzed in the treatment group they were randomized to even if they received incorrect study drug. The FAS will be the primary analysis set for efficacy analyses.

Per Protocol Analysis Set

The per protocol (PP) analysis set is a subset of the FAS, and will consist of all randomized patients for whom no major protocol violation (no violation which may affect the study efficacy outcome) is documented. The PP analysis set will provide supportive data for the efficacy analyses.

Allocation of patients to the PP analysis set will be performed before unblinding of the study.

11.2.2 Methods of Statistical Analyses

11.2.2.1 General Principles

The primary objective of this study is to demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with PFIC Types 1 and 2.

In the EU and RoW, for the primary efficacy variable of fasting bile acid responder endpoint (at least a 70% reduction in fasting s-BA concentration or reaching a level ≤70 µmol/L at the end of treatment), the Cochran Mantel Haenszel (CMH) test stratified by PFIC class and age class will be performed to compare the proportion in fasting bile acid responder endpoint at the end of treatment (Week 22 and 24) in the two A4250 dose groups to placebo group. To ascertain that all data are used in the CMH analysis, neighboring strata will be pooled if all subjects in a stratum have the same response. Dropouts will be treated as non-responders. The proportion together with corresponding 95% CI, odds ratio and corresponding 95% Clopper-Pearson exact CI and p-value for the CMH test will be presented.

In the US, for the primary efficacy variable of the proportion of positive pruritus assessments at subject level over the 24-week treatment period, an analysis of covariance (ANCOVA) will be used. The model will include treatment arm, AM baseline pruritus score, PM baseline pruritus score, and randomization stratification factors, i.e. PFIC class and age class. LS mean(SE) by treatment arm and LS mean difference (SE), 95% confidence intervals, and p-values (where applicable) between treatments (120 µg/kg/day and 40 µg/kg/day, respectively) vs. placebo will be provided. If there are concerns on model assumptions, i.e. normality and homogeneity of variances, a non-parametric ANCOVA based on the rank scores will be used as a sensitivity analysis. LS mean/SE on the outcome by treatment arm and LS mean difference/SE between active dose and placebo will be provided.

The procedure for multiplicity adjustment is specified as follows:

In the closed testing procedure, the low and high dose groups will be pooled to compare with the placebo group first. If the 1-sided p-value is ≤0.025, the 1-sided p-values for low dose vs.

placebo and high dose vs. placebo will be calculated, respectively. If both individual p-values are ≤0.025, a significant treatment effect will be declared on both dose groups. If only one of them is ≤0.025, a significant treatment effect will be declared on the corresponding dose group.

Other secondary and exploratory outcome variables will provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple comparisons when testing secondary and exploratory efficacy variables.

If not otherwise specified, the baseline value of a parameter is defined as the last assessment of that parameter prior to the first intake of study treatment.

11.2.2.2 Missing Data

In the primary analysis of the pruritus endpoint, all intermittently missing assessments will be classified as negative pruritus assessments. Similarly, all planned assessments after the intercurrent events (premature treatment discontinuation, death or initiation of rescue treatments such as biliary diversion surgery or liver transplantation) will be counted as negative pruritus assessments. However, as described above, all 336 assessments planned over the 24-week treatment period will be included in the denominator of the endpoint definition.

In the primary analysis of fasting bile acid responder endpoint, dropouts and patients with a missing average at the end of the treatment will be treated as non-responders. Patients in need of surgical rescue, i.e. biliary diversion and listing for liver transplant will be classified as non-responders. Continued collection of efficacy data for patients who discontinue treatment will be made as far as possible.

11.2.2.3 Demographic and Baseline Characteristics

Descriptive summaries of demographics and other baseline characteristics (including medical and surgical history) will be presented by treatment group and overall using the FAS.

Prior and concomitant medication use during the study will be summarized by treatment group and overall using the safety analysis set.

11.2.2.4 Subject Disposition

The following will be summarized (by treatment group and overall where applicable):

- Patients enrolled (who signed the informed consent)
- Patients randomized
- Patients treated
- Patients completing the study
- Patients withdrawing early (including withdrawal reason)

Additionally, patients enrolled, included in the safety, in the FAS, and in the PP analysis set will be summarized by region.

11.2.2.5 Evaluation of Primary Efficacy Variables

For EU and RoW, the primary responder endpoint based on change in fasting bile acids will be analyzed in terms of the proportion of patients achieving the event of interest. Patients in need of surgical rescue, i.e. biliary diversion and listing for liver transplant, will be classified as non-responders. Dropouts will be treated as non-responders.

CMH stratified by PFIC class and age class will be performed to compare the proportion in fasting bile acid responders at the end of treatment (Week 22 and 24) in the two A4250 dose groups to the placebo group. To ascertain that all data are used in the CMH analysis, neighboring strata will be pooled if all subjects in a stratum have the same response. The proportion together with corresponding 95% CI, odds ratio and corresponding 95% Clopper-Pearson exact CI and p-value for the CMH test will be presented

Logistic regression will be performed as a supportive analysis to compare the proportion in fasting bile acid responders at the end of treatment (Week 22 and 24) in the two A4250 dose groups to the placebo group. The model will include treatment arm, baseline value, and randomization stratification factors, i.e. PFIC class and age class. Baseline value will be calculated as the average of serum bile acid values at randomization and the previous visit (Clinic Visit 2).

In the US, for the primary efficacy variable of the proportion of positive pruritus assessments at the subject level over the 24-week treatment period, ANCOVA will be used to analyze the pruritus endpoint. The model will include treatment arm, AM baseline pruritus score, PM baseline pruritus score, and randomization stratification factors, i.e. PFIC class and age class. LS mean(SE) by treatment arm and LS mean difference (SE), 95% confidence intervals, and p-values (where applicable) between treatments (120 µg/kg/day and 40 µg/kg/day, respectively) vs. placebo will be provided. If there are concerns on model assumptions, i.e. normality and homogeneity of variances, a non-parametric ANCOVA based on the rank scores can be used. LS mean/SE on the outcome by treatment arm and LS mean difference/SE between active dose and placebo will be provided.

Sensitivity analysis will be conducted to assess the impact of intermittently missing data as well as intercurrent events and will be specified in the SAP. Subgroup analyses will also be performed for each of the 3 age groups (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) and for patients who were not included in the previous Study A4250-003.

11.2.2.6 Evaluation of Secondary and Exploratory Efficacy Variables

The change in the secondary endpoints such as serum bile acids, total bilirubin, ALT, growth, Albireo PRO and ObsRO sleep parameters, and patient-reported itch severity will be

summarized by visit using descriptive statistics. Change in growth (weight for age and BMI for age) will also be displayed using graphical presentations.

Proportion of responders at Weeks 12 and 24 based on the PRO/ObsRO instruments estimated from both distribution and anchor-based approaches will be analyzed using the same model as specified for the primary analysis for EU and RoW.

Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval over the 24-week treatment period for AM or PM, respectively will be analyzed using the same model as specified for the primary analysis for US.

Undergoing biliary diversion surgery and/or liver transplant, all-cause mortality, and liver decompensation events will mainly be analyzed by using descriptive statistics. Kaplan-Meier curves will be used when appropriate for time to event data.

Comparisons of the change from baseline at Week 24 in PedsQL between the treatment groups will be conducted using ANCOVA. The model will include terms for baseline, PFIC class, age class, and treatment.

Other exploratory variables (Global Symptom Relief, biochemical markers and measures of bile acid synthesis, additional Albireo PRO and ObsRO sleep parameters, PELD/MELD score, APRI score, and Fib-4 score) will be analyzed descriptively for categorical and continuous data, as applicable. For continuous data, the change from baseline will be analyzed in addition to the actual visit values. For categorical data, shift tables or frequency and percentages of patients will be presented as appropriate. Additional details on secondary and exploratory efficacy variables and subgroup analyses by age class (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) will be specified in the SAP.

11.2.2.7 Evaluation of Safety Variables

Safety data will be analyzed using descriptive statistics and summaries by treatment group of SAEs, AEs, vital signs, clinical laboratory tests (hematology, clinical chemistry and urinalysis), and concomitant medication. Analyses will be performed using the safety analysis set.

Summaries of AEs (coded according to the Medical Dictionary for Regulatory Activities [MedDRA] system organ class [SOC] and MedDRA preferred term) will include the following:

- Overview of the incidence of TEAEs (TEAEs, drug-related TEAEs, TEAEs leading to study discontinuation, and treatment-emergent SAEs)
- TEAEs by SOC and preferred term
- Intensity of TEAEs by SOC and preferred term

- Drug-related TEAEs by SOC and preferred term
- TEAEs leading to study discontinuation by SOC and preferred term
- Treatment-emergent SAEs by SOC and preferred term

Concomitant medication use during the Treatment Period will be summarized by Anatomical Therapeutic Chemical (ATC) class and World Health Organization (WHO) preferred name.

Summaries of vital signs will be presented. For each visit, the actual results and the change from baseline, and the number and percentage of patients with potentially clinically significant (PCS) values observed post-baseline will be presented.

Summaries of clinical safety laboratory data will be presented. For each visit, the actual result and the change from baseline, and the number and percentage of patients with PCS values observed post-baseline will be presented.

Data listings will be provided for each patient for all safety parameters.

11.2.2.8 Compliance and Exposure

Exposure will be analyzed by calculating the number of days with exposure to study drug. Results will be presented by treatment group using the safety analysis set.

The percentage compliance will be described by treatment group and the number of patients with a compliance <80%, between 80% and 120%, and >120% will be presented based on the safety analysis set.

11.2.3 Blinded Analyses to Confirm Responder Definitions

A blinded analysis of Albireo ObsRO and PRO eDiary data will be performed after 50% or more of the planned sample has completed the Week 24 Follow-Up Period. The blinded analysis will be used to estimate a threshold of clinically meaningful change (i.e., responder definition) in Albireo ObsRO and PRO pruritus scores. The analysis will be performed by a group independent from both the study team and Albireo. Blinded data will be used for this analysis; data will be collapsed across treatment groups.

The analyses will include distribution- and anchor-based analyses to examine a responder definition for the Albireo PRO/ObsRO pruritus scores. Distribution-based analyses will include calculation of the SD and standard error of measurement for the baseline Albireo PRO and ObsRO scores. Anchor-based analyses will involve examining the degree of change on the Albireo PRO and ObsRO scores from baseline to Week 24 for patients who experience change in pruritus according to PGIC and symptom items. Details of this analysis will be included in a stand-alone SAP for assessing the measurement characteristics (reliability, validity, sensitivity to change, responder definition) of the Albireo PRO and ObsRO items.

11.2.4 Data Safety Monitoring Board

A DSMB consisting of sponsor-independent clinical and statistical experts will be established. The DSMB will periodically (approximately on a quarterly basis) meet for the review of accumulating study data, including safety (AE and laboratory data) and liver values.

The DSMB will have access to unblinded data. The DSMB will make recommendations for the remaining part of the study (further details will be provided in the DSMB charter). The DSMB may recommend continuing with the study as planned or stopping the study early for safety reasons. The DSMB will submit its recommendations in writing to Albireo Medical/[redacted] who are responsible for responding to the recommendations of the DSMB and taking appropriate action. The investigators will only be informed by Albireo Medical/[redacted] if the study requires a protocol amendment or is stopped. The DSMB may choose to make additional evaluations at any time if they feel this is warranted from a safety point of view.

The DSMB will act according to its own written standard operating procedure described in a charter and will prepare written minutes of its meetings. The charter of the DSMB will be stored in the trial master file. The DSMB will maintain records of its meetings and these will become part of the study file when the study is complete. In order not to disseminate unblinded data and to ensure that all staff involved in the conduct and final analysis of the study remain blind to the data, only the members of the DSMB and the unblinded DSMB statistician will have access to these data.

12 DIRECT ACCESS TO SOURCE DATA/NOTES

The investigator/institution shall provide direct access to source data/documents for study-related monitoring, audits, IEC/Institutional Review Board (IRB) review and regulatory inspection.

13 QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Conduct of the Study

Albireo/[redacted] shall implement and maintain quality control and quality assurance procedures with written standard operating procedures to ensure that the study is conducted and data are generated, documented, and reported in compliance with the protocol, ICH GCP and applicable regulatory requirements.

This study shall be conducted in accordance with the provisions of the Declaration of Helsinki (October 1996) and all revisions thereof, and in accordance with United States Food and Drug Administration (FDA) regulations (Code of Federal Regulations, Sections 312.50 and 312.56) and with ICH GCP (CPMP 135/95).

The investigator may not deviate from the protocol without a formal protocol amendment having been established and approved by an appropriate competent authority and IEC/IRB, except when necessary to eliminate immediate hazards to the patient or when the change(s) involve(s) only logistical or administrative aspects of the study. Any deviations may result in the patient having to be withdrawn from the study and render that patient non-evaluable.

13.2 Study Monitoring

Before study initiation, at a site initiation visit or at an investigator's meeting, an Albireo representative or designee will review the protocol and eCRF with the investigators and the investigative staff. During the study, the clinical monitor (clinical research associate [CRA]) will visit the site regularly to check the completeness of patient records, the accuracy of entries on the eCRFs, the adherence to the protocol and to GCP, the progress of enrollment, and to ensure that study drug is being stored, dispensed, and accounted for according to specifications.

The investigator must ensure that eCRFs are completed within a timely period of patient visits, as per individual site agreements, and must allow the CRA and Albireo representative or designee periodic access to patient records and all study-related materials, including relevant hospital or clinical records, to confirm their consistency with the eCRF entries. No information in these records about the identity of the patients will leave the study center. Albireo monitoring standards require full verification for the presence of the signed ICF, adherence to the inclusion/exclusion criteria, documentation of SAEs, and recording of primary efficacy and safety variables. The CRA will review source data compared with the eCRFs and will verify source data according to the study-specific monitoring plan. The design of the study, the frequency of patient visits, and the site enrollment rate will determine the frequency of monitoring visits. Upon study completion, the CRA will visit the site to conduct a study termination visit, which will include collection of any outstanding documentation.

It is recommended that the investigator/investigative staff log into the eCRF system every working day or at minimum twice weekly in order to provide a prompt response to queries. The investigator/investigative staff should respond to queries and make any relevant changes to the study data within 3 working days.

14 ETHICS

14.1 Independent Ethics Committee/Institutional Review Board

Prior to the start of the study, the investigator is responsible for ensuring that the protocol and consent form have been reviewed and approved by a relevant IEC/IRB. The IEC/IRB shall be appropriately constituted and perform its functions in accordance with FDA ICH GCP and local requirements as applicable.

The IEC/IRB shall approve all protocol amendments (except for logistical or administrative changes), written informed consent documents and document updates, patient recruitment procedures (e.g., advertisements), written information to be provided to the patients and caregivers, IB, available safety information, information about payment and compensation available to patients, the investigator's curriculum vitae and/or other evidence of qualifications and any other documents requested by the IEC/IRB and regulatory authority (competent authority) as applicable.

14.2 Written Informed Consents and Assents

The investigator (physician) or investigative staff, in accordance with local regulations, will explain to each patient (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, alternative treatment, the potential risks and benefits involved, and any discomfort that may occur. Each caregiver/patient will be informed that participation in the study is voluntary and that he/she or their child may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

Caregivers/patients will be informed that they/their children may experience side-effects or be at risk for symptoms, illnesses, or complications that cannot be foreseen by Albireo. As with other medications, people treated with A4250 may be at risk of developing allergic reactions or anaphylaxis. Caregivers/patients will be advised that study procedures include regular blood sampling for measurement of safety parameters and biological markers and that some minor risks are associated with these procedures.

This informed consent should be given by means of a signed ICF, written in non-technical language in accordance with ICH GCP, the Declaration of Helsinki, and regulatory authorities. The caregiver(s)/patient should read and consider the statements before signing and dating them and should be given a copy of each signed document. If written consent is not possible, oral consent can be obtained if witnessed and followed by a signed statement from one or more persons not involved in the study, indicating why the patient was unable to sign the form. No patient can enter the study before his/her or caregiver(s) informed consent has been obtained, as required by country regulations.

The ICF is part of the protocol and must be submitted by the investigator/investigative staff with the protocol for IEC approval. Albireo will supply an ICF which complies with

regulatory requirements and country laws and is considered appropriate for the study. Any changes to the ICF suggested by the investigator must be agreed to by Albireo before submission to the IEC and a copy of the approved version must be provided to the clinical monitor after IEC approval.

15 DATA HANDLING AND RECORD KEEPING

15.1 Case Report Forms/Source Data Handling

The investigator shall be provided with standardized eCRFs and shall ensure that all data from patient visits are promptly entered into the eCRFs in accordance with the specific instructions given. The investigator must sign each eCRF to verify the integrity of the data recorded.

A list of the normal ranges for all laboratory tests to be undertaken forms part of the documentation to be collated prior to study start. As a central laboratory has been selected to conduct any or all tests, it is essential that all samples be analyzed at that laboratory.

The investigator must maintain source documents such as laboratory reports, consultation reports, and complete medical history and physical examination reports.

As no ethnicity data can be collected in the source document for study sites in France, the eCRF will be the Source Document for “origines ethniques”,

15.2 Retention of Essential Documents

Essential documents, as defined by ICH GCP, include: the signed protocol and any amendment(s); copies of the completed eCRFs (for site archiving, compact discs of eCRF data for specific patients will be provided); signed ICFs; hospital records and other source documents; IEC approvals and all related correspondence including approved documents; drug accountability records; study correspondence; and a list of patients’ names and addresses.

The investigator/investigative staff must retain copies of these essential documents for the period specified by ICH GCP and by applicable regulatory requirements. The investigator/investigative staff will inform Albireo of the location where the essential documents are stored and must contact Albireo for approval before disposing of any essential documents. The investigator/investigative staff should take measures to prevent accidental or premature destruction of these documents.

16 FINANCING AND INSURANCE

Financing and insurance are addressed in a separate agreement.

17 PUBLICATION POLICY

Albireo will retain the ownership of all data. When the study is complete, Albireo shall arrange the analysis, tabulation of data and preparation of a clinical study report. Albireo may also use the data for publication, presentation at scientific meetings and submission to regulatory authorities. All proposed publications based on this study must be subject to the sponsor's approval requirements.

18 REFERENCE LIST

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19 APPENDICES

Appendix 1 Concomitant Medication Guidelines

Appendix 2 Diary Questions including Albireo Patient-Reported Outcomes/Observer-Reported Outcome Instrument

Appendix 3 Patient Global Impression of Change (PGIC) and Patient Global Impression of Symptoms (PGIS)

Appendix 4 Pediatric Quality of Life Inventory (PedQL) Questionnaire

Appendix 5 Guideline for Fat-Soluble Vitamin Supplementation

Appendix 6 Contraceptive Requirements

Appendix 7 Blood Volumes

CLINICAL STUDY PROTOCOL A4250-005

A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

IND Number:	130591
EudraCT Number	2017-002338-21
Test Product:	A4250
Indication:	Progressive Familial Intrahepatic Cholestasis (PFIC)
Sponsor:	Albireo AB
Development Phase:	Phase 3
Sponsor Signatory:	[redacted]
Sponsor Medical Monitor:	[redacted]
Principal Investigator:	[redacted]
Date of the Protocol:	29 April 2019
Version of the Protocol:	Final Amendment 05

Confidential

The information in this document contains trade secrets and commercial information that are privileged or confidential and may not be used, divulged, published, or otherwise disclosed without the written consent of Albireo AB. These restrictions on disclosure apply equally to all future information supplied to you or generated by you in connection with the study.

SPONSOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

Albireo AB

[redacted]

29 April 2019
Date (day/month/year)

INVESTIGATOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

I have read this protocol and agree that it contains all necessary details for performing this study. I will conduct the study as outlined herein and will complete the study within the time designated, in accordance with all stipulations of the protocol and in accordance with Good Clinical Practice (GCP), local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study drug and the conduct of the study.

I will use only the informed consent approved by the Independent Ethics Committee (IEC) and will fulfil all responsibilities for submitting pertinent information to the IEC responsible for this study.

I agree that the sponsor (Albireo AB) shall have access to any source documents from which case report form information may have been generated.

I further agree not to originate or use the name of Albireo AB, A4250 in any publicity, news release, or other public announcement, written or oral, whether to the public, press, or otherwise, relating to this protocol, to any amendment to the protocol, or to the performance of this protocol without the prior written consent of Albireo AB.

Name of Investigator

Signature

Date (day/month/year)

1 ADMINISTRATIVE INFORMATION

A Double-blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Protocol No.:	A4250-005
Date of the initial Protocol:	20 September 2017
Date and Number of Amendment(s):	Amendment 01, 06 December 2017 Amendment 02, 10 May 2018 Amendment 03, 22 November 2018 Amendment 04, 01 March 2019 Amendment 05, 29 April 2019
Sponsor:	[redacted]
Clinical Research Organization:	
Sponsor Signatory:	
Sponsor Medical Monitor:	
Principal Investigator:	

2 STUDY SYNOPSIS

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250
Title of Study: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)		
Principal Investigator: [redacted]		
Study Centers: Up to 50 sites will be initiated for this study in the United States (US), Canada, European Union (EU), Middle East, and Australia.		
Publication(s): None.		
Planned Study Period: Q2 2018 to Q4 2019	**Development Phase:** Phase 3	

Objectives:

Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with progressive familial intrahepatic cholestasis (PFIC) Types 1 and 2, as determined by the following:

- Proportion of patients experiencing at least a 70% reduction in fasting serum bile acid (s-BA) concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- The proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period

Secondary Objectives

- To evaluate the effect of A4250 on serum alanine aminotransferase (ALT) concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

Methodology:

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of familial intrahepatic cholestasis-1 or bile salt export pump).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-up Period. After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo. Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008) in which all patients will receive active treatment.

There will be up to 10 clinic visits during the study and one scheduled telephone contact.

Name of Sponsor/Company:	**Name of Product:**	**Name of Active Ingredient:**
Albireo	A4250	A4250

Number of Patients:

Approximately 60 patients diagnosed with PFIC-1 or PFIC-2 will be randomized. The study will target 15% of enrolled patients to be PFIC-1 patients. If enrollment of all PFIC-2 patients is completed and the 15% enrollment of PFIC-1 patients has not yet been achieved, then enrollment of PFIC-2 patients will continue in order to reach the total study target enrollment.

Diagnosis and Main Criteria for Inclusion:

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have clinical genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples at least 7 days apart (Visits 1 and 2) prior to randomization
4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the electronic diary (eDiary) average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients are expected to have a consistent caregiver for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

Test Product, Dose and Mode of Administration:

A4250, 40 µg/kg/day or 120 µg/kg/day, orally administered

Reference Therapy, Dose and Duration of Administration:

Matching placebo, orally administered

Duration of Treatment:

24 weeks

Variables

Efficacy:

Primary Efficacy Endpoints

The primary efficacy endpoints are:

- **EU and rest of world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.
- **US**: Proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period.

Secondary Efficacy Endpoints

Secondary efficacy endpoints include the following:

- **EU and RoW**: Proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period.
- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

All Regions:

All secondary endpoints will be compared to placebo.

- Change from baseline to Week 12 and to Week 24 in fasting s-BA
- Change from baseline to Week 12 and to Week 24 in serum ALT concentration

Name of Sponsor/Company:	Name of Product:	Name of Active Ingredient:
Albireo	A4250	A4250

- Change in growth from baseline to Week 12 and Week 24
- Proportion of responders for pruritus scores at Weeks 12 and 24 based on the Albireo PRO and ObsRO instruments
- Change in sleep parameters measured with the Albireo PRO and ObsRO instruments from baseline over the 24-week Treatment Period
- Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level over the 24-week Treatment Period. A positive pruritus assessment includes an itch score ≤1, or at least a one-point drop from baseline based on the Albireo PRO instrument; only patients ≥8 years of age will complete the Albireo PRO instrument
- Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Proportion of individual AM assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Proportion of individual PM assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Number of patients undergoing biliary diversion surgery or liver transplantation

Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the patient global impression of change (PGIC) items
- Change from baseline in fasting s-BA at Week 4
- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline by each 4 week interval of the Treatment Period as measured by the Albireo ObsRO instrument
- Change from baseline by each 4-week interval of the Treatment Period in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline by each 4-week interval of the Treatment Period in patient-reported and observer-reported morning time itching and scratching severity scores, respectively
- Change from baseline by each 4-week interval of the Treatment Period in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline by each 4-week interval of the Treatment Period in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in Pediatric Quality of Life Inventory (PedsQL)
- Change from baseline in serum ALT concentration at Week 4
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST and gamma-glutamyl transferase at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, plasma 7α-hydroxy-4-cholesten-3-one concentration [p-C4]) at Week 24
- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events.
- All-cause mortality

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Albireo	A4250	A4250

Safety:

Safety criteria are as follows:

- Occurrence of treatment-emergent adverse events including severity and relatedness to study drug at all visits
- The incidence of treatment-emergent serious adverse events (SAEs), based upon information from patient reports, including the description, incidence, and severity of an SAE
- Trends evaluated for the following assessments: physical examinations, concomitant medications, vital signs, laboratory test results (including clinical chemistry, hematology, urinalysis, alfa-fetoprotein, vitamins A and E, 25-hydroxy vitamin D and INR), and abdominal ultrasound

Statistical Methods:

The primary objective of this study is to demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with PFIC Types 1 and 2.

The Cochran Mantel Haenzel (CMH) test stratified by PFIC class and age class will be performed to compare the proportion in fasting bile acid responders at the end of treatment (Week 22 and 24) in the two A4250 dose groups to the placebo group. To ascertain that all data are used in the CMH analysis, neighboring strata will be pooled if all subjects in a stratum have the same response. The proportion together with the corresponding 95% CI, odds ratio and corresponding 95% Clopper-Pearson exact CI and p-value for the CMH test will be presented.

For the primary efficacy variable of the proportion of positive pruritus assessments at subject level over the 24-week treatment period, an ANCOVA model will be used to analyse the comparisons between the treatment groups. The model will include treatment arm, AM baseline pruritus score, PM baseline pruritus score, and randomization stratification factors, i.e. PFIC class and age class. LS mean (SE) by treatment arm and LS mean difference (SE), 95% confidence intervals, and p-values (where applicable) between treatments (120 µg/kg/day and 40 µg/kg/day, respectively) vs. placebo will be provided. If there are concerns on model assumptions, i.e. normality and homogeneity of variances, a non-parametric ANCOVA based on the rank scores can be used as a sensitivity analysis. LS mean/SE on the outcome by treatment arm and LS mean difference/SE between active dose and placebo will be provided.

For each primary endpoint by region (EU & RoW and US), a pooled analysis for the closed testing procedure will be applied to control the 1-sided overall type I error rate for two treatment comparisons vs. the placebo at the 0.025 level, as specified below:

In the closed testing procedure, the low and high dose groups will be pooled to compare with the placebo group first. If the 1-sided p-value is ≤0.025, the 1-sided p-values for low dose vs. placebo and high dose vs. placebo will be calculated respectively. If both individual p-values are ≤0.025, a significant treatment effect will be declared on both dose groups. If only one of them is ≤0.025, a significant treatment effect will be declared on the corresponding dose group.

Other secondary and exploratory outcome variables will provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple comparisons when testing secondary and exploratory efficacy variables.

Date of the Protocol: 29 April 2019

3 TABLE OF CONTENTS

3.1 List of Tables

3.2 List of Figures

3.3 List of Appendices

4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADME	absorption, distribution, metabolism, and excretion
AE(s)	adverse event(s)
AFP	alfa-fetoprotein
ALT	alanine aminotransferase
APRI	AST to platelet ratio index
ASBT	apical sodium-dependent bile transporter (also known as IBAT)
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	body mass index
BSEP	bile salt export pump
CPK	creatine phosphokinase
CRA	clinical research associate
CYP	cytochrome P450
DSMB	Data and Safety Monitoring Board
eCRF	electronic case report form
eDiary	electronic diary
EOT	End of Treatment
EU	European Union
EudraCT	European Union drug regulatory agency clinical trial
FAS	full analysis set
FDA	United States Food and Drug Administration
FIC1	familial intrahepatic cholestasis-1
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
GI	gastrointestinal
GLMM	generalized linear mixed modelling
IB	Investigator's Brochure
IBAT	ileal bile acid transporter (also known as ASBT)
ICF	informed consent form
ICH	International Council for Harmonisation
[redacted]	[redacted]
IEC	Independent Ethics Committee
INR	international normalized ratio
IRB	Institutional Review Board

IWRS	Interactive Web Response System
LDH	lactate dehydrogenase
LFT	liver function test
LPLV	last patient last visit
MDR3	multidrug-resistance protein 3
MedDRA	Medical Dictionary for Regulatory Activities
MELD	model for end-stage liver disease
MMRM	mixed model repeated measures
ObsRO	observer-reported outcome
PBC	primary biliary cholangitis
p-C4	plasma 7α-hydroxy-4-cholesten-3-one concentration
PCS	potentially clinically significant
PedsQL	Pediatric Quality of Life Inventory
PELD	pediatric end-stage liver disease
PFIC	progressive familial intrahepatic cholestasis
PGIC	patient global impression of change
PGIS	patient global impression of symptoms
PK	pharmacokinetic(s)
PP	per protocol
PRO	patient-reported outcome
PT	prothrombin time
PVM	pharmacovigilance manager
QoL	quality of life
RoW	rest of world
RR	respiratory rate
SAE(s)	serious adverse event(s)
SAP	statistical analysis plan
s-BA	serum bile acid
SD	standard deviation
s-FGF19	serum fibroblast growth factor 19 concentration
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction
TEAE(s)	treatment-emergent adverse event(s)
ULN	upper limit of normal
US	United States
WHO	World Health Organization

5 INTRODUCTION

5.1 Investigational Medicinal Product

A4250 is a small molecule and a potent selective inhibitor of the ileal bile acid transporter (IBAT), also known as the apical sodium-dependent bile acid transporter (ASBT). IBAT is an integral brush border membrane glycoprotein that co-transports sodium and bile acids and appears to be a critical component in the regulation of the bile acid pool size in animals and man. This transporter, expressed in the distal ileum, is a key element in the enterohepatic circulation of bile acids since it facilitates the high-affinity, high-capacity reabsorption of bile acids. Indeed, 95% of secreted bile acids are reabsorbed via IBAT [Hofmann 2009; Miethke 2016]. A4250 is orally administered and acts locally in the gut where it binds reversibly to IBAT to decrease the reuptake of bile acids. A4250 has minimal systemic exposure at the predicted therapeutic dose ranges.

No safety signals were found with A4250 in preclinical safety and toxicity studies. In the first clinical study of A4250 in healthy volunteers, A4250 was given as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. A4250 was well tolerated and the only findings were gastrointestinal (GI) related effects, such as diarrhea, at higher doses.

In an exploratory Phase 2 study in children with cholestatic pruritus, A4250 was given as oral doses of 10 to 200 µg/kg/day for 4 weeks. A4250 reduced mean levels of serum bile acids (s-BA) in all cohorts. In 11 of 13 progressive familial intrahepatic cholestasis (PFIC) patients, substantial reductions (ranging from 43% to 98%) were observed. Patient-reported diary data documented improvement of pruritus during treatment. A4250 was well tolerated. No serious adverse events (SAEs) were deemed to be related to study drug, and most adverse events (AEs), including some increased transaminases, were considered unrelated to study drug and transient in nature.

5.2 Background

5.2.1 Progressive Familial Intrahepatic Cholestasis

PFIC is a rare autosomal recessive cholestatic liver disease estimated to affect between 1 in every 50,000 to 100,000 children born worldwide. PFIC represents 10% to 15% of causes of cholestasis in children and 10% to 15% of liver transplantation indications in children. All types of PFIC exist worldwide and both sexes appear to be equally affected.

The common underlying pathogenesis of PFIC is disruption of bile formation and bile transport through the liver [Jacquemin 2000]. The classification of PFIC has evolved over the years. The most commonly used sub-classifications are PFIC Types 1, 2, and 3, which are based on the associated affected gene and described in more detail below.

- PFIC, Type 1: also referred to as “Byler disease” or “familial intrahepatic cholestasis-1 (FIC1) protein deficiency.” FIC1 protein is located on the canalicular

membrane of hepatocytes and facilitates movement of aminophospholipids from the outer to inner leaflet of the plasma membrane of the hepatocyte. The *ATP8B1* gene encodes FIC1 protein. Biallelic pathologic variants in the *ATP8B1* gene are associated with FIC1 dysfunction and classified clinically as PFIC Type 1 disease.

- PFIC, Type 2: also referred to as "Byler syndrome" or "bile salt export pump (BSEP) deficiency." BSEP is a transporter protein that is expressed at the canalicular membrane of hepatocytes and is the primary exporter of bile acids. The *ABCB11* gene encodes the BSEP protein. Biallelic pathologic variations in the *ABCB11* gene are associated with BSEP dysfunction and classified clinically as PFIC Type 2 disease.
- PFIC, Type 3: is caused by a deficiency of the multidrug-resistance protein 3 (MDR3) due to mutations in the *ABCB4* gene. MDR3 is a phospholipid translocase critical for phospholipid secretion.

Severe pruritus is common in children diagnosed with PFIC. Itching (and subsequent scratching) is a significant morbidity for these patients and their families [Suchy 2007]. A more severe degree of pruritus is experienced compared to patients with other forms of liver disease and to other pruritic conditions such as atopic dermatitis [Murray 2011]. In patients with PFIC, liver biopsy reveals canalicular cholestasis and, later, the appearance of portal fibrosis. Serum biochemistry indicates cholestasis with hyperbilirubinemia, elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The concentrations of bile acids in serum are very high, while serum gamma-glutamyl transferase (GGT) activity (exception being MDR3 variants) and cholesterol are normal [Hori 2010]. Symptoms of portal hypertension and liver failure will develop during the course of the disease [Davit-Spraul 2009; Alissa 2008]. Symptoms develop early; median age at onset of symptoms is 2 months, and 78% of PFIC patients present with jaundice [Pawlikowska 2010]. The life-threatening and debilitating nature of PFIC is reflected by the fact that survival in those not resorting to surgery is 50% at 10 years of age and almost zero at 20 years of age. Approximately half of PFIC patients undergo liver transplantation [Davit-Spraul 2009] and treatment-resistant pruritus is the leading indication for the surgical procedure partial external biliary diversion, mostly in PFIC Type 1 and 2 patients.

PFIC is life-threatening and debilitating. Currently, the treatment for PFIC is palliative and there is no pharmaceutical treatment approved for use in PFIC. The therapeutic choices are restricted to non-specific therapy of the symptoms and signs of the disease such as nutritional support, preventing vitamin deficiencies, and treatment of extrahepatic features. Medical treatment options include off-label use of ursodeoxycholic acid, rifampin, antihistamines, and naltrexone. A minority of patients respond nominally and transiently to these interventions. Biliary diversion is used to decrease systemic bile acids through interruption of the enterohepatic circulation and so avoid transplantation. Liver transplantation is typically only viewed as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

5.2.2 Summary of Clinical and Nonclinical Studies

This is a summary of nonclinical and clinical studies. More detail is provided in the Investigator's Brochure ([IB] see A4250 IB).

A4250 is a potent, selective IBAT inhibitor with no safety signals seen in the safety pharmacological studies. In addition, no safety signals were seen in repeat-dose oral toxicity studies in rats with up to 26 weeks of daily dosing and in dogs with up to 39 weeks of daily dosing. No safety signals were identified when A4250 was administered daily to juvenile rats from age 14 days to 63 days.

A4250 is minimally absorbed with very low systemic exposure in both humans and other species. In blood, protein binding is high (>99.7%). Systemically absorbed A4250 is slowly metabolized in humans and in preclinical studies by hydroxylation only, a metabolite that retains IBAT activity. A4250 did not show any significant inhibition of cytochrome P450 (CYP) enzymes in the rat. A4250 inhibited human CYP3A4 and CYP2D6 (IC_{50}: 16 µmol/L for both enzymes), and CYP2C9 (IC_{50}:1.2 µmol/L).

To date, A4250 has been studied in 3 Albireo-sponsored clinical studies: a study in healthy volunteers evaluating single and multiple administration of A4250; a single-dose absorption, distribution, metabolism, and excretion (ADME) study; and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with primary biliary cholangitis (PBC).

In the first clinical study (A4250-001), A4250 was given to healthy volunteers as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. Following a single oral dose of 10 mg, the median maximum drug concentration was 0.2 nmol/L and the area under the curve was 0.8 nmol × h/L (n=6). Pharmacodynamic evaluation of bile acid synthesis biomarkers (serum fibroblast growth factor 19 [s-FGF19] and plasma 7α Hydroxy 4-cholesten-3-one [p-C4]) identified the expected decrease in s-FGF19 and an increase in p-C4. In addition, total plasma bile acids decreased and total fecal bile acids increased in A4250-treated patients compared to placebo. A4250 was generally well tolerated. The only findings were GI-related effects, such as diarrhea at higher doses.

In the ADME study (A4250-007), healthy subjects were administered a single oral dose of 3 mg [^{14}C]-A4250 capsule. The main excretion route was determined to be fecal. There were no quantifiable concentrations of A4250 in plasma or total radioactivity in plasma or whole blood. A4250 was well tolerated. AEs were reported and most were mild and not related to A4250. The 3 AEs experienced by 2 subjects reported as related to study drug were GI in nature, including diarrhea (2 AEs; 2 subjects) and abdominal pain (1 AE; 1 subject).

In an exploratory Phase 2 study in children with cholestatic pruritus (A4250-003), A4250 was administered as oral daily doses of 10 to 200 µg/kg/day for 4 weeks. Twenty patients (8 females and 12 males) aged 1 to 17 years were enrolled in this open-label dose-escalation study. Four patients were re-enrolled into a second dose level. No

treatment-related SAEs were reported, and most AEs, including some increased transaminases, were transient. Four-week, once-daily treatment with A4250 reduced mean levels of s-BA in all cohorts. Substantial s-BA reductions in 11 of 13 PFIC patients (ranging from 43% to 98%) were observed. A4250 also reduced s-BA in the 11 non-PFIC patients, but to a lesser extent than in PFIC patients. Patient-reported diaries documented improvement of the visual analogue scale itch score in 17 of 24 patients during treatment. The dose with the greatest improvement showed a mean decrease (± standard error of the mean) of 2.9 ±1.1 from baseline. The results from this study form the basis of the current (A4250-005) study design.

5.3 Rationale

In this investigational study, A4250 is under development for treatment of PFIC and is also being considered for other orphan cholestatic liver diseases.

There is currently no medical treatment approved for PFIC. In PFIC patients with severe pruritus, biliary diversion surgery is used to decrease systemic bile acids through interruption of the enterohepatic circulation and thereby reducing pruritus [Whitington 1988]. A portion of patients who have biliary diversion surgery continue to have elevated serum bile acids and pruritus. Liver transplantation is typically used as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

By inhibiting IBAT with high selectivity and potency, A4250 has the potential to relieve cholestasis and improve liver function without surgical intervention in patients with PFIC. Since A4250 is minimally absorbed and achieves a substantial reduction of s-BA, it has the potential to be a safer and less invasive alternative to surgical biliary drainage. The rationale for using A4250 is to decrease s-BA levels, and to reduce the major morbidity of pruritus, improving the health and wellbeing of patients affected with PFIC. Ultimately, this noninvasive therapeutic approach has the potential to not only improve quality of life (QoL), but also reduce the need for biliary diversion and subsequent liver failure and liver transplantation. For those patients who have undergone biliary diversion surgery and continue to have elevated serum bile acids and symptomatic pruritus, IBAT inhibition has the potential to reduce both the serum bile acid levels and the pruritus.

The doses selected, 40 and 120 µg/kg/day, were based on the efficacy and safety data generated from patients with PFIC (n=10 + 3 re-entered) and supporting data from all patients (n=20 + 4 re-entered) in the five dose groups dosed in the A4250-003 study. Improvements in s-BA and pruritus were observed with doses from 30 µg/kg to 100 µg/kg, with a dose-related trend. The best dose response in all patients was at the 60 µg/kg/day for s-BA and 100 µg/kg/day for pruritus. The best dose response in the PFIC subgroup was at 30 µg/kg/day for both s-BA and pruritus. Patients with PFIC responded well to the doses given from 30 µg/kg to 200 µg/kg/day; however, a clear dose relationship could not be

established in order to select a single dose for this Phase 3 study. As efficacy was observed at both low and high doses, 40 µg/kg and 120 µg/kg/day are considered to be the most optimal for this study.

This study will be conducted in compliance with the protocol and with the International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP).

Patients completing 24 weeks of double-blind treatment will be offered to enter Study A4250-008 and receive open-label treatment with A4250.

5.3.1 Risk/Benefit

A4250 has been evaluated in three Albireo-sponsored clinical studies: a double-blind placebo-controlled study in healthy volunteers, a single-dose ADME study, and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with PBC.

Healthy subjects have been exposed to up to 10 mg as a single dose and up to 3 mg daily as part of a multiple-dose evaluation. Children with cholestatic liver disease have been treated with up to 0.2 mg/kg/day (200 µg/kg/day) for 4 weeks. In total, 2 SAEs have been reported; both were judged by the physician to be not related to the study drug.

Patients with cholestatic liver diseases suffer from excess bile acids in the liver resulting in tissue damage. A commonly used treatment in these patients is bile diversion surgery, whereby approximately 50% to 100% of the enterohepatic circulation of bile acids is interrupted. Inhibition of IBAT with A4250, thereby interrupting the enterohepatic circulation of bile acids, is therefore a potential medical alternative to surgery which could be of benefit to these patients if shown to be effective and safe. Data from the Phase 2 study showed efficacy of A4250 in reducing s-BA concentrations and pruritus in such patients.

Based on the mode of action of an IBAT inhibitor, loose stools or diarrhea could be expected. However, in the pediatric Phase 2 study with 4 weeks daily treatment, only one patient had mild transient diarrhea after a single dose that did not recur on multiple dosing.

6 STUDY OBJECTIVES

6.1 Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with PFIC Types 1 and 2, as determined by the following:

- The proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- The proportion of positive pruritus assessments at the subject level over the 24-week treatment period.

6.2 Secondary Objectives

- To evaluate the effect of A4250 on serum ALT concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

7 INVESTIGATIONAL PLAN

7.1 Overall Study Design and Plan

7.1.1 Description

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of FIC1 or BSEP).

Study data will be periodically (approximately on a quarterly basis) reviewed by an unblinded Data Safety and Monitoring Board (DSMB).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-Up Period.

After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo.

Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008; EudraCT Number 2017-002325-38, subject to a separate study approval) in which all patients will receive active treatment. A patient who prematurely withdraws due to intolerable symptoms and has completed at least 12 weeks of the Treatment Period and End of Treatment (EOT) Visit, will also be offered the opportunity to enter Study A4250-008.

Visit windows applicable to each visit are presented in the schedule of assessments (Table 1). There will be up to 10 clinic visits during the study (Figure 1) and one scheduled telephone contact, as follows:

- Visit 1: Screening Period start. Patient is considered enrolled after signing the informed consent form (ICF) (Days -56 to -35)
- Visit 2: second Screening Visit (Days -28 to -7)
- Visit 3: Randomization Visit (Day 0)
- Telephone Contact 1: (Day 14)
- Visit 4: Clinic Visit (Day 28)
- Visit 5: Clinic Visit (Day 56)
- Visit 6: Clinic Visit (Day 84)
- Visit 7: Clinic Visit (Day 126)
- Visit 8: Clinic Visit (Day 154)
- Visit 9: Clinic Visit, EOT (Day 168)

- Visit 10: Follow-Up Visit, 28 days after last dose of study drug (Day 196). All patients continuing in Study A4250-008 will not have this Follow-up Visit.

Additional clinic visits may be required for patients who need direct site assistance, e.g., due to AE monitoring, to fulfil screening requirements, and/or for safety maintenance.

Screening Period

Informed consent must be obtained at Visit 1 prior to performing any study procedures. After signing the ICF, patients will be considered enrolled and evaluated for study eligibility.

Daily recording of pruritus using an electronic diary (eDiary) will be started to determine confirmation of the magnitude and the baseline pruritus symptoms before the study. After consultation with the Medical Monitor, patients failing eligibility criteria may be re-screened. Patients not fulfilling inclusion/exclusion criteria after 3 attempts are not allowed to re-screen.

Caregivers/patients will be instructed on the daily use of the eDiary. The eDiary will include patient-reported outcome (PRO) and observer-reported outcome (ObsRO) items for evaluation of itching (PRO), scratching (ObsRO), and sleep disturbance (PRO and ObsRO) during the Screening Period (study Days -56 to 0), throughout the 24-week Treatment Period (study Days 0 to 168), and Follow-up (Day 196).

The 14 consecutive days prior to randomization will be used for eligibility calculation of the diary entries. If a patient does not meet the minimum number of observations (see Section 9.2.2 for scoring and missing data definitions) to determine eligibility, the patient may be re-screened. Observer-reported outcomes in patients of all ages will be recorded by a caregiver. If possible, the same caregiver will complete the Albireo ObsRO items throughout the study.

At a second Screening Visit (Visit 2) additional laboratory samples will be taken for eligibility assessments.

Treatment Period

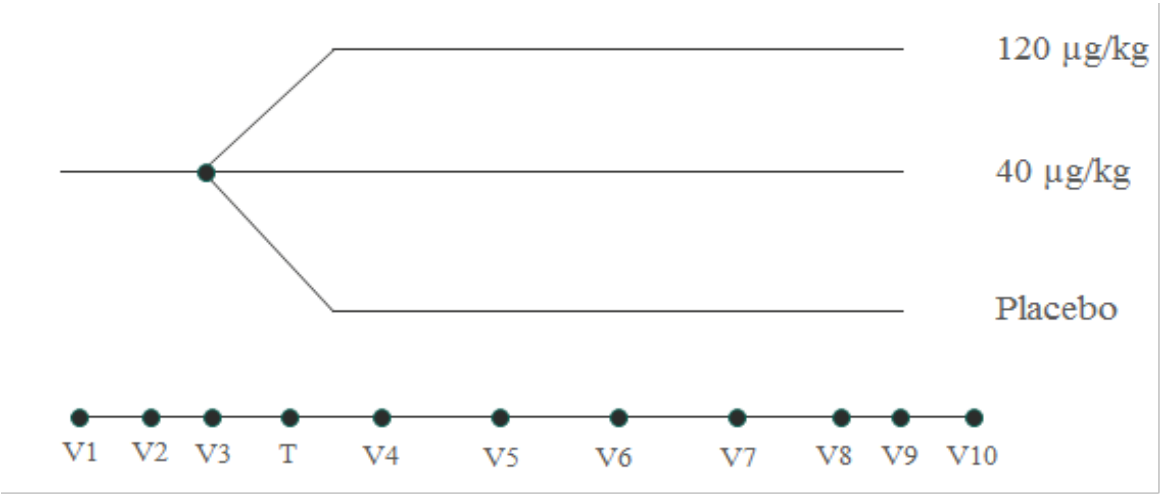
Eligibility for randomization will be determined at Visit 3 using the pruritus (scratching reports from the Albireo ObsRO) data in the 2 weeks before Visit 3, clinical genetic confirmation of diagnosis, and the liver biochemistry evaluations from the previous screening visits. Two weeks after Visit 3, there will be a telephone contact with the caregiver/patient to evaluate any AEs.

The patient will return to the clinic at Visit 4, Visit 5, Visit 6, Visit 7, Visit 8, and Visit 9 for assessments. If a new caregiver will begin to complete the Albireo ObsRO during the course of the trial, they will be provided with training during a clinic visit before they begin to use the eDiary. Additionally, caregivers will be requested to use the diary to report the time each dose of study drug is administered during the Treatment Period.

The last dose of study drug will be administered the day before Visit 9. At study Visit 9, the patient has the option to enroll in an open-label extension study (A4250-008) should they meet the required entry criteria. If the patient is continuing into Study A4250-008 this will be the last visit in this study and the first visit in Study A4250-008. Visit 9/EOT should also be performed at the time patients are prematurely withdrawn from the study.

Clinic Visit 10 will take place 28 days after Visit 9 for all patients not continuing into Study A4250-008.

Figure 1 Study Design



T: telephone contact; V: study visit

End of Study

The end of the study is defined as follows:

a) End of study in one country: last patient last visit (LPLV) and sites are closed

b) End of study globally: LPLV globally and all sites closed

7.1.2 Schedule of Assessments

The schedule of assessments is presented in Table 1. For information on blood volumes, please see Appendix 7.

Table 1 Schedule of Assessments

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Informed consent[c]	X										
Inclusion/exclusion criteria	X		X								
Demographics	X										
Concomitant medication[d]	X	X	X	X	X	X	X	X	X	X	X
Medical and surgical history	X		X								
Physical examination including voluntary photography	X		X				X			X	
Skin examination	X		X		X		X			X	X
Vital signs[e]	X	X	X		X	X	X	X	X	X	X
eDiary: itching, scratching, and sleep scores[f]	Daily diary entry										
Clinical chemistry[g]	X		X		X	X	X	X		X	X
Hematology[g]			X		X	X	X	X		X	X
Urinalysis[g]		X					X			X	
Serum bile acids[h]	X	X	X		X	X	X	X	X	X	X

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
INR		X			X		X		X		X
Autotaxin[i]			X		X					X	
p-C4[i]			X		X					X	
AFP			X							X	
Vitamins A & E[h]		X					X			X	
25-hydroxy vitamin D			X				X			X	
Blood sample for A4250 PK[i]					X					X	
Abdominal Ultrasound			X							X	
Fibroscan® (where available)			X							X	
QoL questionnaire (PedsQL)			X							X	
Patient/Caregiver/Clinician Global Impression of Change					X		X			X	
Patient/Caregiver/Clinician Global Impression of Symptoms			X		X		X			X	
Pregnancy test[j]	X[j]	X	X		X	X	X	X	X	X[j]	X

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Study drug dispensed[k]			X		X	X	X	X	X		
Adverse events[l]	Continuous collection										
Study drug compliance					X	X	X	X	X	X	

AFP: alfa-fetoprotein; BMI: body mass index; eCRF: electronic case report form; eDiary: electronic diary; EOT: End of Treatment; ICF: informed consent form; INR: international normalized ratio; p-C4: plasma 7α Hydroxy 4-cholesten-3-one; PedsQL: Pediatric Quality of Life Inventory; PK: pharmacokinetic; QoL: quality of life; RR: respiratory rate; SAE: serious adverse event; s-BA: serum bile acid.

a Must also be performed at the time a patient is prematurely withdrawn from the study. This visit is the same as Visit 1 in the extension protocol (Study A4250-008).

b Assessments must be performed 28 days following the final dose of study drug. This visit is omitted if patients are entering Study A4250-008.

c The ICF (and assent) must be signed before any study procedures are performed.

d Past (within 1 month prior to Visit 1) and current medication will be documented in the eCRF.

e Includes blood pressure, pulse, RR, temperature, height (or length depending on age), and body weight (using a certified weight scale). BMI will be calculated.

f Includes training on use of the device at Screening (Visit 1) and review of patient daily entries during 14 days prior to Clinic Visit 3 to determine eligibility.

g See Table 3 for detailed parameters.

h Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA and vitamin A. Exceptions can be made for infants, less than 12 months of age, if unable to fast for the full 4 hours. All s-BA results during the treatment period and follow-up will be blinded.

i Samples will not be collected for patients ≤10 kg. In the event of an SAE, a PK sample should be collected as close to the onset of the event as possible.

j For girls who have reached menarche. Serum test at Visit 1; urine test at other visits. A serum pregnancy test will also be conducted at Visit 9 for patients entering Study A4250-008. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy.

k Study drug to be taken once daily on Day 1 through Day 167 as described in Section 8.2.

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**

1 Adverse event information will be collected from the time of signing of the ICF to study discontinuation.

7.1.3 Study Procedures and Assessments

7.1.3.1 Screening Period (Days -56 to 0)

Day -56 to Day -35/Clinic Visit 1

Patients will undergo a Screening Visit up to 56 days prior to the planned first day of study treatment. Screening procedures and assessments are as follows:

- Obtain written informed consent
- Assess inclusion/exclusion criteria (Section 7.2)
- Record demographics (age, full date of birth, gender, race, and ethnicity)
- Document concomitant medications
- Medical and surgical history (date of diagnosis of PFIC Types 1 or 2, prior investigational medications for PFIC, historical liver function test [LFT] values, any surgery performed including biliary diversion (if applicable), any other diagnosis, and historical liver biopsy data)
- Collect, if available, past confirmatory clinical genetic laboratory report for PFIC-1/PFIC-2 and send to central reader for review. If a historical report is equivocal, unavailable, or unobtainable, a blood sample for clinical genetic testing will be collected to determine eligibility
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- eDiary training and compliance requirements; caregivers and, if applicable, patients begin daily recording of pruritus using the eDiary (Appendix 2)
- Clinical chemistry (Section 10.2.1)
- s-BA (Section 9.2.1)
- Serum pregnancy test for girls who have reached menarche. Please see Appendix 6 for contraceptive requirements
- AE monitoring

Day -28 to Day -7/Clinic Visit 2

A second Screening Visit will be performed. Screening procedures and assessments are as follows:

- Review concomitant medications
- Vital signs (Section 10.2.6)

- Albireo PRO/ObsRO eDiary review for compliance
- Urinalysis (Section 10.2.1)
- s-BA (Section 9.2.1)
- International normalized ratio (INR)
- Vitamins A and E
- Urine pregnancy test for girls of who have reached menarche
- AE monitoring

7.1.3.2 Treatment Period (Day 0 to Day 168)

Study Day 0/Clinic Visit 3 (Randomization)

At the randomization visit, the following assessments will be conducted:

- Review inclusion/exclusion criteria (Section 7.2)
- Review concomitant medications, medical and surgical history
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance and entrance criteria requirement (see Inclusion Criteria 4, Section 7.2.1)
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin and p-C4 (samples will not be taken for patients ≤10 kg)
- 25-hydroxy vitamin D
- Alfa-fetoprotein (AFP)
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)
- Administer QoL (Pediatric Quality of Life Inventory [PedsQL]) questionnaire
- Patient/caregiver/clinician complete the patient global impression of symptoms (PGIS)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Study drug is dispensed and patients are instructed to administer daily from Day 1

Study Day 14 (Telephone Contact)

A study nurse will contact the patient/caregiver by telephone for monitoring of the following:

- Review concomitant medications
- Albireo PRO/ObsRO eDiary review for compliance
- AEs

Study Day 28 and Day 84/Clinic Visits 4 and 6

The following procedures and assessments will be performed:

- Review of concomitant medications
- Physical examination, including voluntary photography (at Clinic Visit 6 only; Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- Urinalysis (at Clinic Visit 6 only; Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- Autotaxin, p-C4, and A4250 pharmacokinetic (PK) assessment (at Clinic Visit 4 only; samples will not be taken for patients ≤10 kg)
- Vitamins A and E, and 25-hydroxy vitamin D (at Clinic Visit 6 only)
- Patient/caregiver/clinician complete the patient global impression of change (PGIC) and PGIS
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 56 and Day 126/Clinic Visits 5 and 7

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance

- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 154/Clinic Visit 8

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- s-BA (Section 9.2.1)
- INR
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 168/Clinic Visit 9/EOT

The last dose of study drug is taken in the morning the day before Clinic Visit 9, and the following procedures and assessments will be performed (also performed at the time a patient prematurely withdraws):

- Review of concomitant medications
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry, hematology, and urinalysis (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin, p-C4, and A4250 PK assessment (samples will not be taken for patients ≤10 kg)

- AFP
- Vitamins A, E, and 25-hydroxy vitamin D
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)
- Administer PedsQL questionnaire
- Patient/caregiver/clinician complete the PGIC and PGIS
- Urine pregnancy test for girls who have reached menarche, or serum pregnancy test for girls continuing to study A4250-008
- AE monitoring
- Evaluation of study drug compliance

7.1.3.3 Follow-Up Period

Study Day 196/Clinic Visit 10

All patients not continuing into Study A4250-008 will return to the study site 28 days after Clinic Visit 9 or the last dose of study drug for the following assessments:

- Review of concomitant medications
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- Urine pregnancy test for girls who have reached menarche
- AE monitoring

7.2 Study Population

A total of approximately 60 patients with a clinical diagnosis of PFIC Type 1 or 2 that have been genetically confirmed will be randomized. The study will target 15% of enrolled patients to be PFIC-1 patients. If enrollment of all PFIC-2 patients is completed and the 15% enrollment of PFIC-1 patients has not yet been achieved, then enrollment of PFIC-2 patients will continue in order to reach the total study target enrollment.

An attempt will be made to randomize a minimum of 10 patients from each age category (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years).

A maximum of 5 patients that were treated in Study A4250-003 will be allowed to participate in this study.

7.2.1 Inclusion Criteria

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have clinical genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples at least 7 days apart (Visits 1 and 2) prior to randomization
4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the eDiary average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients will be expected to have a consistent caregiver for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

7.2.2 Exclusion Criteria

1. Patient with pathologic variations of the *ABCB11* gene that predict complete absence of the BSEP protein
2. Patient with past medical history or ongoing presence of other types of liver disease including, but not limited to, the following:
   a) Biliary atresia of any kind
   b) Benign recurrent intrahepatic cholestasis, indicated by any history of normal s-BAs
   c) Suspected or proven liver cancer or metastasis to the liver on imaging studies
   d) Histopathology on liver biopsy is suggestive of alternate non-PFIC related etiology of cholestasis
3. Patient with a past medical history or ongoing presence of any other disease or condition known to interfere with the absorption, distribution, metabolism (specifically bile acid metabolism), or excretion of drugs in the intestine, including but not limited to, inflammatory bowel disease

4. Patient with past medical history or ongoing chronic (i.e., >3 months) diarrhea requiring intravenous fluid or nutritional intervention for treatment of the diarrhea and/or its sequelae
5. Patient has a confirmed past diagnosis of infection with human immunodeficiency virus or other present and active, clinically significant, acute, or chronic infection, or past medical history of any major episode of infection requiring hospitalization or treatment with parenteral anti-infective treatment within 4 weeks of treatment start (study Day 1) or completion of oral anti-infective treatment within 2 weeks prior to start of Screening Period
6. Any patient with suspected or confirmed cancers except for basal cell carcinoma, and non-liver cancers treated at least 5 years prior to Screening with no evidence of recurrence
7. Patient with a past medical history of chronic kidney disease with an impaired renal function and a glomerular filtration rate <70 mL/min/1.73 m^2
8. Patient with surgical history of disruption of the enterohepatic circulation (biliary diversion surgery) within 6 months prior to start of Screening Period.
9. Patient has had a liver transplant or a liver transplant is planned within 6 months of randomization
10. Decompensated liver disease, coagulopathy, history or presence of clinically significant ascites, variceal hemorrhage, and/or encephalopathy
11. INR >1.4 (the patient may be treated with Vitamin K intravenously, and if INR is ≤1.4 at resampling the patient may be randomized)
12. Serum ALT >10 × upper limit of normal (ULN) at Screening
13. Serum ALT >15 × ULN at any time point during the last 6 months unless an alternate etiology was confirmed for the elevation
14. Total bilirubin >10 × ULN at Screening
15. Patient suffers from uncontrolled, recalcitrant pruritic condition other than PFIC. Examples include, but not limited to, refractory atopic dermatitis or other primary pruritic skin diseases
16. Any patient who is pregnant or lactating or who is planning to become pregnant within 24 weeks of randomization
17. Sexually active males and females who are not using a reliable contraceptive method with ≤1% failure rate (such as hormonal contraception, intrauterine device, or complete abstinence) throughout the duration of the study and 90 days thereafter (from signed

informed consent through 90 days after last dose of study drug). See Appendix 6 for further details

18. Patient with a past medical history of alcohol or substance abuse will be excluded. Patient must agree to refrain from illicit drug and alcohol use during the study
19. Administration of bile acid or lipid binding resins and medications that slow GI motility (Refer to Appendix 1 – Concomitant Medications Guidelines)
20. Patient has had investigational exposure to a drug, biologic agent, or medical device within 30 days prior to Screening, or 5 half-lives of the study agent, whichever is longer
21. Patient who has been previously treated with an IBAT inhibitor whose pruritus has not responded to treatment
22. Any other conditions or abnormalities which, in the opinion of the investigator or Medical Monitor, may compromise the safety of the patient, or interfere with the patient participating in or completing the study

7.2.3 Withdrawal of Patients

Patients/caregivers will be informed that they have the right to withdraw from the study at any time, without prejudice to their medical care, and that they are not obliged to state their reasons.

Any withdrawal, and reasons for withdrawal, must be fully documented in the electronic case report form (eCRF) and source documents and the patient followed by the investigator/investigative staff. Even after the study is completed at the Follow-up Visit, the investigator/investigative staff will, regardless of reason for withdrawal, record any patient data they receive concerning SAEs, and all drug-related non-serious AEs, and subsequently report these according to Section 10.1. Withdrawn patients will not be replaced.

Patients will be withdrawn in the following circumstances:

- A patient's/caregiver's desire for withdrawal for any reason
- Lost to follow-up (every effort must be made to contact the patient/caregiver; a certified letter must be sent)
- An AE that, in the opinion of the investigator, necessitates withdrawal
- A worsening of the symptoms related to diarrhea occurs
- Death
- Patient/caregiver estimation of intolerable symptoms
- A patient's/caregiver's substantial noncompliance (eDiary and study drug compliance) or protocol violation

- An investigator's opinion that continuing the patient in the study is not appropriate. The investigator may withdraw a patient at any time, if it is considered to be in the patient's best interest

A patient who prematurely withdraws due to intolerable symptoms and has completed at least 12 weeks treatment in the double-blind study, will be offered the opportunity to enter open-label Study A4250-008. A patient who withdraws from treatment prematurely and does not enter Study A4250-008 will have EOT (Visit 9) assessments at the time of withdrawal and a follow-up assessment (Visit 10) 28 days following the last dose of study drug, and the patient will be recorded as withdrawn.

7.2.4 Study Termination by Sponsor

This study may be terminated at any time by Albireo if serious side effects should occur, if the investigator does not adhere to the protocol; or if, in the sponsor's judgment, there are no further benefits to be achieved from the study. In this event, Albireo/designee will inform the study investigators, institutions, and all regulatory authorities.

Albireo may temporarily or permanently discontinue the study at an investigative site at any time for safety, ethical, compliance, or other reasons. If this is necessary, Albireo will endeavor to provide advance notification to the site. If a site or the study is suspended or discontinued, the investigator/investigative staff will be responsible for promptly informing the Independent Ethics Committee (IEC). If required by local regulations, Albireo/designee will be responsible for informing the IEC and the regulatory authority of study or site discontinuation. In such an event, all study data and unused study drug must be returned to Albireo.

8 TREATMENT OF PATIENTS

8.1 Identity of Study Drug

A4250 and placebo will be supplied as capsules for oral administration. White opaque capsules filled with pellets containing A4250 or placebo will be provided. Two different capsule sizes will be available, as follows:

- Capsule size 0 that can be opened
- Capsule size 3 that should be swallowed intact. These size 3 capsules may be opened only under exceptional circumstances, e.g., patient cannot swallow capsule intact

The content of the A4250 and placebo capsules will be identical in appearance. Capsule filling weight will also be identical for high dose, low dose, and placebo. Bottles with 34 capsules will be given to the patient at each visit. A patient who requires 2 or more capsules per day will be given multiple bottles. Refer to the investigational product manual.

8.2 Administration of Study Drug

Patients will be dosed with A4250 at a dose of 40 µg/kg/day, 120 µg/kg/day, or placebo for 24 weeks. Study drug will be dispensed to the patient at Visits 3, 4, 5, 6, 7, and 8, together with instructions on how to store and take the drug. Study drug administration data, including whether each patient took each dose or partial doses of study drug, whether there were any delayed or missed doses, and whether the capsule was opened or swallowed whole will be documented through the eDiary and transferred to the study database.

A4250 should be taken in the morning, together with food. On clinic visits days when laboratory assessments are conducted, study drug should be taken after the visit and after laboratory samples are taken. Patients should not crush or chew the capsule(s). When swallowing the capsule intact, the patient should administer the dose with a glass of water.

For a patient unable to swallow capsule intact, the capsule can be opened and the contents sprinkled and mixed in a small amount of soft room-temperature yogurt, apple sauce, or fruit purée. A list of food options can be found in the Pharmacy manual. The amount of food should be less than what the patient/infant is expected to eat and should be followed by water.

1. Hold the capsule over the prepared food, gently twist open and allow the content to fall into food
2. Mix the entire capsule content with 1 or 2 tablespoons (15 to 30 mL) of soft food (apple sauce, fruit purée, or yogurt)
3. Administer the mixture immediately after mixing.

The number of capsules provided to the patient will be based on body weight at Visit 3. At Visit 6, if a patient's weight has shifted to another weight category (identified in Table 2), the number of capsules per day may be adjusted.

Table 2 Dosing and Capsule Strength

Body Weight (kg)	Capsule Size	Number of Capsules per Day	Capsule Strength, Low Dose (µg)	Total Dose (µg)	Capsule Strength, High Dose (µg)	Total Dose (µg)	Capsule Size, Placebo (µg)
5.0 to <7.5	0	1	200	200	600	600	0
7.5 to <12.5	0	2	200	400	600	1200	0
12.5 to <17.5	0	3	200	600	600	1800	0
17.5 to <19.5	0	4	200	800	600	2400	0
19.5 to <25.5	3	2	400	800	1200	2400	3
25.5 to <35.5	3	3	400	1200	1200	3600	3
35.5 to <45.5	3	4	400	1600	1200	4800	3
45.5 to 55.5	3	5	400	2000	1200	6000	3
>55.5	3	6	400	2400	1200	7200	3

8.3 Study Treatment Packaging and Labeling

8.3.1 Packaging and Labeling

The capsules will be packed in high-density polyethylene containers, with child-proof polypropylene caps. Study drug capsules containing A4250 in the strength specified in Table 2 above will be manufactured.

Packaging and labeling will be prepared to comply with applicable regulatory requirements.

8.3.2 Storage

Treatment packs containing A4250 capsules should be stored and dispensed in accordance with regulations in their original containers. The storage facility at the investigative site should be locked and the storage temperature should be between 15°C and 25°C.

Patients/caregivers should be informed of appropriate storage conditions (i.e., room temperature, between 15°C and 25°C).

Any deviations from the recommended storage conditions should be immediately reported to Albireo and the study drug should not be used until authorization has been given by Albireo.

8.3.3 Blinding and Randomization of Study Drug

A double-blind design is employed so that both the investigators and the patients will be unaware of the treatment assignment during the study.

After written informed consent is obtained from an eligible patient, an 8-digit patient identification number will be assigned. The first 2 digits will denote the country, followed by a 3-digit site number and a 3-digit patient sequence number. This number will be created and

allocated by the Interactive Web Response System (IWRS), when the patient first enters the system at Screening. The randomization codes will be computer-generated by [redacted] Biostatistics and kept by a statistician independent from the project team. The randomization will be done in blocks and stratified according to PFIC class (Type 1 and 2) and age class (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) to ensure approximate balance between dose schemes (1:1:1). A separate randomization list will be prepared for the maximum 5 patients from Study A4250-003 regardless of stratification. Randomization codes will be assigned sequentially as patients become eligible for randomization.

Patients who are eligible for randomization will be assigned a unique 4-digit randomization number by IWRS. This randomization number identifies which treatment will be allocated to the patient. Patients who withdraw from the study after randomization visit are not to be replaced and their randomization number will not be reused.

To ensure blinding, the study drug and the matching placebo have the same shape and size. Patients will receive capsule(s) of A4250 according to their dose group or capsule(s) of matching placebo once a day during the double-blind Treatment Period. Labels on the study drug containers will not identify the treatment that a patient is randomized to. Traceability of the treatment is ensured by the study drug number.

The 5-digit study drug number will identify study drug packs and will be detailed on the study drug label. Dispensing of study drug will be coordinated by IWRS.

The system will assign a study drug number corresponding to the randomization arm. The randomization number will be used in the background only to ensure no unblinding and will not be displayed to end-users in the IWRS.

8.4 Procedure for Breaking the Randomization Code

Should a situation arise where unblinding is urgently required, i.e., only where knowledge of the study drug is required to adequately manage a life-threatening situation, the investigator at that study site may perform immediate unblinding through IWRS. The responsibility to break the treatment code in emergency situations resides solely with the investigator. The investigator should make every effort to discuss the rationale (status and outcome) for emergency unblinding with the sponsor Medical Monitor or the [redacted] Medical Monitor as soon as possible to review the individual patient details.

The study site investigator and appropriate [redacted] project team members will be authorized to access the emergency unblinding functionality within the IWRS. Emergency unblinding takes place in the IWRS via a dedicated emergency unblinding module. The system will require the user to enter their emergency unblinding, which is typically provided at the same time login credentials are distributed. The exact description of the treatment assigned to the individual patient then will be accessible to the user. Once the randomization code is broken for a patient, he/she must be withdrawn from the study and all assessments and procedures at EOT should be performed. As a follow-up, Visit 10, will be performed according to the time

schedule. Once a randomization code has been broken, the investigator must inform the [redacted] and sponsor Medical Monitor in writing within 24 hours. In case of technical issues accessing the system online, please see the IWRS site user manual for country-specific contact telephone numbers for the [redacted] HelpDesk 24/7 system support.

8.5 Patient Compliance

The study nurse will monitor eDiary compliance by routine review of the CRF health website. If both diary entries on a day are missing, the study nurse will call the patient/caregiver to remind them to complete all scheduled entries. Any noncompliance will be documented and explained in the source documents.

Study drug compliance will also be checked through diary responses. Additionally, patients will return all unused study drug at Visits 4, 5, 6, 7, 8, and 9. The study site staff will count all returned drug, assess compliance, and record details in the eCRF.

Treatment Compliance = 100 × ((Number of study drug dispensed – number of study drug returned)/number of study drug that should be taken).

Treatment compliance between 80% and 120% will be acceptable.

8.6 Study Drug Accountability

Records shall be maintained of the delivery of study treatment(s) to the study site(s), of the inventory at the study site(s), of each use of the study treatment(s) for each patient, and of the return of unused study treatment(s) to Albireo.

These records shall include dates, quantities, batch numbers, expiry dates, and the unique code numbers assigned to the study drug and to the study patients.

The investigator will be responsible for ensuring that the records adequately document that the patients were provided the quantities specified in the protocol and that all study drug received from Albireo is reconciled.

8.7 Concomitant Therapy and Prohibited Medications

The investigator will note all ongoing medication and any medication recently stopped (within 1 month prior to Visit 1) in the eCRF. At Visits 2 through 10, all changes in medication (stopping or starting new medication or changes in dose) will be recorded in the eCRF. All medications (prescribed or over the counter) for pruritus and sleep will be recorded.

All medications taken by a patient within 3 months prior to the first intake of study drug are regarded as prior medication.

All medications taken by a patient on or after the first intake of study drug, and which continue to be taken during the study, are regarded as concomitant medication.

Prohibited medications are listed in Appendix 1.

9 ASSESSMENT OF EFFICACY

9.1 Efficacy Endpoints

9.1.1 Primary Efficacy Endpoint

The primary efficacy endpoints are as follows:

- **European Union (EU) and rest of the world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  Fasting s-BA baseline will be calculated as the average of the value at randomization and the previous visit (Clinic Visit 2). The end value is the average of the values at Weeks 22 and 24 after the start of double-blind treatment.

- **United States (US)**: Proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period. A positive pruritus assessment is defined as a scratching score of ≤1 or at least a one-point drop from baseline on the Albireo ObsRO instrument. At each assessment, the AM score will be compared to the AM baseline, and the PM score will be compared to the PM baseline. Both AM baseline and PM baseline pruritus assessments will be included in the analysis of this endpoint. AM scores from the 14 days prior to the first dose of study medications will be averaged as AM baseline. PM scores from the 14 days prior to the first dose will be averaged as PM baseline.

9.1.2 Secondary Efficacy Endpoint

Secondary efficacy endpoints include the following:

- **EU and RoW**: Proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period. A positive pruritus assessment is defined as a scratching score of ≤1 or at least a one-point drop from baseline on the Albireo ObsRO instrument. At each assessment, the AM score will be compared to the AM baseline, and the PM score will be compared to the PM baseline. Both AM baseline and PM baseline pruritus assessments will be included in the analysis of this endpoint. AM scores from the 14 days prior to the first dose of study medications will be averaged as AM baseline. PM scores from the 14 days prior to the first dose will be averaged as PM baseline.

- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

- **All Regions:**

All secondary endpoints are compared to placebo.

- Change from baseline to Week 12 and to Week 24 in fasting serum bile acids
- Change from baseline to Week 12 and to Week 24 in serum ALT concentration
- Change in growth from baseline to Week 12 and to Week 24
- Proportion of responders for pruritus scores at Weeks 12 and 24 based on the Albireo PRO and ObsRO instruments
- Change in sleep parameters measured with the Albireo PRO and ObsRO instruments from baseline over the 24-week Treatment Period
- Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level over the 24-week Treatment Period. A positive pruritus assessment includes an itch score ≤1, or at least a one-point drop from baseline based on the Albireo PRO instrument; only patients ≥8 years of age will complete the Albireo PRO instrument
- Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Proportion of individual AM assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Proportion of individual PM assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Number of patients undergoing biliary diversion surgery or liver transplantation

9.1.3 Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the PGIC items (Appendix 3)
- Change from baseline in fasting s-BA at Week 4

- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline by each 4 week-interval of the Treatment Period as measured by the Albireo ObsRO instrument
- Change from baseline by each 4-week interval of the Treatment Period in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline by each 4-week interval of the Treatment Period in patient-reported and observer-reported morning time itching and scratching severity scores, respectively
- Change from baseline by each 4-week interval of the Treatment Period in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline by each 4-week interval of the Treatment Period in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in PedsQL (Appendix 4)
- Change from baseline in serum ALT concentration at Week 4
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST and GGT at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, p-C4) at Week 24
- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events
- All-cause mortality

9.2 Efficacy Assessments

9.2.1 Serum Bile Acids

Blood samples for analysis of fasting total s-BA will be drawn at all visits. Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA. Exceptions can be made for infants, <12 months of age, if unable to fast for the full 4 hours. Any visit at which a bile acid sample result is unreportable, an additional unscheduled visit for a repeat sample collection may be scheduled. All s-BA results during

the treatment period and follow-up will be blinded. Samples will be processed and transported to a central laboratory per instructions in the laboratory manual.

9.2.2 Itching, Scratching and Sleep Score

Itching, observed scratching, and sleep disturbance will be recorded twice each day via eDiary. Patients and/or caregivers will be instructed to complete the eDiary every day in the morning after the patient wakes and in the evening just before the patient goes to sleep throughout the study. Patients and caregivers will receive a formal training session during Visit 1.

The eDiary includes Albireo ObsRO and PRO items. Patients <8 years of age will not be asked to complete the Albireo PRO items; only the Albireo ObsRO will be completed by caregivers of patients in this age group. Older patients, 8 to 18 years of age, will complete the Albireo PRO items and the caregiver will complete the Albireo ObsRO items. The Albireo PRO items assess severity of itch, aspects of sleep disturbance (morning diary only), and tiredness. For patients 8 to 12 years of age, the caregiver will read the Albireo PRO items along with the child and record the child's response. A guide will be provided to the caregivers that provides standardized explanations of the Albireo PRO items, in case the patient is confused or requires clarification about the meaning of a question. The Albireo ObsRO items assess severity of observed scratching, aspects of observed sleep disturbance (morning diary only), and observed signs of tiredness (evening diary only). The Albireo ObsRO and PRO scratching and itch severity items use 0 to 4 response scales, where each response is distinguished by a unique facial expression, verbal anchor, number, and color code (Appendix 2). To meet eligibility criteria, a scratch score will be calculated from daily diary entries (ObsRO items) the 14 consecutive days prior to randomization.

A daily AM & PM score for the Albireo ObsRO scratching item will be averaged from the 2 ratings for each day. A daily score will be considered missing if both of the daily assessments are missing. A weekly score will be calculated by averaging the daily scratching scores. A weekly score will be considered missing if ≥4 out of 7 days a week of data are missing. For AM & PM baseline, a 14-day daily score of AM & PM prior to the first dose of study medications will be averaged as the baseline score.

For AM baseline, a 14-day AM score prior to the first dose of study medications will be averaged as the AM baseline score. The same approach will be used for PM baseline and to calculate a patient-reported itch severity score. The handling of missing data is described in Section 11.2.2.2.

9.2.3 Growth

Growth will be measured as height (velocity) and weight (Z-score) using a certified weight scale. BMI will be calculated by weight (kg) / height $(m)^2$. Change will be defined as linear growth deficit (weight and BMI for age) compared to a standard growth curve (Z-score, SD from P50).

9.2.4 Quality of Life Questionnaire (PedsQL)

Patients and caregivers will be asked to complete a QoL questionnaire (PedsQL) at Visit 3 and Visit 9. Details of the questions included on the questionnaire are provided in Appendix 4.

9.2.5 Biomarkers and PK Samples

Blood samples for autotaxin and p-C4 will be drawn at Visits 3, 4, and 9 only for children with body weight >10 kg. Blood for A4250 PK assessment will only be drawn at Visits 4 and 9 (only for children with body weight >10 kg), and as close to the onset of an SAE as possible, should an SAE occur. Samples will be processed and transported to a laboratory per instructions in the laboratory manual.

9.2.6 PELD/MELD Score

At randomization and end of treatment, PELD score will be calculated for children up to 12 years of age. For children 13 years or older, the MELD score will be calculated.

PELD Score = 4.80 * ln(total bilirubin) + 18.57 * ln(INR) - 6.87 * ln(albumin) + 4.36 (if patient <1 year: scores for patients listed for liver transplantation before the patient's first birthday continue to include the value assigned for age (<1 year) until the patient reaches the age of 24 months) + 6.67 (if the patient has growth failure [≤2 SD])

Laboratory values less than 1.0 will be set to 1.0 for the calculation of the PELD score.

MELD Score = 9.57 * ln(creatinine) + 3.78 * ln(total bilirubin) + 11.2 * ln(INR) + 6.43

Laboratory values less than 1.0 will be set to 1.0 and serum creatinine values greater than 4.0 mg/dL (equivalent to 353.6 µmol/L) will be set to 4.0 for calculation of the MELD score.

9.2.7 Fibroscan®

Where available, Fibroscan® will be performed as per institution standard practice at Visits 3 and 9.

9.2.8 Markers of Fibrosis

APRI score and Fib-4 score will be calculated at randomization and at end of treatment.

APRI = [(AST in U/L) / (AST ULN in U/L)] / (Platelets in 10^9/L)

Fibrosis 4 Score = (Age*AST in U/L) / (Platelets in 10^9/L * √(ALT in U/L)).

9.2.9 Global Impression of Change and Global Impression of Symptom Measures

Patients (≥8 years of age), caregivers, and clinicians will complete the PGIC and the PGIS measures (Appendix 3) at randomization (Visit 3; PGIS only), Visits 4 and 6, and at EOT (Visit 9).

The PGIC items assess change in itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) since starting the study drug. The PGIS items assess itch

(patient version), scratching (caregiver and clinician versions), and sleep (all versions) in the past week. Caregivers and clinicians will complete the PGIC and PGIS for all patients; those patients ≥8 years of age will complete the patient version.

10 ASSESSMENT OF SAFETY

The timing and frequency of safety assessments are described in Section 7.1.3 and in Table 1 above.

The primary safety analysis for this study will include the incidence of total treatment-emergent adverse events (TEAE) and TEAEs categorized by causality, severity, and seriousness assessments made by the investigator by comparing study drug exposure to placebo.

Trends in safety will also be evaluated for the following assessments:

- Physical examinations
- Concomitant medications
- Vital signs
- Laboratory test results (including clinical chemistry, hematology, urinalysis, AFP, and vitamins A and E, 25-hydroxy vitamin D, and INR)
- Abdominal ultrasound
- Discontinuations due to AEs

10.1 Adverse Events

10.1.1 Definitions and Investigator Assessments

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 unfavorable and unintended sign, symptom, or disease that occurs once a patient is enrolled (ICF is signed) in the study until the patient is discharged from the study, whether or not related to the study drug.

10.1.1.1 Clinical Significance

Clinical significance is defined as any abnormal finding that results in further clinical investigation(s), treatment(s), or the diagnosis of new condition. Patient-reported events and protocol-mandated laboratory values, vital signs, and physical examination findings can be considered clinically significant (i.e., an AE) if there is a deterioration as compared to baseline. Examples of clinically significant worsening from baseline could include, but is not limited to, events causing withdrawal from the study and events requiring medical intervention outside of the study causing apparent clinical manifestations, or judged relevant by the investigator.

10.1.1.2 Serious Adverse Events

Serious criteria are applied by the investigator to each AE as specifically defined below. These criteria are used to determine whether an AE is serious or non-serious. The assessment

is made independently of severity assessment (Section 10.1.1.3). For example, the development of a severe rash that occurs after signing of informed consent may not meet serious criteria as defined below and therefore would be considered a severe, non-serious AE.

Any AE that meets any 1 of the following 6 criteria is considered an SAE:

- The outcome of the AE is **death**
- The AE is immediately **life-threatening**. Life-threatening means that the patient is, in the opinion of the investigator, at immediate risk of death from the reaction as it occurred. This does not include an AE that, if more severe, might have caused death
- The AE results in persistent or significant **disability/incapacity**. Disability means a substantial disruption of a person's ability to conduct normal life functions
- The AE requires or prolongs **hospitalization**
- The AE results in a **congenital anomaly/birth defect**
- **The AE is an important medical event**. Important medical events may meet serious criteria should the investigator assess that they may significantly jeopardize the patient, represent a significant hazard, or requires medical/surgical intervention to prevent one of the outcomes listed above. Examples of potential SAEs based on this criteria include, but are not limited to, allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions even if they do not result in inpatient hospitalization, or the development of drug dependency and drug abuse.

10.1.1.3 Severity Assessment

Severity assessments are based on the intensity of the event in relation to expectation. The investigator will assess the intensity of AEs based on the following definitions:

- Mild (awareness of sign or symptom, but easily tolerated)
- Moderate (discomfort sufficient to cause interference with normal activities)
- Severe (incapacitating, with inability to perform normal activities)

Severity is a measure of intensity where seriousness is defined by the criteria outlined in Section 10.1.1.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not meet serious criteria, and therefore would be assessed as a severe AE but not an SAE.

10.1.1.4 Causality Assessment

The investigator determines the causality of all AEs to the study drug using medical judgment and considering all relevant factors such as (but not limited to) the underlying study indication, coexisting disease, concomitant medication, relevant history, pattern of the AE, temporal relationship to the study drug, and de-challenge or re-challenge. The causality assessment of the AE/SAE is to be made as follows.

Related to study drug (possibly, probably, or definitely related)

Based on medical judgment, there is at least a reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event follows a reasonable temporal sequence from administration of study drug
- The event could not be reasonably attributed to the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient
- The event follows a known pattern of response to study drug
- The event disappears or decreases on cessation or reduction in dose of the study drug. (It should be noted that in some situations an AE will not disappear or decrease in intensity upon discontinuation of study drug despite other clear indications of relatedness)
- The event reappears or worsens when the study drug is re-administered

Unrelated to study drug (unlikely or unrelated)

Based on medical judgment there is no reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event does not follow a reasonable temporal sequence from administration of study drug
- The event could be reasonably attributed to the known characteristics of the patient's clinical state, concurrent illness, environment or toxic factors, or other modes of therapy administered to the patient
- The event does not follow a known pattern of response to study drug
- The event does not disappear or decrease on cessation or reduction in dose of the study drug, and it does not reappear or worsen when the study drug is re-administered

10.1.2 Recording of Adverse Events

It is the investigator's responsibility to assess whether each untoward event is a clinically significant worsening from baseline, thereby considered an AE. For all AEs, the severity, seriousness, and causality to study drug for each AE as outlined in Section 10.1.1 will be assessed and recorded in the eCRF.

All serious and non-serious AEs are collected once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10) or 28 days after the last dose of study drug.

Any AEs or SAEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator until resolution or stabilization up to the database lock and recorded in the eCRF. Albireo retains the right to request additional information for any patient with ongoing AE(s) or SAE(s) at the end of the study.

TEAEs are defined as any AE that occurs after randomization (Day 0). All AEs that occur in the Screening Period, i.e., after enrollment and prior to randomization, will be collected on the eCRF as non-treatment-emergent AEs.

If there is a clinically significant deterioration of a laboratory value/vital sign or other routine study assessment that is associated with a diagnosis, the clinical diagnosis will be reported as an AE and the associated signs and symptoms will be considered additional information unless the sign or symptom is more severe than expected given the diagnosis. For example, if an investigator diagnoses a study patient with hepatitis during the study period, hepatitis would be considered the AE and the concomitant signs and symptoms of abdominal pain, vomiting, and elevated ALT and AST would not be reported separately unless, in the opinion of the investigator, one of these signs or symptoms is more severe than expected and therefore a separate AE assessment is indicated.

10.1.3 Recording and Reporting of Serious Adverse Events

Every SAE (regardless of severity and causality) that occurs once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10), and 28 days after the final dose of study drug, should immediately and not later than within 24 hours of knowledge of the event, be reported by the investigator or delegate in the SAE report form.

Report of a SAE must include at least the following information:

- Patient identification information (study number, site number, initials, and date of birth [as per local country requirements for data protection])
- The last study drug administration date
- The diagnosis of the event with the description (or a brief description of signs/symptoms/clinical course if the diagnosis is not available) and the date of onset
- The action used to treat the event (i.e., treatment medications, temporary discontinuation)
- The reason(s) for considering the event serious
- The relationship of the event to the study drug or to the study procedure (e.g., the investigator's assessment of causality)
- A brief narrative of the SAE

Follow-up reports including all new information obtained of the subsequent course of the SAE must be prepared and the information collected in the SAE submitted to [redacted] (Pharmacovigilance and Safety Services Department) by e-mail within 24 hours of knowledge of the event.

The pharmacovigilance manager (PVM) may contact the investigator to obtain further information on a reported SAE. The investigator/investigative staff must respond to any

request for follow-up information or answers to questions regarding the SAE within the same timelines as for initial reports.

The PVM reports the occurrence of the SAE and follow-up to the Albireo medical monitor for medical assessment of the case.

Should an outcome of death occur within the study period or within 28 days after the last administration of study drug, an SAE form should be completed including the main and contributory causes of death.

All SAE reports must be e-mailed to the following e-mail address within **24 hours**:

[redacted]

If email is unavailable, SAEs may be transmitted via fax to the following number:

[redacted]

In a study-related medical emergency situation, when the assigned medical monitors for the study cannot be reached by a caller, an on call physician can be reached 24 hours per day, 7 days per week, via the [redacted] call center. The following number is a chargeable telephone number allowing a global reach from both landlines and mobile phones and the internet page indicated below contains a list of country-specific toll-free telephone numbers that is provided. It should be noted that not all countries globally have access to toll-free numbers as indicated on the "24/7 Medical Help desk" index. Countries without toll-free numbers need to dial the chargeable number as indicated below. Furthermore, toll-free numbers might not be available from mobile phones.

[redacted]

10.1.4 Reporting of Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reaction (SUSAR) is an SAE that occurs in a patient, the nature or severity of which is not expected per the applicable product information (e.g., the IB for an unauthorized investigational product or summary of product characteristics for an authorized product).

Reporting and tracking of SUSARs will be in accordance to all applicable competent authority regulations. The Ethics Committee and all investigators involved in this study will be informed according to national and international requirements.

10.2 Laboratory Values/Vital Signs/Physical Examinations and Other Safety Assessments

10.2.1 Laboratory Assessments

Samples will be collected for clinical chemistry, hematology, and urinalysis testing at the time points specified in Table 1. The parameters assessed are presented in Table 3.

Blood for AFP will be drawn at Visits 3 and 9. Fat-soluble vitamin levels including vitamins A and E, and 25-hydroxy vitamin D will be drawn at Visit 2 (vitamins A & E only), Visit 3 (25-hydroxy vitamin D only), Visit 6, and Visit 9. Blood for INR (surrogate for vitamin K) will be drawn at Visits 2, 4, 6, 8, and 10. Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for vitamin A. Exceptions can be made for infants, <12 months of age, if unable to fast for the full 4 hours. If a patient has any vitamin level(s) that are outside of the reference range, vitamin supplementation adjustments may be required. Target ranges and supplementation strategy guidelines can be found in Appendix 5.

A serum pregnancy test will be performed at Visit 1 and Visit 9 if the patient is entering the A4250-008 study. A urine pregnancy test will be collected at all other visits for girls who have reached menarche. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy. Study drug administration will be held pending the confirmatory results. If the serum pregnancy test is negative, the patient can resume dosing. If the serum pregnancy test is positive, the patient should be discontinued (see Section 10.2.9).

All samples will be processed and transported to a laboratory per instructions in the laboratory manual.

The observed values will be recorded and assessed as “normal” or “abnormal not clinically significant” or “abnormal clinically significant”.

Additional safety blood samples may be needed due to follow-up of an abnormal value or analysis failure. The blood samples collected for safety laboratory analysis will be destroyed after the analyses have been completed.

Table 3 Routine Laboratory Parameters

Clinical Chemistry	Hematology	Urinalysis
• Albumin • ALT • Alkaline phosphatase • AST • Bilirubin – total and direct • Calcium • Chloride • Creatinine • Creatine kinase • Gamma-glutamyl transferase • Potassium • Sodium	• Hematocrit • Hemoglobin • Platelet count • Red blood cell count • White blood cell count and differential (neutrophils, eosinophils, basophils, lymphocytes, and monocytes)	• Blood • Glucose • Ketones • Leukocytes • Nitrites • pH • Protein

10.2.2 Individual Patient Safety Monitoring

10.2.2.1 Liver Monitoring

Strategies to monitor markers of liver disease throughout the study are outlined below where the ULN will be based on central laboratory reference values for age and gender.

If isolated transaminase elevations are observed, defined as:

1. Normal bilirubin AND absence of clinical hepatitis symptoms AND
   - ALT or AST ≥5 × ULN (if normal at baseline) or an absolute threshold of 800 U/L, whichever comes first
   - OR ALT or AST ≥3 × baseline (if abnormal at baseline) or an absolute threshold of 800 U/L, whichever comes first

Then:

a) Repeat liver profile (AST, ALT, bilirubin, and prothrombin time [PT] or INR) within 2 to 3 days

b) Evaluate creatine phosphokinase (CPK) and lactate dehydrogenase (LDH)

c) As needed (for example, persistent ALT/total bilirubin elevation or worsening of liver function), consider evaluation for alternative etiologies

Monitor the patient using close observation found in Section 10.2.2.2

If any ONE of the following criteria are met:

1. Transaminases (ALT or AST ≥3 × baseline or 800 U/L, whichever comes first) AND bilirubin (total bilirubin > 2 × ULN) elevations

2. Transaminase elevations alone (ALT or AST >10 × ULN or 5 × baseline or absolute threshold of 800 U/L, whichever comes first) in presence of normal LDH and CPK
3. Total bilirubin increased, unrelated to hemolysis (elevated reticulocyte count) or established genetic diseases, such as Gilbert's syndrome
   a. Doubling if total bilirubin was <3 mg/dL (equivalent to 51.3 µmol/L) at baseline
   b. OR Increase by >3 mg/dL (equivalent to 51.3 µmol/L) if total bilirubin was ≥3 mg/dL (equivalent to 51.3 µmol/L) at baseline
4. INR increase refractory to vitamin K administration
   a. INR >1.5 if INR was normal at baseline
   b. OR Increase by >0.4 if INR was abnormal at baseline
5. Any increase in total bilirubin and transaminases if accompanied by EITHER a symptom of clinical hepatitis (e.g., vomiting, nausea, right upper quadrant pain) OR immunological reaction (rash or >5% eosinophilia)

Then:

a) Interrupt study medication

b) Initiate drug-induced liver injury work-up for alternative etiologies

c) Repeat liver profile (AST, ALT, total bilirubin, direct bilirubin) and PT or INR within 48 to 72 hours

d) Monitor the patient using close observation found in Section 10.2.2.2

e) If a patient lives in a remote area, they may be tested locally and the results communicated to the investigator site promptly

10.2.2.2 Close Observation

- Repeat liver enzyme and serum bilirubin tests two or three times weekly. Frequency of re-testing can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the subject is asymptomatic
- Obtain a more detailed history of symptoms and prior or concurrent diseases
- Obtain a history of concomitant drug use (including non-prescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets
- Consider ruling out alternate etiology including acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; non-alcoholic steatohepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease
- Obtain a history of exposure to environmental chemical agents.

- Obtain additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin)
- Consider gastroenterology or hepatology consultations.

10.2.2.3 De-challenge/Re-challenge for Liver and Clinical Hepatitis Monitoring

1. Re-challenge is not recommended:
   a. If a patient has had possible/probable drug-induced liver injury
   b. If a decompensation event has occurred (i.e., variceal hemorrhage, ascites, hepatic encephalopathy, etc.).
2. If due to underlying cholestatic liver disease variability or another alternative etiology is identified AND liver tests returned to baseline, re-challenge may be considered after consultation with the sponsor Medical Monitor.
3. If the ALT/total bilirubin elevations are observed after re-challenge, then repeat re-challenge is discouraged.

If patient is permanently discontinued, monitoring should be continued as outlined in Section 10.2.2.5.

10.2.2.4 Diarrhea

Study drug should be discontinued if a patient develops diarrhea with at least 1 of the following concomitant signs or symptoms: grossly bloody stools, vomiting, dehydration requiring treatment with oral or intravenous rehydration and/or electrolyte imbalances, fever (≥38 C) and/or the diarrhea persists for 7 or more days.

If there is gross blood in stool, INR and platelets should be measured.

Study drug will be reintroduced (re-challenge) when the symptoms have resolved. If the diarrhea re-occurs within 1 week after the re-challenge with no alternate etiology, the patient will be permanently discontinued and monitored as outlined in Section 10.2.2.5.

10.2.2.5 Monitoring after Permanent Discontinuation of Study Drug Due to Safety

Once study drug is permanently discontinued for liver, hepatitis, diarrhea, or other severe AE related to study drug, the patient will be monitored weekly until the laboratory and clinical parameters have normalized/stabilized.

10.2.3 Demographics/Medical and Surgical History

Demographic information per country regulations (age, full date of birth, gender, race, and ethnicity), along with medical and surgical history, will be obtained and recorded in the eCRF at Visit 1.

Medical and surgical history will be entered in the eCRF at Visit 1. This includes date of diagnosis of PFIC, prior investigational medications for PFIC, historical (3 months prior to

Screening) LFT values (AST, ALT, and total bilirubin), ongoing medication, any surgery performed, any other diagnosis, and historical biopsy data.

If a liver biopsy has been performed within 1 year prior to Screening, or during the study, the results will be recorded in the eCRF.

10.2.4 Clinical Genetic Testing

For previous confirmatory clinical genetic testing results for PFIC Type 1 or 2 performed prior to Screening Visit 1, the confirmatory clinical genetic testing laboratory report will be verified to determine eligibility.

If the historical clinical genetic result is equivocal, unavailable, or unobtainable, clinical genetic analysis to confirm pathologic biallelic variations in the *ATP8B1* or *ABCB11* genes will be performed and verified to determine eligibility. No other diagnostic genetic testing will be offered.

10.2.5 Physical Examination

A physician or suitably trained qualified assistant will perform a complete physical examination, including voluntary photography of skin lesions due to scratching, at Visits 1, 3, 6, and 9. A skin examination will be conducted at Visits 1, 3, 4, 6, 9, and 10 (see Table 1).

A complete physical examination will include assessment of general appearance, eyes, ears, nose, throat, head/neck/thyroid, lymph nodes, cardiovascular, lungs/chest, abdomen, genitourinary, extremities, skin, musculoskeletal, neurologic, and other.

Skin will be examined thoroughly and excoriations/scratch marks recorded.

10.2.6 Vital Signs

Evaluation of vital signs will be performed at all visits. This includes blood pressure (systolic and diastolic), pulse, respiratory rate, temperature, height (or length depending on age), and body weight (using a certified weight scale) at clinic visits. BMI will be calculated.

10.2.7 Abdominal Ultrasound

Ultrasound of the liver and spleen will be performed at randomization (Visit 3) and at EOT (Visit 9). Liver size, echogenicity, and presence of masses/nodules as well as spleen size will be recorded.

10.2.8 Overdose

A4250 is minimally absorbed and has a very low systemic availability. Based on toxicology data, for study purposes any dose exceeding a total of >3 mg/kg body weight of A4250 taken as a single dose or as a cumulative dose within 24 hours is defined as an overdose.

The no-adverse-effect dose level in the most sensitive species (defined as 20 mg/kg/day) in the rat 1 month toxicity study gives a human equivalent dose of approximately 194 mg/day in

a 60 kg person. This dose is approximately 10-fold higher than the maximum possible dose predicted in human studies (20 mg).

The investigator/investigative staff should immediately and not later than within 24 hours of knowledge of the overdose, be reported by the investigator or delegate on the SAE report form. In the event of an A4250 overdose, the patient should be monitored closely.

10.2.9 Pregnancy

If a pregnancy is discovered in a female patient enrolled in the study before the end of dosing, the patient will be immediately discontinued from the study and will attend the same visits as a prematurely withdrawn patient. If the pregnancy is discovered in a female patient enrolled in the study after the end of dosing, the patient will continue in the study per protocol. If a pregnancy occurs in a male patient's partner at any time during the study, the pregnancy should also be reported and followed.

Pregnancy is not considered to be an AE. However, if the patient has been dosed with the study drug, the pregnancy must be reported on the Paper Pregnancy Notification Form immediately within 24 hours after recognition to Safety Manager (please refer to Section 10.1.3). Date of exposure and as far as possible, details of the period of gestation at the time of exposure must be given.

The pregnancy should be followed to determine outcome, including spontaneous termination, details of birth, and presence of any birth defects, congenital anomalies or newborn or maternal complication. The infant who was exposed in-utero will be followed for up to 2 years after delivery. Individual cases with an abnormal outcome in association with the study drug should be reported on an expedited basis, i.e., reported rapidly to a competent authority.

11 STATISTICAL EVALUATION

All personnel involved with the analysis of the study will remain blinded until database lock for the double-blind Treatment Period and until major protocol violations have been identified.

All statistical analyses will be performed using SAS version 9.3 or higher.

11.1 Sample Size and Power

For the EU and RoW, the primary analysis will relate to the serum bile acid responder endpoint. For the US, the primary analysis will relate to the pruritus endpoint. For each primary endpoint, a pooled analysis for the closed testing procedure will be applied to control the 1-sided overall type I error rate for two treatment comparisons vs. the placebo at the 0.025 level (Kane et al, 2015, Lehmacher et al, 1991, and Marcus et al, 1976). The study will enroll 60 to 70 patients in order to obtain at least least 20 evaluable patients in each arm. For each primary endpoint, simulations with 5000 iterations using 20 patients per arm were conducted to estimate the power after multiplicity adjustment, resulting in a standard error of <0.7% for each estimated power.

Based on the Phase 2 A4250-003 study data both dose groups are predicted to have similar treatment effects for both serum bile acids and pruritus. For serum bile acids, simulated proportions were analyzed using the CMH test to generate the following 1-sided p-values: both A4250 arms pooled vs. placebo, low dose vs. placebo, and high dose vs. placebo. Assuming 60% responders in the A4250 arms and 10% responders in the placebo arm, the power to claim significance for a particular A4250 arm after multiplicity adjustment is approximately 94%. The power to claim significance for at least one arm or both arms are approximately 99% and 91%, respectively.

For the proportion of positive pruritus assessments at the subject level in pruritus scores, simulated proportions were analyzed using ANCOVA to generate the following 1-sided p-values: both A4250 arms pooled vs. placebo, low dose vs. placebo, and high dose vs. placebo. With an effect size of 1.0526, the simulated power to claim significance for a particular arm after multiplicity adjustment is approximately 89%. The probability to claim significance for at least one arm or both arms are approximately 95% and 83%, respectively.

11.2 Statistical Methods

11.2.1 Statistical Analysis Sets

Safety Analysis Set

The safety analysis set will consist of all patients who received at least 1 dose of study drug. Patients will be analyzed according to the treatment they actually received. The safety analysis set will be the primary analysis set for safety analyses.

Full Analysis Set

The full analysis set (FAS) will consist of all randomized patients who received at least 1 dose of assigned treatment. Patients will be analyzed in the treatment group they were randomized to even if they received incorrect study drug. The FAS will be the primary analysis set for efficacy analyses.

Per Protocol Analysis Set

The per protocol (PP) analysis set is a subset of the FAS, and will consist of all randomized patients for whom no major protocol violation (no violation which may affect the study efficacy outcome) is documented. The PP analysis set will provide supportive data for the efficacy analyses.

Allocation of patients to the PP analysis set will be performed before unblinding of the study.

11.2.2 Methods of Statistical Analyses

11.2.2.1 General Principles

The primary objective of this study is to demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with PFIC Types 1 and 2.

In the EU and RoW, for the primary efficacy variable of fasting bile acid responder endpoint (at least a 70% reduction in fasting s-BA concentration or reaching a level ≤70 µmol/L at the end of treatment), the Cochran Mantel Haenszel (CMH) test stratified by PFIC class and age class will be performed to compare the proportion in fasting bile acid responder endpoint at the end of treatment (Week 22 and 24) in the two A4250 dose groups to placebo group. To ascertain that all data are used in the CMH analysis, neighboring strata will be pooled if all subjects in a stratum have the same response. Dropouts will be treated as non-responders. The proportion together with corresponding 95% CI, odds ratio and corresponding 95% Clopper-Pearson exact CI and p-value for the CMH test will be presented.

In the US, for the primary efficacy variable of the proportion of positive pruritus assessments at subject level over the 24-week treatment period, an analysis of covariance (ANCOVA) will be used. The model will include treatment arm, AM baseline pruritus score, PM baseline pruritus score, and randomization stratification factors, i.e. PFIC class and age class. LS mean(SE) by treatment arm and LS mean difference (SE), 95% confidence intervals, and p-values (where applicable) between treatments (120 µg/kg/day and 40 µg/kg/day, respectively) vs. placebo will be provided. If there are concerns on model assumptions, i.e. normality and homogeneity of variances, a non-parametric ANCOVA based on the rank scores will be used as a sensitivity analysis. LS mean/SE on the outcome by treatment arm and LS mean difference/SE between active dose and placebo will be provided.

The procedure for multiplicity adjustment is specified as follows:

In the closed testing procedure, the low and high dose groups will be pooled to compare with the placebo group first. If the 1-sided p-value is ≤0.025, the 1-sided p-values for low dose vs.

placebo and high dose vs. placebo will be calculated, respectively. If both individual p-values are ≤0.025, a significant treatment effect will be declared on both dose groups. If only one of them is ≤0.025, a significant treatment effect will be declared on the corresponding dose group.

Other secondary and exploratory outcome variables will provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple comparisons when testing secondary and exploratory efficacy variables.

If not otherwise specified, the baseline value of a parameter is defined as the last assessment of that parameter prior to the first intake of study treatment.

11.2.2.2 Missing Data

In the primary analysis of the pruritus endpoint, all intermittently missing assessments will be classified as negative pruritus assessments. Similarly, all planned assessments after the intercurrent events (premature treatment discontinuation, death or initiation of rescue treatments such as biliary diversion surgery or liver transplantation) will be counted as negative pruritus assessments. However, as described above, all 336 assessments planned over the 24-week treatment period will be included in the denominator of the endpoint definition.

In the primary analysis of fasting bile acid responder endpoint, dropouts and patients with a missing average at the end of the treatment will be treated as non-responders. Patients in need of surgical rescue, i.e. biliary diversion and listing for liver transplant will be classified as non-responders. Continued collection of efficacy data for patients who discontinue treatment will be made as far as possible.

11.2.2.3 Demographic and Baseline Characteristics

Descriptive summaries of demographics and other baseline characteristics (including medical and surgical history) will be presented by treatment group and overall using the FAS.

Prior and concomitant medication use during the study will be summarized by treatment group and overall using the safety analysis set.

11.2.2.4 Subject Disposition

The following will be summarized (by treatment group and overall where applicable):

- Patients enrolled (who signed the informed consent)
- Patients randomized
- Patients treated
- Patients completing the study
- Patients withdrawing early (including withdrawal reason)

Additionally, patients enrolled, included in the safety, in the FAS, and in the PP analysis set will be summarized by region.

11.2.2.5 Evaluation of Primary Efficacy Variables

For EU and RoW, the primary responder endpoint based on change in fasting bile acids will be analyzed in terms of the proportion of patients achieving the event of interest. Patients in need of surgical rescue, i.e. biliary diversion and listing for liver transplant, will be classified as non-responders. Dropouts will be treated as non-responders.

CMH stratified by PFIC class and age class will be performed to compare the proportion in fasting bile acid responders at the end of treatment (Week 22 and 24) in the two A4250 dose groups to the placebo group. To ascertain that all data are used in the CMH analysis, neighboring strata will be pooled if all subjects in a stratum have the same response. The proportion together with corresponding 95% CI, odds ratio and corresponding 95% Clopper-Pearson exact CI and p-value for the CMH test will be presented

Logistic regression will be performed as a supportive analysis to compare the proportion in fasting bile acid responders at the end of treatment (Week 22 and 24) in the two A4250 dose groups to the placebo group. The model will include treatment arm, baseline value, and randomization stratification factors, i.e. PFIC class and age class. Baseline value will be calculated as the average of serum bile acid values at randomization and the previous visit (Clinic Visit 2).

In the US, for the primary efficacy variable of the proportion of positive pruritus assessments at the subject level over the 24-week treatment period, ANCOVA will be used to analyze the pruritus endpoint. The model will include treatment arm, AM baseline pruritus score, PM baseline pruritus score, and randomization stratification factors, i.e. PFIC class and age class. LS mean(SE) by treatment arm and LS mean difference (SE), 95% confidence intervals, and p-values (where applicable) between treatments (120 µg/kg/day and 40 µg/kg/day, respectively) vs. placebo will be provided. If there are concerns on model assumptions, i.e. normality and homogeneity of variances, a non-parametric ANCOVA based on the rank scores can be used. LS mean/SE on the outcome by treatment arm and LS mean difference/SE between active dose and placebo will be provided.

Sensitivity analysis will be conducted to assess the impact of intermittently missing data as well as intercurrent events and will be specified in the SAP. Subgroup analyses will also be performed for each of the 3 age groups (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) and for patients who were not included in the previous Study A4250-003.

11.2.2.6 Evaluation of Secondary and Exploratory Efficacy Variables

The change in the secondary endpoints such as serum bile acids, total bilirubin, ALT, growth, Albireo PRO and ObsRO sleep parameters, and patient-reported itch severity will be

summarized by visit using descriptive statistics. Change in growth (weight for age and BMI for age) will also be displayed using graphical presentations.

Proportion of responders at Weeks 12 and 24 based on the PRO/ObsRO instruments estimated from both distribution and anchor-based approaches will be analyzed using the same model as specified for the primary analysis for EU and RoW.

Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval over the 24-week treatment period for AM or PM, respectively will be analyzed using the same model as specified for the primary analysis for US.

Undergoing biliary diversion surgery and/or liver transplant, all-cause mortality, and liver decompensation events will mainly be analyzed by using descriptive statistics. Kaplan-Meier curves will be used when appropriate for time to event data.

Comparisons of the change from baseline at Week 24 in PedsQL between the treatment groups will be conducted using ANCOVA. The model will include terms for baseline, PFIC class, age class, and treatment.

Other exploratory variables (Global Symptom Relief, biochemical markers and measures of bile acid synthesis, additional Albireo PRO and ObsRO sleep parameters, PELD/MELD score, APRI score, and Fib-4 score) will be analyzed descriptively for categorical and continuous data, as applicable. For continuous data, the change from baseline will be analyzed in addition to the actual visit values. For categorical data, shift tables or frequency and percentages of patients will be presented as appropriate. Additional details on secondary and exploratory efficacy variables and subgroup analyses by age class (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) will be specified in the SAP.

11.2.2.7 Evaluation of Safety Variables

Safety data will be analyzed using descriptive statistics and summaries by treatment group of SAEs, AEs, vital signs, clinical laboratory tests (hematology, clinical chemistry and urinalysis), and concomitant medication. Analyses will be performed using the safety analysis set.

Summaries of AEs (coded according to the Medical Dictionary for Regulatory Activities [MedDRA] system organ class [SOC] and MedDRA preferred term) will include the following:

- Overview of the incidence of TEAEs (TEAEs, drug-related TEAEs, TEAEs leading to study discontinuation, and treatment-emergent SAEs)
- TEAEs by SOC and preferred term
- Intensity of TEAEs by SOC and preferred term

- Drug-related TEAEs by SOC and preferred term
- TEAEs leading to study discontinuation by SOC and preferred term
- Treatment-emergent SAEs by SOC and preferred term

Concomitant medication use during the Treatment Period will be summarized by Anatomical Therapeutic Chemical (ATC) class and World Health Organization (WHO) preferred name.

Summaries of vital signs will be presented. For each visit, the actual results and the change from baseline, and the number and percentage of patients with potentially clinically significant (PCS) values observed post-baseline will be presented.

Summaries of clinical safety laboratory data will be presented. For each visit, the actual result and the change from baseline, and the number and percentage of patients with PCS values observed post-baseline will be presented.

Data listings will be provided for each patient for all safety parameters.

11.2.2.8 Compliance and Exposure

Exposure will be analyzed by calculating the number of days with exposure to study drug. Results will be presented by treatment group using the safety analysis set.

The percentage compliance will be described by treatment group and the number of patients with a compliance <80%, between 80% and 120%, and >120% will be presented based on the safety analysis set.

11.2.3 Blinded Analyses to Confirm Responder Definitions

A blinded analysis of Albireo ObsRO and PRO eDiary data will be performed after 50% or more of the planned sample has completed the Week 24 Follow-Up Period. The blinded analysis will be used to estimate a threshold of clinically meaningful change (i.e., responder definition) in Albireo ObsRO and PRO pruritus scores. The analysis will be performed by a group independent from both the study team and Albireo. Blinded data will be used for this analysis; data will be collapsed across treatment groups.

The analyses will include distribution- and anchor-based analyses to examine a responder definition for the Albireo PRO/ObsRO pruritus scores. Distribution-based analyses will include calculation of the SD and standard error of measurement for the baseline Albireo PRO and ObsRO scores. Anchor-based analyses will involve examining the degree of change on the Albireo PRO and ObsRO scores from baseline to Week 24 for patients who experience change in pruritus according to PGIC and symptom items. Details of this analysis will be included in a stand-alone SAP for assessing the measurement characteristics (reliability, validity, sensitivity to change, responder definition) of the Albireo PRO and ObsRO items.

11.2.4 Data Safety Monitoring Board

A DSMB consisting of sponsor-independent clinical and statistical experts will be established. The DSMB will periodically (approximately on a quarterly basis) meet for the review of accumulating study data, including safety (AE and laboratory data) and liver values.

The DSMB will have access to unblinded data. The DSMB will make recommendations for the remaining part of the study (further details will be provided in the DSMB charter). The DSMB may recommend continuing with the study as planned or stopping the study early for safety reasons. The DSMB will submit its recommendations in writing to Albireo Medical/[redacted] who are responsible for responding to the recommendations of the DSMB and taking appropriate action. The investigators will only be informed by Albireo Medical/[redacted] if the study requires a protocol amendment or is stopped. The DSMB may choose to make additional evaluations at any time if they feel this is warranted from a safety point of view.

The DSMB will act according to its own written standard operating procedure described in a charter and will prepare written minutes of its meetings. The charter of the DSMB will be stored in the trial master file. The DSMB will maintain records of its meetings and these will become part of the study file when the study is complete. In order not to disseminate unblinded data and to ensure that all staff involved in the conduct and final analysis of the study remain blind to the data, only the members of the DSMB and the unblinded DSMB statistician will have access to these data.

12 DIRECT ACCESS TO SOURCE DATA/NOTES

The investigator/institution shall provide direct access to source data/documents for study-related monitoring, audits, IEC/Institutional Review Board (IRB) review and regulatory inspection.

13 QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Conduct of the Study

Albireo/[redacted] shall implement and maintain quality control and quality assurance procedures with written standard operating procedures to ensure that the study is conducted and data are generated, documented, and reported in compliance with the protocol, ICH GCP and applicable regulatory requirements.

This study shall be conducted in accordance with the provisions of the Declaration of Helsinki (October 1996) and all revisions thereof, and in accordance with United States Food and Drug Administration (FDA) regulations (Code of Federal Regulations, Sections 312.50 and 312.56) and with ICH GCP (CPMP 135/95).

The investigator may not deviate from the protocol without a formal protocol amendment having been established and approved by an appropriate competent authority and IEC/IRB, except when necessary to eliminate immediate hazards to the patient or when the change(s) involve(s) only logistical or administrative aspects of the study. Any deviations may result in the patient having to be withdrawn from the study and render that patient non-evaluable.

13.2 Study Monitoring

Before study initiation, at a site initiation visit or at an investigator's meeting, an Albireo representative or designee will review the protocol and eCRF with the investigators and the investigative staff. During the study, the clinical monitor (clinical research associate [CRA]) will visit the site regularly to check the completeness of patient records, the accuracy of entries on the eCRFs, the adherence to the protocol and to GCP, the progress of enrollment, and to ensure that study drug is being stored, dispensed, and accounted for according to specifications.

The investigator must ensure that eCRFs are completed within a timely period of patient visits, as per individual site agreements, and must allow the CRA and Albireo representative or designee periodic access to patient records and all study-related materials, including relevant hospital or clinical records, to confirm their consistency with the eCRF entries. No information in these records about the identity of the patients will leave the study center. Albireo monitoring standards require full verification for the presence of the signed ICF, adherence to the inclusion/exclusion criteria, documentation of SAEs, and recording of primary efficacy and safety variables. The CRA will review source data compared with the eCRFs and will verify source data according to the study-specific monitoring plan. The design of the study, the frequency of patient visits, and the site enrollment rate will determine the frequency of monitoring visits. Upon study completion, the CRA will visit the site to conduct a study termination visit, which will include collection of any outstanding documentation.

It is recommended that the investigator/investigative staff log into the eCRF system every working day or at minimum twice weekly in order to provide a prompt response to queries. The investigator/investigative staff should respond to queries and make any relevant changes to the study data within 3 working days.

14 ETHICS

14.1 Independent Ethics Committee/Institutional Review Board

Prior to the start of the study, the investigator is responsible for ensuring that the protocol and consent form have been reviewed and approved by a relevant IEC/IRB. The IEC/IRB shall be appropriately constituted and perform its functions in accordance with FDA ICH GCP and local requirements as applicable.

The IEC/IRB shall approve all protocol amendments (except for logistical or administrative changes), written informed consent documents and document updates, patient recruitment procedures (e.g., advertisements), written information to be provided to the patients and caregivers, IB, available safety information, information about payment and compensation available to patients, the investigator's curriculum vitae and/or other evidence of qualifications and any other documents requested by the IEC/IRB and regulatory authority (competent authority) as applicable.

14.2 Written Informed Consents and Assents

The investigator (physician) or investigative staff, in accordance with local regulations, will explain to each patient (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, alternative treatment, the potential risks and benefits involved, and any discomfort that may occur. Each caregiver/patient will be informed that participation in the study is voluntary and that he/she or their child may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

Caregivers/patients will be informed that they/their children may experience side-effects or be at risk for symptoms, illnesses, or complications that cannot be foreseen by Albireo. As with other medications, people treated with A4250 may be at risk of developing allergic reactions or anaphylaxis. Caregivers/patients will be advised that study procedures include regular blood sampling for measurement of safety parameters and biological markers and that some minor risks are associated with these procedures.

This informed consent should be given by means of a signed ICF, written in non-technical language in accordance with ICH GCP, the Declaration of Helsinki, and regulatory authorities. The caregiver(s)/patient should read and consider the statements before signing and dating them and should be given a copy of each signed document. If written consent is not possible, oral consent can be obtained if witnessed and followed by a signed statement from one or more persons not involved in the study, indicating why the patient was unable to sign the form. No patient can enter the study before his/her or caregiver(s) informed consent has been obtained, as required by country regulations.

The ICF is part of the protocol and must be submitted by the investigator/investigative staff with the protocol for IEC approval. Albireo will supply an ICF which complies with

regulatory requirements and country laws and is considered appropriate for the study. Any changes to the ICF suggested by the investigator must be agreed to by Albireo before submission to the IEC and a copy of the approved version must be provided to the clinical monitor after IEC approval.

15 DATA HANDLING AND RECORD KEEPING

15.1 Case Report Forms/Source Data Handling

The investigator shall be provided with standardized eCRFs and shall ensure that all data from patient visits are promptly entered into the eCRFs in accordance with the specific instructions given. The investigator must sign each eCRF to verify the integrity of the data recorded.

A list of the normal ranges for all laboratory tests to be undertaken forms part of the documentation to be collated prior to study start. As a central laboratory has been selected to conduct any or all tests, it is essential that all samples be analyzed at that laboratory.

The investigator must maintain source documents such as laboratory reports, consultation reports, and complete medical history and physical examination reports.

As no ethnicity data can be collected in the source document for study sites in France, the eCRF will be the Source Document for “origines ethniques”,

15.2 Retention of Essential Documents

Essential documents, as defined by ICH GCP, include: the signed protocol and any amendment(s); copies of the completed eCRFs (for site archiving, compact discs of eCRF data for specific patients will be provided); signed ICFs; hospital records and other source documents; IEC approvals and all related correspondence including approved documents; drug accountability records; study correspondence; and a list of patients’ names and addresses.

The investigator/investigative staff must retain copies of these essential documents for the period specified by ICH GCP and by applicable regulatory requirements. The investigator/investigative staff will inform Albireo of the location where the essential documents are stored and must contact Albireo for approval before disposing of any essential documents. The investigator/investigative staff should take measures to prevent accidental or premature destruction of these documents.

16 FINANCING AND INSURANCE

Financing and insurance are addressed in a separate agreement.

17 PUBLICATION POLICY

Albireo will retain the ownership of all data. When the study is complete, Albireo shall arrange the analysis, tabulation of data and preparation of a clinical study report. Albireo may also use the data for publication, presentation at scientific meetings and submission to regulatory authorities. All proposed publications based on this study must be subject to the sponsor's approval requirements.

18 REFERENCE LIST

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19 APPENDICES

Appendix 1 Concomitant Medication Guidelines

Appendix 2 Diary Questions including Albireo Patient-Reported Outcomes/Observer-Reported Outcome Instrument

Appendix 3 Patient Global Impression of Change (PGIC) and Patient Global Impression of Symptoms (PGIS)

Appendix 4 Pediatric Quality of Life Inventory (PedQL) Questionnaire

Appendix 5 Guideline for Fat-Soluble Vitamin Supplementation

Appendix 6 Contraceptive Requirements

Appendix 7 Blood Volumes

CLINICAL STUDY PROTOCOL A4250-005

A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

IND Number:	130591
EudraCT Number	2017-002338-21
Test Product:	A4250
Indication:	Progressive Familial Intrahepatic Cholestasis (PFIC)
Sponsor:	Albireo AB
Development Phase:	Phase 3
Sponsor Signatory:	[redacted]
Sponsor Medical Monitor:	[redacted]
Principal Investigator:	[redacted]
Date of the Protocol:	01 March 2019
Version of the Protocol:	Final Amendment 04

Confidential

The information in this document contains trade secrets and commercial information that are privileged or confidential and may not be used, divulged, published, or otherwise disclosed without the written consent of Albireo AB. These restrictions on disclosure apply equally to all future information supplied to you or generated by you in connection with the study.

SPONSOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

Albireo AB

01-March-2019

Date (day/month/year)

INVESTIGATOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

I have read this protocol and agree that it contains all necessary details for performing this study. I will conduct the study as outlined herein and will complete the study within the time designated, in accordance with all stipulations of the protocol and in accordance with Good Clinical Practice (GCP), local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study drug and the conduct of the study.

I will use only the informed consent approved by the Independent Ethics Committee (IEC) and will fulfil all responsibilities for submitting pertinent information to the IEC responsible for this study.

I agree that the sponsor (Albireo AB) shall have access to any source documents from which case report form information may have been generated.

I further agree not to originate or use the name of Albireo AB, A4250 in any publicity, news release, or other public announcement, written or oral, whether to the public, press, or otherwise, relating to this protocol, to any amendment to the protocol, or to the performance of this protocol without the prior written consent of Albireo AB.

Name of Investigator

Signature

Date (day/month/year)

1 ADMINISTRATIVE INFORMATION

A Double-blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Protocol No.:	A4250-005
Date of the initial Protocol:	20 September 2017
Date and Number of Amendment(s):	Amendment 01, 06 December 2017 Amendment 02, 10 May 2018 Amendment 03, 22 November 2018 Amendment 04, 01 March 2019
Sponsor:	Albireo AB Arvid Wallgrens backe 20 413 46 Göteborg Sweden
Clinical Research Organization:	[redacted]
Sponsor Signatory:	
Sponsor Medical Monitor:	
Principal Investigator:	

2 STUDY SYNOPSIS

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250

Title of Study:
A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Principal Investigator:
[redacted]

Study Centers:
Up to 50 sites will be initiated for this study in the United States (US), Canada, European Union (EU), Middle East, and Australia.

Publication(s):
None.

Planned Study Period:	**Development Phase:**
Q2 2018 to Q4 2019	Phase 3

Objectives:

Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with progressive familial intrahepatic cholestasis (PFIC) Types 1 and 2, as determined by the following:

- Proportion of patients experiencing at least a 70% reduction in fasting serum bile acid (s-BA) concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- The proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period

Secondary Objectives

- To evaluate the effect of A4250 on serum alanine aminotransferase (ALT) concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

Methodology:

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of familial intrahepatic cholestasis-1 or bile salt export pump).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-up Period. After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo. Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008) in which all patients will receive active treatment.

There will be up to 10 clinic visits during the study and one scheduled telephone contact.

Name of Sponsor/Company:	**Name of Product:**	**Name of Active Ingredient:**
Albireo	A4250	A4250

Number of Patients:

Approximately 60 patients diagnosed with PFIC-1 or PFIC-2 will be randomized. The study will target 15% of enrolled patients to be PFIC-1 patients. If enrollment of all PFIC-2 patients is completed and the 15% enrollment of PFIC-1 patients has not yet been achieved, then enrollment of PFIC-2 patients will continue in order to reach the total study target enrollment.

Diagnosis and Main Criteria for Inclusion:

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have clinical genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples at least 7 days apart (Visits 1 and 2) prior to randomization
4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the electronic diary (eDiary) average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients are expected to have a consistent caregiver for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

Test Product, Dose and Mode of Administration:

A4250, 40 µg/kg/day or 120 µg/kg/day, orally administered

Reference Therapy, Dose and Duration of Administration:

Matching placebo, orally administered

Duration of Treatment:

24 weeks

Variables

Efficacy:

Primary Efficacy Endpoints

The primary efficacy endpoints are:

- **EU and rest of world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.
- **US**: Proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period.

Secondary Efficacy Endpoints

Secondary efficacy endpoints include the following:

- **EU and RoW**: Proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period.
- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

All Regions:

All secondary endpoints will be compared to placebo.

- Change from baseline to Week 12 and to Week 24 in fasting s-BA
- Change from baseline to Week 12 and to Week 24 in serum ALT concentration

Name of Sponsor/Company:	Name of Product:	Name of Active Ingredient:
Albireo	A4250	A4250

- Change in growth from baseline to Week 12 and Week 24
- Proportion of responders for pruritus scores at Weeks 12 and 24 based on the Albireo PRO and ObsRO instruments
- Change in sleep parameters measured with the Albireo PRO and ObsRO instruments from baseline over the 24-week Treatment Period
- Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level over the 24-week Treatment Period. A positive pruritus assessment includes an itch score ≤1, or at least a one-point drop from baseline based on the Albireo PRO instrument; only patients ≥8 years of age will complete the Albireo PRO instrument
- Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Proportion of individual AM assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Proportion of individual PM assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Number of patients undergoing biliary diversion surgery or liver transplantation

Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the patient global impression of change (PGIC) items
- Change from baseline in fasting s-BA at Week 4
- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline by each 4 week interval of the Treatment Period as measured by the Albireo ObsRO instrument
- Change from baseline by each 4-week interval of the Treatment Period in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline by each 4-week interval of the Treatment Period in patient-reported and observer-reported morning time itching and scratching severity scores, respectively
- Change from baseline by each 4-week interval of the Treatment Period in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline by each 4-week interval of the Treatment Period in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in Pediatric Quality of Life Inventory (PedsQL)
- Change from baseline in serum ALT concentration at Week 4
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST and gamma-glutamyl transferase at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, plasma 7α-hydroxy-4-cholesten-3-one concentration [p-C4]) at Week 24
- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events.
- All-cause mortality

Name of Sponsor/Company:	Name of Product:	Name of Active Ingredient:
Albireo	A4250	A4250

Safety:

Safety criteria are as follows:

- Occurrence of treatment-emergent adverse events including severity and relatedness to study drug at all visits
- The incidence of treatment-emergent serious adverse events (SAEs), based upon information from patient reports, including the description, incidence, and severity of an SAE
- Trends evaluated for the following assessments: physical examinations, concomitant medications, vital signs, laboratory test results (including clinical chemistry, hematology, urinalysis, alfa-fetoprotein, vitamins A and E, 25-hydroxy vitamin D and INR), and abdominal ultrasound

Statistical Methods:

The primary objective of this study is to demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with PFIC Types 1 and 2.

The Cochran Mantel Haenzel (CMH) test stratified by PFIC class and age class will be performed to compare the proportion in fasting bile acid responders at the end of treatment (Week 22 and 24) in the two A4250 dose groups to the placebo group. To ascertain that all data are used in the CMH analysis, neighboring strata will be pooled if all subjects in a stratum have the same response. The proportion together with the corresponding 95% CI, odds ratio and corresponding 95% Clopper-Pearson exact CI and p-value for the CMH test will be presented.

For the primary efficacy variable of the proportion of positive pruritus assessments at subject level over the 24-week treatment period, an ANCOVA model will be used to analyse the comparisons between the treatment groups. The model will include treatment arm, AM baseline pruritus score, PM baseline pruritus score, and randomization stratification factors, i.e. PFIC class and age class. LS mean (SE) by treatment arm and LS mean difference (SE), 95% confidence intervals, and p-values (where applicable) between treatments (120 µg/kg/day and 40 µg/kg/day, respectively) vs. placebo will be provided. If there are concerns on model assumptions, i.e. normality and homogeneity of variances, a non-parametric ANCOVA based on the rank scores can be used as a sensitivity analysis. LS mean/SE on the outcome by treatment arm and LS mean difference/SE between active dose and placebo will be provided.

For each primary endpoint by region (EU & RoW and US), a pooled analysis for the closed testing procedure will be applied to control the 1-sided overall type I error rate for two treatment comparisons vs. the placebo at the 0.025 level, as specified below:

In the closed testing procedure, the low and high dose groups will be pooled to compare with the placebo group first. If the 1-sided p-value is ≤0.025, the 1-sided p-values for low dose vs. placebo and high dose vs. placebo will be calculated respectively. If both individual p-values are ≤0.025, a significant treatment effect will be declared on both dose groups. If only one of them is ≤0.025, a significant treatment effect will be declared on the corresponding dose group.

Other secondary and exploratory outcome variables will provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple comparisons when testing secondary and exploratory efficacy variables.

Date of the Protocol: 01 March 2019

3 TABLE OF CONTENTS

3.1 List of Tables

3.2 List of Figures

3.3 List of Appendices

4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADME	absorption, distribution, metabolism, and excretion
AE(s)	adverse event(s)
AFP	alfa-fetoprotein
ALT	alanine aminotransferase
APRI	AST to platelet ratio index
ASBT	apical sodium-dependent bile transporter (also known as IBAT)
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	body mass index
BSEP	bile salt export pump
CPK	creatine phosphokinase
CRA	clinical research associate
CYP	cytochrome P450
DSMB	Data and Safety Monitoring Board
eCRF	electronic case report form
eDiary	electronic diary
EOT	End of Treatment
EU	European Union
EudraCT	European Union drug regulatory agency clinical trial
FAS	full analysis set
FDA	United States Food and Drug Administration
FIC1	familial intrahepatic cholestasis-1
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
GI	gastrointestinal
GLMM	generalized linear mixed modelling
IB	Investigator's Brochure
IBAT	ileal bile acid transporter (also known as ASBT)
ICF	informed consent form
ICH	International Council for Harmonisation
[redacted]	[redacted]
IEC	Independent Ethics Committee
INR	international normalized ratio
IRB	Institutional Review Board

IWRS	Interactive Web Response System
LDH	lactate dehydrogenase
LFT	liver function test
LPLV	last patient last visit
MDR3	multidrug-resistance protein 3
MedDRA	Medical Dictionary for Regulatory Activities
MELD	model for end-stage liver disease
MMRM	mixed model repeated measures
ObsRO	observer-reported outcome
PBC	primary biliary cholangitis
p-C4	plasma 7α-hydroxy-4-cholesten-3-one concentration
PCS	potentially clinically significant
PedsQL	Pediatric Quality of Life Inventory
PELD	pediatric end-stage liver disease
PFIC	progressive familial intrahepatic cholestasis
PGIC	patient global impression of change
PGIS	patient global impression of symptoms
PK	pharmacokinetic(s)
PP	per protocol
PRO	patient-reported outcome
PT	prothrombin time
PVM	pharmacovigilance manager
QoL	quality of life
RoW	rest of world
RR	respiratory rate
SAE(s)	serious adverse event(s)
SAP	statistical analysis plan
s-BA	serum bile acid
SD	standard deviation
s-FGF19	serum fibroblast growth factor 19 concentration
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction
TEAE(s)	treatment-emergent adverse event(s)
ULN	upper limit of normal
US	United States
WHO	World Health Organization

5 INTRODUCTION

5.1 Investigational Medicinal Product

A4250 is a small molecule and a potent selective inhibitor of the ileal bile acid transporter (IBAT), also known as the apical sodium-dependent bile acid transporter (ASBT). IBAT is an integral brush border membrane glycoprotein that co-transports sodium and bile acids and appears to be a critical component in the regulation of the bile acid pool size in animals and man. This transporter, expressed in the distal ileum, is a key element in the enterohepatic circulation of bile acids since it facilitates the high-affinity, high-capacity reabsorption of bile acids. Indeed, 95% of secreted bile acids are reabsorbed via IBAT [Hofmann 2009; Miethke 2016]. A4250 is orally administered and acts locally in the gut where it binds reversibly to IBAT to decrease the reuptake of bile acids. A4250 has minimal systemic exposure at the predicted therapeutic dose ranges.

No safety signals were found with A4250 in preclinical safety and toxicity studies. In the first clinical study of A4250 in healthy volunteers, A4250 was given as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. A4250 was well tolerated and the only findings were gastrointestinal (GI) related effects, such as diarrhea, at higher doses.

In an exploratory Phase 2 study in children with cholestatic pruritus, A4250 was given as oral doses of 10 to 200 µg/kg/day for 4 weeks. A4250 reduced mean levels of serum bile acids (s-BA) in all cohorts. In 11 of 13 progressive familial intrahepatic cholestasis (PFIC) patients, substantial reductions (ranging from 43% to 98%) were observed. Patient-reported diary data documented improvement of pruritus during treatment. A4250 was well tolerated. No serious adverse events (SAEs) were deemed to be related to study drug, and most adverse events (AEs), including some increased transaminases, were considered unrelated to study drug and transient in nature.

5.2 Background

5.2.1 Progressive Familial Intrahepatic Cholestasis

PFIC is a rare autosomal recessive cholestatic liver disease estimated to affect between 1 in every 50,000 to 100,000 children born worldwide. PFIC represents 10% to 15% of causes of cholestasis in children and 10% to 15% of liver transplantation indications in children. All types of PFIC exist worldwide and both sexes appear to be equally affected.

The common underlying pathogenesis of PFIC is disruption of bile formation and bile transport through the liver [Jacquemin 2000]. The classification of PFIC has evolved over the years. The most commonly used sub-classifications are PFIC Types 1, 2, and 3, which are based on the associated affected gene and described in more detail below.

- PFIC, Type 1: also referred to as “Byler disease” or “familial intrahepatic cholestasis-1 (FIC1) protein deficiency.” FIC1 protein is located on the canalicular

membrane of hepatocytes and facilitates movement of aminophospholipids from the outer to inner leaflet of the plasma membrane of the hepatocyte. The *ATP8B1* gene encodes FIC1 protein. Biallelic pathologic variants in the *ATP8B1* gene are associated with FIC1 dysfunction and classified clinically as PFIC Type 1 disease.

- PFIC, Type 2: also referred to as "Byler syndrome" or "bile salt export pump (BSEP) deficiency." BSEP is a transporter protein that is expressed at the canalicular membrane of hepatocytes and is the primary exporter of bile acids. The *ABCB11* gene encodes the BSEP protein. Biallelic pathologic variations in the *ABCB11* gene are associated with BSEP dysfunction and classified clinically as PFIC Type 2 disease.
- PFIC, Type 3: is caused by a deficiency of the multidrug-resistance protein 3 (MDR3) due to mutations in the *ABCB4* gene. MDR3 is a phospholipid translocase critical for phospholipid secretion.

Severe pruritus is common in children diagnosed with PFIC. Itching (and subsequent scratching) is a significant morbidity for these patients and their families [Suchy 2007]. A more severe degree of pruritus is experienced compared to patients with other forms of liver disease and to other pruritic conditions such as atopic dermatitis [Murray 2011]. In patients with PFIC, liver biopsy reveals canalicular cholestasis and, later, the appearance of portal fibrosis. Serum biochemistry indicates cholestasis with hyperbilirubinemia, elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The concentrations of bile acids in serum are very high, while serum gamma-glutamyl transferase (GGT) activity (exception being MDR3 variants) and cholesterol are normal [Hori 2010]. Symptoms of portal hypertension and liver failure will develop during the course of the disease [Davit-Spraul 2009; Alissa 2008]. Symptoms develop early; median age at onset of symptoms is 2 months, and 78% of PFIC patients present with jaundice [Pawlikowska 2010]. The life-threatening and debilitating nature of PFIC is reflected by the fact that survival in those not resorting to surgery is 50% at 10 years of age and almost zero at 20 years of age. Approximately half of PFIC patients undergo liver transplantation [Davit-Spraul 2009] and treatment-resistant pruritus is the leading indication for the surgical procedure partial external biliary diversion, mostly in PFIC Type 1 and 2 patients.

PFIC is life-threatening and debilitating. Currently, the treatment for PFIC is palliative and there is no pharmaceutical treatment approved for use in PFIC. The therapeutic choices are restricted to non-specific therapy of the symptoms and signs of the disease such as nutritional support, preventing vitamin deficiencies, and treatment of extrahepatic features. Medical treatment options include off-label use of ursodeoxycholic acid, rifampin, antihistamines, and naltrexone. A minority of patients respond nominally and transiently to these interventions. Biliary diversion is used to decrease systemic bile acids through interruption of the enterohepatic circulation and so avoid transplantation. Liver transplantation is typically only viewed as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

5.2.2 Summary of Clinical and Nonclinical Studies

This is a summary of nonclinical and clinical studies. More detail is provided in the Investigator's Brochure ([IB] see A4250 IB).

A4250 is a potent, selective IBAT inhibitor with no safety signals seen in the safety pharmacological studies. In addition, no safety signals were seen in repeat-dose oral toxicity studies in rats with up to 26 weeks of daily dosing and in dogs with up to 39 weeks of daily dosing. No safety signals were identified when A4250 was administered daily to juvenile rats from age 14 days to 63 days.

A4250 is minimally absorbed with very low systemic exposure in both humans and other species. In blood, protein binding is high (>99.7%). Systemically absorbed A4250 is slowly metabolized in humans and in preclinical studies by hydroxylation only, a metabolite that retains IBAT activity. A4250 did not show any significant inhibition of cytochrome P450 (CYP) enzymes in the rat. A4250 inhibited human CYP3A4 and CYP2D6 (IC_{50}: 16 µmol/L for both enzymes), and CYP2C9 (IC_{50}:1.2 µmol/L).

To date, A4250 has been studied in 3 Albireo-sponsored clinical studies: a study in healthy volunteers evaluating single and multiple administration of A4250; a single-dose absorption, distribution, metabolism, and excretion (ADME) study; and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with primary biliary cholangitis (PBC).

In the first clinical study (A4250-001), A4250 was given to healthy volunteers as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. Following a single oral dose of 10 mg, the median maximum drug concentration was 0.2 nmol/L and the area under the curve was 0.8 nmol × h/L (n=6). Pharmacodynamic evaluation of bile acid synthesis biomarkers (serum fibroblast growth factor 19 [s-FGF19] and plasma 7α Hydroxy 4-cholesten-3-one [p-C4]) identified the expected decrease in s-FGF19 and an increase in p-C4. In addition, total plasma bile acids decreased and total fecal bile acids increased in A4250-treated patients compared to placebo. A4250 was generally well tolerated. The only findings were GI-related effects, such as diarrhea at higher doses.

In the ADME study (A4250-007), healthy subjects were administered a single oral dose of 3 mg [^{14}C]-A4250 capsule. The main excretion route was determined to be fecal. There were no quantifiable concentrations of A4250 in plasma or total radioactivity in plasma or whole blood. A4250 was well tolerated. AEs were reported and most were mild and not related to A4250. The 3 AEs experienced by 2 subjects reported as related to study drug were GI in nature, including diarrhea (2 AEs; 2 subjects) and abdominal pain (1 AE; 1 subject).

In an exploratory Phase 2 study in children with cholestatic pruritus (A4250-003), A4250 was administered as oral daily doses of 10 to 200 µg/kg/day for 4 weeks. Twenty patients (8 females and 12 males) aged 1 to 17 years were enrolled in this open-label dose-escalation study. Four patients were re-enrolled into a second dose level. No

treatment-related SAEs were reported, and most AEs, including some increased transaminases, were transient. Four-week, once-daily treatment with A4250 reduced mean levels of s-BA in all cohorts. Substantial s-BA reductions in 11 of 13 PFIC patients (ranging from 43% to 98%) were observed. A4250 also reduced s-BA in the 11 non-PFIC patients, but to a lesser extent than in PFIC patients. Patient-reported diaries documented improvement of the visual analogue scale itch score in 17 of 24 patients during treatment. The dose with the greatest improvement showed a mean decrease (± standard error of the mean) of 2.9 ±1.1 from baseline. The results from this study form the basis of the current (A4250-005) study design.

5.3 Rationale

In this investigational study, A4250 is under development for treatment of PFIC and is also being considered for other orphan cholestatic liver diseases.

There is currently no medical treatment approved for PFIC. In PFIC patients with severe pruritus, biliary diversion surgery is used to decrease systemic bile acids through interruption of the enterohepatic circulation and thereby reducing pruritus [Whitington 1988]. A portion of patients who have biliary diversion surgery continue to have elevated serum bile acids and pruritus. Liver transplantation is typically used as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

By inhibiting IBAT with high selectivity and potency, A4250 has the potential to relieve cholestasis and improve liver function without surgical intervention in patients with PFIC. Since A4250 is minimally absorbed and achieves a substantial reduction of s-BA, it has the potential to be a safer and less invasive alternative to surgical biliary drainage. The rationale for using A4250 is to decrease s-BA levels, and to reduce the major morbidity of pruritus, improving the health and wellbeing of patients affected with PFIC. Ultimately, this noninvasive therapeutic approach has the potential to not only improve quality of life (QoL), but also reduce the need for biliary diversion and subsequent liver failure and liver transplantation. For those patients who have undergone biliary diversion surgery and continue to have elevated serum bile acids and symptomatic pruritus, IBAT inhibition has the potential to reduce both the serum bile acid levels and the pruritus.

The doses selected, 40 and 120 µg/kg/day, were based on the efficacy and safety data generated from patients with PFIC (n=10 + 3 re-entered) and supporting data from all patients (n=20 + 4 re-entered) in the five dose groups dosed in the A4250-003 study. Improvements in s-BA and pruritus were observed with doses from 30 µg/kg to 100 µg/kg, with a dose-related trend. The best dose response in all patients was at the 60 µg/kg/day for s-BA and 100 µg/kg/day for pruritus. The best dose response in the PFIC subgroup was at 30 µg/kg/day for both s-BA and pruritus. Patients with PFIC responded well to the doses given from 30 µg/kg to 200 µg/kg/day; however, a clear dose relationship could not be

established in order to select a single dose for this Phase 3 study. As efficacy was observed at both low and high doses, 40 µg/kg and 120 µg/kg/day are considered to be the most optimal for this study.

This study will be conducted in compliance with the protocol and with the International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP).

Patients completing 24 weeks of double-blind treatment will be offered to enter Study A4250-008 and receive open-label treatment with A4250.

5.3.1 Risk/Benefit

A4250 has been evaluated in three Albireo-sponsored clinical studies: a double-blind placebo-controlled study in healthy volunteers, a single-dose ADME study, and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with PBC.

Healthy subjects have been exposed to up to 10 mg as a single dose and up to 3 mg daily as part of a multiple-dose evaluation. Children with cholestatic liver disease have been treated with up to 0.2 mg/kg/day (200 µg/kg/day) for 4 weeks. In total, 2 SAEs have been reported; both were judged by the physician to be not related to the study drug.

Patients with cholestatic liver diseases suffer from excess bile acids in the liver resulting in tissue damage. A commonly used treatment in these patients is bile diversion surgery, whereby approximately 50% to 100% of the enterohepatic circulation of bile acids is interrupted. Inhibition of IBAT with A4250, thereby interrupting the enterohepatic circulation of bile acids, is therefore a potential medical alternative to surgery which could be of benefit to these patients if shown to be effective and safe. Data from the Phase 2 study showed efficacy of A4250 in reducing s-BA concentrations and pruritus in such patients.

Based on the mode of action of an IBAT inhibitor, loose stools or diarrhea could be expected. However, in the pediatric Phase 2 study with 4 weeks daily treatment, only one patient had mild transient diarrhea after a single dose that did not recur on multiple dosing.

6 STUDY OBJECTIVES

6.1 Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with PFIC Types 1 and 2, as determined by the following:

- The proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- The proportion of positive pruritus assessments at the subject level over the 24-week treatment period.

6.2 Secondary Objectives

- To evaluate the effect of A4250 on serum ALT concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

7 INVESTIGATIONAL PLAN

7.1 Overall Study Design and Plan

7.1.1 Description

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of FIC1 or BSEP).

Study data will be periodically (approximately on a quarterly basis) reviewed by an unblinded Data Safety and Monitoring Board (DSMB).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-Up Period.

After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo.

Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008; EudraCT Number 2017-002325-38, subject to a separate study approval) in which all patients will receive active treatment. A patient who prematurely withdraws due to intolerable symptoms and has completed at least 12 weeks of the Treatment Period and End of Treatment (EOT) Visit, will also be offered the opportunity to enter Study A4250-008.

Visit windows applicable to each visit are presented in the schedule of assessments (Table 1). There will be up to 10 clinic visits during the study (Figure 1) and one scheduled telephone contact, as follows:

- Visit 1: Screening Period start. Patient is considered enrolled after signing the informed consent form (ICF) (Days -56 to -35)
- Visit 2: second Screening Visit (Days -28 to -7)
- Visit 3: Randomization Visit (Day 0)
- Telephone Contact 1: (Day 14)
- Visit 4: Clinic Visit (Day 28)
- Visit 5: Clinic Visit (Day 56)
- Visit 6: Clinic Visit (Day 84)
- Visit 7: Clinic Visit (Day 126)
- Visit 8: Clinic Visit (Day 154)
- Visit 9: Clinic Visit, EOT (Day 168)

- Visit 10: Follow-Up Visit, 28 days after last dose of study drug (Day 196). All patients continuing in Study A4250-008 will not have this Follow-up Visit.

Additional clinic visits may be required for patients who need direct site assistance, e.g., due to AE monitoring, to fulfil screening requirements, and/or for safety maintenance.

Screening Period

Informed consent must be obtained at Visit 1 prior to performing any study procedures. After signing the ICF, patients will be considered enrolled and evaluated for study eligibility.

Daily recording of pruritus using an electronic diary (eDiary) will be started to determine confirmation of the magnitude and the baseline pruritus symptoms before the study. After consultation with the Medical Monitor, patients failing eligibility criteria may be re-screened after 2 months. Patients not fulfilling inclusion/exclusion criteria after 3 attempts are not allowed to re-screen.

Caregivers/patients will be instructed on the daily use of the eDiary. The eDiary will include patient-reported outcome (PRO) and observer-reported outcome (ObsRO) items for evaluation of itching (PRO), scratching (ObsRO), and sleep disturbance (PRO and ObsRO) during the Screening Period (study Days -56 to 0), throughout the 24-week Treatment Period (study Days 0 to 168), and Follow-up (Day 196).

The 14 consecutive days prior to randomization will be used for eligibility calculation of the diary entries. If a patient does not meet the minimum number of observations (see Section 9.2.2 for scoring and missing data definitions) to determine eligibility, the patient may be re-screened. Observer-reported outcomes in patients of all ages will be recorded by a caregiver. If possible, the same caregiver will complete the Albireo ObsRO items throughout the study.

At a second Screening Visit (Visit 2) additional laboratory samples will be taken for eligibility assessments.

Treatment Period

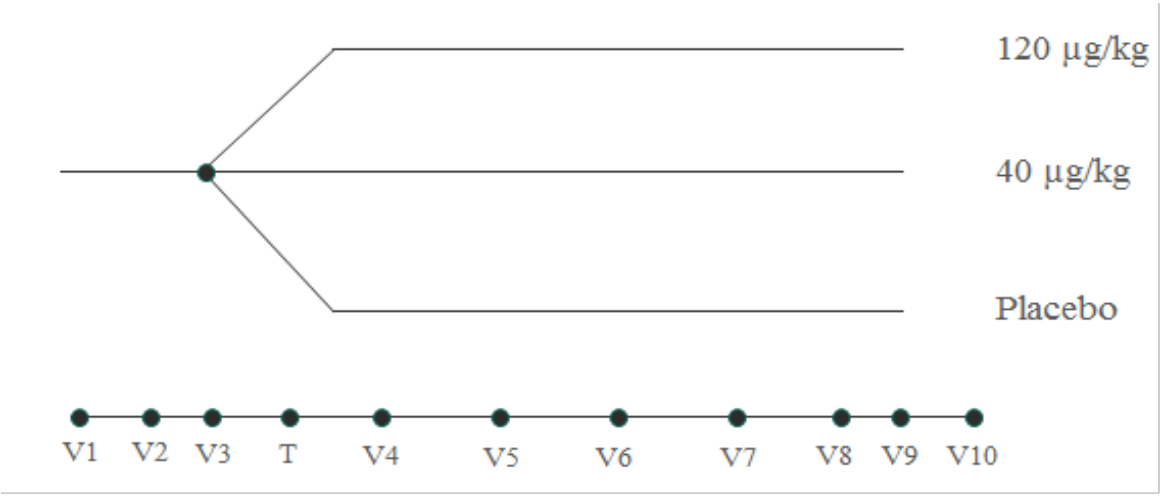
Eligibility for randomization will be determined at Visit 3 using the pruritus (scratching reports from the Albireo ObsRO) data in the 2 weeks before Visit 3, clinical genetic confirmation of diagnosis, and the liver biochemistry evaluations from the previous screening visits. Two weeks after Visit 3, there will be a telephone contact with the caregiver/patient to evaluate any AEs.

The patient will return to the clinic at Visit 4, Visit 5, Visit 6, Visit 7, Visit 8, and Visit 9 for assessments. If a new caregiver will begin to complete the Albireo ObsRO during the course of the trial, they will be provided with training during a clinic visit before they begin to use the eDiary. Additionally, caregivers will be requested to use the diary to report the time each dose of study drug is administered during the Treatment Period.

The last dose of study drug will be administered the day before Visit 9. At study Visit 9, the patient has the option to enroll in an open-label extension study (A4250-008) should they meet the required entry criteria. If the patient is continuing into Study A4250-008 this will be the last visit in this study and the first visit in Study A4250-008. Visit 9/EOT should also be performed at the time patients are prematurely withdrawn from the study.

Clinic Visit 10 will take place 28 days after Visit 9 for all patients not continuing into Study A4250-008.

Figure 1 Study Design



T: telephone contact; V: study visit

End of Study

The end of the study is defined as follows:

a) End of study in one country: last patient last visit (LPLV) and sites are closed

b) End of study globally: LPLV globally and all sites closed

7.1.2 Schedule of Assessments

The schedule of assessments is presented in Table 1. For information on blood volumes, please see Appendix 7.

Table 1 Schedule of Assessments

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Informed consent[c]	X										
Inclusion/exclusion criteria	X		X								
Demographics	X										
Concomitant medication[d]	X	X	X	X	X	X	X	X	X	X	X
Medical and surgical history	X		X								
Physical examination including voluntary photography	X		X				X			X	
Skin examination	X		X		X		X			X	X
Vital signs[e]	X	X	X		X	X	X	X	X	X	X
eDiary: itching, scratching, and sleep scores[f]	Daily diary entry										
Clinical chemistry[g]	X		X		X	X	X	X		X	X
Hematology[g]			X		X	X	X	X		X	X
Urinalysis[g]		X					X			X	
Serum bile acids[h]	X	X	X		X	X	X	X	X	X	X

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
INR		X			X		X		X		X
Autotaxin[i]			X		X					X	
p-C4[i]			X		X					X	
AFP			X							X	
Vitamins A & E[h]		X					X			X	
25-hydroxy vitamin D			X				X			X	
Blood sample for A4250 PK[i]					X					X	
Abdominal Ultrasound			X							X	
Fibroscan® (where available)			X							X	
QoL questionnaire (PedsQL)			X							X	
Patient/Caregiver/Clinician Global Impression of Change					X		X			X	
Patient/Caregiver/Clinician Global Impression of Symptoms			X		X		X			X	
Pregnancy test[j]	X[j]	X	X		X	X	X	X	X	X[j]	X

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Study drug dispensed[k]			X		X	X	X	X	X		
Adverse events[l]	Continuous collection										
Study drug compliance					X	X	X	X	X	X	

AFP: alfa-fetoprotein; BMI: body mass index; eCRF: electronic case report form; eDiary: electronic diary; EOT: End of Treatment; ICF: informed consent form; INR: international normalized ratio; p-C4: plasma 7α Hydroxy 4-cholesten-3-one; PedsQL: Pediatric Quality of Life Inventory; PK: pharmacokinetic; QoL: quality of life; RR: respiratory rate; SAE: serious adverse event; s-BA: serum bile acid.

a Must also be performed at the time a patient is prematurely withdrawn from the study. This visit is the same as Visit 1 in the extension protocol (Study A4250-008).

b Assessments must be performed 28 days following the final dose of study drug. This visit is omitted if patients are entering Study A4250-008.

c The ICF (and assent) must be signed before any study procedures are performed.

d Past (within 1 month prior to Visit 1) and current medication will be documented in the eCRF.

e Includes blood pressure, pulse, RR, temperature, height (or length depending on age), and body weight (using a certified weight scale). BMI will be calculated.

f Includes training on use of the device at Screening (Visit 1) and review of patient daily entries during 14 days prior to Clinic Visit 3 to determine eligibility.

g See Table 3 for detailed parameters.

h Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA and vitamin A. Exceptions can be made for infants, less than 12 months of age, if unable to fast for the full 4 hours. All s-BA results during the treatment period and follow-up will be blinded.

i Samples will not be collected for patients ≤10 kg. In the event of an SAE, a PK sample should be collected as close to the onset of the event as possible.

j For girls who have reached menarche. Serum test at Visit 1; urine test at other visits. A serum pregnancy test will also be conducted at Visit 9 for patients entering Study A4250-008. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy.

k Study drug to be taken once daily on Day 1 through Day 167 as described in Section 8.2.

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**

1 Adverse event information will be collected from the time of signing of the ICF to study discontinuation.

7.1.3 Study Procedures and Assessments

7.1.3.1 Screening Period (Days -56 to 0)

Day -56 to Day -35/Clinic Visit 1

Patients will undergo a Screening Visit up to 56 days prior to the planned first day of study treatment. Screening procedures and assessments are as follows:

- Obtain written informed consent
- Assess inclusion/exclusion criteria (Section 7.2)
- Record demographics (age, full date of birth, gender, race, and ethnicity)
- Document concomitant medications
- Medical and surgical history (date of diagnosis of PFIC Types 1 or 2, prior investigational medications for PFIC, historical liver function test [LFT] values, any surgery performed including biliary diversion (if applicable), any other diagnosis, and historical liver biopsy data)
- Collect, if available, past confirmatory clinical genetic laboratory report for PFIC-1/PFIC-2 and send to central reader for review. If a historical report is equivocal, unavailable, or unobtainable, a blood sample for clinical genetic testing will be collected to determine eligibility
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- eDiary training and compliance requirements; caregivers and, if applicable, patients begin daily recording of pruritus using the eDiary (Appendix 2)
- Clinical chemistry (Section 10.2.1)
- s-BA (Section 9.2.1)
- Serum pregnancy test for girls who have reached menarche. Please see Appendix 6 for contraceptive requirements
- AE monitoring

Day -28 to Day -7/Clinic Visit 2

A second Screening Visit will be performed. Screening procedures and assessments are as follows:

- Review concomitant medications
- Vital signs (Section 10.2.6)

- Albireo PRO/ObsRO eDiary review for compliance
- Urinalysis (Section 10.2.1)
- s-BA (Section 9.2.1)
- International normalized ratio (INR)
- Vitamins A and E
- Urine pregnancy test for girls of who have reached menarche
- AE monitoring

7.1.3.2 Treatment Period (Day 0 to Day 168)

Study Day 0/Clinic Visit 3 (Randomization)

At the randomization visit, the following assessments will be conducted:

- Review inclusion/exclusion criteria (Section 7.2)
- Review concomitant medications, medical and surgical history
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance and entrance criteria requirement (see Inclusion Criteria 4, Section 7.2.1)
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin and p-C4 (samples will not be taken for patients ≤10 kg)
- 25-hydroxy vitamin D
- Alfa-fetoprotein (AFP)
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)
- Administer QoL (Pediatric Quality of Life Inventory [PedsQL]) questionnaire
- Patient/caregiver/clinician complete the patient global impression of symptoms (PGIS)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Study drug is dispensed and patients are instructed to administer daily from Day 1

Study Day 14 (Telephone Contact)

A study nurse will contact the patient/caregiver by telephone for monitoring of the following:

- Review concomitant medications
- Albireo PRO/ObsRO eDiary review for compliance
- AEs

Study Day 28 and Day 84/Clinic Visits 4 and 6

The following procedures and assessments will be performed:

- Review of concomitant medications
- Physical examination, including voluntary photography (at Clinic Visit 6 only; Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- Urinalysis (at Clinic Visit 6 only; Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- Autotaxin, p-C4, and A4250 pharmacokinetic (PK) assessment (at Clinic Visit 4 only; samples will not be taken for patients ≤10 kg)
- Vitamins A and E, and 25-hydroxy vitamin D (at Clinic Visit 6 only)
- Patient/caregiver/clinician complete the patient global impression of change (PGIC) and PGIS
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 56 and Day 126/Clinic Visits 5 and 7

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance

- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 154/Clinic Visit 8

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- s-BA (Section 9.2.1)
- INR
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 168/Clinic Visit 9/EOT

The last dose of study drug is taken in the morning the day before Clinic Visit 9, and the following procedures and assessments will be performed (also performed at the time a patient prematurely withdraws):

- Review of concomitant medications
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry, hematology, and urinalysis (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin, p-C4, and A4250 PK assessment (samples will not be taken for patients ≤10 kg)

- AFP
- Vitamins A, E, and 25-hydroxy vitamin D
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)
- Administer PedsQL questionnaire
- Patient/caregiver/clinician complete the PGIC and PGIS
- Urine pregnancy test for girls who have reached menarche, or serum pregnancy test for girls continuing to study A4250-008
- AE monitoring
- Evaluation of study drug compliance

7.1.3.3 Follow-Up Period

Study Day 196/Clinic Visit 10

All patients not continuing into Study A4250-008 will return to the study site 28 days after Clinic Visit 9 or the last dose of study drug for the following assessments:

- Review of concomitant medications
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- Urine pregnancy test for girls who have reached menarche
- AE monitoring

7.2 Study Population

A total of approximately 60 patients with a clinical diagnosis of PFIC Type 1 or 2 that have been genetically confirmed will be randomized. The study will target 15% of enrolled patients to be PFIC-1 patients. If enrollment of all PFIC-2 patients is completed and the 15% enrollment of PFIC-1 patients has not yet been achieved, then enrollment of PFIC-2 patients will continue in order to reach the total study target enrollment.

An attempt will be made to randomize a minimum of 10 patients from each age category (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years).

A maximum of 5 patients that were treated in Study A4250-003 will be allowed to participate in this study.

7.2.1 Inclusion Criteria

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have clinical genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples at least 7 days apart (Visits 1 and 2) prior to randomization
4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the eDiary average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients will be expected to have a consistent caregiver for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

7.2.2 Exclusion Criteria

1. Patient with pathologic variations of the *ABCB11* gene that predict complete absence of the BSEP protein
2. Patient with past medical history or ongoing presence of other types of liver disease including, but not limited to, the following:
   a) Biliary atresia of any kind
   b) Benign recurrent intrahepatic cholestasis, indicated by any history of normal s-BAs
   c) Suspected or proven liver cancer or metastasis to the liver on imaging studies
   d) Histopathology on liver biopsy is suggestive of alternate non-PFIC related etiology of cholestasis
3. Patient with a past medical history or ongoing presence of any other disease or condition known to interfere with the absorption, distribution, metabolism (specifically bile acid metabolism), or excretion of drugs in the intestine, including but not limited to, inflammatory bowel disease

4. Patient with past medical history or ongoing chronic (i.e., >3 months) diarrhea requiring intravenous fluid or nutritional intervention for treatment of the diarrhea and/or its sequelae
5. Patient has a confirmed past diagnosis of infection with human immunodeficiency virus or other present and active, clinically significant, acute, or chronic infection, or past medical history of any major episode of infection requiring hospitalization or treatment with parenteral anti-infective treatment within 4 weeks of treatment start (study Day 1) or completion of oral anti-infective treatment within 2 weeks prior to start of Screening Period
6. Any patient with suspected or confirmed cancers except for basal cell carcinoma, and non-liver cancers treated at least 5 years prior to Screening with no evidence of recurrence
7. Patient with a past medical history of chronic kidney disease with an impaired renal function and a glomerular filtration rate <70 mL/min/1.73 m^2
8. Patient with surgical history of disruption of the enterohepatic circulation (biliary diversion surgery) within 6 months prior to start of Screening Period.
9. Patient has had a liver transplant or a liver transplant is planned within 6 months of randomization
10. Decompensated liver disease, coagulopathy, history or presence of clinically significant ascites, variceal hemorrhage, and/or encephalopathy
11. INR >1.4 (the patient may be treated with Vitamin K intravenously, and if INR is ≤1.4 at resampling the patient may be randomized)
12. Serum ALT >10 × upper limit of normal (ULN) at Screening
13. Serum ALT >15 × ULN at any time point during the last 6 months unless an alternate etiology was confirmed for the elevation
14. Total bilirubin >5 × ULN at Screening
15. Patient suffers from uncontrolled, recalcitrant pruritic condition other than PFIC. Examples include, but not limited to, refractory atopic dermatitis or other primary pruritic skin diseases
16. Any patient who is pregnant or lactating or who is planning to become pregnant within 24 weeks of randomization
17. Sexually active males and females who are not using a reliable contraceptive method with ≤1% failure rate (such as hormonal contraception, intrauterine device, or complete abstinence) throughout the duration of the study and 90 days thereafter (from signed

informed consent through 90 days after last dose of study drug). See Appendix 6 for further details

18. Patient with a past medical history of alcohol or substance abuse will be excluded. Patient must agree to refrain from illicit drug and alcohol use during the study
19. Administration of bile acid or lipid binding resins and medications that slow GI motility (Refer to Appendix 1 – Concomitant Medications Guidelines)
20. Patient has had investigational exposure to a drug, biologic agent, or medical device within 30 days prior to Screening, or 5 half-lives of the study agent, whichever is longer
21. Patient who has been previously treated with an IBAT inhibitor whose pruritus has not responded to treatment
22. Any other conditions or abnormalities which, in the opinion of the investigator or Medical Monitor, may compromise the safety of the patient, or interfere with the patient participating in or completing the study

7.2.3 Withdrawal of Patients

Patients/caregivers will be informed that they have the right to withdraw from the study at any time, without prejudice to their medical care, and that they are not obliged to state their reasons.

Any withdrawal, and reasons for withdrawal, must be fully documented in the electronic case report form (eCRF) and source documents and the patient followed by the investigator/investigative staff. Even after the study is completed at the Follow-up Visit, the investigator/investigative staff will, regardless of reason for withdrawal, record any patient data they receive concerning SAEs, and all drug-related non-serious AEs, and subsequently report these according to Section 10.1. Withdrawn patients will not be replaced.

Patients will be withdrawn in the following circumstances:

- A patient's/caregiver's desire for withdrawal for any reason
- Lost to follow-up (every effort must be made to contact the patient/caregiver; a certified letter must be sent)
- An AE that, in the opinion of the investigator, necessitates withdrawal
- A worsening of the symptoms related to diarrhea occurs
- Death
- Patient/caregiver estimation of intolerable symptoms
- A patient's/caregiver's substantial noncompliance (eDiary and study drug compliance) or protocol violation

- An investigator's opinion that continuing the patient in the study is not appropriate. The investigator may withdraw a patient at any time, if it is considered to be in the patient's best interest

A patient who prematurely withdraws due to intolerable symptoms and has completed at least 12 weeks treatment in the double-blind study, will be offered the opportunity to enter open-label Study A4250-008. A patient who withdraws from treatment prematurely and does not enter Study A4250-008 will have EOT (Visit 9) assessments at the time of withdrawal and a follow-up assessment (Visit 10) 28 days following the last dose of study drug, and the patient will be recorded as withdrawn.

7.2.4 Study Termination by Sponsor

This study may be terminated at any time by Albireo if serious side effects should occur, if the investigator does not adhere to the protocol; or if, in the sponsor's judgment, there are no further benefits to be achieved from the study. In this event, Albireo/designee will inform the study investigators, institutions, and all regulatory authorities.

Albireo may temporarily or permanently discontinue the study at an investigative site at any time for safety, ethical, compliance, or other reasons. If this is necessary, Albireo will endeavor to provide advance notification to the site. If a site or the study is suspended or discontinued, the investigator/investigative staff will be responsible for promptly informing the Independent Ethics Committee (IEC). If required by local regulations, Albireo/designee will be responsible for informing the IEC and the regulatory authority of study or site discontinuation. In such an event, all study data and unused study drug must be returned to Albireo.

8 TREATMENT OF PATIENTS

8.1 Identity of Study Drug

A4250 and placebo will be supplied as capsules for oral administration. White opaque capsules filled with pellets containing A4250 or placebo will be provided. Two different capsule sizes will be available, as follows:

- Capsule size 0 that can be opened
- Capsule size 3 that should be swallowed intact. These size 3 capsules may be opened only under exceptional circumstances, e.g., patient cannot swallow capsule intact

The content of the A4250 and placebo capsules will be identical in appearance. Capsule filling weight will also be identical for high dose, low dose, and placebo. Bottles with 34 capsules will be given to the patient at each visit. A patient who requires 2 or more capsules per day will be given multiple bottles. Refer to the investigational product manual.

8.2 Administration of Study Drug

Patients will be dosed with A4250 at a dose of 40 µg/kg/day, 120 µg/kg/day, or placebo for 24 weeks. Study drug will be dispensed to the patient at Visits 3, 4, 5, 6, 7, and 8, together with instructions on how to store and take the drug. Study drug administration data, including whether each patient took each dose or partial doses of study drug, whether there were any delayed or missed doses, and whether the capsule was opened or swallowed whole will be documented through the eDiary and transferred to the study database.

A4250 should be taken in the morning, together with food. On clinic visits days when laboratory assessments are conducted, study drug should be taken after the visit and after laboratory samples are taken. Patients should not crush or chew the capsule(s). When swallowing the capsule intact, the patient should administer the dose with a glass of water.

For a patient unable to swallow capsule intact, the capsule can be opened and the contents sprinkled and mixed in a small amount of soft room-temperature yogurt, apple sauce, or fruit purée. A list of food options can be found in the Pharmacy manual. The amount of food should be less than what the patient/infant is expected to eat and should be followed by water.

1. Hold the capsule over the prepared food, gently twist open and allow the content to fall into food
2. Mix the entire capsule content with 1 or 2 tablespoons (15 to 30 mL) of soft food (apple sauce, fruit purée, or yogurt)
3. Administer the mixture immediately after mixing.

The number of capsules provided to the patient will be based on body weight at Visit 3. At Visit 6, if a patient's weight has shifted to another weight category (identified in Table 2), the number of capsules per day may be adjusted.

Table 2 Dosing and Capsule Strength

Body Weight (kg)	Capsule Size	Number of Capsules per Day	Capsule Strength, Low Dose (µg)	Total Dose (µg)	Capsule Strength, High Dose (µg)	Total Dose (µg)	Capsule Size, Placebo (µg)
5.0 to <7.5	0	1	200	200	600	600	0
7.5 to <12.5	0	2	200	400	600	1200	0
12.5 to <17.5	0	3	200	600	600	1800	0
17.5 to <19.5	0	4	200	800	600	2400	0
19.5 to <25.5	3	2	400	800	1200	2400	3
25.5 to <35.5	3	3	400	1200	1200	3600	3
35.5 to <45.5	3	4	400	1600	1200	4800	3
45.5 to 55.5	3	5	400	2000	1200	6000	3
>55.5	3	6	400	2400	1200	7200	3

8.3 Study Treatment Packaging and Labeling

8.3.1 Packaging and Labeling

The capsules will be packed in high-density polyethylene containers, with child-proof polypropylene caps. Study drug capsules containing A4250 in the strength specified in Table 2 above will be manufactured.

Packaging and labeling will be prepared to comply with applicable regulatory requirements.

8.3.2 Storage

Treatment packs containing A4250 capsules should be stored and dispensed in accordance with regulations in their original containers. The storage facility at the investigative site should be locked and the storage temperature should be between 15°C and 25°C.

Patients/caregivers should be informed of appropriate storage conditions (i.e., room temperature, between 15°C and 25°C).

Any deviations from the recommended storage conditions should be immediately reported to Albireo and the study drug should not be used until authorization has been given by Albireo.

8.3.3 Blinding and Randomization of Study Drug

A double-blind design is employed so that both the investigators and the patients will be unaware of the treatment assignment during the study.

After written informed consent is obtained from an eligible patient, an 8-digit patient identification number will be assigned. The first 2 digits will denote the country, followed by a 3-digit site number and a 3-digit patient sequence number. This number will be created and

allocated by the Interactive Web Response System (IWRS), when the patient first enters the system at Screening. The randomization codes will be computer-generated by [redacted] Biostatistics and kept by a statistician independent from the project team. The randomization will be done in blocks and stratified according to PFIC class (Type 1 and 2) and age class (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) to ensure approximate balance between dose schemes (1:1:1). A separate randomization list will be prepared for the maximum 5 patients from Study A4250-003 regardless of stratification. Randomization codes will be assigned sequentially as patients become eligible for randomization.

Patients who are eligible for randomization will be assigned a unique 4-digit randomization number by IWRS. This randomization number identifies which treatment will be allocated to the patient. Patients who withdraw from the study after randomization visit are not to be replaced and their randomization number will not be reused.

To ensure blinding, the study drug and the matching placebo have the same shape and size. Patients will receive capsule(s) of A4250 according to their dose group or capsule(s) of matching placebo once a day during the double-blind Treatment Period. Labels on the study drug containers will not identify the treatment that a patient is randomized to. Traceability of the treatment is ensured by the study drug number.

The 5-digit study drug number will identify study drug packs and will be detailed on the study drug label. Dispensing of study drug will be coordinated by IWRS.

The system will assign a study drug number corresponding to the randomization arm. The randomization number will be used in the background only to ensure no unblinding and will not be displayed to end-users in the IWRS.

8.4 Procedure for Breaking the Randomization Code

Should a situation arise where unblinding is urgently required, i.e., only where knowledge of the study drug is required to adequately manage a life-threatening situation, the investigator at that study site may perform immediate unblinding through IWRS. The responsibility to break the treatment code in emergency situations resides solely with the investigator. The investigator should make every effort to discuss the rationale (status and outcome) for emergency unblinding with the sponsor Medical Monitor or the [redacted] Medical Monitor as soon as possible to review the individual patient details.

The study site investigator and appropriate [redacted] project team members will be authorized to access the emergency unblinding functionality within the IWRS. Emergency unblinding takes place in the IWRS via a dedicated emergency unblinding module. The system will require the user to enter their emergency unblinding, which is typically provided at the same time login credentials are distributed. The exact description of the treatment assigned to the individual patient then will be accessible to the user. Once the randomization code is broken for a patient, he/she must be withdrawn from the study and all assessments and procedures at EOT should be performed. As a follow-up, Visit 10, will be performed according to the time

schedule. Once a randomization code has been broken, the investigator must inform the [redacted] and sponsor Medical Monitor in writing within 24 hours. In case of technical issues accessing the system online, please see the IWRS site user manual for country-specific contact telephone numbers for the [redacted] HelpDesk 24/7 system support.

8.5 Patient Compliance

The study nurse will monitor eDiary compliance by routine review of the CRF health website. If both diary entries on a day are missing, the study nurse will call the patient/caregiver to remind them to complete all scheduled entries. Any noncompliance will be documented and explained in the source documents.

Study drug compliance will also be checked through diary responses. Additionally, patients will return all unused study drug at Visits 4, 5, 6, 7, 8, and 9. The study site staff will count all returned drug, assess compliance, and record details in the eCRF.

Treatment Compliance = 100 × ((Number of study drug dispensed – number of study drug returned)/number of study drug that should be taken).

Treatment compliance between 80% and 120% will be acceptable.

8.6 Study Drug Accountability

Records shall be maintained of the delivery of study treatment(s) to the study site(s), of the inventory at the study site(s), of each use of the study treatment(s) for each patient, and of the return of unused study treatment(s) to Albireo.

These records shall include dates, quantities, batch numbers, expiry dates, and the unique code numbers assigned to the study drug and to the study patients.

The investigator will be responsible for ensuring that the records adequately document that the patients were provided the quantities specified in the protocol and that all study drug received from Albireo is reconciled.

8.7 Concomitant Therapy and Prohibited Medications

The investigator will note all ongoing medication and any medication recently stopped (within 1 month prior to Visit 1) in the eCRF. At Visits 2 through 10, all changes in medication (stopping or starting new medication or changes in dose) will be recorded in the eCRF. All medications (prescribed or over the counter) for pruritus and sleep will be recorded.

All medications taken by a patient within 3 months prior to the first intake of study drug are regarded as prior medication.

All medications taken by a patient on or after the first intake of study drug, and which continue to be taken during the study, are regarded as concomitant medication.

Prohibited medications are listed in Appendix 1.

9 ASSESSMENT OF EFFICACY

9.1 Efficacy Endpoints

9.1.1 Primary Efficacy Endpoint

The primary efficacy endpoints are as follows:

- **European Union (EU) and rest of the world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  Fasting s-BA baseline will be calculated as the average of the value at randomization and the previous visit (Clinic Visit 2). The end value is the average of the values at Weeks 22 and 24 after the start of double-blind treatment.

- **United States (US)**: Proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period. A positive pruritus assessment is defined as a scratching score of ≤1 or at least a one-point drop from baseline on the Albireo ObsRO instrument. At each assessment, the AM score will be compared to the AM baseline, and the PM score will be compared to the PM baseline. Both AM baseline and PM baseline pruritus assessments will be included in the analysis of this endpoint. AM scores from the 14 days prior to the first dose of study medications will be averaged as AM baseline. PM scores from the 14 days prior to the first dose will be averaged as PM baseline.

9.1.2 Secondary Efficacy Endpoint

Secondary efficacy endpoints include the following:

- **EU and RoW**: Proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period. A positive pruritus assessment is defined as a scratching score of ≤1 or at least a one-point drop from baseline on the Albireo ObsRO instrument. At each assessment, the AM score will be compared to the AM baseline, and the PM score will be compared to the PM baseline. Both AM baseline and PM baseline pruritus assessments will be included in the analysis of this endpoint. AM scores from the 14 days prior to the first dose of study medications will be averaged as AM baseline. PM scores from the 14 days prior to the first dose will be averaged as PM baseline.

- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

- **All Regions:**

All secondary endpoints are compared to placebo.

- Change from baseline to Week 12 and to Week 24 in fasting serum bile acids
- Change from baseline to Week 12 and to Week 24 in serum ALT concentration
- Change in growth from baseline to Week 12 and to Week 24
- Proportion of responders for pruritus scores at Weeks 12 and 24 based on the Albireo PRO and ObsRO instruments
- Change in sleep parameters measured with the Albireo PRO and ObsRO instruments from baseline over the 24-week Treatment Period
- Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level over the 24-week Treatment Period. A positive pruritus assessment includes an itch score ≤1, or at least a one-point drop from baseline based on the Albireo PRO instrument; only patients ≥8 years of age will complete the Albireo PRO instrument
- Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Proportion of individual AM assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Proportion of individual PM assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Number of patients undergoing biliary diversion surgery or liver transplantation

9.1.3 Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the PGIC items (Appendix 3)
- Change from baseline in fasting s-BA at Week 4

- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline by each 4 week-interval of the Treatment Period as measured by the Albireo ObsRO instrument
- Change from baseline by each 4-week interval of the Treatment Period in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline by each 4-week interval of the Treatment Period in patient-reported and observer-reported morning time itching and scratching severity scores, respectively
- Change from baseline by each 4-week interval of the Treatment Period in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline by each 4-week interval of the Treatment Period in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in PedsQL (Appendix 4)
- Change from baseline in serum ALT concentration at Week 4
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST and GGT at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, p-C4) at Week 24
- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events
- All-cause mortality

9.2 Efficacy Assessments

9.2.1 Serum Bile Acids

Blood samples for analysis of fasting total s-BA will be drawn at all visits. Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA. Exceptions can be made for infants, <12 months of age, if unable to fast for the full 4 hours. Any visit at which a bile acid sample result is unreportable, an additional unscheduled visit for a repeat sample collection may be scheduled. All s-BA results during

the treatment period and follow-up will be blinded. Samples will be processed and transported to a central laboratory per instructions in the laboratory manual.

9.2.2 Itching, Scratching and Sleep Score

Itching, observed scratching, and sleep disturbance will be recorded twice each day via eDiary. Patients and/or caregivers will be instructed to complete the eDiary every day in the morning after the patient wakes and in the evening just before the patient goes to sleep throughout the study. Patients and caregivers will receive a formal training session during Visit 1.

The eDiary includes Albireo ObsRO and PRO items. Patients <8 years of age will not be asked to complete the Albireo PRO items; only the Albireo ObsRO will be completed by caregivers of patients in this age group. Older patients, 8 to 18 years of age, will complete the Albireo PRO items and the caregiver will complete the Albireo ObsRO items. The Albireo PRO items assess severity of itch, aspects of sleep disturbance (morning diary only), and tiredness. For patients 8 to 12 years of age, the caregiver will read the Albireo PRO items along with the child and record the child's response. A guide will be provided to the caregivers that provides standardized explanations of the Albireo PRO items, in case the patient is confused or requires clarification about the meaning of a question. The Albireo ObsRO items assess severity of observed scratching, aspects of observed sleep disturbance (morning diary only), and observed signs of tiredness (evening diary only). The Albireo ObsRO and PRO scratching and itch severity items use 0 to 4 response scales, where each response is distinguished by a unique facial expression, verbal anchor, number, and color code (Appendix 2). To meet eligibility criteria, a scratch score will be calculated from daily diary entries (ObsRO items) the 14 consecutive days prior to randomization.

A daily AM & PM score for the Albireo ObsRO scratching item will be averaged from the 2 ratings for each day. A daily score will be considered missing if both of the daily assessments are missing. A weekly score will be calculated by averaging the daily scratching scores. A weekly score will be considered missing if ≥4 out of 7 days a week of data are missing. For AM & PM baseline, a 14-day daily score of AM & PM prior to the first dose of study medications will be averaged as the baseline score.

For AM baseline, a 14-day AM score prior to the first dose of study medications will be averaged as the AM baseline score. The same approach will be used for PM baseline and to calculate a patient-reported itch severity score. The handling of missing data is described in Section 11.2.2.2.

9.2.3 Growth

Growth will be measured as height (velocity) and weight (Z-score) using a certified weight scale. BMI will be calculated by weight (kg) / height $(m)^2$. Change will be defined as linear growth deficit (weight and BMI for age) compared to a standard growth curve (Z-score, SD from P50).

9.2.4 Quality of Life Questionnaire (PedsQL)

Patients and caregivers will be asked to complete a QoL questionnaire (PedsQL) at Visit 3 and Visit 9. Details of the questions included on the questionnaire are provided in Appendix 4.

9.2.5 Biomarkers and PK Samples

Blood samples for autotaxin and p-C4 will be drawn at Visits 3, 4, and 9 only for children with body weight >10 kg. Blood for A4250 PK assessment will only be drawn at Visits 4 and 9 (only for children with body weight >10 kg), and as close to the onset of an SAE as possible, should an SAE occur. Samples will be processed and transported to a laboratory per instructions in the laboratory manual.

9.2.6 PELD/MELD Score

At randomization and end of treatment, PELD score will be calculated for children up to 12 years of age. For children 13 years or older, the MELD score will be calculated.

PELD Score = 4.80 * ln(total bilirubin) + 18.57 * ln(INR) - 6.87 * ln(albumin) + 4.36 (if patient <1 year: scores for patients listed for liver transplantation before the patient's first birthday continue to include the value assigned for age (<1 year) until the patient reaches the age of 24 months) + 6.67 (if the patient has growth failure [≤2 SD])

Laboratory values less than 1.0 will be set to 1.0 for the calculation of the PELD score.

MELD Score = 9.57 * ln(creatinine) + 3.78 * ln(total bilirubin) + 11.2 * ln(INR) + 6.43

Laboratory values less than 1.0 will be set to 1.0 and serum creatinine values greater than 4.0 mg/dL (equivalent to 353.6 µmol/L) will be set to 4.0 for calculation of the MELD score.

9.2.7 Fibroscan®

Where available, Fibroscan® will be performed as per institution standard practice at Visits 3 and 9.

9.2.8 Markers of Fibrosis

APRI score and Fib-4 score will be calculated at randomization and at end of treatment.

APRI = [(AST in U/L) / (AST ULN in U/L)] / (Platelets in 10^9/L)

Fibrosis 4 Score = (Age*AST in U/L) / (Platelets in 10^9/L * √(ALT in U/L)).

9.2.9 Global Impression of Change and Global Impression of Symptom Measures

Patients (≥8 years of age), caregivers, and clinicians will complete the PGIC and the PGIS measures (Appendix 3) at randomization (Visit 3; PGIS only), Visits 4 and 6, and at EOT (Visit 9).

The PGIC items assess change in itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) since starting the study drug. The PGIS items assess itch

(patient version), scratching (caregiver and clinician versions), and sleep (all versions) in the past week. Caregivers and clinicians will complete the PGIC and PGIS for all patients; those patients ≥8 years of age will complete the patient version.

10 ASSESSMENT OF SAFETY

The timing and frequency of safety assessments are described in Section 7.1.3 and in Table 1 above.

The primary safety analysis for this study will include the incidence of total treatment-emergent adverse events (TEAE) and TEAEs categorized by causality, severity, and seriousness assessments made by the investigator by comparing study drug exposure to placebo.

Trends in safety will also be evaluated for the following assessments:

- Physical examinations
- Concomitant medications
- Vital signs
- Laboratory test results (including clinical chemistry, hematology, urinalysis, AFP, and vitamins A and E, 25-hydroxy vitamin D, and INR)
- Abdominal ultrasound
- Discontinuations due to AEs

10.1 Adverse Events

10.1.1 Definitions and Investigator Assessments

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 unfavorable and unintended sign, symptom, or disease that occurs once a patient is enrolled (ICF is signed) in the study until the patient is discharged from the study, whether or not related to the study drug.

10.1.1.1 Clinical Significance

Clinical significance is defined as any abnormal finding that results in further clinical investigation(s), treatment(s), or the diagnosis of new condition. Patient-reported events and protocol-mandated laboratory values, vital signs, and physical examination findings can be considered clinically significant (i.e., an AE) if there is a deterioration as compared to baseline. Examples of clinically significant worsening from baseline could include, but is not limited to, events causing withdrawal from the study and events requiring medical intervention outside of the study causing apparent clinical manifestations, or judged relevant by the investigator.

10.1.1.2 Serious Adverse Events

Serious criteria are applied by the investigator to each AE as specifically defined below. These criteria are used to determine whether an AE is serious or non-serious. The assessment

is made independently of severity assessment (Section 10.1.1.3). For example, the development of a severe rash that occurs after signing of informed consent may not meet serious criteria as defined below and therefore would be considered a severe, non-serious AE.

Any AE that meets any 1 of the following 6 criteria is considered an SAE:

- The outcome of the AE is **death**
- The AE is immediately **life-threatening**. Life-threatening means that the patient is, in the opinion of the investigator, at immediate risk of death from the reaction as it occurred. This does not include an AE that, if more severe, might have caused death
- The AE results in persistent or significant **disability/incapacity**. Disability means a substantial disruption of a person's ability to conduct normal life functions
- The AE requires or prolongs **hospitalization**
- The AE results in a **congenital anomaly/birth defect**
- **The AE is an important medical event**. Important medical events may meet serious criteria should the investigator assess that they may significantly jeopardize the patient, represent a significant hazard, or requires medical/surgical intervention to prevent one of the outcomes listed above. Examples of potential SAEs based on this criteria include, but are not limited to, allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions even if they do not result in inpatient hospitalization, or the development of drug dependency and drug abuse.

10.1.1.3 Severity Assessment

Severity assessments are based on the intensity of the event in relation to expectation. The investigator will assess the intensity of AEs based on the following definitions:

- Mild (awareness of sign or symptom, but easily tolerated)
- Moderate (discomfort sufficient to cause interference with normal activities)
- Severe (incapacitating, with inability to perform normal activities)

Severity is a measure of intensity where seriousness is defined by the criteria outlined in Section 10.1.1.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not meet serious criteria, and therefore would be assessed as a severe AE but not an SAE.

10.1.1.4 Causality Assessment

The investigator determines the causality of all AEs to the study drug using medical judgment and considering all relevant factors such as (but not limited to) the underlying study indication, coexisting disease, concomitant medication, relevant history, pattern of the AE, temporal relationship to the study drug, and de-challenge or re-challenge. The causality assessment of the AE/SAE is to be made as follows.

Related to study drug (possibly, probably, or definitely related)

Based on medical judgment, there is at least a reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event follows a reasonable temporal sequence from administration of study drug
- The event could not be reasonably attributed to the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient
- The event follows a known pattern of response to study drug
- The event disappears or decreases on cessation or reduction in dose of the study drug. (It should be noted that in some situations an AE will not disappear or decrease in intensity upon discontinuation of study drug despite other clear indications of relatedness)
- The event reappears or worsens when the study drug is re-administered

Unrelated to study drug (unlikely or unrelated)

Based on medical judgment there is no reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event does not follow a reasonable temporal sequence from administration of study drug
- The event could be reasonably attributed to the known characteristics of the patient's clinical state, concurrent illness, environment or toxic factors, or other modes of therapy administered to the patient
- The event does not follow a known pattern of response to study drug
- The event does not disappear or decrease on cessation or reduction in dose of the study drug, and it does not reappear or worsen when the study drug is re-administered

10.1.2 Recording of Adverse Events

It is the investigator's responsibility to assess whether each untoward event is a clinically significant worsening from baseline, thereby considered an AE. For all AEs, the severity, seriousness, and causality to study drug for each AE as outlined in Section 10.1.1 will be assessed and recorded in the eCRF.

All serious and non-serious AEs are collected once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10) or 28 days after the last dose of study drug.

Any AEs or SAEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator until resolution or stabilization up to the database lock and recorded in the eCRF. Albireo retains the right to request additional information for any patient with ongoing AE(s) or SAE(s) at the end of the study.

TEAEs are defined as any AE that occurs after randomization (Day 0). All AEs that occur in the Screening Period, i.e., after enrollment and prior to randomization, will be collected on the eCRF as non-treatment-emergent AEs.

If there is a clinically significant deterioration of a laboratory value/vital sign or other routine study assessment that is associated with a diagnosis, the clinical diagnosis will be reported as an AE and the associated signs and symptoms will be considered additional information unless the sign or symptom is more severe than expected given the diagnosis. For example, if an investigator diagnoses a study patient with hepatitis during the study period, hepatitis would be considered the AE and the concomitant signs and symptoms of abdominal pain, vomiting, and elevated ALT and AST would not be reported separately unless, in the opinion of the investigator, one of these signs or symptoms is more severe than expected and therefore a separate AE assessment is indicated.

10.1.3 Recording and Reporting of Serious Adverse Events

Every SAE (regardless of severity and causality) that occurs once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10), and 28 days after the final dose of study drug, should immediately and not later than within 24 hours of knowledge of the event, be reported by the investigator or delegate in the SAE report form.

Report of a SAE must include at least the following information:

- Patient identification information (study number, site number, initials, and date of birth [as per local country requirements for data protection])
- The last study drug administration date
- The diagnosis of the event with the description (or a brief description of signs/symptoms/clinical course if the diagnosis is not available) and the date of onset
- The action used to treat the event (i.e., treatment medications, temporary discontinuation)
- The reason(s) for considering the event serious
- The relationship of the event to the study drug or to the study procedure (e.g., the investigator's assessment of causality)
- A brief narrative of the SAE

Follow-up reports including all new information obtained of the subsequent course of the SAE must be prepared and the information collected in the SAE submitted to [redacted] (Pharmacovigilance and Safety Services Department) by e-mail within 24 hours of knowledge of the event.

The pharmacovigilance manager (PVM) may contact the investigator to obtain further information on a reported SAE. The investigator/investigative staff must respond to any

request for follow-up information or answers to questions regarding the SAE within the same timelines as for initial reports.

The PVM reports the occurrence of the SAE and follow-up to the Albireo medical monitor for medical assessment of the case.

Should an outcome of death occur within the study period or within 28 days after the last administration of study drug, an SAE form should be completed including the main and contributory causes of death.

All SAE reports must be e-mailed to the following e-mail address within **24 hours**:

[redacted]

If email is unavailable, SAEs may be transmitted via fax to the following number:

[redacted]

In a study-related medical emergency situation, when the assigned medical monitors for the study cannot be reached by a caller, an on call physician can be reached 24 hours per day, 7 days per week, via the [redacted] call center. The following number is a chargeable telephone number allowing a global reach from both landlines and mobile phones and the internet page indicated below contains a list of country-specific toll-free telephone numbers that is provided. It should be noted that not all countries globally have access to toll-free numbers as indicated on the "24/7 Medical Help desk" index. Countries without toll-free numbers need to dial the chargeable number as indicated below. Furthermore, toll-free numbers might not be available from mobile phones.

[redacted]

10.1.4 Reporting of Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reaction (SUSAR) is an SAE that occurs in a patient, the nature or severity of which is not expected per the applicable product information (e.g., the IB for an unauthorized investigational product or summary of product characteristics for an authorized product).

Reporting and tracking of SUSARs will be in accordance to all applicable competent authority regulations. The Ethics Committee and all investigators involved in this study will be informed according to national and international requirements.

10.2 Laboratory Values/Vital Signs/Physical Examinations and Other Safety Assessments

10.2.1 Laboratory Assessments

Samples will be collected for clinical chemistry, hematology, and urinalysis testing at the time points specified in Table 1. The parameters assessed are presented in Table 3.

Blood for AFP will be drawn at Visits 3 and 9. Fat-soluble vitamin levels including vitamins A and E, and 25-hydroxy vitamin D will be drawn at Visit 2 (vitamins A & E only), Visit 3 (25-hydroxy vitamin D only), Visit 6, and Visit 9. Blood for INR (surrogate for vitamin K) will be drawn at Visits 2, 4, 6, 8, and 10. Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for vitamin A. Exceptions can be made for infants, <12 months of age, if unable to fast for the full 4 hours. If a patient has any vitamin level(s) that are outside of the reference range, vitamin supplementation adjustments may be required. Target ranges and supplementation strategy guidelines can be found in Appendix 5.

A serum pregnancy test will be performed at Visit 1 and Visit 9 if the patient is entering the A4250-008 study. A urine pregnancy test will be collected at all other visits for girls who have reached menarche. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy. Study drug administration will be held pending the confirmatory results. If the serum pregnancy test is negative, the patient can resume dosing. If the serum pregnancy test is positive, the patient should be discontinued (see Section 10.2.9).

All samples will be processed and transported to a laboratory per instructions in the laboratory manual.

The observed values will be recorded and assessed as “normal” or “abnormal not clinically significant” or “abnormal clinically significant”.

Additional safety blood samples may be needed due to follow-up of an abnormal value or analysis failure. The blood samples collected for safety laboratory analysis will be destroyed after the analyses have been completed.

Table 3 Routine Laboratory Parameters

Clinical Chemistry	Hematology	Urinalysis
• Albumin • ALT • Alkaline phosphatase • AST • Bilirubin – total and direct • Calcium • Chloride • Creatinine • Creatine kinase • Gamma-glutamyl transferase • Potassium • Sodium	• Hematocrit • Hemoglobin • Platelet count • Red blood cell count • White blood cell count and differential (neutrophils, eosinophils, basophils, lymphocytes, and monocytes)	• Blood • Glucose • Ketones • Leukocytes • Nitrites • pH • Protein

10.2.2 Individual Patient Safety Monitoring

10.2.2.1 Liver Monitoring

Strategies to monitor markers of liver disease throughout the study are outlined below where the ULN will be based on central laboratory reference values for age and gender.

If isolated transaminase elevations are observed, defined as:

1. Normal bilirubin AND absence of clinical hepatitis symptoms AND
   - ALT or AST ≥5 × ULN (if normal at baseline) or an absolute threshold of 800 U/L, whichever comes first
   - OR ALT or AST ≥3 × baseline (if abnormal at baseline) or an absolute threshold of 800 U/L, whichever comes first

Then:

a) Repeat liver profile (AST, ALT, bilirubin, and prothrombin time [PT] or INR) within 2 to 3 days

b) Evaluate creatine phosphokinase (CPK) and lactate dehydrogenase (LDH)

c) As needed (for example, persistent ALT/total bilirubin elevation or worsening of liver function), consider evaluation for alternative etiologies

Monitor the patient using close observation found in Section 10.2.2.2

If any ONE of the following criteria are met:

1. Transaminases (ALT or AST ≥3 × baseline or 800 U/L, whichever comes first) AND bilirubin (total bilirubin > 2 × ULN) elevations

2. Transaminase elevations alone (ALT or AST >10 × ULN or 5 × baseline or absolute threshold of 800 U/L, whichever comes first) in presence of normal LDH and CPK
3. Total bilirubin increased, unrelated to hemolysis (elevated reticulocyte count) or established genetic diseases, such as Gilbert's syndrome
   a. Doubling if total bilirubin was <3 mg/dL (equivalent to 51.3 µmol/L) at baseline
   b. OR Increase by >3 mg/dL (equivalent to 51.3 µmol/L) if total bilirubin was ≥3 mg/dL (equivalent to 51.3 µmol/L) at baseline
4. INR increase refractory to vitamin K administration
   a. INR >1.5 if INR was normal at baseline
   b. OR Increase by >0.4 if INR was abnormal at baseline
5. Any increase in total bilirubin and transaminases if accompanied by EITHER a symptom of clinical hepatitis (e.g., vomiting, nausea, right upper quadrant pain) OR immunological reaction (rash or >5% eosinophilia)

Then:

a) Interrupt study medication

b) Initiate drug-induced liver injury work-up for alternative etiologies

c) Repeat liver profile (AST, ALT, total bilirubin, direct bilirubin) and PT or INR within 48 to 72 hours

d) Monitor the patient using close observation found in Section 10.2.2.2

e) If a patient lives in a remote area, they may be tested locally and the results communicated to the investigator site promptly

10.2.2.2 Close Observation

- Repeat liver enzyme and serum bilirubin tests two or three times weekly. Frequency of re-testing can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the subject is asymptomatic
- Obtain a more detailed history of symptoms and prior or concurrent diseases
- Obtain a history of concomitant drug use (including non-prescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets
- Consider ruling out alternate etiology including acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; non-alcoholic steatohepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease
- Obtain a history of exposure to environmental chemical agents.

- Obtain additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin)
- Consider gastroenterology or hepatology consultations.

10.2.2.3 De-challenge/Re-challenge for Liver and Clinical Hepatitis Monitoring

1. Re-challenge is not recommended:
   a. If a patient has had possible/probable drug-induced liver injury
   b. If a decompensation event has occurred (i.e., variceal hemorrhage, ascites, hepatic encephalopathy, etc.).
2. If due to underlying cholestatic liver disease variability or another alternative etiology is identified AND liver tests returned to baseline, re-challenge may be considered after consultation with the sponsor Medical Monitor.
3. If the ALT/total bilirubin elevations are observed after re-challenge, then repeat re-challenge is discouraged.

If patient is permanently discontinued, monitoring should be continued as outlined in Section 10.2.2.5.

10.2.2.4 Diarrhea

Study drug should be discontinued if a patient develops diarrhea with at least 1 of the following concomitant signs or symptoms: grossly bloody stools, vomiting, dehydration requiring treatment with oral or intravenous rehydration and/or electrolyte imbalances, fever (≥38 C) and/or the diarrhea persists for 7 or more days.

If there is gross blood in stool, INR and platelets should be measured.

Study drug will be reintroduced (re-challenge) when the symptoms have resolved. If the diarrhea re-occurs within 1 week after the re-challenge with no alternate etiology, the patient will be permanently discontinued and monitored as outlined in Section 10.2.2.5.

10.2.2.5 Monitoring after Permanent Discontinuation of Study Drug Due to Safety

Once study drug is permanently discontinued for liver, hepatitis, diarrhea, or other severe AE related to study drug, the patient will be monitored weekly until the laboratory and clinical parameters have normalized/stabilized.

10.2.3 Demographics/Medical and Surgical History

Demographic information per country regulations (age, full date of birth, gender, race, and ethnicity), along with medical and surgical history, will be obtained and recorded in the eCRF at Visit 1.

Medical and surgical history will be entered in the eCRF at Visit 1. This includes date of diagnosis of PFIC, prior investigational medications for PFIC, historical (3 months prior to

Screening) LFT values (AST, ALT, and total bilirubin), ongoing medication, any surgery performed, any other diagnosis, and historical biopsy data.

If a liver biopsy has been performed within 1 year prior to Screening, or during the study, the results will be recorded in the eCRF.

10.2.4 Clinical Genetic Testing

For previous confirmatory clinical genetic testing results for PFIC Type 1 or 2 performed prior to Screening Visit 1, the confirmatory clinical genetic testing laboratory report will be verified to determine eligibility.

If the historical clinical genetic result is equivocal, unavailable, or unobtainable, clinical genetic analysis to confirm pathologic biallelic variations in the *ATP8B1* or *ABCB11* genes will be performed and verified to determine eligibility. No other diagnostic genetic testing will be offered.

10.2.5 Physical Examination

A physician or suitably trained qualified assistant will perform a complete physical examination, including voluntary photography of skin lesions due to scratching, at Visits 1, 3, 6, and 9. A skin examination will be conducted at Visits 1, 3, 4, 6, 9, and 10 (see Table 1).

A complete physical examination will include assessment of general appearance, eyes, ears, nose, throat, head/neck/thyroid, lymph nodes, cardiovascular, lungs/chest, abdomen, genitourinary, extremities, skin, musculoskeletal, neurologic, and other.

Skin will be examined thoroughly and excoriations/scratch marks recorded.

10.2.6 Vital Signs

Evaluation of vital signs will be performed at all visits. This includes blood pressure (systolic and diastolic), pulse, respiratory rate, temperature, height (or length depending on age), and body weight (using a certified weight scale) at clinic visits. BMI will be calculated.

10.2.7 Abdominal Ultrasound

Ultrasound of the liver and spleen will be performed at randomization (Visit 3) and at EOT (Visit 9). Liver size, echogenicity, and presence of masses/nodules as well as spleen size will be recorded.

10.2.8 Overdose

A4250 is minimally absorbed and has a very low systemic availability. Based on toxicology data, for study purposes any dose exceeding a total of >3 mg/kg body weight of A4250 taken as a single dose or as a cumulative dose within 24 hours is defined as an overdose.

The no-adverse-effect dose level in the most sensitive species (defined as 20 mg/kg/day) in the rat 1 month toxicity study gives a human equivalent dose of approximately 194 mg/day in

a 60 kg person. This dose is approximately 10-fold higher than the maximum possible dose predicted in human studies (20 mg).

The investigator/investigative staff should immediately and not later than within 24 hours of knowledge of the overdose, be reported by the investigator or delegate on the SAE report form. In the event of an A4250 overdose, the patient should be monitored closely.

10.2.9 Pregnancy

If a pregnancy is discovered in a female patient enrolled in the study before the end of dosing, the patient will be immediately discontinued from the study and will attend the same visits as a prematurely withdrawn patient. If the pregnancy is discovered in a female patient enrolled in the study after the end of dosing, the patient will continue in the study per protocol. If a pregnancy occurs in a male patient's partner at any time during the study, the pregnancy should also be reported and followed.

Pregnancy is not considered to be an AE. However, if the patient has been dosed with the study drug, the pregnancy must be reported on the Paper Pregnancy Notification Form immediately within 24 hours after recognition to Safety Manager (please refer to Section 10.1.3). Date of exposure and as far as possible, details of the period of gestation at the time of exposure must be given.

The pregnancy should be followed to determine outcome, including spontaneous termination, details of birth, and presence of any birth defects, congenital anomalies or newborn or maternal complication. The infant who was exposed in-utero will be followed for up to 2 years after delivery. Individual cases with an abnormal outcome in association with the study drug should be reported on an expedited basis, i.e., reported rapidly to a competent authority.

11 STATISTICAL EVALUATION

All personnel involved with the analysis of the study will remain blinded until database lock for the double-blind Treatment Period and until major protocol violations have been identified.

All statistical analyses will be performed using SAS version 9.3 or higher.

11.1 Sample Size and Power

For the EU and RoW, the primary analysis will relate to the serum bile acid responder endpoint. For the US, the primary analysis will relate to the pruritus endpoint. For each primary endpoint, a pooled analysis for the closed testing procedure will be applied to control the 1-sided overall type I error rate for two treatment comparisons vs. the placebo at the 0.025 level (Kane et al, 2015, Lehmacher et al, 1991, and Marcus et al, 1976). The study will enroll 60 to 70 patients in order to obtain at least least 20 evaluable patients in each arm. For each primary endpoint, simulations with 5000 iterations using 20 patients per arm were conducted to estimate the power after multiplicity adjustment, resulting in a standard error of <0.7% for each estimated power.

Based on the Phase 2 A4250-003 study data both dose groups are predicted to have similar treatment effects for both serum bile acids and pruritus. For serum bile acids, simulated proportions were analyzed using the CMH test to generate the following 1-sided p-values: both A4250 arms pooled vs. placebo, low dose vs. placebo, and high dose vs. placebo. Assuming 60% responders in the A4250 arms and 10% responders in the placebo arm, the power to claim significance for a particular A4250 arm after multiplicity adjustment is approximately 94%. The power to claim significance for at least one arm or both arms are approximately 99% and 91%, respectively.

For the proportion of positive pruritus assessments at the subject level in pruritus scores, simulated proportions were analyzed using ANCOVA to generate the following 1-sided p-values: both A4250 arms pooled vs. placebo, low dose vs. placebo, and high dose vs. placebo. With an effect size of 1.0526, the simulated power to claim significance for a particular arm after multiplicity adjustment is approximately 89%. The probability to claim significance for at least one arm or both arms are approximately 95% and 83%, respectively.

11.2 Statistical Methods

11.2.1 Statistical Analysis Sets

Safety Analysis Set

The safety analysis set will consist of all patients who received at least 1 dose of study drug. Patients will be analyzed according to the treatment they actually received. The safety analysis set will be the primary analysis set for safety analyses.

Full Analysis Set

The full analysis set (FAS) will consist of all randomized patients who received at least 1 dose of assigned treatment. Patients will be analyzed in the treatment group they were randomized to even if they received incorrect study drug. The FAS will be the primary analysis set for efficacy analyses.

Per Protocol Analysis Set

The per protocol (PP) analysis set is a subset of the FAS, and will consist of all randomized patients for whom no major protocol violation (no violation which may affect the study efficacy outcome) is documented. The PP analysis set will provide supportive data for the efficacy analyses.

Allocation of patients to the PP analysis set will be performed before unblinding of the study.

11.2.2 Methods of Statistical Analyses

11.2.2.1 General Principles

The primary objective of this study is to demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with PFIC Types 1 and 2.

In the EU and RoW, for the primary efficacy variable of fasting bile acid responder endpoint (at least a 70% reduction in fasting s-BA concentration or reaching a level ≤70 µmol/L at the end of treatment), the Cochran Mantel Haenszel (CMH) test stratified by PFIC class and age class will be performed to compare the proportion in fasting bile acid responder endpoint at the end of treatment (Week 22 and 24) in the two A4250 dose groups to placebo group. To ascertain that all data are used in the CMH analysis, neighboring strata will be pooled if all subjects in a stratum have the same response. Dropouts will be treated as non-responders. The proportion together with corresponding 95% CI, odds ratio and corresponding 95% Clopper-Pearson exact CI and p-value for the CMH test will be presented.

In the US, for the primary efficacy variable of the proportion of positive pruritus assessments at subject level over the 24-week treatment period, an analysis of covariance (ANCOVA) will be used. The model will include treatment arm, AM baseline pruritus score, PM baseline pruritus score, and randomization stratification factors, i.e. PFIC class and age class. LS mean(SE) by treatment arm and LS mean difference (SE), 95% confidence intervals, and p-values (where applicable) between treatments (120 µg/kg/day and 40 µg/kg/day, respectively) vs. placebo will be provided. If there are concerns on model assumptions, i.e. normality and homogeneity of variances, a non-parametric ANCOVA based on the rank scores will be used as a sensitivity analysis. LS mean/SE on the outcome by treatment arm and LS mean difference/SE between active dose and placebo will be provided.

The procedure for multiplicity adjustment is specified as follows:

In the closed testing procedure, the low and high dose groups will be pooled to compare with the placebo group first. If the 1-sided p-value is ≤0.025, the 1-sided p-values for low dose vs.

placebo and high dose vs. placebo will be calculated, respectively. If both individual p-values are ≤0.025, a significant treatment effect will be declared on both dose groups. If only one of them is ≤0.025, a significant treatment effect will be declared on the corresponding dose group.

Other secondary and exploratory outcome variables will provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple comparisons when testing secondary and exploratory efficacy variables.

If not otherwise specified, the baseline value of a parameter is defined as the last assessment of that parameter prior to the first intake of study treatment.

11.2.2.2 Missing Data

In the primary analysis of the pruritus endpoint, all intermittently missing assessments will be classified as negative pruritus assessments. Similarly, all planned assessments after the intercurrent events (premature treatment discontinuation, death or initiation of rescue treatments such as biliary diversion surgery or liver transplantation) will be counted as negative pruritus assessments. However, as described above, all 336 assessments planned over the 24-week treatment period will be included in the denominator of the endpoint definition.

In the primary analysis of fasting bile acid responder endpoint, dropouts and patients with a missing average at the end of the treatment will be treated as non-responders. Patients in need of surgical rescue, i.e. biliary diversion and listing for liver transplant will be classified as non-responders. Continued collection of efficacy data for patients who discontinue treatment will be made as far as possible.

11.2.2.3 Demographic and Baseline Characteristics

Descriptive summaries of demographics and other baseline characteristics (including medical and surgical history) will be presented by treatment group and overall using the FAS.

Prior and concomitant medication use during the study will be summarized by treatment group and overall using the safety analysis set.

11.2.2.4 Subject Disposition

The following will be summarized (by treatment group and overall where applicable):

- Patients enrolled (who signed the informed consent)
- Patients randomized
- Patients treated
- Patients completing the study
- Patients withdrawing early (including withdrawal reason)

Additionally, patients enrolled, included in the safety, in the FAS, and in the PP analysis set will be summarized by region.

11.2.2.5 Evaluation of Primary Efficacy Variables

For EU and RoW, the primary responder endpoint based on change in fasting bile acids will be analyzed in terms of the proportion of patients achieving the event of interest. Patients in need of surgical rescue, i.e. biliary diversion and listing for liver transplant, will be classified as non-responders. Dropouts will be treated as non-responders.

CMH stratified by PFIC class and age class will be performed to compare the proportion in fasting bile acid responders at the end of treatment (Week 22 and 24) in the two A4250 dose groups to the placebo group. To ascertain that all data are used in the CMH analysis, neighboring strata will be pooled if all subjects in a stratum have the same response. The proportion together with corresponding 95% CI, odds ratio and corresponding 95% Clopper-Pearson exact CI and p-value for the CMH test will be presented

Logistic regression will be performed as a supportive analysis to compare the proportion in fasting bile acid responders at the end of treatment (Week 22 and 24) in the two A4250 dose groups to the placebo group. The model will include treatment arm, baseline value, and randomization stratification factors, i.e. PFIC class and age class. Baseline value will be calculated as the average of serum bile acid values at randomization and the previous visit (Clinic Visit 2).

In the US, for the primary efficacy variable of the proportion of positive pruritus assessments at the subject level over the 24-week treatment period, ANCOVA will be used to analyze the pruritus endpoint. The model will include treatment arm, AM baseline pruritus score, PM baseline pruritus score, and randomization stratification factors, i.e. PFIC class and age class. LS mean(SE) by treatment arm and LS mean difference (SE), 95% confidence intervals, and p-values (where applicable) between treatments (120 µg/kg/day and 40 µg/kg/day, respectively) vs. placebo will be provided. If there are concerns on model assumptions, i.e. normality and homogeneity of variances, a non-parametric ANCOVA based on the rank scores can be used. LS mean/SE on the outcome by treatment arm and LS mean difference/SE between active dose and placebo will be provided.

Sensitivity analysis will be conducted to assess the impact of intermittently missing data as well as intercurrent events and will be specified in the SAP. Subgroup analyses will also be performed for each of the 3 age groups (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) and for patients who were not included in the previous Study A4250-003.

11.2.2.6 Evaluation of Secondary and Exploratory Efficacy Variables

The change in the secondary endpoints such as serum bile acids, total bilirubin, ALT, growth, Albireo PRO and ObsRO sleep parameters, and patient-reported itch severity will be

summarized by visit using descriptive statistics. Change in growth (weight for age and BMI for age) will also be displayed using graphical presentations.

Proportion of responders at Weeks 12 and 24 based on the PRO/ObsRO instruments estimated from both distribution and anchor-based approaches will be analyzed using the same model as specified for the primary analysis for EU and RoW.

Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval over the 24-week treatment period for AM or PM, respectively will be analyzed using the same model as specified for the primary analysis for US.

Undergoing biliary diversion surgery and/or liver transplant, all-cause mortality, and liver decompensation events will mainly be analyzed by using descriptive statistics. Kaplan-Meier curves will be used when appropriate for time to event data.

Comparisons of the change from baseline at Week 24 in PedsQL between the treatment groups will be conducted using ANCOVA. The model will include terms for baseline, PFIC class, age class, and treatment.

Other exploratory variables (Global Symptom Relief, biochemical markers and measures of bile acid synthesis, additional Albireo PRO and ObsRO sleep parameters, PELD/MELD score, APRI score, and Fib-4 score) will be analyzed descriptively for categorical and continuous data, as applicable. For continuous data, the change from baseline will be analyzed in addition to the actual visit values. For categorical data, shift tables or frequency and percentages of patients will be presented as appropriate. Additional details on secondary and exploratory efficacy variables and subgroup analyses by age class (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) will be specified in the SAP.

11.2.2.7 Evaluation of Safety Variables

Safety data will be analyzed using descriptive statistics and summaries by treatment group of SAEs, AEs, vital signs, clinical laboratory tests (hematology, clinical chemistry and urinalysis), and concomitant medication. Analyses will be performed using the safety analysis set.

Summaries of AEs (coded according to the Medical Dictionary for Regulatory Activities [MedDRA] system organ class [SOC] and MedDRA preferred term) will include the following:

- Overview of the incidence of TEAEs (TEAEs, drug-related TEAEs, TEAEs leading to study discontinuation, and treatment-emergent SAEs)
- TEAEs by SOC and preferred term
- Intensity of TEAEs by SOC and preferred term

- Drug-related TEAEs by SOC and preferred term
- TEAEs leading to study discontinuation by SOC and preferred term
- Treatment-emergent SAEs by SOC and preferred term

Concomitant medication use during the Treatment Period will be summarized by Anatomical Therapeutic Chemical (ATC) class and World Health Organization (WHO) preferred name.

Summaries of vital signs will be presented. For each visit, the actual results and the change from baseline, and the number and percentage of patients with potentially clinically significant (PCS) values observed post-baseline will be presented.

Summaries of clinical safety laboratory data will be presented. For each visit, the actual result and the change from baseline, and the number and percentage of patients with PCS values observed post-baseline will be presented.

Data listings will be provided for each patient for all safety parameters.

11.2.2.8 Compliance and Exposure

Exposure will be analyzed by calculating the number of days with exposure to study drug. Results will be presented by treatment group using the safety analysis set.

The percentage compliance will be described by treatment group and the number of patients with a compliance <80%, between 80% and 120%, and >120% will be presented based on the safety analysis set.

11.2.3 Blinded Analyses to Confirm Responder Definitions

A blinded analysis of Albireo ObsRO and PRO eDiary data will be performed after 50% or more of the planned sample has completed the Week 24 Follow-Up Period. The blinded analysis will be used to estimate a threshold of clinically meaningful change (i.e., responder definition) in Albireo ObsRO and PRO pruritus scores. The analysis will be performed by a group independent from both the study team and Albireo. Blinded data will be used for this analysis; data will be collapsed across treatment groups.

The analyses will include distribution- and anchor-based analyses to examine a responder definition for the Albireo PRO/ObsRO pruritus scores. Distribution-based analyses will include calculation of the SD and standard error of measurement for the baseline Albireo PRO and ObsRO scores. Anchor-based analyses will involve examining the degree of change on the Albireo PRO and ObsRO scores from baseline to Week 24 for patients who experience change in pruritus according to PGIC and symptom items. Details of this analysis will be included in a stand-alone SAP for assessing the measurement characteristics (reliability, validity, sensitivity to change, responder definition) of the Albireo PRO and ObsRO items.

11.2.4 Data Safety Monitoring Board

A DSMB consisting of sponsor-independent clinical and statistical experts will be established. The DSMB will periodically (approximately on a quarterly basis) meet for the review of accumulating study data, including safety (AE and laboratory data) and liver values.

The DSMB will have access to unblinded data. The DSMB will make recommendations for the remaining part of the study (further details will be provided in the DSMB charter). The DSMB may recommend continuing with the study as planned or stopping the study early for safety reasons. The DSMB will submit its recommendations in writing to Albireo Medical/[redacted] who are responsible for responding to the recommendations of the DSMB and taking appropriate action. The investigators will only be informed by Albireo Medical/[redacted] if the study requires a protocol amendment or is stopped. The DSMB may choose to make additional evaluations at any time if they feel this is warranted from a safety point of view.

The DSMB will act according to its own written standard operating procedure described in a charter and will prepare written minutes of its meetings. The charter of the DSMB will be stored in the trial master file. The DSMB will maintain records of its meetings and these will become part of the study file when the study is complete. In order not to disseminate unblinded data and to ensure that all staff involved in the conduct and final analysis of the study remain blind to the data, only the members of the DSMB and the unblinded DSMB statistician will have access to these data.

12 DIRECT ACCESS TO SOURCE DATA/NOTES

The investigator/institution shall provide direct access to source data/documents for study-related monitoring, audits, IEC/Institutional Review Board (IRB) review and regulatory inspection.

13 QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Conduct of the Study

Albireo/[redacted] shall implement and maintain quality control and quality assurance procedures with written standard operating procedures to ensure that the study is conducted and data are generated, documented, and reported in compliance with the protocol, ICH GCP and applicable regulatory requirements.

This study shall be conducted in accordance with the provisions of the Declaration of Helsinki (October 1996) and all revisions thereof, and in accordance with United States Food and Drug Administration (FDA) regulations (Code of Federal Regulations, Sections 312.50 and 312.56) and with ICH GCP (CPMP 135/95).

The investigator may not deviate from the protocol without a formal protocol amendment having been established and approved by an appropriate competent authority and IEC/IRB, except when necessary to eliminate immediate hazards to the patient or when the change(s) involve(s) only logistical or administrative aspects of the study. Any deviations may result in the patient having to be withdrawn from the study and render that patient non-evaluable.

13.2 Study Monitoring

Before study initiation, at a site initiation visit or at an investigator's meeting, an Albireo representative or designee will review the protocol and eCRF with the investigators and the investigative staff. During the study, the clinical monitor (clinical research associate [CRA]) will visit the site regularly to check the completeness of patient records, the accuracy of entries on the eCRFs, the adherence to the protocol and to GCP, the progress of enrollment, and to ensure that study drug is being stored, dispensed, and accounted for according to specifications.

The investigator must ensure that eCRFs are completed within a timely period of patient visits, as per individual site agreements, and must allow the CRA and Albireo representative or designee periodic access to patient records and all study-related materials, including relevant hospital or clinical records, to confirm their consistency with the eCRF entries. No information in these records about the identity of the patients will leave the study center. Albireo monitoring standards require full verification for the presence of the signed ICF, adherence to the inclusion/exclusion criteria, documentation of SAEs, and recording of primary efficacy and safety variables. The CRA will review source data compared with the eCRFs and will verify source data according to the study-specific monitoring plan. The design of the study, the frequency of patient visits, and the site enrollment rate will determine the frequency of monitoring visits. Upon study completion, the CRA will visit the site to conduct a study termination visit, which will include collection of any outstanding documentation.

It is recommended that the investigator/investigative staff log into the eCRF system every working day or at minimum twice weekly in order to provide a prompt response to queries. The investigator/investigative staff should respond to queries and make any relevant changes to the study data within 3 working days.

14 ETHICS

14.1 Independent Ethics Committee/Institutional Review Board

Prior to the start of the study, the investigator is responsible for ensuring that the protocol and consent form have been reviewed and approved by a relevant IEC/IRB. The IEC/IRB shall be appropriately constituted and perform its functions in accordance with FDA ICH GCP and local requirements as applicable.

The IEC/IRB shall approve all protocol amendments (except for logistical or administrative changes), written informed consent documents and document updates, patient recruitment procedures (e.g., advertisements), written information to be provided to the patients and caregivers, IB, available safety information, information about payment and compensation available to patients, the investigator's curriculum vitae and/or other evidence of qualifications and any other documents requested by the IEC/IRB and regulatory authority (competent authority) as applicable.

14.2 Written Informed Consents and Assents

The investigator (physician) or investigative staff, in accordance with local regulations, will explain to each patient (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, alternative treatment, the potential risks and benefits involved, and any discomfort that may occur. Each caregiver/patient will be informed that participation in the study is voluntary and that he/she or their child may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

Caregivers/patients will be informed that they/their children may experience side-effects or be at risk for symptoms, illnesses, or complications that cannot be foreseen by Albireo. As with other medications, people treated with A4250 may be at risk of developing allergic reactions or anaphylaxis. Caregivers/patients will be advised that study procedures include regular blood sampling for measurement of safety parameters and biological markers and that some minor risks are associated with these procedures.

This informed consent should be given by means of a signed ICF, written in non-technical language in accordance with ICH GCP, the Declaration of Helsinki, and regulatory authorities. The caregiver(s)/patient should read and consider the statements before signing and dating them and should be given a copy of each signed document. If written consent is not possible, oral consent can be obtained if witnessed and followed by a signed statement from one or more persons not involved in the study, indicating why the patient was unable to sign the form. No patient can enter the study before his/her or caregiver(s) informed consent has been obtained, as required by country regulations.

The ICF is part of the protocol and must be submitted by the investigator/investigative staff with the protocol for IEC approval. Albireo will supply an ICF which complies with

regulatory requirements and country laws and is considered appropriate for the study. Any changes to the ICF suggested by the investigator must be agreed to by Albireo before submission to the IEC and a copy of the approved version must be provided to the clinical monitor after IEC approval.

15 DATA HANDLING AND RECORD KEEPING

15.1 Case Report Forms/Source Data Handling

The investigator shall be provided with standardized eCRFs and shall ensure that all data from patient visits are promptly entered into the eCRFs in accordance with the specific instructions given. The investigator must sign each eCRF to verify the integrity of the data recorded.

A list of the normal ranges for all laboratory tests to be undertaken forms part of the documentation to be collated prior to study start. As a central laboratory has been selected to conduct any or all tests, it is essential that all samples be analyzed at that laboratory.

The investigator must maintain source documents such as laboratory reports, consultation reports, and complete medical history and physical examination reports.

As no ethnicity data can be collected in the source document for study sites in France, the eCRF will be the Source Document for “origines ethniques”,

15.2 Retention of Essential Documents

Essential documents, as defined by ICH GCP, include: the signed protocol and any amendment(s); copies of the completed eCRFs (for site archiving, compact discs of eCRF data for specific patients will be provided); signed ICFs; hospital records and other source documents; IEC approvals and all related correspondence including approved documents; drug accountability records; study correspondence; and a list of patients’ names and addresses.

The investigator/investigative staff must retain copies of these essential documents for the period specified by ICH GCP and by applicable regulatory requirements. The investigator/investigative staff will inform Albireo of the location where the essential documents are stored and must contact Albireo for approval before disposing of any essential documents. The investigator/investigative staff should take measures to prevent accidental or premature destruction of these documents.

16 FINANCING AND INSURANCE

Financing and insurance are addressed in a separate agreement.

17 PUBLICATION POLICY

Albireo will retain the ownership of all data. When the study is complete, Albireo shall arrange the analysis, tabulation of data and preparation of a clinical study report. Albireo may also use the data for publication, presentation at scientific meetings and submission to regulatory authorities. All proposed publications based on this study must be subject to the sponsor's approval requirements.

18 REFERENCE LIST

1. A4250 Investigator's Brochure.
2. Alissa FT, Jaffe R, Shneider BL. Update on progressive familial intrahepatic cholestasis. J Pediatr Gastroenterol Nutr. 2008;46(3):241-52.
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5. Hori T, Justin H, Nguyen JH and Uemoto S. Progressive familial intrahepatic cholestasis. Hepatobiliary pancreat Dis Int. 2010; 9(6):570-8.
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12. Venkat VL, Shneider BL, Magee JC, et al. Total Serum Bilirubin Predicts Fat-Soluble Vitamin Deficiency Better Than Serum Bile Acids in Infants with Biliary Atresia. J Pediatr Gastroenterol Nutr. 2014;59(6):702-7.
13. Whitington PF, Whitington GL. Partial external diversion of bile for the treatment of intractable pruritus associated with intrahepatic cholestasis. Gastroenterology. 1988;95(1):130-6.

19 APPENDICES

Appendix 1 Concomitant Medication Guidelines

Appendix 2 Diary Questions including Albireo Patient-Reported Outcomes/Observer-Reported Outcome Instrument

Appendix 3 Patient Global Impression of Change (PGIC) and Patient Global Impression of Symptoms (PGIS)

Appendix 4 Pediatric Quality of Life Inventory (PedQL) Questionnaire

Appendix 5 Guideline for Fat-Soluble Vitamin Supplementation

Appendix 6 Contraceptive Requirements

Appendix 7 Blood Volumes

CLINICAL STUDY PROTOCOL A4250-005

A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

IND Number:	130591
EudraCT Number	2017-002338-21
Test Product:	A4250
Indication:	Progressive Familial Intrahepatic Cholestasis (PFIC)
Sponsor:	Albireo AB
Development Phase:	Phase 3
Sponsor Signatory:	[redacted]
Sponsor Medical Monitor:	[redacted]
Principal Investigator:	[redacted]
Date of the Protocol:	22 November 2018
Version of the Protocol:	Final Amendment 03

Confidential

The information in this document contains trade secrets and commercial information that are privileged or confidential and may not be used, divulged, published, or otherwise disclosed without the written consent of Albireo AB. These restrictions on disclosure apply equally to all future information supplied to you or generated by you in connection with the study.

SPONSOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

Albireo AB

[redacted]

22-Nov-2018
Date (day/month/year)

INVESTIGATOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

I have read this protocol and agree that it contains all necessary details for performing this study. I will conduct the study as outlined herein and will complete the study within the time designated, in accordance with all stipulations of the protocol and in accordance with Good Clinical Practice (GCP), local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study drug and the conduct of the study.

I will use only the informed consent approved by the Independent Ethics Committee (IEC) and will fulfil all responsibilities for submitting pertinent information to the IEC responsible for this study.

I agree that the sponsor (Albireo AB) shall have access to any source documents from which case report form information may have been generated.

I further agree not to originate or use the name of Albireo AB, A4250 in any publicity, news release, or other public announcement, written or oral, whether to the public, press, or otherwise, relating to this protocol, to any amendment to the protocol, or to the performance of this protocol without the prior written consent of Albireo AB.

Name of Investigator

Signature

Date (day/month/year)

1 ADMINISTRATIVE INFORMATION

A Double-blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Protocol No.:	A4250-005
Date of the initial Protocol:	20 September 2017
Date and Number of Amendment(s):	Amendment 01, 06 December 2017 Amendment 02, 10 May 2018 Amendment 03, 22 November 2018
Sponsor:	Albireo AB Arvid Wallgrens backe 20 413 46 Göteborg Sweden
Clinical Research Organization:	[redacted]
Sponsor Signatory:	
Sponsor Medical Monitor:	
Principal Investigator:	

2 STUDY SYNOPSIS

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250

Title of Study:
A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Principal Investigator:
[redacted]

Study Centers:
Up to 50 sites will be initiated for this study in the United States (US), Canada, European Union (EU), Middle East, and Australia.

Publication(s):
None.

Planned Study Period:	**Development Phase:**
Q2 2018 to Q4 2019	Phase 3

Objectives:

Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with progressive familial intrahepatic cholestasis (PFIC) Types 1 and 2, as determined by the following:

- Proportion of patients experiencing at least a 70% reduction in fasting serum bile acid (s-BA) concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- The proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period

Secondary Objectives

- To evaluate the effect of A4250 on serum alanine aminotransferase (ALT) concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

Methodology:

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of familial intrahepatic cholestasis-1 or bile salt export pump).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-up Period. After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo. Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008) in which all patients will receive active treatment.

There will be up to 10 clinic visits during the study and one scheduled telephone contact.

Name of Sponsor/Company:	**Name of Product:**	**Name of Active Ingredient:**
Albireo	A4250	A4250

Number of Patients:

Approximately 60 patients diagnosed with PFIC-1 or PFIC-2 will be randomized. The study will target 15% of enrolled patients to be PFIC-1 patients. If enrollment of all PFIC-2 patients is completed and the 15% enrollment of PFIC-1 patients has not yet been achieved, then enrollment of PFIC-2 patients will continue in order to reach the total study target enrollment.

Diagnosis and Main Criteria for Inclusion:

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have clinical genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples at least 7 days apart (Visits 1 and 2) prior to randomization
4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the electronic diary (eDiary) average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients are expected to have a consistent caregiver for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

Test Product, Dose and Mode of Administration:

A4250, 40 µg/kg/day or 120 µg/kg/day, orally administered

Reference Therapy, Dose and Duration of Administration:

Matching placebo, orally administered

Duration of Treatment:

24 weeks

Variables

Efficacy:

Primary Efficacy Endpoints

The primary efficacy endpoints are:

- **EU and rest of world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.
- **US**: Proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period.

Secondary Efficacy Endpoints

Secondary efficacy endpoints include the following:

- **EU and RoW**: Proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period.
- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

All Regions:

All secondary endpoints will be compared to placebo.

- Change from baseline to Week 12 and to Week 24 in fasting s-BA
- Change from baseline to Week 12 and to Week 24 in serum ALT concentration

Name of Sponsor/Company:	Name of Product:	Name of Active Ingredient:
Albireo	A4250	A4250

- Change in growth from baseline to Week 12 and Week 24
- Proportion of responders for pruritus scores at Weeks 12 and 24 based on the Albireo PRO and ObsRO instruments
- Change in sleep parameters measured with the Albireo PRO and ObsRO instruments from baseline over the 24-week Treatment Period
- Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level over the 24-week Treatment Period. A positive pruritus assessment includes an itch score ≤1, or at least a one-point drop from baseline based on the Albireo PRO instrument; only patients ≥8 years of age will complete the Albireo PRO instrument
- Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Proportion of individual AM assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Proportion of individual PM assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Number of patients undergoing biliary diversion surgery or being listed for liver transplantation

Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the patient global impression of change (PGIC) items
- Change from baseline in fasting s-BA at Week 4 Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline by each 4 week interval of the Treatment Period as measured by the Albireo ObsRO instrument
- Change from baseline by each 4-week interval of the Treatment Period in patient-reported and observer-reported night time itching and scratching severity scores, respectively Change from baseline by each 4-week interval of the Treatment Period in patient-reported and observer-reported morning time itching and scratching severity scores, respectively
- Change from baseline by each 4-week interval of the Treatment Period in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline by each 4-week interval of the Treatment Period in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in Pediatric Quality of Life Inventory (PedsQL)
- Change from baseline in serum ALT concentration at Week 4
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST and gamma-glutamyl transferase at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, plasma 7α-hydroxy-4-cholesten-3-one concentration [p-C4]) at Week 24
- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events.
- All-cause mortality

Safety:

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250

Safety criteria are as follows:

- Occurrence of treatment-emergent adverse events including severity and relatedness to study drug at all visits
- The incidence of treatment-emergent serious adverse events (SAEs), based upon information from patient reports, including the description, incidence, and severity of an SAE
- Trends evaluated for the following assessments: physical examinations, concomitant medications, vital signs, laboratory test results (including clinical chemistry, hematology, urinalysis, alfa-fetoprotein, vitamins A and E, 25-hydroxy vitamin D and INR), and abdominal ultrasound

Statistical Methods:

The primary objective of this study is to show superiority in efficacy of A4250 (120 µg/kg/day and 40 µg/kg/day) compared to placebo in patients with PFIC Types 1 and 2.

The Cochran Mantel Haenzel (CMH) test stratified by PFIC class and age class will be performed to compare the proportion in fasting bile acid responders at the end of treatment (Week 22 and 24) in the two A4250 dose groups to the placebo group. To ascertain that all data are used in the CMH analysis, neighboring strata will be pooled if all subjects in a stratum have the same response. The proportion together with the corresponding 95% CI, odds ratio and corresponding 95% Clopper-Pearson exact CI and p-value for the CMH test will be presented.

For the primary efficacy variable of the proportion of positive pruritus assessments at subject level over the 24-week treatment period, an ANCOVA model will be used to analyse the comparisons between the treatment groups. The model will include treatment arm, the averaged AM and PM baseline pruritus scores, and randomization stratification factors, i.e. PFIC class and age class. LS mean(SE) by treatment arm and LS mean difference (SE), 95% confidence intervals, and p-values (where applicable) between treatments (120 µg/kg/day and 40 µg/kg/day, respectively) vs. placebo will be provided. If there are concerns on model assumptions, i.e. normality and homogeneity of variances, a non-parametric ANCOVA based on the rank scores can be used as a sensitivity analysis. LS mean/SE on the outcome by treatment arm and LS mean difference/SE between active dose and placebo will be provided.

For each primary endpoint by region (EU & RoW and US), a pooled analysis for the closed testing procedure will be applied to control the 1-sided overall type I error rate for two treatment comparisons vs. the placebo at the 0.025 level, as specified below:

In the closed testing procedure, the low and high dose groups will be pooled to compare with the placebo group first. If the 1-sided p-value is ≤ 0.025, the 1-sided p-values for low dose vs. placebo and high dose vs. placebo will be calculated respectively. If both individual p-values are ≤ 0.025, a significant treatment effect will be declared on both dose groups. If only one of them is ≤ 0.025, a significant treatment effect will be declared on the corresponding dose group.

Other secondary and exploratory outcome variables will provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple comparisons when testing secondary and exploratory efficacy variables.

Date of the Protocol: 22 November 2018

3 TABLE OF CONTENTS

3.1 List of Tables

3.2 List of Figures

3.3 List of Appendices

4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADME	absorption, distribution, metabolism, and excretion
AE(s)	adverse event(s)
AFP	alfa-fetoprotein
ALT	alanine aminotransferase
APRI	AST to platelet ratio index
ASBT	apical sodium-dependent bile transporter (also known as IBAT)
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	body mass index
BSEP	bile salt export pump
CPK	creatine phosphokinase
CRA	clinical research associate
CYP	cytochrome P450
DSMB	Data and Safety Monitoring Board
eCRF	electronic case report form
eDiary	electronic diary
EOT	End of Treatment
EU	European Union
EudraCT	European Union drug regulatory agency clinical trial
FAS	full analysis set
FDA	United States Food and Drug Administration
FIC1	familial intrahepatic cholestasis-1
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
GI	gastrointestinal
GLMM	generalized linear mixed modelling
IB	Investigator's Brochure
IBAT	ileal bile acid transporter (also known as ASBT)
ICF	informed consent form
ICH	International Council for Harmonisation
[redacted]	[redacted]
IEC	Independent Ethics Committee
INR	international normalized ratio
IRB	Institutional Review Board

IWRS	Interactive Web Response System
LDH	lactate dehydrogenase
LFT	liver function test
LPLV	last patient last visit
MDR3	multidrug-resistance protein 3
MedDRA	Medical Dictionary for Regulatory Activities
MELD	model for end-stage liver disease
MMRM	mixed model repeated measures
ObsRO	observer-reported outcome
PBC	primary biliary cholangitis
p-C4	plasma 7α-hydroxy-4-cholesten-3-one concentration
PCS	potentially clinically significant
PedsQL	Pediatric Quality of Life Inventory
PELD	pediatric end-stage liver disease
PFIC	progressive familial intrahepatic cholestasis
PGIC	patient global impression of change
PGIS	patient global impression of symptoms
PK	pharmacokinetic(s)
PP	per protocol
PRO	patient-reported outcome
PT	prothrombin time
PVM	pharmacovigilance manager
QoL	quality of life
RoW	rest of world
RR	respiratory rate
SAE(s)	serious adverse event(s)
SAP	statistical analysis plan
s-BA	serum bile acid
SD	standard deviation
s-FGF19	serum fibroblast growth factor 19 concentration
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction
TEAE(s)	treatment-emergent adverse event(s)
ULN	upper limit of normal
US	United States
WHO	World Health Organization

5 INTRODUCTION

5.1 Investigational Medicinal Product

A4250 is a small molecule and a potent selective inhibitor of the ileal bile acid transporter (IBAT), also known as the apical sodium-dependent bile acid transporter (ASBT). IBAT is an integral brush border membrane glycoprotein that co-transports sodium and bile acids and appears to be a critical component in the regulation of the bile acid pool size in animals and man. This transporter, expressed in the distal ileum, is a key element in the enterohepatic circulation of bile acids since it facilitates the high-affinity, high-capacity reabsorption of bile acids. Indeed, 95% of secreted bile acids are reabsorbed via IBAT [Hofmann 2009; Miethke 2016]. A4250 is orally administered and acts locally in the gut where it binds reversibly to IBAT to decrease the reuptake of bile acids. A4250 has minimal systemic exposure at the predicted therapeutic dose ranges.

No safety signals were found with A4250 in preclinical safety and toxicity studies. In the first clinical study of A4250 in healthy volunteers, A4250 was given as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. A4250 was well tolerated and the only findings were gastrointestinal (GI) related effects, such as diarrhea, at higher doses.

In an exploratory Phase 2 study in children with cholestatic pruritus, A4250 was given as oral doses of 10 to 200 µg/kg/day for 4 weeks. A4250 reduced mean levels of serum bile acids (s-BA) in all cohorts. In 11 of 13 progressive familial intrahepatic cholestasis (PFIC) patients, substantial reductions (ranging from 43% to 98%) were observed. Patient-reported diary data documented improvement of pruritus during treatment. A4250 was well tolerated. No serious adverse events (SAEs) were deemed to be related to study drug, and most adverse events (AEs), including some increased transaminases, were considered unrelated to study drug and transient in nature.

5.2 Background

5.2.1 Progressive Familial Intrahepatic Cholestasis

PFIC is a rare autosomal recessive cholestatic liver disease estimated to affect between 1 in every 50,000 to 100,000 children born worldwide. PFIC represents 10% to 15% of causes of cholestasis in children and 10% to 15% of liver transplantation indications in children. All types of PFIC exist worldwide and both sexes appear to be equally affected.

The common underlying pathogenesis of PFIC is disruption of bile formation and bile transport through the liver [Jacquemin 2000]. The classification of PFIC has evolved over the years. The most commonly used sub-classifications are PFIC Types 1, 2, and 3, which are based on the associated affected gene and described in more detail below.

- PFIC, Type 1: also referred to as "Byler disease" or "familial intrahepatic cholestasis-1 (FIC1) protein deficiency." FIC1 protein is located on the canalicular

membrane of hepatocytes and facilitates movement of aminophospholipids from the outer to inner leaflet of the plasma membrane of the hepatocyte. The *ATP8B1* gene encodes FIC1 protein. Biallelic pathologic variants in the *ATP8B1* gene are associated with FIC1 dysfunction and classified clinically as PFIC Type 1 disease.

- PFIC, Type 2: also referred to as "Byler syndrome" or "bile salt export pump (BSEP) deficiency." BSEP is a transporter protein that is expressed at the canalicular membrane of hepatocytes, and is the primary exporter of bile acids. The *ABCB11* gene encodes the BSEP protein. Biallelic pathologic variations in the *ABCB11* gene are associated with BSEP dysfunction and classified clinically as PFIC Type 2 disease.
- PFIC, Type 3: is caused by a deficiency of the multidrug-resistance protein 3 (MDR3) due to mutations in the *ABCB4* gene. MDR3 is a phospholipid translocase critical for phospholipid secretion.

Severe pruritus is common in children diagnosed with PFIC. Itching (and subsequent scratching) is a significant morbidity for these patients and their families [Suchy 2007]. A more severe degree of pruritus is experienced compared to patients with other forms of liver disease and to other pruritic conditions such as atopic dermatitis [Murray 2011]. In patients with PFIC, liver biopsy reveals canalicular cholestasis and, later, the appearance of portal fibrosis. Serum biochemistry indicates cholestasis with hyperbilirubinemia, elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The concentrations of bile acids in serum are very high, while serum gamma-glutamyl transferase (GGT) activity (exception being MDR3 variants) and cholesterol are normal [Hori 2010]. Symptoms of portal hypertension and liver failure will develop during the course of the disease [Davit-Spraul 2009; Alissa 2008]. Symptoms develop early; median age at onset of symptoms is 2 months, and 78% of PFIC patients present with jaundice [Pawlikowska 2010]. The life-threatening and debilitating nature of PFIC is reflected by the fact that survival in those not resorting to surgery is 50% at 10 years of age and almost zero at 20 years of age. Approximately half of PFIC patients undergo liver transplantation [Davit-Spraul 2009] and treatment-resistant pruritus is the leading indication for the surgical procedure partial external biliary diversion, mostly in PFIC Type 1 and 2 patients.

PFIC is life-threatening and debilitating. Currently, the treatment for PFIC is palliative and there is no pharmaceutical treatment approved for use in PFIC. The therapeutic choices are restricted to non-specific therapy of the symptoms and signs of the disease such as nutritional support, preventing vitamin deficiencies, and treatment of extrahepatic features. Medical treatment options include off-label use of ursodeoxycholic acid, rifampin, antihistamines, and naltrexone. A minority of patients respond nominally and transiently to these interventions. Biliary diversion is used to decrease systemic bile acids through interruption of the enterohepatic circulation and so avoid transplantation. Liver transplantation is typically only viewed as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

5.2.2 Summary of Clinical and Nonclinical Studies

This is a summary of nonclinical and clinical studies. More detail is provided in the Investigator's Brochure ([IB] see A4250 IB).

A4250 is a potent, selective IBAT inhibitor with no safety signals seen in the safety pharmacological studies. In addition, no safety signals were seen in repeat-dose oral toxicity studies in rats with up to 26 weeks of daily dosing and in dogs with up to 39 weeks of daily dosing. No safety signals were identified when A4250 was administered daily to juvenile rats from age 14 days to 63 days.

A4250 is minimally absorbed with very low systemic exposure in both humans and other species. In blood, protein binding is high (>99.7%). Systemically absorbed A4250 is slowly metabolized in humans and in preclinical studies by hydroxylation only, a metabolite that retains IBAT activity. A4250 did not show any significant inhibition of cytochrome P450 (CYP) enzymes in the rat. A4250 inhibited human CYP3A4 and CYP2D6 (IC_{50}: 16 µmol/L for both enzymes), and CYP2C9 (IC_{50}:1.2 µmol/L).

To date, A4250 has been studied in 3 Albireo-sponsored clinical studies: a study in healthy volunteers evaluating single and multiple administration of A4250; a single-dose absorption, distribution, metabolism, and excretion (ADME) study; and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with primary biliary cholangitis (PBC). In total, 98 subjects have been exposed to A4250.

In the first clinical study (A4250-001), A4250 was given to healthy volunteers as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. Following a single oral dose of 10 mg, the median maximum drug concentration was 0.2 nmol/L and the area under the curve was 0.8 nmol × h/L (n=6). Pharmacodynamic evaluation of bile acid synthesis biomarkers (serum fibroblast growth factor 19 [s-FGF19] and plasma 7α Hydroxy 4-cholesten-3-one [p-C4]) identified the expected decrease in s-FGF19 and an increase in p-C4. In addition, total plasma bile acids decreased and total fecal bile acids increased in A4250-treated patients compared to placebo. A4250 was generally well tolerated. The only findings were GI-related effects, such as diarrhea at higher doses.

In the ADME study (A4250-007), healthy subjects were administered a single oral dose of 3 mg [^{14}C]-A4250 capsule. The main excretion route was determined to be fecal. There were no quantifiable concentrations of A4250 in plasma or total radioactivity in plasma or whole blood. A4250 was well tolerated. AEs were reported and most were mild and not related to A4250. The 3 AEs experienced by 2 subjects reported as related to study drug were GI in nature, including diarrhea (2 AEs; 2 subjects) and abdominal pain (1 AE; 1 subject).

In an exploratory Phase 2 study in children with cholestatic pruritus (A4250-003), A4250 was administered as oral daily doses of 10 to 200 µg/kg/day for 4 weeks. Twenty patients (8 females and 12 males) aged 1 to 17 years were enrolled in this open-label

dose-escalation study. Four patients were re-enrolled into a second dose level. No treatment-related SAEs were reported, and most AEs, including some increased transaminases, were transient. Four-week, once-daily treatment with A4250 reduced mean levels of s-BA in all cohorts. Substantial s-BA reductions in 11 of 13 PFIC patients (ranging from 43% to 98%) were observed. A4250 also reduced s-BA in the 11 non-PFIC patients, but to a lesser extent than in PFIC patients. Patient-reported diaries documented improvement of the visual analogue scale itch score in 17 of 24 patients during treatment. The dose with the greatest improvement showed a mean decrease (± standard error of the mean) of 2.9 ±1.1 from baseline. The results from this study form the basis of the current (A4250-005) study design.

5.3 Rationale

In this investigational study, A4250 is under development for treatment of PFIC and is also being considered for other orphan cholestatic liver diseases.

There is currently no medical treatment approved for PFIC. In PFIC patients with severe pruritus, biliary diversion surgery is used to decrease systemic bile acids through interruption of the enterohepatic circulation and thereby reducing pruritus [Whitington 1988]. Liver transplantation is typically used as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

By inhibiting IBAT with high selectivity and potency, A4250 has the potential to relieve cholestasis and improve liver function without surgical intervention in patients with PFIC. Since A4250 is minimally absorbed and achieves a substantial reduction of s-BA, it has the potential to be a safer and less invasive alternative to surgical biliary drainage. The rationale for using A4250 is to decrease s-BA levels, and to reduce the major morbidity of pruritus, improving the health and wellbeing of patients affected with PFIC. Ultimately, this noninvasive therapeutic approach has the potential to not only improve quality of life (QoL), but also reduce the need for biliary diversion and subsequent liver failure and liver transplantation.

The doses selected, 40 and 120 µg/kg/day, were based on the efficacy and safety data generated from patients with PFIC (n=10 + 3 re-entered) and supporting data from all patients (n=20 + 4 re-entered) in the five dose groups dosed in the A4250-003 study. Improvements in s-BA and pruritus were observed with doses from 30 µg/kg to 100 µg/kg, with a dose-related trend. The best dose response in all patients was at the 60 µg/kg/day for s-BA and 100 µg/kg/day for pruritus. The best dose response in the PFIC subgroup was at 30 µg/kg/day for both s-BA and pruritus. Patients with PFIC responded well to the doses given from 30 µg/kg to 200 µg/kg/day; however, a clear dose relationship could not be established in order to select a single dose for this Phase 3 study. As efficacy was observed at both low and high doses, 40 µg/kg and 120 µg/kg/day are considered to be the most optimal for this study.

This study will be conducted in compliance with the protocol and with the International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP).

Patients completing 24 weeks of double-blind treatment will be offered to enter Study A4250-008 and receive open-label treatment with A4250.

5.3.1 Risk/Benefit

A4250 has been evaluated in three Albireo-sponsored clinical studies: a double-blind placebo-controlled study in healthy volunteers, a single-dose ADME study, and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with PBC.

A total of 98 subjects/patients have been exposed to A4250. Healthy subjects have been exposed to up to 10 mg as a single dose and up to 3 mg daily as part of a multiple-dose evaluation. Children with cholestatic liver disease have been treated with up to 0.2 mg/kg/day (200 µg/kg/day) for 4 weeks. In total, 2 SAEs have been reported; both were judged by the physician to be not related to the study drug.

Patients with cholestatic liver diseases suffer from excess bile acids in the liver resulting in tissue damage. A commonly used treatment in these patients is bile diversion surgery, whereby approximately 50% to 100% of the enterohepatic circulation of bile acids is interrupted. Inhibition of IBAT with A4250, thereby interrupting the enterohepatic circulation of bile acids, is therefore a potential medical alternative to surgery which could be of benefit to these patients if shown to be effective and safe. Data from the Phase 2 study showed efficacy of A4250 in reducing s-BA concentrations and pruritus in such patients.

Based on the mode of action of an IBAT inhibitor, loose stools or diarrhea could be expected. However, in the pediatric Phase 2 study with 4 weeks daily treatment, only one patient had mild transient diarrhea after a single dose that did not recur on multiple dosing.

6 STUDY OBJECTIVES

6.1 Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with PFIC Types 1 and 2, as determined by the following:

- The proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- The proportion of positive pruritus assessments at the subject level over the 24-week treatment period.

6.2 Secondary Objectives

- To evaluate the effect of A4250 on serum ALT concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

7 INVESTIGATIONAL PLAN

7.1 Overall Study Design and Plan

7.1.1 Description

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of FIC1 or BSEP).

Study data will be periodically (approximately on a quarterly basis) reviewed by an unblinded Data Safety and Monitoring Board (DSMB).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-Up Period.

After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo.

Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008; EudraCT Number 2017-002325-38, subject to a separate study approval) in which all patients will receive active treatment. A patient who prematurely withdraws due to intolerable symptoms, and has completed at least 12 weeks of the Treatment Period and End of Treatment (EOT) Visit, will also be offered the opportunity to enter Study A4250-008.

Visit windows applicable to each visit are presented in the schedule of assessments (Table 1). There will be up to 10 clinic visits during the study (Figure 1) and one scheduled telephone contact, as follows:

- Visit 1: Screening Period start. Patient is considered enrolled after signing the informed consent form (ICF) (Days -56 to -35)
- Visit 2: second Screening Visit (Days -28 to -7)
- Visit 3: Randomization Visit (Day 0)
- Telephone Contact 1: (Day 14)
- Visit 4: Clinic Visit (Day 28)
- Visit 5: Clinic Visit (Day 56)
- Visit 6: Clinic Visit (Day 84)
- Visit 7: Clinic Visit (Day 126)
- Visit 8: Clinic Visit (Day 154)
- Visit 9: Clinic Visit, EOT (Day 168)

- Visit 10: Follow-Up Visit, 28 days after last dose of study drug (Day 196). All patients continuing in Study A4250-008 will not have this Follow-up Visit.

Additional clinic visits may be required for patients who need direct site assistance, e.g., due to AE monitoring, to fulfil screening requirements, and/or for safety maintenance.

Screening Period

Informed consent must be obtained at Visit 1 prior to performing any study procedures. After signing the ICF, patients will be considered enrolled and evaluated for study eligibility.

Daily recording of pruritus using an electronic diary (eDiary) will be started to determine confirmation of the magnitude and the baseline pruritus symptoms before the study. After consultation with the Medical Monitor, patients failing eligibility criteria may be re-screened after 2 months. Patients not fulfilling inclusion/exclusion criteria after 3 attempts are not allowed to re-screen.

Caregivers/patients will be instructed on the daily use of the eDiary. The eDiary will include patient-reported outcome (PRO) and observer-reported outcome (ObsRO) items for evaluation of itching (PRO), scratching (ObsRO), and sleep disturbance (PRO and ObsRO) during the Screening Period (study Days -56 to 0), throughout the 24-week Treatment Period (study Days 0 to 168), and Follow-up (Day 196).

The 14 consecutive days prior to randomization will be used for eligibility calculation of the diary entries. If a patient does not meet the minimum number of observations (see Section 9.2.2 for scoring and missing data definitions) to determine eligibility, the patient may be re-screened. Observer-reported outcomes in patients of all ages will be recorded by a caregiver. If possible, the same caregiver will complete the Albireo ObsRO items throughout the study.

At a second Screening Visit (Visit 2) additional laboratory samples will be taken for eligibility assessments.

Treatment Period

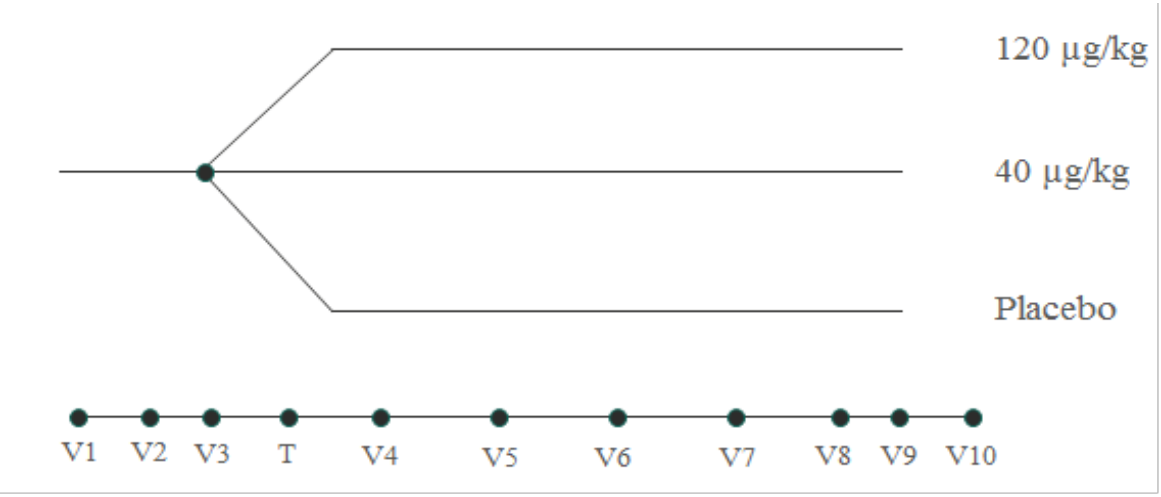
Eligibility for randomization will be determined at Visit 3 using the pruritus (scratching reports from the Albireo ObsRO) data in the 2 weeks before Visit 3, clinical genetic confirmation of diagnosis, and the liver biochemistry evaluations from the previous screening visits. Two weeks after Visit 3, there will be a telephone contact with the caregiver/patient to evaluate any AEs.

The patient will return to the clinic at Visit 4, Visit 5, Visit 6, Visit 7, Visit 8, and Visit 9 for assessments. If a new caregiver will begin to complete the Albireo ObsRO during the course of the trial, they will be provided with training during a clinic visit before they begin to use the eDiary. Additionally, caregivers will be requested to use the diary to report the time each dose of study drug is administered during the Treatment Period.

The last dose of study drug will be administered the day before Visit 9. At study Visit 9, the patient has the option to enroll in an open-label extension study (A4250-008) should they meet the required entry criteria. If the patient is continuing into Study A4250-008 this will be the last visit in this study and the first visit in Study A4250-008. Visit 9/EOT should also be performed at the time patients are prematurely withdrawn from the study.

Clinic Visit 10 will take place 28 days after Visit 9 for all patients not continuing into Study A4250-008.

Figure 1 Study Design



T: telephone contact; V: study visit

End of Study

The end of the study is defined as follows:

a) End of study in one country: last patient last visit (LPLV) and sites are closed

b) End of study globally: LPLV globally and all sites closed

7.1.2 Schedule of Assessments

The schedule of assessments is presented in Table 1. For information on blood volumes, please see Appendix 7.

Table 1 Schedule of Assessments

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Informed consent[c]	X										
Inclusion/exclusion criteria	X		X								
Demographics	X										
Concomitant medication[d]	X	X	X	X	X	X	X	X	X	X	X
Medical and surgical history	X		X								
Physical examination including voluntary photography	X		X				X			X	
Skin examination	X		X		X		X			X	X
Vital signs[e]	X	X	X		X	X	X	X	X	X	X
eDiary: itching, scratching, and sleep scores[f]	Daily diary entry										
Clinical chemistry[g]	X		X		X	X	X	X		X	X
Hematology[g]			X		X	X	X	X		X	X
Urinalysis[g]		X					X			X	
Serum bile acids[h]	X	X	X		X	X	X	X	X	X	X

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
INR		X			X		X		X		X
Autotaxin[i]			X		X					X	
p-C4[i]			X		X					X	
AFP			X							X	
Vitamins A & E[h]		X					X			X	
25-hydroxy vitamin D			X				X			X	
Blood sample for A4250 PK[i]					X					X	
Abdominal Ultrasound			X							X	
Fibroscan® (where available)			X							X	
QoL questionnaire (PedsQL)			X							X	
Patient/Caregiver/Clinician Global Impression of Change					X		X			X	
Patient/Caregiver/Clinician Global Impression of Symptoms			X		X		X			X	
Pregnancy test[j]	X[j]	X	X		X	X	X	X	X	X[j]	X

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Study drug dispensed[k]			X		X	X	X	X	X		
Adverse events[l]	Continuous collection										
Study drug compliance					X	X	X	X	X	X	

AFP: alfa-fetoprotein; BMI: body mass index; eCRF: electronic case report form; eDiary: electronic diary; EOT: End of Treatment; ICF: informed consent form; INR: international normalized ratio; p-C4: plasma 7α Hydroxy 4-cholesten-3-one; PedsQL: Pediatric Quality of Life Inventory; PK: pharmacokinetic; QoL: quality of life; RR: respiratory rate; SAE: serious adverse event; s-BA: serum bile acid.

a Must also be performed at the time a patient is prematurely withdrawn from the study. This visit is the same as Visit 1 in the extension protocol (Study A4250-008).

b Assessments must be performed 28 days following the final dose of study drug. This visit is omitted if patients are entering Study A4250-008.

c The ICF (and assent) must be signed before any study procedures are performed.

d Past (within 1 month prior to Visit 1) and current medication will be documented in the eCRF.

e Includes blood pressure, pulse, RR, temperature, height (or length depending on age), and body weight (using a certified weight scale). BMI will be calculated.

f Includes training on use of the device at Screening (Visit 1) and review of patient daily entries during 14 days prior to Clinic Visit 3 to determine eligibility.

g See Table 3 for detailed parameters.

h Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA and vitamin A. Exceptions can be made for infants, less than 12 months of age, if unable to fast for the full 4 hours. All s-BA results during the treatment period and follow-up will be blinded.

i Samples will not be collected for patients ≤10 kg. In the event of an SAE, a PK sample should be collected as close to the onset of the event as possible.

j For girls who have reached menarche. Serum test at Visit 1; urine test at other visits. A serum pregnancy test will also be conducted at Visit 9 for patients entering Study A4250-008. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy.

k Study drug to be taken once daily on Day 1 through Day 167 as described in Section 8.2.

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**

l Adverse event information will be collected from the time of signing of the ICF to study discontinuation.

7.1.3 Study Procedures and Assessments

7.1.3.1 Screening Period (Days -56 to 0)

Day -56 to Day -35/Clinic Visit 1

Patients will undergo a Screening Visit up to 56 days prior to the planned first day of study treatment. Screening procedures and assessments are as follows:

- Obtain written informed consent
- Assess inclusion/exclusion criteria (Section 7.2)
- Record demographics (age, full date of birth, gender, race, and ethnicity)
- Document concomitant medications
- Medical and surgical history (date of diagnosis of PFIC Types 1 or 2, prior investigational medications for PFIC, historical liver function test [LFT] values, any surgery performed, any other diagnosis, and historical liver biopsy data)
- Collect, if available, past confirmatory clinical genetic laboratory report for PFIC-1/PFIC-2 and send to central reader for review. If a historical report is equivocal, unavailable, or unobtainable, a blood sample for clinical genetic testing will be collected to determine eligibility
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- eDiary training and compliance requirements; caregivers and, if applicable, patients begin daily recording of pruritus using the eDiary (Appendix 2)
- Clinical chemistry (Section 10.2.1)
- s-BA (Section 9.2.1)
- Serum pregnancy test for girls who have reached menarche. Please see Appendix 6 for contraceptive requirements
- AE monitoring

Day -28 to Day -7/Clinic Visit 2

A second Screening Visit will be performed. Screening procedures and assessments are as follows:

- Review concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance

- Urinalysis (Section 10.2.1)
- s-BA (Section 9.2.1)
- International normalized ratio (INR)
- Vitamins A and E
- Urine pregnancy test for girls of who have reached menarche
- AE monitoring

7.1.3.2 Treatment Period (Day 0 to Day 168)

Study Day 0/Clinic Visit 3 (Randomization)

At the randomization visit, the following assessments will be conducted:

- Review inclusion/exclusion criteria (Section 7.2)
- Review concomitant medications, medical and surgical history
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance and entrance criteria requirement (see Inclusion Criteria 4, Section 7.2.1)
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin and p-C4 (samples will not be taken for patients ≤10 kg)
- 25-hydroxy vitamin D
- Alfa-fetoprotein (AFP)
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)
- Administer QoL (Pediatric Quality of Life Inventory [PedsQL]) questionnaire
- Patient/caregiver/clinician complete the patient global impression of symptoms (PGIS)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Study drug is dispensed and patients are instructed to administer daily from Day 1

Study Day 14 (Telephone Contact)

A study nurse will contact the patient/caregiver by telephone for monitoring of the following:

- Review concomitant medications
- Albireo PRO/ObsRO eDiary review for compliance
- AEs

Study Day 28 and Day 84/Clinic Visits 4 and 6

The following procedures and assessments will be performed:

- Review of concomitant medications
- Physical examination, including voluntary photography (at Clinic Visit 6 only; Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- Urinalysis (at Clinic Visit 6 only; Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- Autotaxin, p-C4, and A4250 pharmacokinetic (PK) assessment (at Clinic Visit 4 only; samples will not be taken for patients ≤10 kg)
- Vitamins A and E, and 25-hydroxy vitamin D (at Clinic Visit 6 only)
- Patient/caregiver/clinician complete the patient global impression of change (PGIC) and PGIS
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 56 and Day 126/Clinic Visits 5 and 7

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)

- s-BA (Section 9.2.1)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 154/Clinic Visit 8

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- s-BA (Section 9.2.1)
- INR
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 168/Clinic Visit 9/EOT

The last dose of study drug is taken in the morning the day before Clinic Visit 9, and the following procedures and assessments will be performed (also performed at the time a patient prematurely withdraws):

- Review of concomitant medications
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry, hematology, and urinalysis (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin, p-C4, and A4250 PK assessment (samples will not be taken for patients ≤10 kg)
- AFP

- Vitamins A, E, and 25-hydroxy vitamin D
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)
- Administer PedsQL questionnaire
- Patient/caregiver/clinician complete the PGIC and PGIS
- Urine pregnancy test for girls who have reached menarche, or serum pregnancy test for girls continuing to study A4250-008
- AE monitoring
- Evaluation of study drug compliance

7.1.3.3 Follow-Up Period

Study Day 196/Clinic Visit 10

All patients not continuing into Study A4250-008 will return to the study site 28 days after Clinic Visit 9 or the last dose of study drug for the following assessments:

- Review of concomitant medications
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- Urine pregnancy test for girls who have reached menarche
- AE monitoring

7.2 Study Population

A total of approximately 60 patients with a clinical diagnosis of PFIC Type 1 or 2 that have been genetically confirmed will be randomized. The study will target 15% of enrolled patients to be PFIC-1 patients. If enrollment of all PFIC-2 patients is completed and the 15% enrollment of PFIC-1 patients has not yet been achieved, then enrollment of PFIC-2 patients will continue in order to reach the total study target enrollment.

An attempt will be made to randomize a minimum of 10 patients from each age category (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years).

A maximum of 5 patients that were treated in Study A4250-003 will be allowed to participate in this study.

7.2.1 Inclusion Criteria

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have clinical genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples at least 7 days apart (Visits 1 and 2) prior to randomization
4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the eDiary average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients will be expected to have a consistent caregiver for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

7.2.2 Exclusion Criteria

1. Patient with pathologic variations of the *ABCB11* gene that predict complete absence of the BSEP protein
2. Patient with past medical history or ongoing presence of other types of liver disease including, but not limited to, the following:
   a) Biliary atresia of any kind
   b) Benign recurrent intrahepatic cholestasis, indicated by any history of normal s-BAs
   c) Suspected or proven liver cancer or metastasis to the liver on imaging studies
   d) Histopathology on liver biopsy is suggestive of alternate non-PFIC related etiology of cholestasis
3. Patient with a past medical history or ongoing presence of any other disease or condition known to interfere with the absorption, distribution, metabolism (specifically bile acid metabolism), or excretion of drugs in the intestine, including but not limited to, inflammatory bowel disease
4. Patient with past medical history or ongoing chronic (i.e., >3 months) diarrhea requiring intravenous fluid or nutritional intervention for treatment of the diarrhea and/or its sequelae

5. Patient has a confirmed past diagnosis of infection with human immunodeficiency virus or other present and active, clinically significant, acute, or chronic infection, or past medical history of any major episode of infection requiring hospitalization or treatment with parenteral anti-infective treatment within 4 weeks of treatment start (study Day 1) or completion of oral anti-infective treatment within 2 weeks prior to start of Screening Period
6. Any patient with suspected or confirmed cancers except for basal cell carcinoma, and non-liver cancers treated at least 5 years prior to Screening with no evidence of recurrence
7. Patient with a past medical history of chronic kidney disease with an impaired renal function and a glomerular filtration rate <70 mL/min/1.73 m^2
8. Patient with surgical history of disruption of the enterohepatic circulation excluding those who have undergone a successful reversal procedure that has permanently restored flow of bile acids from the liver to the duodenum
9. Patient has had a liver transplant or a liver transplant is planned within 6 months of randomization
10. Decompensated liver disease, coagulopathy, history or presence of clinically significant ascites, variceal hemorrhage, and/or encephalopathy
11. INR >1.4 (the patient may be treated with Vitamin K intravenously, and if INR is ≤1.4 at resampling the patient may be randomized)
12. Serum ALT >10 × upper limit of normal (ULN) at Screening
13. Serum ALT >15 × ULN at any time point during the last 6 months unless an alternate etiology was confirmed for the elevation
14. Total bilirubin >5 × ULN at Screening
15. Patient suffers from uncontrolled, recalcitrant pruritic condition other than PFIC. Examples include, but not limited to, refractory atopic dermatitis or other primary pruritic skin diseases
16. Any patient who is pregnant or lactating or who is planning to become pregnant within 24 weeks of randomization
17. Sexually active males and females who are not using a reliable contraceptive method with ≤1% failure rate (such as hormonal contraception, intrauterine device, or complete abstinence) throughout the duration of the study and 90 days thereafter (from signed informed consent through 90 days after last dose of study drug). See Appendix 6 for further details

18. Patient with a past medical history of alcohol or substance abuse will be excluded. Patient must agree to refrain from illicit drug and alcohol use during the study
19. Administration of bile acid or lipid binding resins and medications that slow GI motility (Refer to Appendix 1 – Concomitant Medications Guidelines)
20. Patient has had investigational exposure to a drug, biologic agent, or medical device within 30 days prior to Screening, or 5 half-lives of the study agent, whichever is longer
21. Patient who has been previously treated with an IBAT inhibitor whose pruritus has not responded to treatment
22. Any other conditions or abnormalities which, in the opinion of the investigator or Medical Monitor, may compromise the safety of the patient, or interfere with the patient participating in or completing the study

7.2.3 Withdrawal of Patients

Patients/caregivers will be informed that they have the right to withdraw from the study at any time, without prejudice to their medical care, and that they are not obliged to state their reasons.

Any withdrawal, and reasons for withdrawal, must be fully documented in the electronic case report form (eCRF) and source documents and the patient followed by the investigator/investigative staff. Even after the study is completed at the Follow-up Visit, the investigator/investigative staff will, regardless of reason for withdrawal, record any patient data they receive concerning SAEs, and all drug-related non-serious AEs, and subsequently report these according to Section 10.1. Withdrawn patients will not be replaced.

Patients will be withdrawn in the following circumstances:

- A patient's/caregiver's desire for withdrawal for any reason
- Lost to follow-up (every effort must be made to contact the patient/caregiver; a certified letter must be sent)
- An AE that, in the opinion of the investigator, necessitates withdrawal
- A worsening of the symptoms related to diarrhea occurs
- Death
- Patient/caregiver estimation of intolerable symptoms
- A patient's/caregiver's substantial noncompliance (eDiary and study drug compliance) or protocol violation
- An investigator's opinion that continuing the patient in the study is not appropriate. The investigator may withdraw a patient at any time, if it is considered to be in the patient's best interest

A patient who prematurely withdraws due to intolerable symptoms, and has completed at least 12 weeks treatment in the double-blind study, will be offered the opportunity to enter open-label Study A4250-008. A patient who withdraws from treatment prematurely and does not enter Study A4250-008 will have EOT (Visit 9) assessments at the time of withdrawal and a follow-up assessment (Visit 10) 28 days following the last dose of study drug, and the patient will be recorded as withdrawn.

7.2.4 Study Termination by Sponsor

This study may be terminated at any time by Albireo if serious side effects should occur, if the investigator does not adhere to the protocol; or if, in the sponsor's judgment, there are no further benefits to be achieved from the study. In this event, Albireo/designee will inform the study investigators, institutions, and all regulatory authorities.

Albireo may temporarily or permanently discontinue the study at an investigative site at any time for safety, ethical, compliance, or other reasons. If this is necessary, Albireo will endeavor to provide advance notification to the site. If a site or the study is suspended or discontinued, the investigator/investigative staff will be responsible for promptly informing the Independent Ethics Committee (IEC). If required by local regulations, Albireo/designee will be responsible for informing the IEC and the regulatory authority of study or site discontinuation. In such an event, all study data and unused study drug must be returned to Albireo.

8 TREATMENT OF PATIENTS

8.1 Identity of Study Drug

A4250 and placebo will be supplied as capsules for oral administration. White opaque capsules filled with pellets containing A4250 or placebo will be provided. Two different capsule sizes will be available, as follows:

- Capsule size 0 that can be opened
- Capsule size 3 that should be swallowed intact. These size 3 capsules may be opened only under exceptional circumstances, e.g., patient cannot swallow capsule intact

The content of the A4250 and placebo capsules will be identical in appearance. Capsule filling weight will also be identical for high dose, low dose, and placebo. Bottles with 34 capsules will be given to the patient at each visit. A patient who requires 2 or more capsules per day will be given multiple bottles. Refer to the investigational product manual.

8.2 Administration of Study Drug

Patients will be dosed with A4250 at a dose of 40 µg/kg/day, 120 µg/kg/day, or placebo for 24 weeks. Study drug will be dispensed to the patient at Visits 3, 4, 5, 6, 7, and 8, together with instructions on how to store and take the drug. Study drug administration data, including whether each patient took each dose or partial doses of study drug, whether there were any delayed or missed doses, and whether the capsule was opened or swallowed whole will be documented through the eDiary and transferred to the study database.

A4250 should be taken in the morning, together with food. On clinic visits days when laboratory assessments are conducted, study drug should be taken after the visit and after laboratory samples are taken. Patients should not crush or chew the capsule(s). When swallowing the capsule intact, the patient should administer the dose with a glass of water.

For a patient unable to swallow capsule intact, the capsule can be opened and the contents sprinkled and mixed in a small amount of soft room-temperature yogurt, apple sauce, or fruit purée. A list of food options can be found in the Pharmacy manual. The amount of food should be less than what the patient/infant is expected to eat and should be followed by water.

1. Hold the capsule over the prepared food, gently twist open and allow the content to fall into food
2. Mix the entire capsule content with 1 or 2 tablespoons (15 to 30 mL) of soft food (apple sauce, fruit purée, or yogurt)
3. Administer the mixture immediately after mixing.

The number of capsules provided to the patient will be based on body weight at Visit 3. At Visit 6, if a patient's weight has shifted to another weight category (identified in Table 2), the number of capsules per day may be adjusted.

Table 2 Dosing and Capsule Strength

Body Weight (kg)	Capsule Size	Number of Capsules per Day	Capsule Strength, Low Dose (µg)	Total Dose (µg)	Capsule Strength, High Dose (µg)	Total Dose (µg)	Capsule Size, Placebo (µg)
5.0 to <7.5	0	1	200	200	600	600	0
7.5 to <12.5	0	2	200	400	600	1200	0
12.5 to <17.5	0	3	200	600	600	1800	0
17.5 to <19.5	0	4	200	800	600	2400	0
19.5 to <25.5	3	2	400	800	1200	2400	3
25.5 to <35.5	3	3	400	1200	1200	3600	3
35.5 to <45.5	3	4	400	1600	1200	4800	3
45.5 to 55.5	3	5	400	2000	1200	6000	3
>55.5	3	6	400	2400	1200	7200	3

8.3 Study Treatment Packaging and Labeling

8.3.1 Packaging and Labeling

The capsules will be packed in high-density polyethylene containers, with child-proof polypropylene caps. Study drug capsules containing A4250 in the strength specified in Table 2 above will be manufactured.

Packaging and labeling will be prepared to comply with applicable regulatory requirements.

8.3.2 Storage

Treatment packs containing A4250 capsules should be stored and dispensed in accordance with regulations in their original containers. The storage facility at the investigative site should be locked and the storage temperature should be between 15°C and 25°C.

Patients/caregivers should be informed of appropriate storage conditions (i.e., room temperature, between 15°C and 25°C).

Any deviations from the recommended storage conditions should be immediately reported to Albireo and the study drug should not be used until authorization has been given by Albireo.

8.3.3 Blinding and Randomization of Study Drug

A double-blind design is employed so that both the investigators and the patients will be unaware of the treatment assignment during the study.

After written informed consent is obtained from an eligible patient, an 8-digit patient identification number will be assigned. The first 2 digits will denote the country, followed by a 3-digit site number and a 3-digit patient sequence number. This number will be created and

allocated by the Interactive Web Response System (IWRS), when the patient first enters the system at Screening. The randomization codes will be computer-generated by [redacted] Biostatistics, and kept by a statistician independent from the project team. The randomization will be done in blocks and stratified according to PFIC class (Type 1 and 2) and age class (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) to ensure approximate balance between dose schemes (1:1:1). A separate randomization list will be prepared for the maximum 5 patients from Study A4250-003 regardless of stratification. Randomization codes will be assigned sequentially as patients become eligible for randomization.

Patients who are eligible for randomization will be assigned a unique 4-digit randomization number by IWRS. This randomization number identifies which treatment will be allocated to the patient. Patients who withdraw from the study after randomization visit are not to be replaced and their randomization number will not be reused.

To ensure blinding, the study drug and the matching placebo have the same shape and size. Patients will receive capsule(s) of A4250 according to their dose group or capsule(s) of matching placebo once a day during the double-blind Treatment Period. Labels on the study drug containers will not identify the treatment that a patient is randomized to. Traceability of the treatment is ensured by the study drug number.

The 5-digit study drug number will identify study drug packs and will be detailed on the study drug label. Dispensing of study drug will be coordinated by IWRS.

The system will assign a study drug number corresponding to the randomization arm. The randomization number will be used in the background only to ensure no unblinding and will not be displayed to end-users in the IWRS.

8.4 Procedure for Breaking the Randomization Code

Should a situation arise where unblinding is urgently required, i.e., only where knowledge of the study drug is required to adequately manage a life-threatening situation, the investigator at that study site may perform immediate unblinding through IWRS. The responsibility to break the treatment code in emergency situations resides solely with the investigator. The investigator should make every effort to discuss the rationale (status and outcome) for emergency unblinding with the sponsor Medical Monitor or the [redacted] Medical Monitor as soon as possible to review the individual patient details.

The study site investigator and appropriate [redacted] project team members will be authorized to access the emergency unblinding functionality within the IWRS. Emergency unblinding takes place in the IWRS via a dedicated emergency unblinding module. The system will require the user to enter their emergency unblinding, which is typically provided at the same time login credentials are distributed. The exact description of the treatment assigned to the individual patient then will be accessible to the user. Once the randomization code is broken for a patient, he/she must be withdrawn from the study and all assessments and procedures at EOT should be performed. As a follow-up, Visit 10 will be performed according to the time

schedule. Once a randomization code has been broken, the investigator must inform the [redacted] and sponsor Medical Monitor in writing within 24 hours. In case of technical issues accessing the system online, please see the IWRS site user manual for country-specific contact telephone numbers for the [redacted] HelpDesk 24/7 system support.

8.5 Patient Compliance

The study nurse will monitor eDiary compliance by routine review of the CRF health website. If both diary entries on a day are missing, the study nurse will call the patient/caregiver to remind them to complete all scheduled entries. Any noncompliance will be documented and explained in the source documents.

Study drug compliance will also be checked through diary responses. Additionally, patients will return all unused study drug at Visits 4, 5, 6, 7, 8, and 9. The study site staff will count all returned drug, assess compliance, and record details in the eCRF.

Treatment Compliance = 100 × ((Number of study drug dispensed – number of study drug returned)/number of study drug that should be taken).

Treatment compliance between 80% and 120% will be acceptable.

8.6 Study Drug Accountability

Records shall be maintained of the delivery of study treatment(s) to the study site(s), of the inventory at the study site(s), of each use of the study treatment(s) for each patient, and of the return of unused study treatment(s) to Albireo.

These records shall include dates, quantities, batch numbers, expiry dates, and the unique code numbers assigned to the study drug and to the study patients.

The investigator will be responsible for ensuring that the records adequately document that the patients were provided the quantities specified in the protocol and that all study drug received from Albireo is reconciled.

8.7 Concomitant Therapy and Prohibited Medications

The investigator will note all ongoing medication and any medication recently stopped (within 1 month prior to Visit 1) in the eCRF. At Visits 2 through 10, all changes in medication (stopping or starting new medication or changes in dose) will be recorded in the eCRF. All medications (prescribed or over the counter) for pruritus and sleep will be recorded.

All medications taken by a patient within 3 months prior to the first intake of study drug are regarded as prior medication.

All medications taken by a patient on or after the first intake of study drug, and which continue to be taken during the study, are regarded as concomitant medication.

Prohibited medications are listed in Appendix 1.

9 ASSESSMENT OF EFFICACY

9.1 Efficacy Endpoints

9.1.1 Primary Efficacy Endpoint

The primary efficacy endpoints are as follows:

- **European Union (EU) and rest of the world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  Fasting s-BA baseline will be calculated as the average of the value at randomization and the previous visit (Clinic Visit 2). The end value is the average of the values at Weeks 22 and 24 after the start of double-blind treatment.

- **United States (US)**: Proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period. A positive pruritus assessment is defined as a scratching score of ≤1 or at least a one-point drop from baseline on the Albireo ObsRO instrument. At each assessment, the AM score will be compared to the baseline AM average, and the PM score will be compared to the baseline PM average. Both AM and PM pruritus assessments will be included in the analysis of this endpoint. AM scores from the 14 days prior to the first dose of study medications will be averaged as AM baseline. PM scores from the 14 days prior to the first dose will be averaged as PM baseline.

9.1.2 Secondary Efficacy Endpoint

Secondary efficacy endpoints include the following:

- **EU and RoW**: Proportion of positive pruritus assessments at the subject level over the 24-week Treatment Period. A positive pruritus assessment is defined as a scratching score of ≤1 or at least a one-point drop from baseline on the Albireo ObsRO instrument. At each assessment, the AM score will be compared to the baseline AM average, and the PM score will be compared to the baseline PM average. Both AM and PM pruritus assessments will be included in the analysis of this endpoint. AM scores from the 14 days prior to the first dose of study medications will be averaged as AM baseline. PM scores from the 14 days prior to the first dose will be averaged as PM baseline.

- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

- **All Regions:**

All secondary endpoints are compared to placebo.

- Change from baseline to Week 12 and to Week 24 in fasting serum bile acids
- Change from baseline to Week 12 and to Week 24 in serum ALT concentration
- Change in growth from baseline to Week 12 and to Week 24Proportion of responders for pruritus scores at Weeks 12 and 24 based on the Albireo PRO and ObsRO instruments
- Change in sleep parameters measured with the Albireo PRO and ObsRO instruments from baseline over the 24-week Treatment Period
- Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level over the 24-week Treatment Period. A positive pruritus assessment includes an itch score ≤1, or at least a one-point drop from baseline based on the Albireo PRO instrument; only patients ≥8 years of age will complete the Albireo PRO instrument
- Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Proportion of individual AM assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Proportion of individual PM assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval.
- Number of patients undergoing biliary diversion surgery or being listed for liver transplantation

9.1.3 Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the PGIC items (Appendix 3)
- Change from baseline in fasting s-BA at Week 4

- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline by each 4 week-interval of the Treatment Period as measured by the Albireo ObsRO instrument
- Change from baseline by each 4-week interval of the Treatment Period in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline by each 4-week interval of the Treatment Period in patient-reported and observer-reported morning time itching and scratching severity scores, respectively
- Change from baseline by each 4-week interval of the Treatment Period in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline by each 4-week interval of the Treatment Period in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in PedsQL (Appendix 4)
- Change from baseline in serum ALT concentration at Week 4
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST and GGT at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, p-C4) at Week 24
- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events
- All-cause mortality

9.2 Efficacy Assessments

9.2.1 Serum Bile Acids

Blood samples for analysis of fasting total s-BA will be drawn at all visits. Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA. Exceptions can be made for infants, <12 months of age, if unable to fast for the full 4 hours. Any visit at which a bile acid sample result is unreportable, an additional unscheduled visit for a repeat sample collection may be scheduled. All s-BA results during

the treatment period and follow-up will be blinded. Samples will be processed and transported to a central laboratory per instructions in the laboratory manual.

9.2.2 Itching, Scratching and Sleep Score

Itching, observed scratching, and sleep disturbance will be recorded twice each day via eDiary. Patients and/or caregivers will be instructed to complete the eDiary every day in the morning after the patient wakes and in the evening just before the patient goes to sleep throughout the study. Patients and caregivers will receive a formal training session during Visit 1.

The eDiary includes Albireo ObsRO and PRO items. Patients <8 years of age will not be asked to complete the Albireo PRO items; only the Albireo ObsRO will be completed by caregivers of patients in this age group. Older patients, 8 to 18 years of age, will complete the Albireo PRO items and the caregiver will complete the Albireo ObsRO items. The Albireo PRO items assess severity of itch, aspects of sleep disturbance (morning diary only), and tiredness. For patients 8 to 12 years of age, the caregiver will read the Albireo PRO items along with the child and record the child's response. A guide will be provided to the caregivers that provides standardized explanations of the Albireo PRO items, in case the patient is confused or requires clarification about the meaning of a question. The Albireo ObsRO items assess severity of observed scratching, aspects of observed sleep disturbance (morning diary only), and observed signs of tiredness (evening diary only). The Albireo ObsRO and PRO scratching and itch severity items use 0 to 4 response scales, where each response is distinguished by a unique facial expression, verbal anchor, number, and color code (Appendix 2). To meet eligibility criteria, a scratch score will be calculated from daily diary entries (ObsRO items) the 14 consecutive days prior to randomization.

A daily AM & PM score for the Albireo ObsRO scratching item will be averaged from the 2 ratings for each day. A daily score will be considered missing if both of the daily assessments are missing. A weekly score will be calculated by averaging the daily scratching scores. A weekly score will be considered missing if ≥4 out of 7 days a week of data are missing. For AM & PM baseline, a 14-day daily score of AM & PM prior to the first dose of study medications will be averaged as the baseline score.

For AM baseline, a 14-day AM score prior to the first dose of study medications will be averaged as the baseline AM score. The same approach will be used for PM baseline and to calculate a patient-reported itch severity score. The handling of missing data is described in Section 11.2.2.2.

9.2.3 Growth

Growth will be measured as height (velocity) and weight (Z-score) using a certified weight scale. BMI will be calculated by weight (kg) / height $(m)^2$. Change will be defined as linear growth deficit (weight and BMI for age) compared to a standard growth curve (Z-score, SD from P50).

9.2.4 Quality of Life Questionnaire (PedsQL)

Patients and caregivers will be asked to complete a QoL questionnaire (PedsQL) at Visit 3 and Visit 9. Details of the questions included on the questionnaire are provided in Appendix 4.

9.2.5 Biomarkers and PK Samples

Blood samples for autotaxin and p-C4 will be drawn at Visits 3, 4, and 9 only for children with body weight >10 kg. Blood for A4250 PK assessment will only be drawn at Visits 4 and 9 (only for children with body weight >10 kg), and as close to the onset of an SAE as possible, should an SAE occur. Samples will be processed and transported to a laboratory per instructions in the laboratory manual.

9.2.6 PELD/MELD Score

At randomization and end of treatment, PELD score will be calculated for children up to 12 years of age. For children 13 years or older, the MELD score will be calculated.

PELD Score = 4.80 * ln(total bilirubin) + 18.57 * ln(INR) - 6.87 * ln(albumin) + 4.36 (if patient <1 year: scores for patients listed for liver transplantation before the patient's first birthday continue to include the value assigned for age (<1 year) until the patient reaches the age of 24 months) + 6.67 (if the patient has growth failure [≤2 SD])

Laboratory values less than 1.0 will be set to 1.0 for the calculation of the PELD score.

MELD Score = 9.57 * ln(creatinine) + 3.78 * ln(total bilirubin) + 11.2 * ln(INR) + 6.43

Laboratory values less than 1.0 will be set to 1.0 and serum creatinine values greater than 4.0 mg/dL (equivalent to 353.6 µmol/L)will be set to 4.0 for calculation of the MELD score.

9.2.7 Fibroscan®

Where available, Fibroscan® will be performed as per institution standard practice at Visits 3 and 9.

9.2.8 Markers of Fibrosis

APRI score and Fib-4 score will be calculated at randomization and at end of treatment.

APRI = [(AST in U/L) / (AST ULN in U/L)] / (Platelets in 10^9/L)

Fibrosis 4 Score = (Age*AST in U/L) / (Platelets in 10^9/L * √(ALT in U/L)).

9.2.9 Global Impression of Change and Global Impression of Symptom Measures

Patients (≥8 years of age), caregivers, and clinicians will complete the PGIC and the PGIS measures (Appendix 3) at randomization (Visit 3; PGIS only), Visits 4 and 6, and at EOT (Visit 9).

The PGIC items assess change in itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) since starting the study drug. The PGIS items assess itch

(patient version), scratching (caregiver and clinician versions), and sleep (all versions) in the past week. Caregivers and clinicians will complete the PGIC and PGIS for all patients; those patients ≥8 years of age will complete the patient version.

10 ASSESSMENT OF SAFETY

The timing and frequency of safety assessments are described in Section 7.1.3 and in Table 1 above.

The primary safety analysis for this study will include the incidence of total treatment-emergent adverse events (TEAE) and TEAEs categorized by causality, severity, and seriousness assessments made by the investigator by comparing study drug exposure to placebo.

Trends in safety will also be evaluated for the following assessments:

- Physical examinations
- Concomitant medications
- Vital signs
- Laboratory test results (including clinical chemistry, hematology, urinalysis, AFP, and vitamins A and E, 25-hydroxy vitamin D, and INR)
- Abdominal ultrasound
- Discontinuations due to AEs

10.1 Adverse Events

10.1.1 Definitions and Investigator Assessments

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 unfavorable and unintended sign, symptom, or disease that occurs once a patient is enrolled (ICF is signed) in the study until the patient is discharged from the study, whether or not related to the study drug.

10.1.1.1 Clinical Significance

Clinical significance is defined as any abnormal finding that results in further clinical investigation(s), treatment(s), or the diagnosis of new condition. Patient-reported events and protocol-mandated laboratory values, vital signs, and physical examination findings can be considered clinically significant (i.e., an AE) if there is a deterioration as compared to baseline. Examples of clinically significant worsening from baseline could include, but is not limited to, events causing withdrawal from the study and events requiring medical intervention outside of the study causing apparent clinical manifestations, or judged relevant by the investigator.

10.1.1.2 Serious Adverse Events

Serious criteria are applied by the investigator to each AE as specifically defined below. These criteria are used to determine whether an AE is serious or non-serious. The assessment

is made independently of severity assessment (Section 10.1.1.3). For example, the development of a severe rash that occurs after signing of informed consent may not meet serious criteria as defined below and therefore would be considered a severe, non-serious AE.

Any AE that meets any 1 of the following 6 criteria is considered an SAE:

- The outcome of the AE is **death**
- The AE is immediately **life-threatening**. Life-threatening means that the patient is, in the opinion of the investigator, at immediate risk of death from the reaction as it occurred. This does not include an AE that, if more severe, might have caused death
- The AE results in persistent or significant **disability/incapacity**. Disability means a substantial disruption of a person's ability to conduct normal life functions
- The AE requires or prolongs **hospitalization**
- The AE results in a **congenital anomaly/birth defect**
- **The AE is an important medical event**. Important medical events may meet serious criteria should the investigator assess that they may significantly jeopardize the patient, represent a significant hazard, or requires medical/surgical intervention to prevent one of the outcomes listed above. Examples of potential SAEs based on this criteria include, but are not limited to, allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions even if they do not result in inpatient hospitalization, or the development of drug dependency and drug abuse.

10.1.1.3 Severity Assessment

Severity assessments are based on the intensity of the event in relation to expectation. The investigator will assess the intensity of AEs based on the following definitions:

- Mild (awareness of sign or symptom, but easily tolerated)
- Moderate (discomfort sufficient to cause interference with normal activities)
- Severe (incapacitating, with inability to perform normal activities)

Severity is a measure of intensity where seriousness is defined by the criteria outlined in Section 10.1.1.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not meet serious criteria, and therefore would be assessed as a severe AE but not an SAE.

10.1.1.4 Causality Assessment

The investigator determines the causality of all AEs to the study drug using medical judgment and considering all relevant factors such as (but not limited to) the underlying study indication, coexisting disease, concomitant medication, relevant history, pattern of the AE, temporal relationship to the study drug, and de-challenge or re-challenge. The causality assessment of the AE/SAE is to be made as follows.

Related to study drug (possibly, probably, or definitely related)

Based on medical judgment, there is at least a reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event follows a reasonable temporal sequence from administration of study drug
- The event could not be reasonably attributed to the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient
- The event follows a known pattern of response to study drug
- The event disappears or decreases on cessation or reduction in dose of the study drug. (It should be noted that in some situations an AE will not disappear or decrease in intensity upon discontinuation of study drug despite other clear indications of relatedness)
- The event reappears or worsens when the study drug is re-administered

Unrelated to study drug (unlikely or unrelated)

Based on medical judgment there is no reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event does not follow a reasonable temporal sequence from administration of study drug
- The event could be reasonably attributed to the known characteristics of the patient's clinical state, concurrent illness, environment or toxic factors, or other modes of therapy administered to the patient
- The event does not follow a known pattern of response to study drug
- The event does not disappear or decrease on cessation or reduction in dose of the study drug, and it does not reappear or worsen when the study drug is re-administered

10.1.2 Recording of Adverse Events

It is the investigator's responsibility to assess whether each untoward event is a clinically significant worsening from baseline, thereby considered an AE. For all AEs, the severity, seriousness, and causality to study drug for each AE as outlined in Section 10.1.1 will be assessed and recorded in the eCRF.

All serious and non-serious AEs are collected once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10) or 28 days after the last dose of study drug.

Any AEs or SAEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator until resolution or stabilization up to the database lock and recorded in the eCRF. Albireo retains the right to request additional information for any patient with ongoing AE(s) or SAE(s) at the end of the study.

TEAEs are defined as any AE that occurs after randomization (Day 0). All AEs that occur in the Screening Period, i.e., after enrollment and prior to randomization, will be collected on the eCRF as non-treatment-emergent AEs.

If there is a clinically significant deterioration of a laboratory value/vital sign or other routine study assessment that is associated with a diagnosis, the clinical diagnosis will be reported as an AE and the associated signs and symptoms will be considered additional information unless the sign or symptom is more severe than expected given the diagnosis. For example, if an investigator diagnoses a study patient with hepatitis during the study period, hepatitis would be considered the AE and the concomitant signs and symptoms of abdominal pain, vomiting, and elevated ALT and AST would not be reported separately unless, in the opinion of the investigator, one of these signs or symptoms is more severe than expected and therefore a separate AE assessment is indicated.

10.1.3 Recording and Reporting of Serious Adverse Events

Every SAE (regardless of severity and causality) that occurs once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10), and 28 days after the final dose of study drug, should immediately and not later than within 24 hours of knowledge of the event, be reported by the investigator or delegate in the SAE report form.

Report of a SAE must include at least the following information:

- Patient identification information (study number, site number, initials, and date of birth [as per local country requirements for data protection])
- The last study drug administration date
- The diagnosis of the event with the description (or a brief description of signs/symptoms/clinical course if the diagnosis is not available) and the date of onset
- The action used to treat the event (i.e., treatment medications, temporary discontinuation)
- The reason(s) for considering the event serious
- The relationship of the event to the study drug or to the study procedure (e.g., the investigator's assessment of causality)
- A brief narrative of the SAE

Follow-up reports including all new information obtained of the subsequent course of the SAE must be prepared and the information collected in the SAE submitted to [redacted] (Pharmacovigilance and Safety Services Department) by e-mail within 24 hours of knowledge of the event.

The pharmacovigilance manager (PVM) may contact the investigator to obtain further information on a reported SAE. The investigator/investigative staff must respond to any

request for follow-up information or answers to questions regarding the SAE within the same timelines as for initial reports.

The PVM reports the occurrence of the SAE and follow-up to the Albireo medical monitor for medical assessment of the case.

Should an outcome of death occur within the study period or within 28 days after the last administration of study drug, an SAE form should be completed including the main and contributory causes of death.

All SAE reports must be e-mailed to the following e-mail address within **24 hours**:

[redacted]

If email is unavailable, SAEs may be transmitted via fax to the following number:

[redacted]

In a study-related medical emergency situation, when the assigned medical monitors for the study cannot be reached by a caller, an on call physician can be reached 24 hours per day, 7 days per week, via the [redacted] call center. The following number is a chargeable telephone number allowing a global reach from both landlines and mobile phones and the internet page indicated below contains a list of country-specific toll-free telephone numbers that is provided. It should be noted that not all countries globally have access to toll-free numbers as indicated on the "24/7 Medical Help desk" index. Countries without toll-free numbers need to dial the chargeable number as indicated below. Furthermore, toll-free numbers might not be available from mobile phones.

[redacted]

10.1.4 Reporting of Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reaction (SUSAR) is an SAE that occurs in a patient, the nature or severity of which is not expected per the applicable product information (e.g., the IB for an unauthorized investigational product or summary of product characteristics for an authorized product).

Reporting and tracking of SUSARs will be in accordance to all applicable competent authority regulations. The Ethics Committee and all investigators involved in this study will be informed according to national and international requirements.

10.2 Laboratory Values/Vital Signs/Physical Examinations and Other Safety Assessments

10.2.1 Laboratory Assessments

Samples will be collected for clinical chemistry, hematology, and urinalysis testing at the time points specified in Table 1. The parameters assessed are presented in Table 3.

Blood for AFP will be drawn at Visits 3 and 9. Fat-soluble vitamin levels including vitamins A and E, and 25-hydroxy vitamin D will be drawn at Visit 2 (vitamins A & E only), Visit 3 (25-hydroxy vitamin D only), Visit 6, and Visit 9. Blood for INR (surrogate for vitamin K) will be drawn at Visits 2, 4, 6, 8, and 10. Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for vitamin A. Exceptions can be made for infants, <12 months of age, if unable to fast for the full 4 hours. If a patient has any vitamin level(s) that are outside of the reference range, vitamin supplementation adjustments may be required. Target ranges and supplementation strategy guidelines can be found in Appendix 5.

A serum pregnancy test will be performed at Visit 1 and Visit 9 if the patient is entering the A4250-008 study. A urine pregnancy test will be collected at all other visits for girls who have reached menarche. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy. Study drug administration will be held pending the confirmatory results. If the serum pregnancy test is negative, the patient can resume dosing. If the serum pregnancy test is positive, the patient should be discontinued (see Section 10.2.9).

All samples will be processed and transported to a laboratory per instructions in the laboratory manual.

The observed values will be recorded and assessed as “normal” or “abnormal not clinically significant” or “abnormal clinically significant”.

Additional safety blood samples may be needed due to follow-up of an abnormal value or analysis failure. The blood samples collected for safety laboratory analysis will be destroyed after the analyses have been completed.

Table 3 Routine Laboratory Parameters

Clinical Chemistry	Hematology	Urinalysis
• Albumin • ALT • Alkaline phosphatase • AST • Bilirubin – total and direct • Calcium • Chloride • Creatinine • Creatine kinase • Gamma-glutamyl transferase • Potassium • Sodium	• Hematocrit • Hemoglobin • Platelet count • Red blood cell count • White blood cell count and differential (neutrophils, eosinophils, basophils, lymphocytes, and monocytes)	• Blood • Glucose • Ketones • Leukocytes • Nitrites • pH • Protein

10.2.2 Individual Patient Safety Monitoring

10.2.2.1 Liver Monitoring

Strategies to monitor markers of liver disease throughout the study are outlined below where the ULN will be based on central laboratory reference values for age and gender.

If isolated transaminase elevations are observed, defined as:

1. Normal bilirubin AND absence of clinical hepatitis symptoms AND
   - ALT or AST ≥5 × ULN (if normal at baseline) or an absolute threshold of 800 U/L, whichever comes first
   - OR ALT or AST ≥3 × baseline (if abnormal at baseline) or an absolute threshold of 800 U/L, whichever comes first

Then:

a) Repeat liver profile (AST, ALT, bilirubin, and prothrombin time [PT] or INR) within 2 to 3 days

b) Evaluate creatine phosphokinase (CPK) and lactate dehydrogenase (LDH)

c) As needed (for example, persistent ALT/total bilirubin elevation or worsening of liver function), consider evaluation for alternative etiologies

Monitor the patient using close observation found in Section 10.2.2.2

If any ONE of the following criteria are met:

1. Transaminases (ALT or AST ≥3 × baseline or 800 U/L, whichever comes first) AND bilirubin (total bilirubin > 2 × ULN) elevations

2. Transaminase elevations alone (ALT or AST >10 × ULN or 5 × baseline or absolute threshold of 800 U/L, whichever comes first) in presence of normal LDH and CPK
3. Total bilirubin increased, unrelated to hemolysis (elevated reticulocyte count) or established genetic diseases, such as Gilbert's syndrome
   a. Doubling if total bilirubin was <3 mg/dL (equivalent to 51.3 µmol/L) at baseline
   b. OR Increase by >3 mg/dL (equivalent to 51.3 µmol/L) if total bilirubin was ≥3 mg/dL (equivalent to 51.3 µmol/L) at baseline
4. INR increase refractory to vitamin K administration
   a. INR >1.5 if INR was normal at baseline
   b. OR Increase by >0.4 if INR was abnormal at baseline
5. Any increase in total bilirubin and transaminases if accompanied by EITHER a symptom of clinical hepatitis (e.g., vomiting, nausea, right upper quadrant pain) OR immunological reaction (rash or >5% eosinophilia)

Then:

a) Interrupt study medication

b) Initiate drug-induced liver injury work-up for alternative etiologies

c) Repeat liver profile (AST, ALT, total bilirubin, direct bilirubin) and PT or INR within 48 to 72 hours

d) Monitor the patient using close observation found in Section 10.2.2.2

e) If a patient lives in a remote area, they may be tested locally and the results communicated to the investigator site promptly

10.2.2.2 Close Observation

- Repeat liver enzyme and serum bilirubin tests two or three times weekly. Frequency of re-testing can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the subject is asymptomatic
- Obtain a more detailed history of symptoms and prior or concurrent diseases
- Obtain a history of concomitant drug use (including non-prescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets
- Consider ruling out alternate etiology including acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; non-alcoholic steatohepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease
- Obtain a history of exposure to environmental chemical agents.

- Obtain additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin)
- Consider gastroenterology or hepatology consultations.

10.2.2.3 De-challenge/Re-challenge for Liver and Clinical Hepatitis Monitoring

1. Re-challenge is not recommended:
   a. If a patient has had possible/probable drug-induced liver injury
   b. If a decompensation event has occurred (i.e., variceal hemorrhage, ascites, hepatic encephalopathy, etc.).
2. If due to underlying cholestatic liver disease variability or another alternative etiology is identified AND liver tests returned to baseline, re-challenge may be considered after consultation with the sponsor Medical Monitor.
3. If the ALT/total bilirubin elevations are observed after re-challenge, then repeat re-challenge is discouraged.

If patient is permanently discontinued, monitoring should be continued as outlined in Section 10.2.2.5.

10.2.2.4 Diarrhea

Study drug should be discontinued if a patient develops diarrhea with at least 1 of the following concomitant signs or symptoms: grossly bloody stools, vomiting, dehydration requiring treatment with oral or intravenous rehydration and/or electrolyte imbalances, fever (≥38 C) and/or the diarrhea persists for 7 or more days.

If there is gross blood in stool, INR and platelets should be measured.

Study drug will be reintroduced (re-challenge) when the symptoms have resolved. If the diarrhea re-occurs within 1 week after the re-challenge with no alternate etiology, the patient will be permanently discontinued and monitored as outlined in Section 10.2.2.5.

10.2.2.5 Monitoring after Permanent Discontinuation of Study Drug Due to Safety

Once study drug is permanently discontinued for liver, hepatitis, diarrhea, or other severe AE related to study drug, the patient will be monitored weekly until the laboratory and clinical parameters have normalized/stabilized.

10.2.3 Demographics/Medical and Surgical History

Demographic information per country regulations (age, full date of birth, gender, race, and ethnicity), along with medical and surgical history, will be obtained and recorded in the eCRF at Visit 1.

Medical and surgical history will be entered in the eCRF at Visit 1. This includes date of diagnosis of PFIC, prior investigational medications for PFIC, historical (3 months prior to

Screening) LFT values (AST, ALT, and total bilirubin), ongoing medication, any surgery performed, any other diagnosis, and historical biopsy data.

If a liver biopsy has been performed within 1 year prior to Screening, or during the study, the results will be recorded in the eCRF.

10.2.4 Clinical Genetic Testing

For previous confirmatory clinical genetic testing results for PFIC Type 1 or 2 performed prior to Screening Visit 1, the confirmatory clinical genetic testing laboratory report will be verified to determine eligibility.

If the historical clinical genetic result is equivocal, unavailable, or unobtainable, clinical genetic analysis to confirm pathologic biallelic variations in the *ATP8B1* or *ABCB11* genes will be performed and verified to determine eligibility. No other diagnostic genetic testing will be offered.

10.2.5 Physical Examination

A physician or suitably trained qualified assistant will perform a complete physical examination, including voluntary photography of skin lesions due to scratching, at Visits 1, 3, 6, and 9. A skin examination will be conducted at Visits 1, 3, 4, 6, 9, and 10 (see Table 1).

A complete physical examination will include assessment of general appearance, eyes, ears, nose, throat, head/neck/thyroid, lymph nodes, cardiovascular, lungs/chest, abdomen, genitourinary, extremities, skin, musculoskeletal, neurologic, and other.

Skin will be examined thoroughly and excoriations/scratch marks recorded.

10.2.6 Vital Signs

Evaluation of vital signs will be performed at all visits. This includes blood pressure (systolic and diastolic), pulse, respiratory rate, temperature, height (or length depending on age), and body weight (using a certified weight scale) at clinic visits. BMI will be calculated.

10.2.7 Abdominal Ultrasound

Ultrasound of the liver and spleen will be performed at randomization (Visit 3) and at EOT (Visit 9). Liver size, echogenicity, and presence of masses/nodules as well as spleen size will be recorded.

10.2.8 Overdose

A4250 is minimally absorbed and has a very low systemic availability. Based on toxicology data, for study purposes any dose exceeding a total of >3 mg/kg body weight of A4250 taken as a single dose or as a cumulative dose within 24 hours is defined as an overdose.

The no-adverse-effect dose level in the most sensitive species (defined as 20 mg/kg/day) in the rat 1 month toxicity study gives a human equivalent dose of approximately 194 mg/day in

a 60 kg person. This dose is approximately 10-fold higher than the maximum possible dose predicted in human studies (20 mg).

The investigator/investigative staff should immediately and not later than within 24 hours of knowledge of the overdose, be reported by the investigator or delegate on the SAE report form. In the event of an A4250 overdose, the patient should be monitored closely.

10.2.9 Pregnancy

If a pregnancy is discovered in a female patient enrolled in the study before the end of dosing, the patient will be immediately discontinued from the study and will attend the same visits as a prematurely withdrawn patient. If the pregnancy is discovered in a female patient enrolled in the study after the end of dosing, the patient will continue in the study per protocol. If a pregnancy occurs in a male patient's partner at any time during the study, the pregnancy should also be reported and followed.

Pregnancy is not considered to be an AE. However, if the patient has been dosed with the study drug, the pregnancy must be reported on the Paper Pregnancy Notification Form immediately within 24 hours after recognition to Safety Manager (please refer to Section 10.1.3). Date of exposure and as far as possible, details of the period of gestation at the time of exposure must be given.

The pregnancy should be followed to determine outcome, including spontaneous termination, details of birth, and presence of any birth defects, congenital anomalies or newborn or maternal complication. The infant who was exposed in-utero will be followed for up to 2 years after delivery. Individual cases with an abnormal outcome in association with the study drug should be reported on an expedited basis, i.e., reported rapidly to a competent authority.

11 STATISTICAL EVALUATION

All personnel involved with the analysis of the study will remain blinded until database lock for the double-blind Treatment Period and until major protocol violations have been identified.

All statistical analyses will be performed using SAS version 9.3 or higher.

11.1 Sample Size and Power

For the EU and RoW, the primary analysis will relate to the serum bile acid responder endpoint. For the US, the primary analysis will relate to the pruritus endpoint. For each primary endpoint, a pooled analysis for the closed testing procedure will be applied to control the 1-sided overall type I error rate for two treatment comparisons vs. the placebo at the 0.025 level (Kane et al, 2015, Lehmacher et al, 1991, and Marcus et al, 1976). The study will enroll 60 to 70 patients in order to obtain at least least 20 evaluable patients in each arm. For each primary endpoint, simulations with 5000 iterations using 20 patients per arm were conducted to estimate the power after multiplicity adjustment, resulting in a standard error of <0.7% for each estimated power.

Based on the Phase 2 A4250-003 study data both dose groups are predicted to have similar treatment effects for both serum bile acids and pruritus. For serum bile acids, simulated proportions were analyzed using the CMH test to generate the following 1-sided p-values: both A4250 arms pooled vs. placebo, low dose vs. placebo, and high dose vs. placebo. Assuming 60% responders in the A4250 arms and 10% responders in the placebo arm, the power to claim significance for a particular A4250 arm after multiplicity adjustment is approximately 94%. The power to claim significance for at least one arm or both arms are approximately 99% and 91%, respectively.

For the proportion of positive pruritus assessments at the subject level in pruritus scores, simulated proportions were analyzed using ANCOVA to generate the following 1-sided p-values: both A4250 arms pooled vs. placebo, low dose vs. placebo, and high dose vs. placebo. With an effect size of 1.0526, the simulated power to claim significance for a particular arm after multiplicity adjustment is approximately 89%. The probability to claim significance for at least one arm or both arms are approximately 95% and 83%, respectively.

11.2 Statistical Methods

11.2.1 Statistical Analysis Sets

Safety Analysis Set

The safety analysis set will consist of all patients who received at least 1 dose of study drug. Patients will be analyzed according to the treatment they actually received. The safety analysis set will be the primary analysis set for safety analyses.

Full Analysis Set

The full analysis set (FAS) will consist of all randomized patients who received at least 1 dose of assigned treatment. Patients will be analyzed in the treatment group they were randomized to even if they received incorrect study drug. The FAS will be the primary analysis set for efficacy analyses.

Per Protocol Analysis Set

The per protocol (PP) analysis set is a subset of the FAS, and will consist of all randomized patients for whom no major protocol violation (no violation which may affect the study efficacy outcome) is documented. The PP analysis set will provide supportive data for the efficacy analyses.

Allocation of patients to the PP analysis set will be performed before unblinding of the study.

11.2.2 Methods of Statistical Analyses

11.2.2.1 General Principles

The primary objective of this study is to show superiority in efficacy of A4250 (120 µg/kg/day and 40 µg/kg/day) compared to placebo in patients with PFIC Types 1 and 2.

In the EU and RoW, for the primary efficacy variable of fasting bile acid responder endpoint (at least a 70% reduction in fasting s-BA concentration or reaching a level ≤70 µmol/L at the end of treatment), the Cochran Mantel Haenszel (CMH) test stratified by PFIC class and age class will be performed to compare the proportion in fasting bile acid responder endpoint at the end of treatment (Week 22 and 24) in the two A4250 dose groups to placebo group. To ascertain that all data are used in the CMH analysis, neighboring strata will be pooled if all subjects in a stratum have the same response. Dropouts will be treated as non-responders. The proportion together with corresponding 95% CI, odds ratio and corresponding 95% Clopper-Pearson exact CI and p-value for the CMH test will be presented.

In the US, for the primary efficacy variable of the proportion of positive pruritus assessments at subject level over the 24-week treatment period, an analysis of covariance (ANCOVA) will be used. The model will include treatment arm, AM and PM baseline pruritus scores, and randomization stratification factors, i.e. PFIC class and age class. LS mean(SE) by treatment arm and LS mean difference (SE), 95% confidence intervals, and p-values (where applicable) between treatments (120 µg/kg/day and 40 µg/kg/day, respectively) vs. placebo will be provided. If there are concerns on model assumptions, i.e. normality and homogeneity of variances, a non-parametric ANCOVA based on the rank scores will be used as a sensitivity analysis. LS mean/SE on the outcome by treatment arm and LS mean difference/SE between active dose and placebo will be provided.

The procedure for multiplicity adjustment is specified as follows:

In the closed testing procedure, the low and high dose groups will be pooled to compare with the placebo group first. If the 1-sided p-value is ≤0.025, the 1-sided p-values for low dose vs. placebo and high dose vs. placebo will be calculated, respectively. If both individual p-values are ≤0.025, a significant treatment effect will be declared on both dose groups. If only one of them is ≤0.025, a significant treatment effect will be declared on the corresponding dose group.

Other secondary and exploratory outcome variables will provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple comparisons when testing secondary and exploratory efficacy variables.

If not otherwise specified, the baseline value of a parameter is defined as the last assessment of that parameter prior to the first intake of study treatment.

11.2.2.2 Missing Data

In the primary analysis of the pruritus endpoint, all intermittently missing assessments will be classified as negative pruritus assessments. Similarly, all planned assessments after the intercurrent events (premature treatment discontinuation, death or initiation of rescue treatments such as biliary diversion surgery or liver transplantation) will be counted as negative pruritus assessments. However, as described above, all 336 assessments planned over the 24-week treatment period will be included in the denominator of the endpoint definition.

In the primary analysis of fasting bile acid responder endpoint, dropouts and patients with a missing average at the end of the treatment will be treated as non-responders. Patients in need of surgical rescue, i.e. biliary diversion and listing for liver transplant will be classified as non-responders. Continued collection of efficacy data for patients who discontinue treatment will be made as far as possible.

11.2.2.3 Demographic and Baseline Characteristics

Descriptive summaries of demographics and other baseline characteristics (including medical and surgical history) will be presented by treatment group and overall using the FAS.

Prior and concomitant medication use during the study will be summarized by treatment group and overall using the safety analysis set.

11.2.2.4 Subject Disposition

The following will be summarized (by treatment group and overall where applicable):

- Patients enrolled (who signed the informed consent)
- Patients randomized
- Patients treated

- Patients completing the study
- Patients withdrawing early (including withdrawal reason)

Additionally, patients enrolled, included in the safety, in the FAS, and in the PP analysis set will be summarized by region.

11.2.2.5 Evaluation of Primary Efficacy Variables

For EU and RoW, the primary responder endpoint based on change in fasting bile acids will be analyzed in terms of the proportion of patients achieving the event of interest. Patients in need of surgical rescue, i.e. biliary diversion and listing for liver transplant, will be classified as non-responders. Dropouts will be treated as non-responders.

CMH stratified by PFIC class and age class will be performed to compare the proportion in fasting bile acid responders at the end of treatment (Week 22 and 24) in the two A4250 dose groups to the placebo group. To ascertain that all data are used in the CMH analysis, neighboring strata will be pooled if all subjects in a stratum have the same response. The proportion together with corresponding 95% CI, odds ratio and corresponding 95% Clopper-Pearson exact CI and p-value for the CMH test will be presented

Logistic regression will be performed as a supportive analysis to compare the proportion in fasting bile acid responders at the end of treatment (Week 22 and 24) in the two A4250 dose groups to the placebo group. The model will include treatment arm, baseline value, and randomization stratification factors, i.e. PFIC class and age class. Baseline value will be calculated as the average of serum bile acid values at randomization and the previous visit (Clinic Visit 2).

In the US, for the primary efficacy variable of the proportion of positive pruritus assessments at the subject level over the 24-week treatment period, ANCOVA will be used to analyze the pruritus endpoint. The model will include treatment arm, the averaged AM and PM baseline pruritus scores, and randomization stratification factors, i.e. PFIC class and age class. LS mean(SE) by treatment arm and LS mean difference (SE), 95% confidence intervals, and p-values (where applicable) between treatments (120 µg/kg/day and 40 µg/kg/day, respectively) vs. placebo will be provided. If there are concerns on model assumptions, i.e. normality and homogeneity of variances, a non-parametric ANCOVA based on the rank scores can be used. LS mean/SE on the outcome by treatment arm and LS mean difference/SE between active dose and placebo will be provided.

Sensitivity analysis will be conducted to assess the impact of intermittently missing data as well as intercurrent events and will be specified in the SAP. Subgroup analyses will also be performed for each of the 3 age groups (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) and for patients who were not included in the previous Study A4250-003.

11.2.2.6 Evaluation of Secondary and Exploratory Efficacy Variables

The change in the secondary endpoints such as serum bile acids, total bilirubin, ALT, growth, Albireo PRO and ObsRO sleep parameters, and patient-reported itch severity will be summarized by visit using descriptive statistics. Change in growth (weight for age and BMI for age) will also be displayed using graphical presentations.

Proportion of responders at Weeks 12 and 24 based on the PRO/ObsRO instruments estimated from both distribution and anchor-based approaches will be analyzed using the same model as specified for the primary analysis for EU and RoW.

Proportion of individual assessments meeting the definition of a positive pruritus assessment at the subject level from Weeks 0-4, Weeks 0-8, Weeks 0-12, Weeks 0-18, Weeks 0-24, respectively, or the proportion of positive pruritus assessments at each 4-week interval over the 24-week treatment period for AM or PM, respectively will be analyzed using the same model as specified for the primary analysis for US.

Undergoing biliary diversion surgery and/or listed for liver transplant, all-cause mortality, and liver decompensation events will mainly be analyzed by using descriptive statistics. Kaplan-Meier curves will be used when appropriate for time to event data.

Comparisons of the change from baseline at Week 24 in PedsQL between the treatment groups will be conducted using ANCOVA. The model will include terms for baseline, PFIC class, age class, and treatment.

Other exploratory variables (Global Symptom Relief, biochemical markers and measures of bile acid synthesis, additional Albireo PRO and ObsRO sleep parameters, PELD/MELD score, APRI score, and Fib-4 score) will be analyzed descriptively for categorical and continuous data, as applicable. For continuous data, the change from baseline will be analyzed in addition to the actual visit values. For categorical data, shift tables or frequency and percentages of patients will be presented as appropriate. Additional details on secondary and exploratory efficacy variables and subgroup analyses by age class (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) will be specified in the SAP.

11.2.2.7 Evaluation of Safety Variables

Safety data will be analyzed using descriptive statistics and summaries by treatment group of SAEs, AEs, vital signs, clinical laboratory tests (hematology, clinical chemistry and urinalysis), and concomitant medication. Analyses will be performed using the safety analysis set.

Summaries of AEs (coded according to the Medical Dictionary for Regulatory Activities [MedDRA] system organ class [SOC] and MedDRA preferred term) will include the following:

- Overview of the incidence of TEAEs (TEAEs, drug-related TEAEs, TEAEs leading to study discontinuation, and treatment-emergent SAEs)
- TEAEs by SOC and preferred term
- Intensity of TEAEs by SOC and preferred term
- Drug-related TEAEs by SOC and preferred term
- TEAEs leading to study discontinuation by SOC and preferred term
- Treatment-emergent SAEs by SOC and preferred term

Concomitant medication use during the Treatment Period will be summarized by Anatomical Therapeutic Chemical (ATC) class and World Health Organization (WHO) preferred name.

Summaries of vital signs will be presented. For each visit, the actual results and the change from baseline, and the number and percentage of patients with potentially clinically significant (PCS) values observed post-baseline will be presented.

Summaries of clinical safety laboratory data will be presented. For each visit, the actual result and the change from baseline, and the number and percentage of patients with PCS values observed post-baseline will be presented.

Data listings will be provided for each patient for all safety parameters.

11.2.2.8 Compliance and Exposure

Exposure will be analyzed by calculating the number of days with exposure to study drug. Results will be presented by treatment group using the safety analysis set.

The percentage compliance will be described by treatment group and the number of patients with a compliance <80%, between 80% and 120%, and >120% will be presented based on the safety analysis set.

11.2.3 Blinded Analyses to Confirm Responder Definitions

A blinded analysis of Albireo ObsRO and PRO eDiary data will be performed after 50% or more of the planned sample has completed the Week 24 Follow-Up Period. The blinded analysis will be used to estimate a threshold of clinically meaningful change (i.e., responder definition) in Albireo ObsRO and PRO pruritus scores. The analysis will be performed by a group independent from both the study team and Albireo. Blinded data will be used for this analysis; data will be collapsed across treatment groups.

The analyses will include distribution- and anchor-based analyses to examine a responder definition for the Albireo PRO/ObsRO pruritus scores. Distribution-based analyses will include calculation of the SD and standard error of measurement for the baseline Albireo PRO and ObsRO scores. Anchor-based analyses will involve examining the degree of change on the Albireo PRO and ObsRO scores from baseline to Week 24 for patients who experience change in pruritus according to PGIC and symptom items. Details of this analysis

will be included in a stand-alone SAP for assessing the measurement characteristics (reliability, validity, sensitivity to change, responder definition) of the Albireo PRO and ObsRO items.

11.2.4 Data Safety Monitoring Board

A DSMB consisting of sponsor-independent clinical and statistical experts will be established. The DSMB will periodically (approximately on a quarterly basis) meet for the review of accumulating study data, including safety (AE and laboratory data) and liver values.

The DSMB will have access to unblinded data. The DSMB will make recommendations for the remaining part of the study (further details will be provided in the DSMB charter). The DSMB may recommend continuing with the study as planned or stopping the study early for safety reasons. The DSMB will submit its recommendations in writing to Albireo Medical[redacted] who are responsible for responding to the recommendations of the DSMB and taking appropriate action. The investigators will only be informed by Albireo Medical/[redacted] if the study requires a protocol amendment or is stopped. The DSMB may choose to make additional evaluations at any time if they feel this is warranted from a safety point of view.

The DSMB will act according to its own written standard operating procedure described in a charter and will prepare written minutes of its meetings. The charter of the DSMB will be stored in the trial master file. The DSMB will maintain records of its meetings and these will become part of the study file when the study is complete. In order not to disseminate unblinded data and to ensure that all staff involved in the conduct and final analysis of the study remain blind to the data, only the members of the DSMB and the unblinded DSMB statistician will have access to these data.

12 DIRECT ACCESS TO SOURCE DATA/NOTES

The investigator/institution shall provide direct access to source data/documents for study-related monitoring, audits, IEC/Institutional Review Board (IRB) review and regulatory inspection.

13 QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Conduct of the Study

Albireo/[redacted] shall implement and maintain quality control and quality assurance procedures with written standard operating procedures to ensure that the study is conducted and data are generated, documented, and reported in compliance with the protocol, ICH GCP and applicable regulatory requirements.

This study shall be conducted in accordance with the provisions of the Declaration of Helsinki (October 1996) and all revisions thereof, and in accordance with United States Food and Drug Administration (FDA) regulations (Code of Federal Regulations, Sections 312.50 and 312.56) and with ICH GCP (CPMP 135/95).

The investigator may not deviate from the protocol without a formal protocol amendment having been established and approved by an appropriate competent authority and IEC/IRB, except when necessary to eliminate immediate hazards to the patient or when the change(s) involve(s) only logistical or administrative aspects of the study. Any deviations may result in the patient having to be withdrawn from the study and render that patient non-evaluable.

13.2 Study Monitoring

Before study initiation, at a site initiation visit or at an investigator's meeting, an Albireo representative or designee will review the protocol and eCRF with the investigators and the investigative staff. During the study, the clinical monitor (clinical research associate [CRA]) will visit the site regularly to check the completeness of patient records, the accuracy of entries on the eCRFs, the adherence to the protocol and to GCP, the progress of enrollment, and to ensure that study drug is being stored, dispensed, and accounted for according to specifications.

The investigator must ensure that eCRFs are completed within a timely period of patient visits, as per individual site agreements, and must allow the CRA and Albireo representative or designee periodic access to patient records and all study-related materials, including relevant hospital or clinical records, to confirm their consistency with the eCRF entries. No information in these records about the identity of the patients will leave the study center. Albireo monitoring standards require full verification for the presence of the signed ICF, adherence to the inclusion/exclusion criteria, documentation of SAEs, and recording of primary efficacy and safety variables. The CRA will review source data compared with the eCRFs and will verify source data according to the study-specific monitoring plan. The design of the study, the frequency of patient visits, and the site enrollment rate will determine the frequency of monitoring visits. Upon study completion, the CRA will visit the site to conduct a study termination visit, which will include collection of any outstanding documentation.

It is recommended that the investigator/investigative staff log into the eCRF system every working day or at minimum twice weekly in order to provide a prompt response to queries. The investigator/investigative staff should respond to queries and make any relevant changes to the study data within 3 working days.

14 ETHICS

14.1 Independent Ethics Committee/Institutional Review Board

Prior to the start of the study, the investigator is responsible for ensuring that the protocol and consent form have been reviewed and approved by a relevant IEC/IRB. The IEC/IRB shall be appropriately constituted and perform its functions in accordance with FDA ICH GCP and local requirements as applicable.

The IEC/IRB shall approve all protocol amendments (except for logistical or administrative changes), written informed consent documents and document updates, patient recruitment procedures (e.g., advertisements), written information to be provided to the patients and caregivers, IB, available safety information, information about payment and compensation available to patients, the investigator's curriculum vitae and/or other evidence of qualifications and any other documents requested by the IEC/IRB and regulatory authority (competent authority) as applicable.

14.2 Written Informed Consents and Assents

The investigator (physician) or investigative staff, in accordance with local regulations, will explain to each patient (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, alternative treatment, the potential risks and benefits involved, and any discomfort that may occur. Each caregiver/patient will be informed that participation in the study is voluntary and that he/she or their child may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

Caregivers/patients will be informed that they/their children may experience side-effects or be at risk for symptoms, illnesses, or complications that cannot be foreseen by Albireo. As with other medications, people treated with A4250 may be at risk of developing allergic reactions or anaphylaxis. Caregivers/patients will be advised that study procedures include regular blood sampling for measurement of safety parameters and biological markers and that some minor risks are associated with these procedures.

This informed consent should be given by means of a signed ICF, written in non-technical language in accordance with ICH GCP, the Declaration of Helsinki, and regulatory authorities. The caregiver(s)/patient should read and consider the statements before signing and dating them and should be given a copy of each signed document. If written consent is not possible, oral consent can be obtained if witnessed and followed by a signed statement from one or more persons not involved in the study, indicating why the patient was unable to sign the form. No patient can enter the study before his/her or caregiver(s) informed consent has been obtained, as required by country regulations.

The ICF is part of the protocol and must be submitted by the investigator/investigative staff with the protocol for IEC approval. Albireo will supply an ICF which complies with

regulatory requirements and country laws, and is considered appropriate for the study. Any changes to the ICF suggested by the investigator must be agreed to by Albireo before submission to the IEC and a copy of the approved version must be provided to the clinical monitor after IEC approval.

15 DATA HANDLING AND RECORD KEEPING

15.1 Case Report Forms/Source Data Handling

The investigator shall be provided with standardized eCRFs and shall ensure that all data from patient visits are promptly entered into the eCRFs in accordance with the specific instructions given. The investigator must sign each eCRF to verify the integrity of the data recorded.

A list of the normal ranges for all laboratory tests to be undertaken forms part of the documentation to be collated prior to study start. As a central laboratory has been selected to conduct any or all tests, it is essential that all samples be analyzed at that laboratory.

The investigator must maintain source documents such as laboratory reports, consultation reports, and complete medical history and physical examination reports.

As no ethnicity data can be collected in the source document for study sites in France, the eCRF will be the Source Document for “origines ethniques”,

15.2 Retention of Essential Documents

Essential documents, as defined by ICH GCP, include: the signed protocol and any amendment(s); copies of the completed eCRFs (for site archiving, compact discs of eCRF data for specific patients will be provided); signed ICFs; hospital records and other source documents; IEC approvals and all related correspondence including approved documents; drug accountability records; study correspondence; and a list of patients’ names and addresses.

The investigator/investigative staff must retain copies of these essential documents for the period specified by ICH GCP and by applicable regulatory requirements. The investigator/investigative staff will inform Albireo of the location where the essential documents are stored and must contact Albireo for approval before disposing of any essential documents. The investigator/investigative staff should take measures to prevent accidental or premature destruction of these documents.

16 FINANCING AND INSURANCE

Financing and insurance are addressed in a separate agreement.

17 PUBLICATION POLICY

Albireo will retain the ownership of all data. When the study is complete, Albireo shall arrange the analysis, tabulation of data and preparation of a clinical study report. Albireo may also use the data for publication, presentation at scientific meetings and submission to regulatory authorities. All proposed publications based on this study must be subject to the sponsor's approval requirements.

18 REFERENCE LIST

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19 APPENDICES

Appendix 1 Concomitant Medication Guidelines

Appendix 2 Diary Questions including Albireo Patient-Reported Outcomes/Observer-Reported Outcome Instrument

Appendix 3 Patient Global Impression of Change (PGIC) and Patient Global Impression of Symptoms (PGIS)

Appendix 4 Pediatric Quality of Life Inventory (PedQL) Questionnaire

ID# _____

Date:_____

PedsQL™

Pediatric Quality of Life Inventory

Version 4.0

PARENT REPORT for **TODDLERS** (ages **2-4**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

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*In the past **ONE month,** how much of a **problem** has your child had with …*

PHYSICAL FUNCTIONING ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Walking	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in active play or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Bathing	0	1	2	3	4
6. Helping to pick up his or her toys	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Low energy level	0	1	2	3	4

EMOTIONAL FUNCTIONING ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad or blue	0	1	2	3	4
3. Feeling angry	0	1	2	3	4
4. Trouble sleeping	0	1	2	3	4
5. Worrying	0	1	2	3	4

SOCIAL FUNCTIONING ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Playing with other children	0	1	2	3	4
2. Other kids not wanting to play with him or her	0	1	2	3	4
3. Getting teased by other children	0	1	2	3	4
4. Not able to do things that other children his or her age can do	0	1	2	3	4
5. Keeping up when playing with other children	0	1	2	3	4

***Please complete this section if your child attends school or daycare**

SCHOOL FUNCTIONING ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Doing the same school activities as peers	0	1	2	3	4
2. Missing school/daycare because of not feeling well	0	1	2	3	4

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3. Missing school/daycare to go to the doctor or hospital	0	1	2	3	4

ID# _____

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YOUNG CHILD REPORT (ages **5-7**)

Instructions for interviewer:

I am going to ask you some questions about things that might be a problem for some children. I want to know how much of a problem any of these things might be for you.

Show the child the template and point to the responses as you read.

If it is <u>not at all</u> a problem for you, point to the smiling face

If it is <u>sometimes</u> a problem for you, point to the middle face

If it is a problem for you <u>a lot</u>, point to the frowning face

I will read each question. Point to the pictures to show me how much of a problem it is for you. Let's try a practice one first.

	Not at all	Sometimes	A lot
Is it hard for you to snap your fingers	☺	😐	☹

Ask the child to demonstrate snapping his or her fingers to determine whether or not the question was answered correctly. Repeat the question if the child demonstrates a response that is different from his or her action.

Think about how you have been doing for the last few weeks. Please listen carefully to each sentence and tell me how much of a problem this is for you.

After reading the item, gesture to the template. If the child hesitates or does not seem to understand how to answer, read the response options while pointing at the faces.

PHYSICAL FUNCTIONING ***(problems with…)***	**Not at all**	**Some-times**	**A lot**
1. Is it hard for you to walk	0	2	4
2. Is it hard for you to run	0	2	4
3. Is it hard for you to play sports or exercise	0	2	4
4. Is it hard for you to pick up big things	0	2	4
5. Is it hard for you to take a bath or shower	0	2	4
6. Is it hard for you to do chores (like pick up your toys)	0	2	4
7. Do you have hurts or aches (***Where?_____***)	0	2	4
8. Do you ever feel too tired to play	0	2	4

Remember, tell me how much of a problem this has been for you for the last few weeks.

EMOTIONAL FUNCTIONING ***(problems with…)***	**Not at all**	**Some-times**	**A lot**
1. Do you feel scared	0	2	4
2. Do you feel sad	0	2	4
3. Do you feel mad	0	2	4
4. Do you have trouble sleeping	0	2	4
5. Do you worry about what will happen to you	0	2	4

SOCIAL FUNCTIONING ***(problems with…)***	**Not at all**	**Some-times**	**A lot**
1. Is it hard for you to get along with other kids	0	2	4
2. Do other kids say they do not want to play with you	0	2	4
3. Do other kids tease you	0	2	4
4. Can other kids do things that you cannot do	0	2	4
5. Is it hard for you to keep up when you play with other kids	0	2	4

SCHOOL FUNCTIONING ***(problems with…)***	**Not at all**	**Some-times**	**A lot**
1. Is it hard for you to pay attention in school	0	2	4
2. Do you forget things	0	2	4
3. Is it hard to keep up with schoolwork	0	2	4
4. Do you miss school because of not feeling good	0	2	4
5. Do you miss school because you have to go to the doctor's or hospital	0	2	4

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How much of a problem is this for you?

Not at all

Sometimes

A lot

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PARENT REPORT for **YOUNG CHILDREN** (ages **5-7**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

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*In the past **ONE month,** how much of a **problem** has your child had with …*

PHYSICAL FUNCTIONING ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Walking more than one block	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports activity or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Taking a bath or shower by him or herself	0	1	2	3	4
6. Doing chores, like picking up his or her toys	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Low energy level	0	1	2	3	4

EMOTIONAL FUNCTIONING ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad or blue	0	1	2	3	4
3. Feeling angry	0	1	2	3	4
4. Trouble sleeping	0	1	2	3	4
5. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Getting along with other children	0	1	2	3	4
2. Other kids not wanting to be his or her friend	0	1	2	3	4
3. Getting teased by other children	0	1	2	3	4
4. Not able to do things that other children his or her age can do	0	1	2	3	4
5. Keeping up when playing with other children	0	1	2	3	4

SCHOOL FUNCTIONING ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Paying attention in class	0	1	2	3	4
2. Forgetting things	0	1	2	3	4
3. Keeping up with school activities	0	1	2	3	4
4. Missing school because of not feeling well	0	1	2	3	4
5. Missing school to go to the doctor or hospital	0	1	2	3	4

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CHILD REPORT (ages **8-12**)

DIRECTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

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*In the past **ONE month**, how much of a **problem** has this been for you …*

ABOUT MY HEALTH AND ACTIVITIES ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. It is hard for me to walk more than one block	0	1	2	3	4
2. It is hard for me to run	0	1	2	3	4
3. It is hard for me to do sports activity or exercise	0	1	2	3	4
4. It is hard for me to lift something heavy	0	1	2	3	4
5. It is hard for me to take a bath or shower by myself	0	1	2	3	4
6. It is hard for me to do chores around the house	0	1	2	3	4
7. I hurt or ache	0	1	2	3	4
8. I have low energy	0	1	2	3	4

ABOUT MY FEELINGS ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. I feel afraid or scared	0	1	2	3	4
2. I feel sad or blue	0	1	2	3	4
3. I feel angry	0	1	2	3	4
4. I have trouble sleeping	0	1	2	3	4
5. I worry about what will happen to me	0	1	2	3	4

HOW I GET ALONG WITH OTHERS ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. I have trouble getting along with other kids	0	1	2	3	4
2. Other kids do not want to be my friend	0	1	2	3	4
3. Other kids tease me	0	1	2	3	4
4. I cannot do things that other kids my age can do	0	1	2	3	4
5. It is hard to keep up when I play with other kids	0	1	2	3	4

ABOUT SCHOOL ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. It is hard to pay attention in class	0	1	2	3	4
2. I forget things	0	1	2	3	4
3. I have trouble keeping up with my schoolwork	0	1	2	3	4
4. I miss school because of not feeling well	0	1	2	3	4
5. I miss school to go to the doctor or hospital	0	1	2	3	4

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PARENT REPORT for **CHILDREN** (ages **8-12**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

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*In the past **ONE month,** how much of a **problem** has your child had with …*

PHYSICAL FUNCTIONING ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Walking more than one block	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports activity or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Taking a bath or shower by him or herself	0	1	2	3	4
6. Doing chores around the house	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Low energy level	0	1	2	3	4

EMOTIONAL FUNCTIONING ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad or blue	0	1	2	3	4
3. Feeling angry	0	1	2	3	4
4. Trouble sleeping	0	1	2	3	4
5. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Getting along with other children	0	1	2	3	4
2. Other kids not wanting to be his or her friend	0	1	2	3	4
3. Getting teased by other children	0	1	2	3	4
4. Not able to do things that other children his or her age can do	0	1	2	3	4
5. Keeping up when playing with other children	0	1	2	3	4

SCHOOL FUNCTIONING ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Paying attention in class	0	1	2	3	4
2. Forgetting things	0	1	2	3	4
3. Keeping up with schoolwork	0	1	2	3	4
4. Missing school because of not feeling well	0	1	2	3	4
5. Missing school to go to the doctor or hospital	0	1	2	3	4

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Date:_____

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TEEN REPORT (ages **13-18**)

DIRECTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

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*In the past **ONE month**, how much of a **problem** has this been for you …*

ABOUT MY HEALTH AND ACTIVITIES ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. It is hard for me to walk more than one block	0	1	2	3	4
2. It is hard for me to run	0	1	2	3	4
3. It is hard for me to do sports activity or exercise	0	1	2	3	4
4. It is hard for me to lift something heavy	0	1	2	3	4
5. It is hard for me to take a bath or shower by myself	0	1	2	3	4
6. It is hard for me to do chores around the house	0	1	2	3	4
7. I hurt or ache	0	1	2	3	4
8. I have low energy	0	1	2	3	4

ABOUT MY FEELINGS ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. I feel afraid or scared	0	1	2	3	4
2. I feel sad or blue	0	1	2	3	4
3. I feel angry	0	1	2	3	4
4. I have trouble sleeping	0	1	2	3	4
5. I worry about what will happen to me	0	1	2	3	4

HOW I GET ALONG WITH OTHERS ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. I have trouble getting along with other teens	0	1	2	3	4
2. Other teens do not want to be my friend	0	1	2	3	4
3. Other teens tease me	0	1	2	3	4
4. I cannot do things that other teens my age can do	0	1	2	3	4
5. It is hard to keep up with my peers	0	1	2	3	4

ABOUT SCHOOL ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. It is hard to pay attention in class	0	1	2	3	4
2. I forget things	0	1	2	3	4
3. I have trouble keeping up with my schoolwork	0	1	2	3	4
4. I miss school because of not feeling well	0	1	2	3	4
5. I miss school to go to the doctor or hospital	0	1	2	3	4

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01/00
PedsQL-4.0-Core-A – United States/English – Original version
PedsQL-4.0-Core-A_AU4.0_eng-USori

ID#_____

Date:_____

PedsQL™

Pediatric Quality of Life Inventory

Version 4.0

PARENT REPORT for **TEENS** (ages **13-18**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your teen**. Please tell us **how much of a problem** each one has been for **your teen** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the past **ONE month,** how much of a **problem** has your teen had with …*

PHYSICAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Walking more than one block	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports activity or exercise	0	1	2	3	4
4. Lifting something heavy	0	1	2	3	4
5. Taking a bath or shower by him or herself	0	1	2	3	4
6. Doing chores around the house	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Low energy level	0	1	2	3	4

EMOTIONAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad or blue	0	1	2	3	4
3. Feeling angry	0	1	2	3	4
4. Trouble sleeping	0	1	2	3	4
5. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Getting along with other teens	0	1	2	3	4
2. Other teens not wanting to be his or her friend	0	1	2	3	4
3. Getting teased by other teens	0	1	2	3	4
4. Not able to do things that other teens his or her age can do	0	1	2	3	4
5. Keeping up with other teens	0	1	2	3	4

SCHOOL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Paying attention in class	0	1	2	3	4
2. Forgetting things	0	1	2	3	4
3. Keeping up with schoolwork	0	1	2	3	4
4. Missing school because of not feeling well	0	1	2	3	4
5. Missing school to go to the doctor or hospital	0	1	2	3	4

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01/00
PedsQL-4.0-Core-PA – United States/English – Original version
PedsQL-4.0-Core-PA_AU4.0_eng-USori

ID#_____

Date:_____

PedsQL™

Family Impact Module

Version 2.0

PARENT REPORT

DIRECTIONS

Families of children sometimes have special concerns or difficulties because of the child's health. On the following page is a list of things that might be a problem for **you**. Please tell us **how much of a problem** each one has been for **you** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

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*In the past **ONE month**, as a result of your child's health, how much of a problem have **you** had with…*

PHYSICAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. I feel tired during the day	0	1	2	3	4
2. I feel tired when I wake up in the morning	0	1	2	3	4
3. I feel too tired to do the things I like to do	0	1	2	3	4
4. I get headaches	0	1	2	3	4
5. I feel physically weak	0	1	2	3	4
6. I feel sick to my stomach	0	1	2	3	4

EMOTIONAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. I feel anxious	0	1	2	3	4
2. I feel sad	0	1	2	3	4
3. I feel angry	0	1	2	3	4
4. I feel frustrated	0	1	2	3	4
5. I feel helpless or hopeless	0	1	2	3	4

SOCIAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. I feel isolated from others	0	1	2	3	4
2. I have trouble getting support from others	0	1	2	3	4
3. It is hard to find time for social activities	0	1	2	3	4
4. I do not have enough energy for social activities	0	1	2	3	4

COGNITIVE FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard for me to keep my attention on things	0	1	2	3	4
2. It is hard for me to remember what people tell me	0	1	2	3	4
3. It is hard for me to remember what I just heard	0	1	2	3	4
4. It is hard for me to think quickly	0	1	2	3	4
5. I have trouble remembering what I was just thinking	0	1	2	3	4

COMMUNICATION *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. I feel that others do not understand my family's situation	0	1	2	3	4
2. It is hard for me to talk about my child's health with others	0	1	2	3	4

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3. It is hard for me to tell doctors and nurses how I feel	0	1	2	3	4

*In the past **ONE month**, as a result of your child's health, how much of a problem have **you** had with…*

WORRY ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. I worry about whether or not my child's medical treatments are working	0	1	2	3	4
2. I worry about the side effects of my child's medications/medical treatments	0	1	2	3	4
3. I worry about how others will react to my child's condition	0	1	2	3	4
4. I worry about how my child's illness is affecting other family members	0	1	2	3	4
5. I worry about my child's future	0	1	2	3	4

DIRECTIONS

Below is a list of things that might be a problem for **your family**. Please tell us **how much of a problem** each one has been for **your family** during the **past ONE month.**

*In the past **ONE month**, as a result of your child's health, how much of a problem has **your family** had with…*

DAILY ACTIVITIES ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Family activities taking more time and effort	0	1	2	3	4
2. Difficulty finding time to finish household tasks	0	1	2	3	4
3. Feeling too tired to finish household tasks	0	1	2	3	4

FAMILY RELATIONSHIPS ***(problems with…)***	**Never**	**Almost Never**	**Some-times**	**Often**	**Almost Always**
1. Lack of communication between family members	0	1	2	3	4
2. Conflicts between family members	0	1	2	3	4
3. Difficulty making decisions together as a family	0	1	2	3	4
4. Difficulty solving family problems together	0	1	2	3	4
5. Stress or tension between family members	0	1	2	3	4

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Appendix 5 Guideline for Fat-Soluble Vitamin Supplementation

Appendix 5: Guideline for Fat-Soluble Vitamin Supplementation

Cholestasis predisposes to fat-soluble vitamin deficiencies. Fat-soluble vitamin levels, i.e., vitamins A and E, 25-hydroxy vitamin D, and INR (surrogate for vitamin K) are measured routinely during the study. If a patient has any fat-soluble vitamin level(s) that are out of range, vitamin supplementation adjustments may be required. Below are suggested guidelines for fat-soluble vitamin deficiency treatment [Venkat 2014; Shneider 2012]. Additional patient monitoring and/or treatment strategies may be warranted at the discretion of the investigator.

Target Fat-soluble Vitamin Levels and Replacement Regimens

Vitamin	Target Range (Serum Level)	Supplementation Strategy
A (retinol)	19 to 77 µg/dL* retinol:retinol-binding protein molar ratio > 0.8	Increments of 5000 IU (up to 25,000 to 50,000 IU/day) orally or monthly intramuscular administration of 50,000 IU**
D (25-hydroxy vitamin D)	15 to 45 ng/mL*	Increments of 1200 to 8000 IU orally daily of cholecalciferol or ergocalciferol; alternatively calcitriol at 0.05 to 0.2 µg/kg/day*
E (α tocopherol)	3.8 to 20.3 µg/mL vitamin E:total serum lipids ratio >0.6 mg/g	Increments of 25 IU/kg of d-α-tocopheryl polyethylene glycol – 1000 succinate (TPGS) orally daily (to 100 IU/kg/day)
K (phytonadione)	INR ≤1.2	• 1.2 <INR ≤1.5: 2.5 mg vitamin K orally daily • 1.5 <INR ≤1.8: 2.0 to 5.0 mg vitamin K intramuscular and 2.5 mg vitamin K orally daily • INR >1.8: 2.0 to 5.0 mg vitamin K intramuscular and 5.0 mg vitamin K orally daily

***Clinical practice may vary. This is meant as a guidance only and does not override local standard of care. Investigators should provide best case practices for management and treatment**

**** For further detail regarding age related Tolerable Upper Intake (UL) for preformed vitamin A please refer to page 163 in the European Food Safety Authority guidance: *Tolerable Upper Intake Levels for Vitamins and Minerals*; Scientific Committee on food Scientific Panel on Dietetic Products, Nutrition and Allergies**
http://www.efsa.europa.eu/sites/default/files/efsa_rep/blobserver_assets/ndatolerableuil.pdf

Appendix 6 Contraceptive Requirements

Appendix 6: Contraceptive Requirements

A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.

Contraceptive methods, or combinations of contraceptive methods, for males and females that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods and must be used at least to up to 90 days following the last day of treatment.

Such methods include:

- Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
  - oral
  - intravaginal
  - transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation:
  - oral
  - injectable
  - implantable
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion
- Vasectomised partner
- Sexual abstinence

Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire study period up to at least 90 days after the last day of treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

Requirements according to "Recommendations related to contraception and pregnancy testing in clinical trials", HMA CTFG (Clinical Trial Facilitation Group), 2014

Appendix 7 Blood Volumes

Scientific Affairs
Estimated Blood Volume Request

Version: 11.0

SPONSOR: Albireo **Protocol#: A4250-005** 6 mon h to 18 yrs of age

Visit^	Testing^[1-8]	Tube volume (mL)	Tube Type	# of Collection Tubes	# of Aliquots	Volume (mLs)	Volume/ visit (mL)	Volume /visit (tsp)	Volume /visit (TBS)
Visit 1 Screening Study Day -56 - (-35) ± 2	Chemistry, hCG	1 1	SST	1	1	1 1			
	Bile Acids, Total[1]	1 1	SST	1	2	1 1			
	DNA[2]	3 0	EDTA	1	0	3 0			
							5 2	1.05	0.35
Visit 2 Screen-28D Study Day -28 - (-7) ± 2	Coagulation (INR/PT)	1 4	NaCit	1	1	1 4			
	Vitamins, A, E[1]	2 5	SST	1	1	2 5			
	Bile Acids, Total[1]	1 1	SST	1	2	1 1			
							5 0	1.01	0.34
Visit 3 Study Day 0	Chemistry, AFP	1 1	SST	1	1	1 1			
	Hematology	1 0	EDTA	1	0	1 0			
	Autotaxin[7]	1 2	LiHep	1	2	1 2			
	C4 (LC-MS/MS)[4]	1 2	LiHep	1	2	1 2			
	Vitamin D,25OH	1 1	SST	1	1	1 1			
	Bile Acids, Total[1]	1 1	SST	1	2	1 1			
							6 7	1.36	0.45
Visit 4 Study Day 28 ± 3	Chemistry	1 1	SST	1	1	1 1			
	Hematology	1 0	EDTA	1	0	1 0			
	Coagulation (INR/PT)	1 4	NaCit	1	1	1 4			
	Autotaxin[7]	1 2	LiHep	1	2	1 2			
	C4 (LC-MS/MS)[4]	1 2	LiHep	1	2	1 2			
	Bile Acids, Total[1]	1 1	SST	1	2	1 1			
	PK	1 1	Plasma	1	2	1 1			
							8 1	1.64	0.55
Visit 5 Study Day 56 ± 3	Chemistry	1 1	SST	1	1	1 1			
	Hematology	1 0	EDTA	1	0	1 0			
	Bile Acids, Total[1]	1 1	SST	1	2	1 1			
							3 2	0.65	0.22
Visit 6 Study Day 84 ± 3	Chemistry	1 1	SST	1	1	1 1			
	Hematology	1 0	EDTA	1	0	1 0			
	Coagulation (INR/PT)	1 4	NaCit	1	1	1 4			
	Bile Acids, Total[1]	1 1	SST	1	2	1 1			
	Vitamins, A, E[1]	2 5	SST	1	1	2 5			
	Vitamin D,25OH	1 1	SST	1	1	1 1			
							8 2	1.66	0.55
Visit 7 Study Day 126 ± 3	Chemistry	1 1	SST	1	1	1 1			
	Hematology	1 0	EDTA	1	0	1 0			
	Bile Acids, Total[1]	1 1	SST	1	2	1 1			
							3 2	0.65	0.22
Visit 8 Study Day 154 ± 3	Coagulation (INR/PT)	1 4	NaCit	1	1	1 4			
	Bile Acids, Total[1]	1 1	SST	1	2	1 1			
							2 5	0.51	0.17
Visit 9/V9A EOT Study Day 168 ± 3	Chemistry, AFP, hCG[3]	1 1	SST	1	1	1 1			
	Hematology	1 0	EDTA	1	0	1 0			
	Autotaxin[7]	1 2	LiHep	1	2	1 2			
	C4 (LC-MS/MS)[4]	1 2	LiHep	1	2	1 2			

Scientific Affairs
Estimated Blood Volume Request

Version: 11.0

SPONSOR: Albireo **Protocol#: A4250-005** 6 mon h to 18 yrs of age

Visit^	Testing^[1-8]	Tube volume (mL)	Tube Type	# of Collection Tubes	# of Aliquots	Volume (mLs)	Volume/ visit (mL)	Volume /visit (tsp)	Volume /visit (TBS)
	Bile Acids, Total[1]	1 1	SST	1	2	1 1			
	Vitamins, A, E[1]	2 5	SST	1	1	2 5			
	Vitamin D,25OH	1 1	SST	1	1	1 1			
	PK	1 1	Plasma	1	2	1 1			
							10 3	2.09	0.70
Visit 10 Follow-Up Study Day 196 ± 3	Chemistry	1 1	SST	1	1	1 1			
	Hematology	1 0	EDTA	1	0	1 0			
	Coagulation (INR/PT)	1 4	NaCit	1	1	1 4			
	Bile Acids, Total[1]	1 1	SST	1	2	1 1			
							4 6	0.93	0.31
Study Total Volume*							57.0	11.6	3.9

^ Visit Schedule and testing based on CLW v1 09

*Total Volume reflects the volumes required for testing by ICL and 3rd Parties as defined in the test list above

Notes: #1 Bile Acids, Total, Vitamin A, and Vitamin E testing at ARUP Lab, USA
#2 Optional sample Only collected if required
#3 hCG is an optional sample, collected if patient is female and of child bearing potential
#4 C4 will be performed on plasma sample at LGC
#5 The following absolute 'minimum' volume does not allow for repeat testing:
Chemistry, AFP requires minimum – 500uL serum
Bile Acids requires minimum – 500uL serum
Coagulation (PT/INR only) requires minimum – 900uL plasma
#6 Sites must collect Full Draw and measure exact aliquot of sample into a "False Bottom" transport tube
#7 Autotaxin serum samples will go to LGC lab for testing Optional sample Collected if subject meets weight/age/medical history criteria for collection
#8 For subjects requiring optional pregnancy testing, collect 1 1mL SST
#9 PK will be performed on plasma sample at LGC

Created by: Jennifer Rodriguez, Protocol Review
Date: 14-May-18

CLINICAL STUDY PROTOCOL A4250-005

A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

IND Number:	130591
EudraCT Number	2017-002338-21
Test Product:	A4250
Indication:	Progressive Familial Intrahepatic Cholestasis (PFIC)
Sponsor:	Albireo AB
Development Phase:	Phase 3
Sponsor Signatory:	[redacted]
Sponsor Medical Monitor:	[redacted]
Principal Investigator:	[redacted]
Date of the Protocol:	10 May 2018
Version of the Protocol:	Final Amendment 02

Confidential

The information in this document contains trade secrets and commercial information that are privileged or confidential and may not be used, divulged, published, or otherwise disclosed without the written consent of Albireo AB. These restrictions on disclosure apply equally to all future information supplied to you or generated by you in connection with the study.

SPONSOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

Albireo AB

[redacted]

10 May 2018
Date (day/month/year)

CLINICAL RESEARCH ORGANIZATION SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

[redacted]

[redacted]

11 May 2018
Date (day/month/year)

[redacted]

11-May-2018
Date (day/month/year)

INVESTIGATOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

I have read this protocol and agree that it contains all necessary details for performing this study. I will conduct the study as outlined herein and will complete the study within the time designated, in accordance with all stipulations of the protocol and in accordance with Good Clinical Practice (GCP), local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study drug and the conduct of the study.

I will use only the informed consent approved by the Independent Ethics Committee (IEC) and will fulfil all responsibilities for submitting pertinent information to the IEC responsible for this study.

I agree that the sponsor (Albireo AB) shall have access to any source documents from which case report form information may have been generated.

I further agree not to originate or use the name of Albireo AB, A4250 in any publicity, news release, or other public announcement, written or oral, whether to the public, press, or otherwise, relating to this protocol, to any amendment to the protocol, or to the performance of this protocol without the prior written consent of Albireo AB.

Name of Investigator

Signature

Date (day/month/year)

1 ADMINISTRATIVE INFORMATION

A Double-blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Protocol No.:	A4250-005
Date of the initial Protocol:	20 September 2017
Date and Number of Amendment(s):	Amendment 01, 06 December 2017 Amendment 02, 10 May 2018
Sponsor:	Albireo AB Arvid Wallgrens backe 20 413 46 Göteborg Sweden
Clinical Research Organization:	[redacted]
Sponsor Signatory:	[redacted]
Sponsor Medical Monitor:	[redacted]
Principal Investigator:	[redacted]

2 STUDY SYNOPSIS

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250

Title of Study:
A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Principal Investigator:
[redacted]

Study Centers:
Up to 50 sites will be initiated for this study in the United States (US), Canada, European Union (EU), Middle East, and Australia.

Publication(s):
None.

Planned Study Period:	**Development Phase:**
Q2 2018 to Q4 2019	Phase 3

Objectives:

Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with progressive familial intrahepatic cholestasis (PFIC) Types 1 and 2, as determined by the following:

- Proportion of patients experiencing at least a 70% reduction in fasting serum bile acid (s-BA) concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- Change in pruritus compared to placebo over 24 weeks of treatment

Secondary Objectives

- To evaluate the effect of A4250 on serum alanine aminotransferase (ALT) concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

Methodology:

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of familial intrahepatic cholestasis-1 or bile salt export pump).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-up Period. After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo. Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008) in which all patients will receive active treatment.

There will be up to 10 clinic visits during the study and one scheduled telephone contact.

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250

Number of Patients:

Approximately 60 patients diagnosed with PFIC-1 or PFIC-2 will be randomized. The study will target 15% of enrolled patients to be PFIC-1 patients. If enrollment of all PFIC-2 patients is completed and the 15% enrollment of PFIC-1 patients has not yet been achieved, then enrollment of PFIC-2 patients will continue in order to reach the total study target enrollment.

Diagnosis and Main Criteria for Inclusion:

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have clinical genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples 7 to 28 days apart (Visits 1 and 2) prior to randomization
4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the electronic diary (eDiary) average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients are expected to have a consistent caregiver for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

Test Product, Dose and Mode of Administration:

A4250, 40 µg/kg/day or 120 µg/kg/day, orally administered

Reference Therapy, Dose and Duration of Administration:

Matching placebo, orally administered

Duration of Treatment:

24 weeks

Variables

Efficacy:

Primary Efficacy Endpoints

The primary efficacy endpoints are:

- **EU and rest of world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  Fasting s-BA baseline will be calculated as the average of the value at randomization and the previous visit (Clinic Visit 2). The end value is the average of the values at Weeks 22 and 24 after the start of double-blind treatment.
- **US**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline over the last 5 months of the Treatment Period as measured by an overall mean of weekly averages of the worst daily scratch score using the Albireo observer-reported outcome (ObsRO) instrument.

Secondary Efficacy Endpoints

Secondary efficacy endpoints include the following:

- **EU and RoW**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline over the last 5 months of the Treatment Period as measured by an overall mean of weekly averages of the worst daily scratch score using the Albireo ObsRO instrument.
- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

Name of Sponsor/Company:	Name of Product:	Name of Active Ingredient:
Albireo	A4250	A4250

All Regions:

All secondary endpoints will be compared to placebo.

- Change from baseline to Week 24 in fasting s-BA
- Change from baseline to Week 24 in serum ALT concentration
- Change in growth from baseline to Week 24, defined as the linear deficit (weight for age and body mass index) compared to a standard growth curve (Z-score, standard deviation from P50)
- Proportion of patients achieving meaningful reduction in caregiver-reported observed scratching over the Treatment Period
- Change from baseline over the last 5 months of the Treatment Period as measured by an overall mean of weekly averages of the sleep parameters measured with the Albireo patient-reported outcome (PRO) and ObsRO instruments
- Change from baseline over the last 5 months of the Treatment Period as measured by an overall mean of weekly averages of patient-reported itch severity as measured by the average daily score of the Albireo PRO instrument; only patients ≥8 years of age will complete the Albireo PRO instrument
- Number of patients undergoing biliary diversion surgery or being listed for liver transplantation

Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Proportion of patients achieving meaningful reduction in patient-reported itch severity over the Treatment Period
- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the patient global impression of change (PGIC) items
- Change from baseline in fasting s-BA at Weeks 4 and 12
- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 4 of the Treatment Period as measured by the Albireo ObsRO instrument
- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline over the last 3 months of the Treatment Period as measured by an overall mean of weekly averages of the worst daily scratch of the Albireo ObsRO instrument
- Change from baseline over the last 5 months of the Treatment Period in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline over the last 5 months of the Treatment Period in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline over the last 5 months of the Treatment Period in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in Pediatric Quality of Life Inventory (PedsQL)
- Change from baseline in serum ALT concentration at Weeks 4 and 12
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST and gamma-glutamyl transferase at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, plasma 7α-hydroxy-4-cholesten-3-one concentration [p-C4]) at Week 24
- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events.
- All-cause mortality

Safety:

Safety criteria are as follows:

- Occurrence of treatment-emergent adverse events including severity and relatedness to study drug at all

Name of Sponsor/Company:	Name of Product:	Name of Active Ingredient:
Albireo	A4250	A4250

visits

- The incidence of treatment-emergent serious adverse events (SAEs), based upon information from patient reports, including the description, incidence, and severity of an SAE
- Trends evaluated for the following assessments: physical examinations, concomitant medications, vital signs, laboratory test results (including clinical chemistry, hematology, urinalysis, alfa-fetoprotein, vitamins A and E, 25-hydroxy vitamin D and INR), and abdominal ultrasound

Statistical Methods:

The primary objective of this study is to show superiority in efficacy of A4250 (120 µg/kg/day and 40 µg/kg/day) compared to placebo in patients with PFIC Types 1 and 2.

In the EU and RoW, for the primary efficacy variable fasting bile acid responder endpoint (at least a 70% reduction in fasting s-BA concentration or reaching a level ≤70 µmol/L at the end of treatment), the null hypothesis will be tested against the alternative hypothesis (α=0.0125, 1-sided). Generalized linear mixed modelling will be used with a logistic link, to allow for repeated assessments over time. The model will include baseline value, treatment group, PFIC class, age class, region, visit and visit*treatment group interaction. The contrasts of primary interest will be the treatment difference at Week 24 but also the difference at Week 4, Week 8, Week 12, and Week 18 will be estimated and analyzed.

In the US, for the primary efficacy variable pruritus score endpoint (change from baseline over the treatment period in observer-reported scratching), the null hypothesis will be tested against the alternative hypothesis (α=0.0125, 1-sided). The primary analysis of the change from baseline in pruritus score (indexed by observer-reported scratching) will be analyzed using a mixed model repeated measures analysis. The comparison of primary interest will be the treatment differences over the last 5 months of the treatment period estimated by averaging the worst daily itch score means from five 14 day post-treatment intervals (Week 7 to 8, Week 11 to 12, Week 15 to 16, Week 19 to 20, and Week 23 to 24). The treatment difference at each of these post-treatment time intervals will also be estimated and analyzed.

To strictly control overall Type I error rate across the dose groups and also across the primary and the main secondary endpoint to a 1-sided 2.5% level, a Bonferroni-based parallel gate-keeping procedure will be used.

Other secondary and exploratory outcome variables shall provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple-dose comparisons when testing the other secondary and exploratory efficacy variables.

Date of the Protocol: 10 May 2018

3 TABLE OF CONTENTS

3.1 List of Tables

3.2 List of Figures

3.3 List of Appendices

4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADME	absorption, distribution, metabolism, and excretion
AE(s)	adverse event(s)
AFP	alfa-fetoprotein
ALT	alanine aminotransferase
APRI	AST to platelet ratio index
ASBT	apical sodium-dependent bile transporter (also known as IBAT)
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	body mass index
BSEP	bile salt export pump
CPK	creatine phosphokinase
CRA	clinical research associate
CYP	cytochrome P450
DSMB	Data and Safety Monitoring Board
eCRF	electronic case report form
eDiary	electronic diary
EOT	End of Treatment
EU	European Union
EudraCT	European Union drug regulatory agency clinical trial
FAS	full analysis set
FDA	United States Food and Drug Administration
FIC1	familial intrahepatic cholestasis-1
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
GI	gastrointestinal
GLMM	generalized linear mixed modelling
IB	Investigator's Brochure
IBAT	ileal bile acid transporter (also known as ASBT)
ICF	informed consent form
ICH	International Council for Harmonisation
[redacted]	[redacted]
IEC	Independent Ethics Committee
INR	international normalized ratio
IRB	Institutional Review Board
IWRS	Interactive Web Response System

LDH	lactate dehydrogenase
LFT	liver function test
LPLV	last patient last visit
MDR3	multidrug-resistance protein 3
MedDRA	Medical Dictionary for Regulatory Activities
MELD	model for end-stage liver disease
MMRM	mixed model repeated measures
ObsRO	observer-reported outcome
PBC	primary biliary cholangitis
p-C4	plasma 7α-hydroxy-4-cholesten-3-one concentration
PCS	potentially clinically significant
PedsQL	Pediatric Quality of Life Inventory
PELD	pediatric end-stage liver disease
PFIC	progressive familial intrahepatic cholestasis
PGIC	patient global impression of change
PGIS	patient global impression of symptoms
PK	pharmacokinetic(s)
PP	per protocol
PRO	patient-reported outcome
PT	prothrombin time
PVM	pharmacovigilance manager
QoL	quality of life
RoW	rest of world
RR	respiratory rate
SAE(s)	serious adverse event(s)
SAP	statistical analysis plan
s-BA	serum bile acid
SD	standard deviation
s-FGF19	serum fibroblast growth factor 19 concentration
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction
TEAE(s)	treatment-emergent adverse event(s)
ULN	upper limit of normal
US	United States
WHO	World Health Organization

5 INTRODUCTION

5.1 Investigational Medicinal Product

A4250 is a small molecule and a potent selective inhibitor of the ileal bile acid transporter (IBAT), also known as the apical sodium-dependent bile acid transporter (ASBT). IBAT is an integral brush border membrane glycoprotein that co-transports sodium and bile acids and appears to be a critical component in the regulation of the bile acid pool size in animals and man. This transporter, expressed in the distal ileum, is a key element in the enterohepatic circulation of bile acids since it facilitates the high-affinity, high-capacity reabsorption of bile acids. Indeed, 95% of secreted bile acids are reabsorbed via IBAT [Hofmann 2009; Miethke 2016]. A4250 is orally administered and acts locally in the gut where it binds reversibly to IBAT to decrease the reuptake of bile acids. A4250 has minimal systemic exposure at the predicted therapeutic dose ranges.

No safety signals were found with A4250 in preclinical safety and toxicity studies. In the first clinical study of A4250 in healthy volunteers, A4250 was given as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. A4250 was well tolerated and the only findings were gastrointestinal (GI) related effects, such as diarrhea, at higher doses.

In an exploratory Phase 2 study in children with cholestatic pruritus, A4250 was given as oral doses of 10 to 200 µg/kg/day for 4 weeks. A4250 reduced mean levels of serum bile acids (s-BA) in all cohorts. In 11 of 13 progressive familial intrahepatic cholestasis (PFIC) patients, substantial reductions (ranging from 43% to 98%) were observed. Patient-reported diary data documented improvement of pruritus during treatment. A4250 was well tolerated. No serious adverse events (SAEs) were deemed to be related to study drug, and most adverse events (AEs), including some increased transaminases, were considered unrelated to study drug and transient in nature.

5.2 Background

5.2.1 Progressive Familial Intrahepatic Cholestasis

PFIC is a rare autosomal recessive cholestatic liver disease estimated to affect between 1 in every 50,000 to 100,000 children born worldwide. PFIC represents 10% to 15% of causes of cholestasis in children and 10% to 15% of liver transplantation indications in children. All types of PFIC exist worldwide and both sexes appear to be equally affected.

The common underlying pathogenesis of PFIC is disruption of bile formation and bile transport through the liver [Jacquemin 2000]. The classification of PFIC has evolved over the years. The most commonly used sub-classifications are PFIC Types 1, 2, and 3, which are based on the associated affected gene and described in more detail below.

- PFIC, Type 1: also referred to as "Byler disease" or "familial intrahepatic cholestasis-1 (FIC1) protein deficiency." FIC1 protein is located on the canalicular membrane of hepatocytes and facilitates movement of aminophospholipids from the outer

to inner leaflet of the plasma membrane of the hepatocyte. The *ATP8B1* gene encodes FIC1 protein. Biallelic pathologic variants in the *ATP8B1* gene are associated with FIC1 dysfunction and classified clinically as PFIC Type 1 disease.

- PFIC, Type 2: also referred to as "Byler syndrome" or "bile salt export pump (BSEP) deficiency." BSEP is a transporter protein that is expressed at the canalicular membrane of hepatocytes, and is the primary exporter of bile acids. The *ABCB11* gene encodes the BSEP protein. Biallelic pathologic variations in the *ABCB11* gene are associated with BSEP dysfunction and classified clinically as PFIC Type 2 disease.
- PFIC, Type 3: is caused by a deficiency of the multidrug-resistance protein 3 (MDR3) due to mutations in the *ABCB4* gene. MDR3 is a phospholipid translocase critical for phospholipid secretion.

Severe pruritus is common in children diagnosed with PFIC. Itching (and subsequent scratching) is a significant morbidity for these patients and their families [Suchy 2007]. A more severe degree of pruritus is experienced compared to patients with other forms of liver disease and to other pruritic conditions such as atopic dermatitis [Murray 2011]. In patients with PFIC, liver biopsy reveals canalicular cholestasis and, later, the appearance of portal fibrosis. Serum biochemistry indicates cholestasis with hyperbilirubinemia, elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The concentrations of bile acids in serum are very high, while serum gamma-glutamyl transferase (GGT) activity (exception being MDR3 variants) and cholesterol are normal [Hori 2010]. Symptoms of portal hypertension and liver failure will develop during the course of the disease [Davit-Spraul 2009; Alissa 2008]. Symptoms develop early; median age at onset of symptoms is 2 months, and 78% of PFIC patients present with jaundice [Pawlikowska 2010]. The life-threatening and debilitating nature of PFIC is reflected by the fact that survival in those not resorting to surgery is 50% at 10 years of age and almost zero at 20 years of age. Approximately half of PFIC patients undergo liver transplantation [Davit-Spraul 2009] and treatment-resistant pruritus is the leading indication for the surgical procedure partial external biliary diversion, mostly in PFIC Type 1 and 2 patients.

PFIC is life-threatening and debilitating. Currently, the treatment for PFIC is palliative and there is no pharmaceutical treatment approved for use in PFIC. The therapeutic choices are restricted to non-specific therapy of the symptoms and signs of the disease such as nutritional support, preventing vitamin deficiencies, and treatment of extrahepatic features. Medical treatment options include off-label use of ursodeoxycholic acid, rifampin, antihistamines, and naltrexone. A minority of patients respond nominally and transiently to these interventions. Biliary diversion is used to decrease systemic bile acids through interruption of the enterohepatic circulation and so avoid transplantation. Liver transplantation is typically only viewed as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

5.2.2 Summary of Clinical and Nonclinical Studies

This is a summary of nonclinical and clinical studies. More detail is provided in the Investigator's Brochure ([IB] see A4250 IB).

A4250 is a potent, selective IBAT inhibitor with no safety signals seen in the safety pharmacological studies. In addition, no safety signals were seen in repeat-dose oral toxicity studies in rats with up to 26 weeks of daily dosing and in dogs with up to 39 weeks of daily dosing. No safety signals were identified when A4250 was administered daily to juvenile rats from age 14 days to 63 days.

A4250 is minimally absorbed with very low systemic exposure in both humans and other species. In blood, protein binding is high (>99.7%). Systemically absorbed A4250 is slowly metabolized in humans and in preclinical studies by hydroxylation only, a metabolite that retains IBAT activity. A4250 did not show any significant inhibition of cytochrome P450 (CYP) enzymes in the rat. A4250 inhibited human CYP3A4 and CYP2D6 (IC_{50}: 16 µmol/L for both enzymes), and CYP2C9 (IC_{50}:1.2 µmol/L).

To date, A4250 has been studied in 3 Albireo-sponsored clinical studies: a study in healthy volunteers evaluating single and multiple administration of A4250; a single-dose absorption, distribution, metabolism, and excretion (ADME) study; and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with primary biliary cholangitis (PBC). In total, 98 subjects have been exposed to A4250.

In the first clinical study (A4250-001), A4250 was given to healthy volunteers as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. Following a single oral dose of 10 mg, the median maximum drug concentration was 0.2 nmol/L and the area under the curve was 0.8 nmol × h/L (n=6). Pharmacodynamic evaluation of bile acid synthesis biomarkers (serum fibroblast growth factor 19 [s-FGF19] and plasma 7α Hydroxy 4-cholesten-3-one [p-C4]) identified the expected decrease in s-FGF19 and an increase in p-C4. In addition, total plasma bile acids decreased and total fecal bile acids increased in A4250-treated patients compared to placebo. A4250 was generally well tolerated. The only findings were GI-related effects, such as diarrhea at higher doses.

In the ADME study (A4250-007), healthy subjects were administered a single oral dose of 3 mg [^{14}C]-A4250 capsule. The main excretion route was determined to be fecal. There were no quantifiable concentrations of A4250 in plasma or total radioactivity in plasma or whole blood. A4250 was well tolerated. AEs were reported and most were mild and not related to A4250. The 3 AEs experienced by 2 subjects reported as related to study drug were GI in nature, including diarrhea (2 AEs; 2 subjects) and abdominal pain (1 AE; 1 subject).

In an exploratory Phase 2 study in children with cholestatic pruritus (A4250-003), A4250 was administered as oral daily doses of 10 to 200 µg/kg/day for 4 weeks. Twenty patients (8 females and 12 males) aged 1 to 17 years were enrolled in this open-label dose-escalation study. Four patients were re-enrolled into a second dose level. No

treatment-related SAEs were reported, and most AEs, including some increased transaminases, were transient. Four-week, once-daily treatment with A4250 reduced mean levels of s-BA in all cohorts. Substantial s-BA reductions in 11 of 13 PFIC patients (ranging from 43% to 98%) were observed. A4250 also reduced s-BA in the 11 non-PFIC patients, but to a lesser extent than in PFIC patients. Patient-reported diaries documented improvement of the visual analogue scale itch score in 17 of 24 patients during treatment. The dose with the greatest improvement showed a mean decrease (± standard error of the mean) of 2.9 ±1.1 from baseline. The results from this study form the basis of the current (A4250-005) study design.

5.3 Rationale

In this investigational study, A4250 is under development for treatment of PFIC and is also being considered for other orphan cholestatic liver diseases.

There is currently no medical treatment approved for PFIC. In PFIC patients with severe pruritus, biliary diversion surgery is used to decrease systemic bile acids through interruption of the enterohepatic circulation and thereby reducing pruritus [Whitington 1988]. Liver transplantation is typically used as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

By inhibiting IBAT with high selectivity and potency, A4250 has the potential to relieve cholestasis and improve liver function without surgical intervention in patients with PFIC. Since A4250 is minimally absorbed and achieves a substantial reduction of s-BA, it has the potential to be a safer and less invasive alternative to surgical biliary drainage. The rationale for using A4250 is to decrease s-BA levels, and to reduce the major morbidity of pruritus, improving the health and wellbeing of patients affected with PFIC. Ultimately, this noninvasive therapeutic approach has the potential to not only improve quality of life (QoL), but also reduce the need for biliary diversion and subsequent liver failure and liver transplantation.

The doses selected, 40 and 120 µg/kg/day, were based on the efficacy and safety data generated from patients with PFIC (n=10 + 3 re-entered) and supporting data from all patients (n=20 + 4 re-entered) in the five dose groups dosed in the A4250-003 study. Improvements in s-BA and pruritus were observed with doses from 30 µg/kg to 100 µg/kg, with a dose-related trend. The best dose response in all patients was at the 60 µg/kg/day for s-BA and 100 µg/kg/day for pruritus. The best dose response in the PFIC subgroup was at 30 µg/kg/day for both s-BA and pruritus. Patients with PFIC responded well to the doses given from 30 µg/kg to 200 µg/kg/day; however, a clear dose relationship could not be established in order to select a single dose for this Phase 3 study. As efficacy was observed at both low and high doses, 40 µg/kg and 120 µg/kg/day are considered to be the most optimal for this study.

This study will be conducted in compliance with the protocol and with the International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP).

Patients completing 24 weeks of double-blind treatment will be offered to enter Study A4250-008 and receive open-label treatment with A4250.

5.3.1 Risk/Benefit

A4250 has been evaluated in three Albireo-sponsored clinical studies: a double-blind placebo-controlled study in healthy volunteers, a single-dose ADME study, and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with PBC.

A total of 98 subjects/patients have been exposed to A4250. Healthy subjects have been exposed to up to 10 mg as a single dose and up to 3 mg daily as part of a multiple-dose evaluation. Children with cholestatic liver disease have been treated with up to 0.2 mg/kg/day (200 µg/kg/day) for 4 weeks. In total, 2 SAEs have been reported; both were judged by the physician to be not related to the study drug.

Patients with cholestatic liver diseases suffer from excess bile acids in the liver resulting in tissue damage. A commonly used treatment in these patients is bile diversion surgery, whereby approximately 50% to 100% of the enterohepatic circulation of bile acids is interrupted. Inhibition of IBAT with A4250, thereby interrupting the enterohepatic circulation of bile acids, is therefore a potential medical alternative to surgery which could be of benefit to these patients if shown to be effective and safe. Data from the Phase 2 study showed efficacy of A4250 in reducing s-BA concentrations and pruritus in such patients.

Based on the mode of action of an IBAT inhibitor, loose stools or diarrhea could be expected. However, in the pediatric Phase 2 study with 4 weeks daily treatment, only one patient had mild transient diarrhea after a single dose that did not recur on multiple dosing.

6 STUDY OBJECTIVES

6.1 Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with PFIC Types 1 and 2, as determined by the following:

- The proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- Change in pruritus compared to placebo over 24 weeks of treatment

6.2 Secondary Objectives

- To evaluate the effect of A4250 on serum ALT concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

7 INVESTIGATIONAL PLAN

7.1 Overall Study Design and Plan

7.1.1 Description

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of FIC1 or BSEP).

Study data will be periodically reviewed by an unblinded Data Safety and Monitoring Board (DSMB).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-Up Period.

After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo.

Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008; EudraCT Number 2017-002325-38, subject to a separate study approval) in which all patients will receive active treatment. A patient who prematurely withdraws due to no improvement/intolerable symptoms, and has completed at least 12 weeks of the Treatment Period and End of Treatment (EOT) Visit, will also be offered the opportunity to enter Study A4250-008.

Visit windows applicable to each visit are presented in the schedule of assessments (Table 1). There will be up to 10 clinic visits during the study (Figure 1) and one scheduled telephone contact, as follows:

- Visit 1: Screening Period start. Patient is considered enrolled after signing the informed consent form (ICF) (Days -56 to -35)
- Visit 2: second Screening Visit (Days -28 to -7)
- Visit 3: Randomization Visit (Day 0)
- Telephone Contact 1: (Day 14)
- Visit 4: Clinic Visit (Day 28)
- Visit 5: Clinic Visit (Day 56)
- Visit 6: Clinic Visit (Day 84)
- Visit 7: Clinic Visit (Day 126)
- Visit 8: Clinic Visit (Day 154)
- Visit 9: Clinic Visit, EOT (Day 168)

- Visit 10: Follow-Up Visit, 28 days after last dose of study drug (Day 196). All patients continuing in Study A4250-008 will not have this Follow-up Visit.

Additional clinic visits may be required for patients who need direct site assistance, e.g., due to AE monitoring, to fulfil screening requirements, and/or for safety maintenance.

Screening Period

Informed consent must be obtained at Visit 1 prior to performing any study procedures. After signing the ICF, patients will be considered enrolled and evaluated for study eligibility.

Daily recording of pruritus using an electronic diary (eDiary) will be started to determine confirmation of the magnitude and the baseline pruritus symptoms before the study. After consultation with the Medical Monitor, patients failing eligibility criteria may be re-screened after 2 months. Patients not fulfilling inclusion/exclusion criteria after 3 attempts are not allowed to re-screen.

Caregivers/patients will be instructed on the daily use of the eDiary. The eDiary will include patient-reported outcome (PRO) and observer-reported outcome (ObsRO) items for evaluation of itching (PRO), scratching (ObsRO), and sleep disturbance (PRO and ObsRO) during the Screening Period (study Days -56 to 0), throughout the 24-week Treatment Period (study Days 0 to 168), and Follow-up (Day 196).

The 14 consecutive days prior to randomization will be used for eligibility calculation of the diary entries. If a patient does not meet the minimum number of observations (see Section 9.2.2 for scoring and missing data definitions) to determine eligibility, the patient may be re-screened. Observer-reported outcomes in patients of all ages will be recorded by a caregiver. If possible, the same caregiver will complete the Albireo ObsRO items throughout the study.

At a second Screening Visit (Visit 2) additional laboratory samples will be taken for eligibility assessments.

Treatment Period

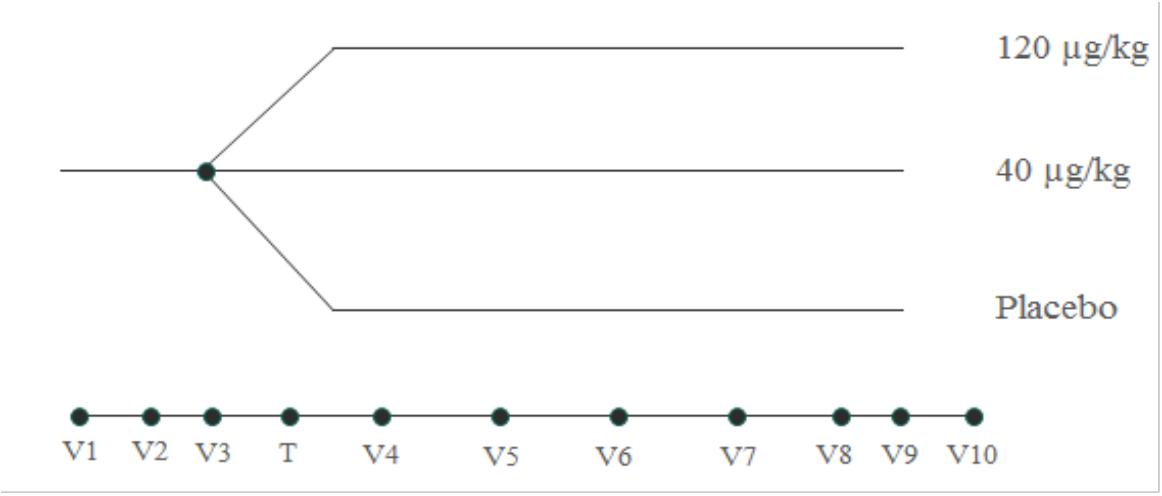
Eligibility for randomization will be determined at Visit 3 using the pruritus (scratching reports from the Albireo ObsRO) data in the 2 weeks before Visit 3, clinical genetic confirmation of diagnosis, and the liver biochemistry evaluations from the previous screening visits. Two weeks after Visit 3, there will be a telephone contact with the caregiver/patient to evaluate any AEs.

The patient will return to the clinic at Visit 4, Visit 5, Visit 6, Visit 7, Visit 8, and Visit 9 for assessments. If a new caregiver will begin to complete the Albireo ObsRO during the course of the trial, they will be provided with training during a clinic visit before they begin to use the eDiary. Additionally, caregivers will be requested to use the diary to report the time each dose of study drug is administered during the Treatment Period.

The last dose of study drug will be administered the day before Visit 9. At study Visit 9, the patient has the option to enroll in an open-label extension study (A4250-008) should they meet the required entry criteria. If the patient is continuing into Study A4250-008 this will be the last visit in this study and the first visit in Study A4250-008. Visit 9/EOT should also be performed at the time patients are prematurely withdrawn from the study.

Clinic Visit 10 will take place 28 days after Visit 9 for all patients not continuing into Study A4250-008.

Figure 1 Study Design



T: telephone contact; V: study visit

End of Study

The end of the study is defined as follows:

a) End of study in one country: last patient last visit (LPLV) and sites are closed

b) End of study globally: LPLV globally and all sites closed

7.1.2 Schedule of Assessments

The schedule of assessments is presented in Table 1. For information on blood volumes, please see Appendix 7.

Table 1 Schedule of Assessments

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Informed consent [c]	X										
Inclusion/exclusion criteria	X		X								
Demographics	X										
Concomitant medication[d]	X	X	X	X	X	X	X	X	X	X	X
Medical and surgical history	X		X								
Physical examination including voluntary photography	X		X				X			X	
Skin examination	X		X		X		X			X	X
Vital signs[e]	X	X	X		X	X	X	X	X	X	X
eDiary: itching, scratching, and sleep scores[f]	Daily diary entry										
Clinical chemistry[g]	X		X		X	X	X	X		X	X
Hematology[g]			X		X	X	X	X		X	X
Urinalysis[g]		X					X			X	
Serum bile acids[h]	X	X	X		X	X	X	X	X	X	X

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
INR		X			X		X		X		X
Autotaxin[i]			X		X					X	
p-C4[i]			X		X					X	
AFP			X							X	
Vitamins A & E[h]		X					X			X	
25-hydroxy vitamin D			X				X			X	
Blood sample for A4250 PK[i]					X					X	
Abdominal Ultrasound			X							X	
Fibroscan® (where available)			X							X	
QoL questionnaire (PedsQL)			X							X	
Patient/Caregiver/Clinician Global Impression of Change					X		X			X	
Patient/Caregiver/Clinician Global Impression of Symptoms			X		X		X			X	
Pregnancy test[j]	X[j]	X	X		X	X	X	X	X	X[j]	X
Study drug dispensed[k]			X		X	X	X	X	X		

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Adverse events[l]	Continuous collection										
Study drug compliance					X	X	X	X	X	X	

AFP: alfa-fetoprotein; BMI: body mass index; eCRF: electronic case report form; eDiary: electronic diary; EOT: End of Treatment; ICF: informed consent form; INR: international normalized ratio; p-C4: plasma 7α Hydroxy 4-cholesten-3-one; PedsQL: Pediatric Quality of Life Inventory; PK: pharmacokinetic; QoL: quality of life; RR: respiratory rate; SAE: serious adverse event; s-BA: serum bile acid.

a Must also be performed at the time a patient is prematurely withdrawn from the study. This visit is the same as Visit 1 in the extension protocol (Study A4250-008).

b Assessments must be performed 28 days following the final dose of study drug. This visit is omitted if patients are entering Study A4250-008.

c The ICF (and assent) must be signed before any study procedures are performed.

d Past (within 1 month prior to Visit 1) and current medication will be documented in the eCRF.

e Includes blood pressure, pulse, RR, temperature, height (or length depending on age), and body weight (using a certified weight scale). BMI will be calculated.

f Includes training on use of the device at Screening (Visit 1) and review of patient daily entries during 14 days prior to Clinic Visit 3 to determine eligibility.

g See Table 3 for detailed parameters.

h Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA and vitamin A. Exceptions can be made for infants, less than 12 months of age, if unable to fast for the full 4 hours. All s-BA results during the treatment period and follow-up will be blinded.

i Samples will not be collected for patients ≤10 kg. In the event of an SAE, a PK sample should be collected as close to the onset of the event as possible.

j For girls who have reached menarche. Serum test at Visit 1; urine test at other visits. A serum pregnancy test will also be conducted at Visit 9 for patients entering Study A4250-008. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy.

k Study drug to be taken once daily on Day 1 through Day 167 as described in Section 8.2.

l Adverse event information will be collected from the time of signing of the ICF to study discontinuation.

7.1.3 Study Procedures and Assessments

7.1.3.1 Screening Period (Days -56 to 0)

Day -56 to Day -35/Clinic Visit 1

Patients will undergo a Screening Visit up to 56 days prior to the planned first day of study treatment. Screening procedures and assessments are as follows:

- Obtain written informed consent
- Assess inclusion/exclusion criteria (Section 7.2)
- Record demographics (age, full date of birth, gender, race, and ethnicity)
- Document concomitant medications
- Medical and surgical history (date of diagnosis of PFIC Types 1 or 2, prior investigational medications for PFIC, historical liver function test [LFT] values, any surgery performed, any other diagnosis, and historical liver biopsy data)
- Collect, if available, past confirmatory clinical genetic laboratory report for PFIC-1/PFIC-2 and send to central reader for review. If a historical report is equivocal, unavailable, or unobtainable, a blood sample for clinical genetic testing will be collected to determine eligibility
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- eDiary training and compliance requirements; caregivers and, if applicable, patients begin daily recording of pruritus using the eDiary (Appendix 2)
- Clinical chemistry (Section 10.2.1)
- s-BA (Section 9.2.1)
- Serum pregnancy test for girls who have reached menarche. Please see Appendix 6 for contraceptive requirements
- AE monitoring

Day -28 to Day -7/Clinic Visit 2

A second Screening Visit will be performed. Screening procedures and assessments are as follows:

- Review concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Urinalysis (Section 10.2.1)

- s-BA (Section 9.2.1)
- International normalized ratio (INR)
- Vitamins A and E
- Urine pregnancy test for girls of who have reached menarche
- AE monitoring

7.1.3.2 Treatment Period (Day 0 to Day 168)

Study Day 0/Clinic Visit 3 (Randomization)

At the randomization visit, the following assessments will be conducted:

- Review inclusion/exclusion criteria (Section 7.2)
- Review concomitant medications, medical and surgical history
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance and entrance criteria requirement (see Inclusion Criteria 4, Section 7.2.1)
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin and p-C4 (samples will not be taken for patients ≤10 kg)
- 25-hydroxy vitamin D
- Alfa-fetoprotein (AFP)
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)
- Administer QoL (Pediatric Quality of Life Inventory [PedsQL]) questionnaire
- Patient/caregiver/clinician complete the patient global impression of symptoms (PGIS)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Study drug is dispensed and patients are instructed to administer daily from Day 1

Study Day 14 (Telephone Contact)

A study nurse will contact the patient/caregiver by telephone for monitoring of the following:

- Review concomitant medications

- Albireo PRO/ObsRO eDiary review for compliance
- AEs

Study Day 28 and Day 84/Clinic Visits 4 and 6

The following procedures and assessments will be performed:

- Review of concomitant medications
- Physical examination, including voluntary photography (at Clinic Visit 6 only; Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- Urinalysis (at Clinic Visit 6 only; Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- Autotaxin, p-C4, and A4250 pharmacokinetic (PK) assessment (at Clinic Visit 4 only; samples will not be taken for patients ≤10 kg)
- Vitamins A and E, and 25-hydroxy vitamin D (at Clinic Visit 6 only)
- Patient/caregiver/clinician complete the patient global impression of change (PGIC) and PGIS
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 56 and Day 126/Clinic Visits 5 and 7

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- Urine pregnancy test for girls who have reached menarche

- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 154/Clinic Visit 8

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- s-BA (Section 9.2.1)
- INR
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 168/Clinic Visit 9/EOT

The last dose of study drug is taken in the morning the day before Clinic Visit 9, and the following procedures and assessments will be performed (also performed at the time a patient prematurely withdraws):

- Review of concomitant medications
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry, hematology, and urinalysis (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin, p-C4, and A4250 PK assessment (samples will not be taken for patients ≤10 kg)
- AFP
- Vitamins A, E, and 25-hydroxy vitamin D
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)

- Administer PedsQL questionnaire
- Patient/caregiver/clinician complete the PGIC and PGIS
- Urine pregnancy test for girls who have reached menarche, or serum pregnancy test for girls continuing to study A4250-008
- AE monitoring
- Evaluation of study drug compliance

7.1.3.3 Follow-Up Period

Study Day 196/Clinic Visit 10

All patients not continuing into Study A4250-008 will return to the study site 28 days after Clinic Visit 9 or the last dose of study drug for the following assessments:

- Review of concomitant medications
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- Urine pregnancy test for girls who have reached menarche
- AE monitoring

7.2 Study Population

A total of approximately 60 patients with a clinical diagnosis of PFIC Type 1 or 2 that have been genetically confirmed will be randomized. The study will target 15% of enrolled patients to be PFIC-1 patients. If enrollment of all PFIC-2 patients is completed and the 15% enrollment of PFIC-1 patients has not yet been achieved, then enrollment of PFIC-2 patients will continue in order to reach the total study target enrollment.

An attempt will be made to randomize a minimum of 10 patients from each age category (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years).

A maximum of 5 patients that were treated in Study A4250-003 will be allowed to participate in this study.

7.2.1 Inclusion Criteria

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg

2. Patient must have clinical genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples 7 to 28 days apart (Visits 1 and 2) prior to randomization
4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the eDiary average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients will be expected to have a consistent caregiver for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

7.2.2 Exclusion Criteria

1. Patient with pathologic variations of the *ABCB11* gene that predict complete absence of the BSEP protein
2. Patient with past medical history or ongoing presence of other types of liver disease including, but not limited to, the following:
   a) Biliary atresia of any kind
   b) Benign recurrent intrahepatic cholestasis, indicated by any history of normal s-BAs
   c) Suspected or proven liver cancer or metastasis to the liver on imaging studies
   d) Histopathology on liver biopsy is suggestive of alternate non-PFIC related etiology of cholestasis
3. Patient with a past medical history or ongoing presence of any other disease or condition known to interfere with the absorption, distribution, metabolism (specifically bile acid metabolism), or excretion of drugs in the intestine, including but not limited to, inflammatory bowel disease
4. Patient with past medical history or ongoing chronic (i.e., >3 months) diarrhea requiring intravenous fluid or nutritional intervention for treatment of the diarrhea and/or its sequelae
5. Patient has a confirmed past diagnosis of infection with human immunodeficiency virus or other present and active, clinically significant, acute, or chronic infection, or past medical history of any major episode of infection requiring hospitalization or treatment with parenteral anti-infective treatment within 4 weeks of treatment start (study Day 1) or

completion of oral anti-infective treatment within 2 weeks prior to start of Screening Period

6. Any patient with suspected or confirmed cancers except for basal cell carcinoma, and non-liver cancers treated at least 5 years prior to Screening with no evidence of recurrence
7. Patient with a past medical history of chronic kidney disease with an impaired renal function and a glomerular filtration rate <70 mL/min/1.73 m^2
8. Patient with surgical history of disruption of the enterohepatic circulation excluding those who have undergone a successful reversal procedure that has permanently restored flow of bile acids from the liver to the duodenum
9. Patient has had a liver transplant or listed for liver transplant, excluding those who have been listed for transplant for pruritus who have since been removed from the transplant list
10. Decompensated liver disease, coagulopathy, history or presence of clinically significant ascites, variceal hemorrhage, and/or encephalopathy
11. INR >1.4 (the patient may be treated with Vitamin K intravenously, and if INR is ≤1.4 at resampling the patient may be randomized)
12. Serum ALT >10 × upper limit of normal (ULN) at Screening
13. Serum ALT >15 × ULN at any time point during the last 6 months unless an alternate etiology was confirmed for the elevation
14. Total bilirubin >5 × ULN at Screening
15. Patient suffers from uncontrolled, recalcitrant pruritic condition other than PFIC. Examples include, but not limited to, refractory atopic dermatitis or other primary pruritic skin diseases
16. Any patient who is pregnant or lactating or who is planning to become pregnant within 24 weeks of randomization
17. Sexually active males and females who are not using a reliable contraceptive method with ≤1% failure rate (such as barrier protection, hormonal contraception, intrauterine device, or complete abstinence) throughout the duration of the study and 90 days thereafter (from signed informed consent through 90 days after last dose of study drug). See Appendix 6 for further details
18. Patient with a past medical history of alcohol or substance abuse will be excluded. Patient must agree to refrain from illicit drug and alcohol use during the study
19. Administration of bile acid or lipid binding resins and medications that slow GI motility (Refer to Appendix 1 – Concomitant Medications Guidelines)

20. Patient has had investigational exposure to a drug, biologic agent, or medical device within 30 days prior to Screening, or 5 half-lives of the study agent, whichever is longer
21. Patient who has been previously treated with an IBAT inhibitor whose pruritus has not responded to treatment
22. Any other conditions or abnormalities which, in the opinion of the investigator or Medical Monitor, may compromise the safety of the patient, or interfere with the patient participating in or completing the study

7.2.3 Withdrawal of Patients

Patients/caregivers will be informed that they have the right to withdraw from the study at any time, without prejudice to their medical care, and that they are not obliged to state their reasons.

Any withdrawal, and reasons for withdrawal, must be fully documented in the electronic case report form (eCRF) and source documents and the patient followed by the investigator/investigative staff. Even after the study is completed at the Follow-up Visit, the investigator/investigative staff will, regardless of reason for withdrawal, record any patient data they receive concerning SAEs, and all drug-related non-serious AEs, and subsequently report these to Albireo. Withdrawn patients will not be replaced.

Patients will be withdrawn in the following circumstances:

- A patient's/caregiver's desire for withdrawal for any reason
- Lost to follow-up (every effort must be made to contact the patient/caregiver; a certified letter must be sent)
- An AE that, in the opinion of the investigator, necessitates withdrawal
- A worsening of the symptoms related to diarrhea occurs
- Death
- Patient/caregiver estimation of lack of improvement/intolerable symptoms
- A patient's/caregiver's substantial noncompliance (eDiary and study drug compliance) or protocol violation
- An investigator's opinion that continuing the patient in the study is not appropriate. The investigator may withdraw a patient at any time, if it is considered to be in the patient's best interest

A patient who prematurely withdraws due to no improvement/intolerable symptoms, and has completed at least 12 weeks treatment in the double-blind study, will be offered the opportunity to enter open-label Study A4250-008. A patient who withdraws from treatment prematurely and does not enter Study A4250-008 will have EOT (Visit 9) assessments at the time of withdrawal and a follow-up assessment (Visit 10) 28 days following the last dose of study drug, and the patient will be recorded as withdrawn.

7.2.4 Study Termination by Sponsor

This study may be terminated at any time by Albireo if serious side effects should occur, if the investigator does not adhere to the protocol; or if, in the sponsor's judgment, there are no further benefits to be achieved from the study. In this event, Albireo/designee will inform the study investigators, institutions, and all regulatory authorities.

Albireo may temporarily or permanently discontinue the study at an investigative site at any time for safety, ethical, compliance, or other reasons. If this is necessary, Albireo will endeavor to provide advance notification to the site. If a site or the study is suspended or discontinued, the investigator/investigative staff will be responsible for promptly informing the Independent Ethics Committee (IEC). If required by local regulations, Albireo/designee will be responsible for informing the IEC and the regulatory authority of study or site discontinuation. In such an event, all study data and unused study drug must be returned to Albireo.

8 TREATMENT OF PATIENTS

8.1 Identity of Study Drug

A4250 and placebo will be supplied as capsules for oral administration. White opaque capsules filled with pellets containing A4250 or placebo will be provided. Two different capsule sizes will be available, as follows:

- Capsule size 0 that can be opened
- Capsule size 3 that should be swallowed intact. These size 3 capsules may be opened only under exceptional circumstances, e.g., patient cannot swallow capsule intact

The content of the A4250 and placebo capsules will be identical in appearance. Capsule filling weight will also be identical for high dose, low dose, and placebo. Bottles with 34 capsules will be given to the patient at each visit. A patient who requires 2 or more capsules per day will be given multiple bottles. Refer to the investigational product manual.

8.2 Administration of Study Drug

Patients will be dosed with A4250 at a dose of 40 µg/kg/day, 120 µg/kg/day, or placebo for 24 weeks. Study drug will be dispensed to the patient at Visits 3, 4, 5, 6, 7, and 8, together with instructions on how to store and take the drug. Study drug administration data, including whether each patient took each dose or partial doses of study drug, whether there were any delayed or missed doses, and whether the capsule was opened or swallowed whole will be documented through the eDiary and transferred to the study database.

A4250 should be taken in the morning, together with food. On clinic visits days when laboratory assessments are conducted, study drug should be taken after the visit and after laboratory samples are taken. Patients should not crush or chew the capsule(s). When swallowing the capsule intact, the patient should administer the dose with a glass of water.

For a patient unable to swallow capsule intact, the capsule can be opened and the contents sprinkled and mixed in a small amount of soft room-temperature yogurt, apple sauce, or fruit purée. The amount of food should be less than what the patient/infant is expected to eat and should be followed by water.

1. Hold the capsule over the prepared food, gently twist open and allow the content to fall into food
2. Mix the entire capsule content with 1 or 2 tablespoons (15 to 30 mL) of soft food (apple sauce, fruit purée, or yogurt)
3. Administer the mixture immediately after mixing.

The number of capsules provided to the patient will be based on body weight at Visit 3. At Visit 6, if a patient's weight has shifted to another weight category (identified in Table 2), the number of capsules per day may be adjusted.

Table 2 Dosing and Capsule Strength

Body Weight (kg)	Capsule Size	Number of Capsules per Day	Capsule Strength, Low Dose (µg)	Total Dose (µg)	Capsule Strength, High Dose (µg)	Total Dose (µg)	Capsule Size, Placebo (µg)
5.0 to <7.5	0	1	200	200	600	600	0
7.5 to <12.5	0	2	200	400	600	1200	0
12.5 to <17.5	0	3	200	600	600	1800	0
17.5 to <19.5	0	4	200	800	600	2400	0
19.5 to <25.5	3	2	400	800	1200	2400	3
25.5 to <35.5	3	3	400	1200	1200	3600	3
35.5 to <45.5	3	4	400	1600	1200	4800	3
45.5 to 55.5	3	5	400	2000	1200	6000	3
>55.5	3	6	400	2400	1200	7200	3

8.3 Study Treatment Packaging and Labeling

8.3.1 Packaging and Labeling

The capsules will be packed in high-density polyethylene containers, with child-proof polypropylene caps. Study drug capsules containing A4250 in the strength specified in Table 2 above will be manufactured.

Packaging and labeling will be prepared to comply with applicable regulatory requirements.

8.3.2 Storage

Treatment packs containing A4250 capsules should be stored and dispensed in accordance with regulations in their original containers. The storage facility at the investigative site should be locked and the storage temperature should be between 15°C and 25°C.

Patients/caregivers should be informed of appropriate storage conditions (i.e., room temperature, between 15°C and 25°C).

Any deviations from the recommended storage conditions should be immediately reported to Albireo and the study drug should not be used until authorization has been given by Albireo.

8.3.3 Blinding and Randomization of Study Drug

A double-blind design is employed so that both the investigators and the patients will be unaware of the treatment assignment during the study.

After written informed consent is obtained from an eligible patient, an 8-digit patient identification number will be assigned. The first 2 digits will denote the country, followed by a 3-digit site number and a 3-digit patient sequence number. This number will be created and allocated by the Interactive Web Response System (IWRS), when the patient first enters the system at Screening. The randomization codes will be computer-generated by [redacted]

Biostatistics, and kept by a statistician independent from the project team. The randomization will be done in blocks and stratified according to PFIC class (Type 1 and 2) and age class (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) to ensure approximate balance between dose schemes (1:1:1). A separate randomization list will be prepared for the maximum 5 patients from Study A4250-003 regardless of stratification. Randomization codes will be assigned sequentially as patients become eligible for randomization.

Patients who are eligible for randomization will be assigned a unique 4-digit randomization number by IWRS. This randomization number identifies which treatment will be allocated to the patient. Patients who withdraw from the study after randomization visit are not to be replaced and their randomization number will not be reused.

To ensure blinding, the study drug and the matching placebo have the same shape and size. Patients will receive capsule(s) of A4250 according to their dose group or capsule(s) of matching placebo once a day during the double-blind Treatment Period. Labels on the study drug containers will not identify the treatment that a patient is randomized to. Traceability of the treatment is ensured by the study drug number.

The 5-digit study drug number will identify study drug packs and will be detailed on the study drug label. Dispensing of study drug will be coordinated by IWRS.

The system will assign a study drug number corresponding to the randomization arm. The randomization number will be used in the background only to ensure no unblinding and will not be displayed to end-users in the IWRS.

8.4 Procedure for Breaking the Randomization Code

Should a situation arise where unblinding is urgently required, i.e., only where knowledge of the study drug is required to adequately manage a life-threatening situation, the investigator at that study site may perform immediate unblinding through IWRS. The responsibility to break the treatment code in emergency situations resides solely with the investigator. The investigator should make every effort to discuss the rationale (status and outcome) for emergency unblinding with the sponsor Medical Monitor or the [redacted] Medical Monitor as soon as possible to review the individual patient details.

The study site investigator and appropriate [redacted] project team members will be authorized to access the emergency unblinding functionality within the IWRS. Emergency unblinding takes place in the IWRS via a dedicated emergency unblinding module. The system will require the user to enter their emergency unblinding, which is typically provided at the same time login credentials are distributed. The exact description of the treatment assigned to the individual patient then will be accessible to the user. Once the randomization code is broken for a patient, he/she must be withdrawn from the study and all assessments and procedures at EOT should be performed. As a follow-up, Visit 10 will be performed according to the time schedule. Once a randomization code has been broken, the investigator must inform the [redacted] and sponsor Medical Monitor in writing within 24 hours. In case of technical issues

accessing the system online, please see the IWRS site user manual for country-specific contact telephone numbers for the [redacted] HelpDesk 24/7 system support.

8.5 Patient Compliance

The study nurse will monitor eDiary compliance by routine review of the CRF health website. If both diary entries on a day are missing, the study nurse will call the patient/caregiver to remind them to complete all scheduled entries. Any noncompliance will be documented and explained in the source documents.

Study drug compliance will also be checked through diary responses. Additionally, patients will return all unused study drug at Visits 4, 5, 6, 7, 8, and 9. The study site staff will count all returned drug, assess compliance, and record details in the eCRF.

Treatment Compliance = 100 × ((Number of study drug dispensed – number of study drug returned)/number of study drug that should be taken).

Treatment compliance between 80% and 120% will be acceptable.

8.6 Study Drug Accountability

Records shall be maintained of the delivery of study treatment(s) to the study site(s), of the inventory at the study site(s), of each use of the study treatment(s) for each patient, and of the return of unused study treatment(s) to Albireo.

These records shall include dates, quantities, batch numbers, expiry dates, and the unique code numbers assigned to the study drug and to the study patients.

The investigator will be responsible for ensuring that the records adequately document that the patients were provided the quantities specified in the protocol and that all study drug received from Albireo is reconciled.

8.7 Concomitant Therapy and Prohibited Medications

The investigator will note all ongoing medication and any medication recently stopped (within 1 month prior to Visit 1) in the eCRF. At Visits 2 through 10, all changes in medication (stopping or starting new medication or changes in dose) will be recorded in the eCRF. All medications (prescribed or over the counter) for pruritus and sleep will be recorded.

All medications taken by a patient within 3 months prior to the first intake of study drug are regarded as prior medication.

All medications taken by a patient on or after the first intake of study drug, and which continue to be taken during the study, are regarded as concomitant medication.

Prohibited medications are listed in Appendix 1.

9 ASSESSMENT OF EFFICACY

9.1 Efficacy Endpoints

9.1.1 Primary Efficacy Endpoint

The primary efficacy endpoints are as follows:

- **European Union (EU) and rest of the world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  Fasting s-BA baseline will be calculated as the average of the value at randomization and the previous visit (Clinic Visit 2). The end value is the average of the values at Weeks 22 and 24 after the start of double-blind treatment.

- **United States (US)**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline over the last 5 months of the Treatment Period as measured by an overall mean of weekly averages of the worst daily scratch score using the Albireo ObsRO instrument (Appendix 2).

9.1.2 Secondary Efficacy Endpoint

Secondary efficacy endpoints include the following:

- **EU and RoW**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline over the last 5 months of the Treatment Period as measured by an overall mean of weekly averages of the worst daily scratch score using the Albireo ObsRO instrument (Appendix 2).
- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.
- **All Regions:**
  - All secondary endpoints are compared to placebo.
    - Change from baseline to Week 24 in fasting s-BA
    - Change from baseline to Week 24 in serum ALT concentration
    - Change in growth from baseline to Week 24, defined as the linear deficit (weight for age, and body mass index [BMI]) compared to a standard growth curve (Z-score, standard deviation [SD] from P50)
    - Proportion of patients achieving meaningful reduction in caregiver-reported observed scratching over the Treatment Period

- Change from baseline over the last 5 months of the Treatment Period as measured by an overall mean of weekly averages of the sleep parameters measured with the Albireo PRO and ObsRO instruments
- Change from baseline over the last 5 months of the Treatment Period as measured by an overall mean of weekly averages of patient-reported itch severity as measured by the average daily score of the Albireo PRO instrument (Appendix 2); only patients ≥8 years of age will complete the Albireo PRO instrument
- Number of patients undergoing biliary diversion surgery or being listed for liver transplantation

9.1.3 Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Proportion of patients achieving meaningful reduction in patient-reported itch severity over the Treatment Period
- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the PGIC items (Appendix 3)
- Change from baseline in fasting s-BA at Weeks 4 and 12
- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 4 of the Treatment Period as measured by the Albireo ObsRO instrument
- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline over the last 3 months of the Treatment Period as measured by an overall mean of weekly averages of the worst daily scratch of the Albireo ObsRO instrument
- Change from baseline over the last 5 months of the Treatment Period in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline over the last 5 months of the Treatment Period in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline over the last 5 months of the Treatment Period in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in PedsQL (Appendix 4)
- Change from baseline in serum ALT concentration at Weeks 4 and 12
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST and GGT at Weeks 4, 12, and 24

- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, p-C4) at Week 24
- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events
- All-cause mortality

9.2 Efficacy Assessments

9.2.1 Serum Bile Acids

Blood samples for analysis of fasting total s-BA will be drawn at all visits. Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA. Exceptions can be made for infants, <12 months of age, if unable to fast for the full 4 hours. Any visit at which a bile acid sample result is unreportable, an additional unscheduled visit for a repeat sample collection may be scheduled. All s-BA results during the treatment period and follow-up will be blinded. Samples will be processed and transported to a central laboratory per instructions in the laboratory manual.

9.2.2 Itching, Scratching and Sleep Score

Itching, observed scratching, and sleep disturbance will be recorded twice each day via eDiary. Patients and/or caregivers will be instructed to complete the eDiary every day in the morning after the patient wakes and in the evening just before the patient goes to sleep throughout the study. Patients and caregivers will receive a formal training session during Visit 1.

The eDiary includes Albireo ObsRO and PRO items. Patients <8 years of age will not be asked to complete the Albireo PRO items; only the Albireo ObsRO will be completed by caregivers of patients in this age group. Older patients, 8 to 18 years of age, will complete the Albireo PRO items and the caregiver will complete the Albireo ObsRO items. The Albireo PRO items assess severity of itch, aspects of sleep disturbance (morning diary only), and tiredness. For patients 8 to 12 years of age, the caregiver will read the Albireo PRO items along with the child and record the child's response. A guide will be provided to the caregivers that provides standardized explanations of the Albireo PRO items, in case the patient is confused or requires clarification about the meaning of a question. The Albireo ObsRO items assess severity of observed scratching, aspects of observed sleep disturbance (morning diary only), and observed signs of tiredness (evening diary only). The Albireo ObsRO and PRO scratching and itch severity items use 0 to 4 response scales, where each

response is distinguished by a unique facial expression, verbal anchor, number, and color code (Appendix 2).

A daily score for the Albireo ObsRO scratching item will be calculated by selecting the maximum scratching severity score from the 2 ratings for each day. A daily score will be considered missing if both of the daily assessments are missing. A weekly score will be calculated by averaging the daily scratching scores. A weekly score will be considered missing if ≥4 out of 7 days a week of data are missing. For baseline, a 14-day score will be calculated by averaging the 2 weekly scores prior to randomization and will be considered missing if one or both weekly scores for that 14-day period are missing. For each treatment month, a 14-day score will be calculated by averaging the 2 last weekly scores and will be considered missing if both weekly scores for that 14-day period are missing. The same approach will be used to calculate a patient-reported itch severity score. The handling of missing data is described in Section 11.2.2.2.

9.2.3 Growth

Growth will be measured as height (velocity) and weight (Z-score) using a certified weight scale. BMI will be calculated by weight (kg) / height $(m)^2$. Change will be defined as linear growth deficit (weight and BMI for age) compared to a standard growth curve (Z-score, SD from P50).

9.2.4 Quality of Life Questionnaire (PedsQL)

Patients and caregivers will be asked to complete a QoL questionnaire (PedsQL) at Visit 3 and Visit 9. Details of the questions included on the questionnaire are provided in Appendix 4.

9.2.5 Biomarkers and PK Samples

Blood samples for autotaxin and p-C4 will be drawn at Visits 3, 4, and 9 only for children with body weight >10 kg. Blood for A4250 PK assessment will only be drawn at Visits 4 and 9 (only for children with body weight >10 kg), and as close to the onset of an SAE as possible, should an SAE occur. Samples will be processed and transported to a laboratory per instructions in the laboratory manual.

9.2.6 PELD/MELD Score

At randomization and end of treatment, PELD score will be calculated for children up to 12 years of age. For children 13 years or older, the MELD score will be calculated.

PELD Score = 4.80 * ln(total bilirubin) + 18.57 * ln(INR) - 6.87 * ln(albumin) + 4.36 (if patient <1 year: scores for patients listed for liver transplantation before the patient's first birthday continue to include the value assigned for age (<1 year) until the patient reaches the age of 24 months) + 6.67 (if the patient has growth failure [≤2 SD])

Laboratory values less than 1.0 will be set to 1.0 for the calculation of the PELD score.

MELD Score = 9.57 * ln(creatinine) + 3.78 * ln(total bilirubin) + 11.2 * ln(INR) + 6.43

Laboratory values less than 1.0 will be set to 1.0 and serum creatinine values greater than 4.0 mg/dL (equivalent to 353.6 µmol/L)will be set to 4.0 for calculation of the MELD score.

9.2.7 Fibroscan®

Where available, Fibroscan® will be performed as per institution standard practice at Visits 3 and 9.

9.2.8 Markers of Fibrosis

APRI score and Fib-4 score will be calculated at randomization and at end of treatment.

APRI = [(AST in U/L) / (AST ULN in U/L)] / (Platelets in 10^9/L)

Fibrosis 4 Score = (Age*AST in U/L) / (Platelets in 10^9/L * √(ALT in U/L)).

9.2.9 Global Impression of Change and Global Impression of Symptom Measures

Patients (≥8 years of age), caregivers, and clinicians will complete the PGIC and the PGIS measures (Appendix 3) at randomization (Visit 3; PGIS only), Visits 4 and 6, and at EOT (Visit 9).

The PGIC items assess change in itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) since starting the study drug. The PGIS items assess itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) in the past week. Caregivers and clinicians will complete the PGIC and PGIS for all patients; those patients ≥8 years of age will complete the patient version.

10 ASSESSMENT OF SAFETY

The timing and frequency of safety assessments are described in Section 7.1.3 and in Table 1 above.

The primary safety analysis for this study will include the incidence of total treatment-emergent adverse events (TEAE) and TEAEs categorized by causality, severity, and seriousness assessments made by the investigator by comparing study drug exposure to placebo.

Trends in safety will also be evaluated for the following assessments:

- Physical examinations
- Concomitant medications
- Vital signs
- Laboratory test results (including clinical chemistry, hematology, urinalysis, AFP, and vitamins A and E, 25-hydroxy vitamin D, and INR)
- Abdominal ultrasound
- Discontinuations due to AEs

10.1 Adverse Events

10.1.1 Definitions and Investigator Assessments

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 unfavorable and unintended sign, symptom, or disease that occurs once a patient is enrolled (ICF is signed) in the study until the patient is discharged from the study, whether or not related to the study drug.

10.1.1.1 Clinical Significance

Clinical significance is defined as any abnormal finding that results in further clinical investigation(s), treatment(s), or the diagnosis of new condition. Patient-reported events and protocol-mandated laboratory values, vital signs, and physical examination findings can be considered clinically significant (i.e., an AE) if there is a deterioration as compared to baseline. Examples of clinically significant worsening from baseline could include, but is not limited to, events causing withdrawal from the study and events requiring medical intervention outside of the study causing apparent clinical manifestations, or judged relevant by the investigator.

10.1.1.2 Serious Adverse Events

Serious criteria are applied by the investigator to each AE as specifically defined below. These criteria are used to determine whether an AE is serious or non-serious. The assessment is made independently of severity assessment (Section 10.1.1.3). For example, the

development of a severe rash that occurs after signing of informed consent may not meet serious criteria as defined below and therefore would be considered a severe, non-serious AE.

Any AE that meets any 1 of the following 6 criteria is considered an SAE:

- The outcome of the AE is **death**
- The AE is immediately **life-threatening**. Life-threatening means that the patient is, in the opinion of the investigator, at immediate risk of death from the reaction as it occurred. This does not include an AE that, if more severe, might have caused death
- The AE results in persistent or significant **disability/incapacity**. Disability means a substantial disruption of a person's ability to conduct normal life functions
- The AE requires or prolongs **hospitalization**
- The AE results in a **congenital anomaly/birth defect**
- **The AE is an important medical event**. Important medical events may meet serious criteria should the investigator assess that they may significantly jeopardize the patient, represent a significant hazard, or requires medical/surgical intervention to prevent one of the outcomes listed above. Examples of potential SAEs based on this criteria include, but are not limited to, allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions even if they do not result in inpatient hospitalization, or the development of drug dependency and drug abuse.

10.1.1.3 Severity Assessment

Severity assessments are based on the intensity of the event in relation to expectation. The investigator will assess the intensity of AEs based on the following definitions:

- Mild (awareness of sign or symptom, but easily tolerated)
- Moderate (discomfort sufficient to cause interference with normal activities)
- Severe (incapacitating, with inability to perform normal activities)

Severity is a measure of intensity where seriousness is defined by the criteria outlined in Section 10.1.1.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not meet serious criteria, and therefore would be assessed as a severe AE but not an SAE.

10.1.1.4 Causality Assessment

The investigator determines the causality of all AEs to the study drug using medical judgment and considering all relevant factors such as (but not limited to) the underlying study indication, coexisting disease, concomitant medication, relevant history, pattern of the AE, temporal relationship to the study drug, and de-challenge or re-challenge. The causality assessment of the AE/SAE is to be made as follows.

Related to study drug (possibly, probably, or definitely related)

Based on medical judgment, there is at least a reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event follows a reasonable temporal sequence from administration of study drug
- The event could not be reasonably attributed to the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient
- The event follows a known pattern of response to study drug
- The event disappears or decreases on cessation or reduction in dose of the study drug. (It should be noted that in some situations an AE will not disappear or decrease in intensity upon discontinuation of study drug despite other clear indications of relatedness)
- The event reappears or worsens when the study drug is re-administered

Unrelated to study drug (unlikely or unrelated)

Based on medical judgment there is no reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event does not follow a reasonable temporal sequence from administration of study drug
- The event could be reasonably attributed to the known characteristics of the patient's clinical state, concurrent illness, environment or toxic factors, or other modes of therapy administered to the patient
- The event does not follow a known pattern of response to study drug
- The event does not disappear or decrease on cessation or reduction in dose of the study drug, and it does not reappear or worsen when the study drug is re-administered

10.1.2 Recording of Adverse Events

It is the investigator's responsibility to assess whether each untoward event is a clinically significant worsening from baseline, thereby considered an AE. For all AEs, the severity, seriousness, and causality to study drug for each AE as outlined in Section 10.1.1 will be assessed and recorded in the eCRF.

All serious and non-serious AEs are collected once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10) or 28 days after the last dose of study drug.

Any AEs or SAEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator until resolution or stabilization up to the database lock and recorded in the eCRF. Albireo retains the right to request additional information for any patient with ongoing AE(s) or SAE(s) at the end of the study.

TEAEs are defined as any AE that occurs after randomization (Day 0). All AEs that occur in the Screening Period, i.e., after enrollment and prior to randomization, will be collected on the eCRF as non-treatment-emergent AEs.

If there is a clinically significant deterioration of a laboratory value/vital sign or other routine study assessment that is associated with a diagnosis, the clinical diagnosis will be reported as an AE and the associated signs and symptoms will be considered additional information unless the sign or symptom is more severe than expected given the diagnosis. For example, if an investigator diagnoses a study patient with hepatitis during the study period, hepatitis would be considered the AE and the concomitant signs and symptoms of abdominal pain, vomiting, and elevated ALT and AST would not be reported separately unless, in the opinion of the investigator, one of these signs or symptoms is more severe than expected and therefore a separate AE assessment is indicated.

10.1.3 Recording and Reporting of Serious Adverse Events

Every SAE (regardless of severity and causality) that occurs once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10), and 28 days after the final dose of study drug, should immediately and not later than within 24 hours of knowledge of the event, be reported by the investigator or delegate in the SAE report form.

Report of a SAE must include at least the following information:

- Patient identification information (study number, site number, initials, and date of birth [as per local country requirements for data protection])
- The last study drug administration date
- The diagnosis of the event with the description (or a brief description of signs/symptoms/clinical course if the diagnosis is not available) and the date of onset
- The action used to treat the event (i.e., treatment medications, temporary discontinuation)
- The reason(s) for considering the event serious
- The relationship of the event to the study drug or to the study procedure (e.g., the investigator's assessment of causality)
- A brief narrative of the SAE

Follow-up reports including all new information obtained of the subsequent course of the SAE must be prepared and the information collected in the SAE submitted to [redacted] (Pharmacovigilance and Safety Services Department) by e-mail within 24 hours of knowledge of the event.

The pharmacovigilance manager (PVM) may contact the investigator to obtain further information on a reported SAE. The investigator/investigative staff must respond to any request for follow-up information or answers to questions regarding the SAE within the same timelines as for initial reports.

The PVM reports the occurrence of the SAE and follow-up to the Albireo medical monitor for medical assessment of the case.

Should an outcome of death occur within the study period or within 28 days after the last administration of study drug, an SAE form should be completed including the main and contributory causes of death.

All SAE reports must be e-mailed to the following e-mail address within **24 hours**:

[redacted]

If email is unavailable, SAEs may be transmitted via fax to the following number:

[redacted]

In a study-related medical emergency situation, when the assigned medical monitors for the study cannot be reached by a caller, an on call physician can be reached 24 hours per day, 7 days per week, via the [redacted] call center. The following number is a chargeable telephone number allowing a global reach from both landlines and mobile phones and the internet page indicated below contains a list of country-specific toll-free telephone numbers that is provided. It should be noted that not all countries globally have access to toll-free numbers as indicated on the "24/7 Medical Help desk" index. Countries without toll-free numbers need to dial the chargeable number as indicated below. Furthermore, toll-free numbers might not be available from mobile phones.

[redacted]

10.1.4 Reporting of Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reaction (SUSAR) is an SAE that occurs in a patient, the nature or severity of which is not expected per the applicable product information (e.g., the IB for an unauthorized investigational product or summary of product characteristics for an authorized product).

Reporting and tracking of SUSARs will be in accordance to all applicable competent authority regulations. The Ethics Committee and all investigators involved in this study will be informed according to national and international requirements.

10.2 Laboratory Values/Vital Signs/Physical Examinations and Other Safety Assessments

10.2.1 Laboratory Assessments

Samples will be collected for clinical chemistry, hematology, and urinalysis testing at the time points specified in Table 1. The parameters assessed are presented in Table 3.

Blood for AFP will be drawn at Visits 3 and 9. Fat-soluble vitamin levels including vitamins A and E, and 25-hydroxy vitamin D will be drawn at Visit 2 (vitamins A & E only), Visit 3 (25-hydroxy vitamin D only), Visit 6, and Visit 9. Blood for INR (surrogate for vitamin K) will be drawn at Visits 2, 4, 6, 8, and 10. Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for vitamin A. Exceptions can be made for infants, <12 months of age, if unable to fast for the full 4 hours. If a patient has any vitamin level(s) that are outside of the reference range, vitamin supplementation adjustments may be required. Target ranges and supplementation strategy guidelines can be found in Appendix 5.

A serum pregnancy test will be performed at Visit 1 and Visit 9 if the patient is entering the A4250-008 study. A urine pregnancy test will be collected at all other visits for girls who have reached menarche. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy. Study drug administration will be held pending the confirmatory results. If the serum pregnancy test is negative, the patient can resume dosing. If the serum pregnancy test is positive, the patient should be discontinued (see Section 10.2.9).

All samples will be processed and transported to a laboratory per instructions in the laboratory manual.

The observed values will be recorded and assessed as "normal" or "abnormal not clinically significant" or "abnormal clinically significant".

Additional safety blood samples may be needed due to follow-up of an abnormal value or analysis failure. The blood samples collected for safety laboratory analysis will be destroyed after the analyses have been completed.

Table 3 Routine Laboratory Parameters

Clinical Chemistry	Hematology	Urinalysis
• Albumin • ALT • Alkaline phosphatase • AST • Bilirubin – total and direct • Calcium • Chloride • Creatinine • Creatine kinase • Gamma-glutamyl transferase • Potassium • Sodium	• Hematocrit • Hemoglobin • Platelet count • Red blood cell count • White blood cell count and differential (neutrophils, eosinophils, basophils, lymphocytes, and monocytes)	• Blood • Glucose • Ketones • Leukocytes • Nitrites • pH • Protein

10.2.2 Individual Patient Safety Monitoring

10.2.2.1 Liver Monitoring

Strategies to monitor markers of liver disease throughout the study are outlined below where the ULN will be based on central laboratory reference values for age and gender.

If isolated transaminase elevations are observed, defined as:

1. Normal bilirubin AND absence of clinical hepatitis symptoms AND
   - ALT or AST ≥5 × ULN (if normal at baseline) or an absolute threshold of 800 U/L, whichever comes first
   - OR ALT or AST ≥3 × baseline (if abnormal at baseline) or an absolute threshold of 800 U/L, whichever comes first

Then:

a) Repeat liver profile (AST, ALT, bilirubin, and prothrombin time [PT] or INR) within 2 to 3 days

b) Evaluate creatine phosphokinase (CPK) and lactate dehydrogenase (LDH)

c) As needed (for example, persistent ALT/total bilirubin elevation or worsening of liver function), consider evaluation for alternative etiologies

Monitor the patient using close observation found in Section 10.2.2.2

If any ONE of the following criteria are met:

1. Transaminases (ALT or AST ≥3 × baseline or 800 U/L, whichever comes first) AND bilirubin (total bilirubin > 2 × ULN) elevations
2. Transaminase elevations alone (ALT or AST >10 × ULN or 5 × baseline or absolute threshold of 800 U/L, whichever comes first) in presence of normal LDH and CPK
3. Total bilirubin increased, unrelated to hemolysis (elevated reticulocyte count) or established genetic diseases, such as Gilbert's syndrome
   a. Doubling if total bilirubin was <3 mg/dL (equivalent to 51.3 µmol/L) at baseline
   b. OR Increase by >3 mg/dL (equivalent to 51.3 µmol/L) if total bilirubin was ≥3 mg/dL (equivalent to 51.3 µmol/L) at baseline
4. INR increase refractory to vitamin K administration
   a. INR >1.5 if INR was normal at baseline
   b. OR Increase by >0.4 if INR was abnormal at baseline
5. Any increase in total bilirubin and transaminases if accompanied by EITHER a symptom of clinical hepatitis (e.g., vomiting, nausea, right upper quadrant pain) OR immunological reaction (rash or >5% eosinophilia)

Then:

a) Interrupt study medication

b) Initiate drug-induced liver injury work-up for alternative etiologies

c) Repeat liver profile (AST, ALT, total bilirubin, direct bilirubin) and PT or INR within 48 to 72 hours

d) Monitor the patient using close observation found in Section 10.2.2.2

e) If a patient lives in a remote area, they may be tested locally and the results communicated to the investigator site promptly

10.2.2.2 Close Observation

- Repeat liver enzyme and serum bilirubin tests two or three times weekly. Frequency of re-testing can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the subject is asymptomatic
- Obtain a more detailed history of symptoms and prior or concurrent diseases
- Obtain a history of concomitant drug use (including non-prescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets
- Consider ruling out alternate etiology including acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; non-alcoholic steatohepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease
- Obtain a history of exposure to environmental chemical agents.
- Obtain additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin)
- Consider gastroenterology or hepatology consultations.

10.2.2.3 De-challenge/Re-challenge for Liver and Clinical Hepatitis Monitoring

1. Re-challenge is not recommended:
   a. If a patient has had possible/probable drug-induced liver injury
   b. If a decompensation event has occurred (i.e., variceal hemorrhage, ascites, hepatic encephalopathy, etc.).
2. If due to underlying cholestatic liver disease variability or another alternative etiology is identified AND liver tests returned to baseline, re-challenge may be considered after consultation with the sponsor Medical Monitor.
3. If the ALT/total bilirubin elevations are observed after re-challenge, then repeat re-challenge is discouraged.

If patient is permanently discontinued, monitoring should be continued as outlined in Section 10.2.2.5.

10.2.2.4 Diarrhea

Study drug should be discontinued if a patient develops diarrhea with at least 1 of the following concomitant signs or symptoms: grossly bloody stools, vomiting, dehydration requiring treatment with oral or intravenous rehydration and/or electrolyte imbalances, fever (≥38 C) and/or the diarrhea persists for 7 or more days.

If there is gross blood in stool, INR and platelets should be measured.

Study drug will be reintroduced (re-challenge) when the symptoms have resolved. If the diarrhea re-occurs within 1 week after the re-challenge with no alternate etiology, the patient will be permanently discontinued and monitored as outlined in Section 10.2.2.5.

10.2.2.5 Monitoring after Permanent Discontinuation of Study Drug Due to Safety

Once study drug is permanently discontinued for liver, hepatitis, diarrhea, or other severe AE related to study drug, the patient will be monitored weekly until the laboratory and clinical parameters have normalized/stabilized.

10.2.3 Demographics/Medical and Surgical History

Demographic information per country regulations (age, full date of birth, gender, race, and ethnicity), along with medical and surgical history, will be obtained and recorded in the eCRF at Visit 1.

Medical and surgical history will be entered in the eCRF at Visit 1. This includes date of diagnosis of PFIC, prior investigational medications for PFIC, historical (3 months prior to Screening) LFT values (AST, ALT, and total bilirubin), ongoing medication, any surgery performed, any other diagnosis, and historical biopsy data.

If a liver biopsy has been performed within 1 year prior to Screening, or during the study, the results will be recorded in the eCRF.

10.2.4 Clinical Genetic Testing

For previous confirmatory clinical genetic testing results for PFIC Type 1 or 2 performed prior to Screening Visit 1, the confirmatory clinical genetic testing laboratory report will be verified to determine eligibility.

If the historical clinical genetic result is equivocal, unavailable, or unobtainable, clinical genetic analysis to confirm pathologic biallelic variations in the *ATP8B1* or *ABCB11* genes will be performed and verified to determine eligibility. No other diagnostic genetic testing will be offered.

10.2.5 Physical Examination

A physician or suitably trained qualified assistant will perform a complete physical examination, including voluntary photography of skin lesions due to scratching, at Visits 1, 3, 6, and 9. A skin examination will be conducted at Visits 1, 3, 4, 6, 9, and 10 (see Table 1).

A complete physical examination will include assessment of general appearance, eyes, ears, nose, throat, head/neck/thyroid, lymph nodes, cardiovascular, lungs/chest, abdomen, genitourinary, extremities, skin, musculoskeletal, neurologic, and other.

Skin will be examined thoroughly and excoriations/scratch marks recorded.

10.2.6 Vital Signs

Evaluation of vital signs will be performed at all visits. This includes blood pressure (systolic and diastolic), pulse, respiratory rate, temperature, height (or length depending on age), and body weight (using a certified weight scale) at clinic visits. BMI will be calculated.

10.2.7 Abdominal Ultrasound

Ultrasound of the liver and spleen will be performed at randomization (Visit 3) and at EOT (Visit 9). Liver size, echogenicity, and presence of masses/nodules as well as spleen size will be recorded.

10.2.8 Overdose

A4250 is minimally absorbed and has a very low systemic availability. Based on toxicology data, for study purposes any dose exceeding a total of >3 mg/kg body weight of A4250 taken as a single dose or as a cumulative dose within 24 hours is defined as an overdose.

The no-adverse-effect dose level in the most sensitive species (defined as 20 mg/kg/day) in the rat 1 month toxicity study gives a human equivalent dose of approximately 194 mg/day in a 60 kg person. This dose is approximately 10-fold higher than the maximum possible dose predicted in human studies (20 mg).

The investigator/investigative staff should immediately and not later than within 24 hours of knowledge of the overdose, be reported by the investigator or delegate on the SAE report form. In the event of an A4250 overdose, the patient should be monitored closely.

10.2.9 Pregnancy

If a pregnancy is discovered in a female patient enrolled in the study before the end of dosing, the patient will be immediately discontinued from the study and will attend the same visits as a prematurely withdrawn patient. If the pregnancy is discovered in a female patient enrolled in the study after the end of dosing, the patient will continue in the study per protocol. If a pregnancy occurs in a male patient's partner at any time during the study, the pregnancy should also be reported and followed.

Pregnancy is not considered to be an AE. However, if the patient has been dosed with the study drug, the pregnancy must be reported on the Paper Pregnancy Notification Form

immediately within 24 hours after recognition to Albireo/Safety Manager (please refer to Section 10.1.3). Date of exposure and as far as possible, details of the period of gestation at the time of exposure must be given.

The pregnancy should be followed to determine outcome, including spontaneous termination, details of birth, and presence of any birth defects, congenital anomalies or newborn or maternal complication. The infant who was exposed in-utero will be followed for up to 2 years after delivery. Individual cases with an abnormal outcome in association with the study drug should be reported on an expedited basis, i.e., reported rapidly to a competent authority.

11 STATISTICAL EVALUATION

All personnel involved with the analysis of the study will remain blinded until database lock for the double-blind Treatment Period and until major protocol violations have been identified.

All statistical analyses will be performed using SAS version 9.3 or higher.

11.1 Sample Size and Power

For the EU and RoW, the primary analysis will relate to the bile acid responder endpoint (at the 1-sided 1.25% level). For the US, the primary analysis will relate to the pruritus endpoint (at the 1-sided 1.25% level). The aim is to enroll 60 to 70 patients in order to get at least 60 evaluable patients.

Assuming 60% responders (patients experiencing at least 70% reduction in fasting s-BA concentration or reaching ≤70 µmol/L) in the A4250 dose group and 10% responders in the placebo group, approximately 20 patients per group are required to achieve a power of 84% with a 1.25% 1-sided Type-1 error.

For pruritus, with approximately 20 patients per treatment group, the power is 83% given an assumed treatment effect difference of -1.0 in change from baseline (-1.6 for A4250 and -0.6 for placebo) on the Albireo ObsRO scratching score with a SD of 0.95 and a Type I error of 1.25% (1-sided).

11.2 Statistical Methods

11.2.1 Statistical Analysis Sets

Safety Analysis Set

The safety analysis set will consist of all patients who received at least 1 dose of study drug. Patients will be analyzed according to the treatment they actually received. The safety analysis set will be the primary analysis set for safety analyses.

Full Analysis Set

The full analysis set (FAS) will consist of all randomized patients who received at least 1 dose of assigned treatment. Patients will be analyzed in the treatment group they were randomized to even if they received incorrect study drug. The FAS will be the primary analysis set for efficacy analyses.

Per Protocol Analysis Set

The per protocol (PP) analysis set is a subset of the FAS, and will consist of all randomized patients for whom no major protocol violation (no violation which may affect the study efficacy outcome) is documented. The PP analysis set will provide supportive data for the efficacy analyses.

Allocation of patients to the PP analysis set will be performed before unblinding of the study.

11.2.2 Methods of Statistical Analyses

11.2.2.1 General Principles

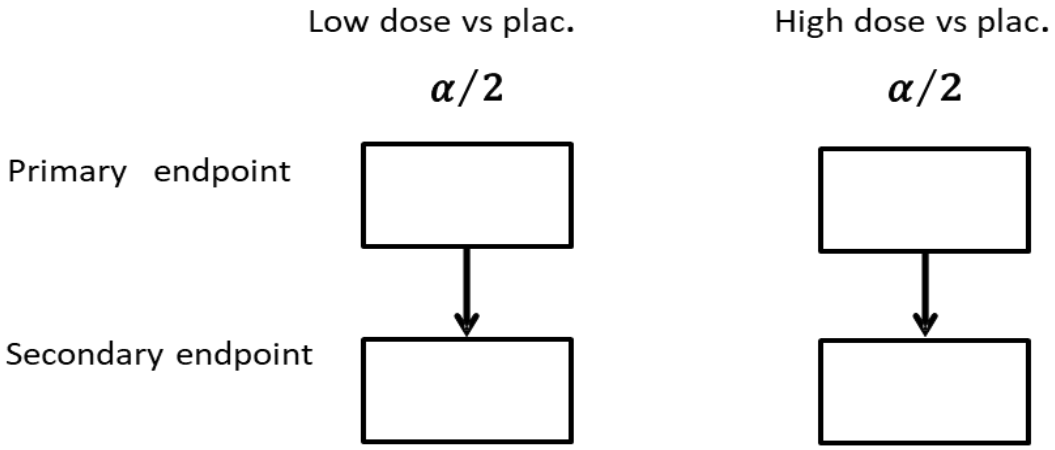
The primary objective of this study is to show superiority in efficacy of A4250 (120 µg/kg/day and 40 µg/kg/day) compared to placebo in patients with PFIC Types 1 and 2.

In the EU and RoW, for the primary efficacy variable fasting bile acid responder endpoint (at least a 70% reduction in fasting s-BA concentration or reaching a level ≤70 µmol/L at the end of treatment), the null hypothesis will be tested against the alternative hypothesis ($\alpha = 0.0125$, 1-sided).

The test will be performed at a Type I error rate of ≤0.0125 1-sided, as described above, based on the least squares means of the treatment difference in mean probability scale.

To strictly control overall Type I error rate across the dose groups and also across the primary and the main secondary endpoint to a 1-sided 2.5% level, a Bonferroni-based parallel gate-keeping procedure will be used. In this procedure the total significance level of 2.5% (1-sided) will be split equally between the dose groups. Only a dose group that is significant for the primary endpoint will be tested, at the nominal significance level of 1.25% (1-sided), for the main secondary endpoint (here the pruritus endpoint). If the previous step was not successful, the analysis of the following step will be considered descriptive. If the procedure reaches the secondary endpoint for both dose groups, these will be evaluated using the Bonferroni Holm procedure. The multiple comparison procedure is presented in Figure 2.

Figure 2 Bonferroni Holm Multiple Comparison Procedure



In the US, for the primary efficacy variable pruritus score endpoint (change from baseline over the Treatment Period in observer-reported scratching), the null hypothesis will be tested against the alternative hypothesis ($\alpha=0.0125$, 1-sided).

The test will be performed at a Type I error rate of ≤0.0125 1-sided, as described above, based on the least squares means of the treatment difference in mean change from week 7 to week 24 (i.e., the average of five 14 day post-treatment intervals).

To strictly control overall Type I error rate across the dose groups and also across the primary and the main secondary endpoint to a one-sided 2.5% level, a Bonferroni-based parallel gate-keeping procedure will be used. In this procedure the total significance level of 2.5% (1-sided) will be split equally between the dose groups. Only a dose group that is significant for the primary endpoint will be tested, at the nominal significance level of 1.25% (1-sided), for the main secondary endpoint (here the bile acid responder endpoint). If the previous step was not successful, the analysis of the following step will be considered descriptive. If the procedure reaches the secondary endpoint for both dose groups, these will be evaluated using the Bonferroni Holm procedure.

Other secondary and exploratory outcome variables shall provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple dose comparisons when testing the other secondary and exploratory efficacy variables.

A more detailed statistical analysis plan (SAP) will be prepared and finalized before study data are locked and unblinded.

If not otherwise specified, the baseline value of a parameter is defined as the last assessment of that parameter prior to the first intake of study treatment.

11.2.2.2 Missing Data

For efficacy variables, with repeated measures, a mixed model will be employed with no additional imputation for missing values, i.e., based on "observed cases", except where otherwise specified. All patients with at least one measurement of change from baseline will be included and contribute to the analysis. The mixed model will handle the missing data, but assumes Missing At Random.

Analyses to investigate the influence of missing values, for example, by using pattern mixture models using multiple imputations and tipping point analysis will be made and detailed in the SAP. The sensitivity analyses addressing the robustness of results to missing data, will consider different approaches imputing data based on Missing Not At Random. Any additional sensitivity analysis that may be done using other data imputation will also be described in the SAP.

Continued collection of efficacy data for patients who discontinue treatment will be made as far as possible.

11.2.2.3 Demographic and Baseline Characteristics

Descriptive summaries of demographics and other baseline characteristics (including medical and surgical history) will be presented by treatment group and overall using the FAS.

Prior medication will be summarized by treatment group and overall using the safety analysis set.

11.2.2.4 Subject Disposition

The following will be summarized (by treatment group and overall where applicable):

- Patients enrolled (who signed the informed consent)
- Patients randomized
- Patients treated
- Patients completing the study/withdrawing early (including withdrawal reason)

Additionally, patients enrolled, included in the safety, in the FAS, and in the PP analysis set will be summarized by region.

11.2.2.5 Evaluation of Primary Efficacy Variables

The responder endpoint based on change in fasting bile acids will be analyzed in terms of the proportion of patients achieving the event of interest. Patients in need of rescue therapy, i.e., biliary diversion will be classified as non-responders after the biliary diversion.

Generalized linear mixed modelling (GLMM) will be used with a logistic link, to allow for repeated assessments over time. The model will include baseline value, treatment group, PFIC class, age class, region, visit, and visit*treatment group interaction. The treatment by visit interaction will remain in the model regardless of significance. Region will be included in the model as a random effect. The contrasts of primary interest will be the treatment differences at Week 24; also, the difference at Week 4, Week 8, Week 12, and Week 18 will be estimated and analyzed. An unstructured covariance matrix will be used to model the within-subject error. Another covariance structure may be chosen if the fit of this structure fails to converge. Model diagnostics checks will be made to ensure appropriate fit.

The primary analysis of the change from baseline in pruritus score (indexed by observer-reported scratching) will be analyzed using a mixed model repeated measures (MMRM) analysis. The comparison of primary interest will be treatment differences over the last 5 months of the Treatment Period estimated by averaging the worst daily itch score means from five 14 day post-treatment intervals (Weeks 7 to 8, Weeks 11 to 12, Weeks 15 to 16, Weeks 19 to 20, and Weeks 23 to 24). The treatment difference at each of these post-treatment time intervals will also be analyzed. Baseline pruritus score will be included as covariate. The model will include baseline value, treatment group, PFIC class, age class, region, time, and time*treatment group interaction. The treatment by time interaction will remain in the model regardless of significance. There will be six time points defined by the last 14 days interval each month (i.e., Weeks 3 to 4, Weeks 7 to 8, Weeks 11 to 12, Weeks 15 to 16, Weeks 19 to 20, and Weeks 23 to 24), but the first of the six time points (Weeks 3 to 4) will not be part of the primary efficacy analysis. Region will be included in the model as a random effect and will be defined on country level. An unstructured covariance matrix will

be used to model the within-subject error. Another covariance structure may be chosen if the fit of this structure fails to converge.

Non-parametric approach may be considered if more appropriate.

The primary analyses will be based on the FAS.

To assess the robustness of the primary efficacy analysis, the following analyses will be performed:

- Primary efficacy analysis using the PP set
- Analyses to investigate the influence of missing values, for example, by using pattern mixture models and tipping point analysis

Subgroup analyses will also be performed for each of the 3 age groups (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) and for patients who were not included in the previous Study A4250-003.

Several sensitivity analyses will be performed, such as the following:

- The change from baseline in pruritus morning diary scores and evening diary scores, respectively, will also be analyzed using the MMRM approach.
- Cochran Mantel Haenzsel test stratified on PFIC class to compare the proportion in fasting bile acid responder endpoint at the end of treatment in the two A4250 dose groups to placebo group. Dropouts will be treated as non-responders
- Based on the primary analysis including the interaction age class*treatment
- Same as primary analysis in which the random factor region is considered as fixed factor. The region*treatment interaction will also be explored
- Same as primary analysis including the interaction PFIC*treatment

Other sensitivity analyses may be performed and will be detailed in the SAP.

11.2.2.6 Evaluation of Secondary and Exploratory Efficacy Variables

The change in the secondary and explorative endpoints such as s-BA, total bilirubin, ALT, growth and patient-reported itch severity will also be analyzed using the MMRM model as specified for the primary analysis. Nonparametric methods may be applied as an alternative approach for some endpoints, if appropriate. Change in growth (weight for age and BMI for age) will also be displayed using graphical presentations.

Proportions of responders (e.g., meaningful reduction in itch severity and scratching at Week 24) will be analyzed using the GLMM model as specified for the primary analysis.

Comparisons of the change from baseline at Week 24 in PedsQL between the treatment groups will be conducted using analysis of covariance. The model will include terms for baseline, PFIC class, age class, region, and treatment.

Undergoing biliary diversion surgery and/or listed for liver transplant, all-cause mortality, and liver decompensation events will mainly be analyzed by using descriptive statistics. Kaplan-Meier curves will be used when appropriate for time to event data.

Other exploratory variables (Global Symptom Relief, biochemical markers and measures of bile acid synthesis, Albireo PRO and ObsRO sleep parameters, PELD/MELD score, APRI score, and Fib-4 score) will be analyzed descriptively for categorical and continuous data, as applicable. For continuous data, the change from baseline will be analyzed in addition to the actual visit values. For categorical data, shift tables or frequency and percentages of patients will be presented as appropriate.

In addition, subgroup analyses will be performed for each of the 3 age classes (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years).

11.2.2.7 Evaluation of Safety Variables

Safety data will be analyzed using descriptive statistics and summaries by treatment group of SAEs, AEs, vital signs, clinical laboratory tests (hematology, clinical chemistry and urinalysis), and concomitant medication. Analyses will be performed using the safety analysis set.

Summaries of AEs (coded according to the Medical Dictionary for Regulatory Activities [MedDRA] system organ class [SOC] and MedDRA preferred term) will include the following:

- Overview of the incidence of TEAEs (TEAEs, drug-related TEAEs, TEAEs leading to study discontinuation, and treatment-emergent SAEs)
- TEAEs by SOC and preferred term
- Intensity of TEAEs by SOC and preferred term
- Drug-related TEAEs by SOC and preferred term
- TEAEs leading to study discontinuation by SOC and preferred term
- Treatment-emergent SAEs by SOC and preferred term

Concomitant medication use during the Treatment Period will be summarized by Anatomical Therapeutic Chemical (ATC) class and World Health Organization (WHO) preferred name.

Summaries of vital signs will be presented. For each visit, the actual results and the change from baseline, and the number and percentage of patients with potentially clinically significant (PCS) values observed post-baseline will be presented.

Summaries of clinical safety laboratory data will be presented. For each visit, the actual result and the change from baseline, and the number and percentage of patients with PCS values observed post-baseline will be presented.

Data listings will be provided for each patient for all safety parameters.

11.2.2.8 Compliance and Exposure

Exposure will be analyzed by calculating the number of days with exposure to study drug. Results will be presented by treatment group using the safety analysis set.

The percentage compliance will be described by treatment group and the number of patients with a compliance <80%, between 80% and 120%, and >120% will be presented based on the safety analysis set.

11.2.3 Blinded Analyses to Confirm Responder Definitions

A blinded analysis of Albireo ObsRO and PRO eDiary data will be performed after 50% or more of the planned sample has completed the Week 24 Follow-Up Period. The blinded analysis will be used to estimate a threshold of clinically meaningful change (i.e., responder definition) in Albireo ObsRO and PRO pruritus scores. The analysis will be performed by a group independent from both the study team and Albireo. Blinded data will be used for this analysis; data will be collapsed across treatment groups.

The analyses will include distribution- and anchor-based analyses to examine a responder definition for the Albireo PRO/ObsRO pruritus scores. Distribution-based analyses will include calculation of the SD and standard error of measurement for the baseline Albireo PRO and ObsRO scores. Anchor-based analyses will involve examining the degree of change on the Albireo PRO and ObsRO scores from baseline to Week 24 for patients who experience change in pruritus according to PGIC and symptom items. Details of this analysis will be included in a stand-alone SAP for assessing the measurement characteristics (reliability, validity, sensitivity to change, responder definition) of the Albireo PRO and ObsRO items.

11.2.4 Data Safety Monitoring Board

A DSMB consisting of sponsor-independent clinical and statistical experts will be established. The DSMB will periodically meet for the review of accumulating study data, including safety (AE and laboratory data) and liver values.

The DSMB will have access to unblinded data. The DSMB will make recommendations for the remaining part of the study (further details will be provided in the DSMB charter). The DSMB may recommend continuing with the study as planned or stopping the study early for safety reasons. The DSMB will submit its recommendations in writing to Albireo Medical/[redacted] who are responsible for responding to the recommendations of the DSMB and taking appropriate action. The investigators will only be informed by Albireo Medical/[redacted] if the study requires a protocol amendment or is stopped. The DSMB may choose to make additional evaluations at any time if they feel this is warranted from a safety point of view.

The DSMB will act according to its own written standard operating procedure described in a charter and will prepare written minutes of its meetings. The charter of the DSMB will be stored in the trial master file. The DSMB will maintain records of its meetings and these will become part of the study file when the study is complete. In order not to disseminate

unblinded data and to ensure that all staff involved in the conduct and final analysis of the study remain blind to the data, only the members of the DSMB and the unblinded DSMB statistician will have access to these data.

12 DIRECT ACCESS TO SOURCE DATA/NOTES

The investigator/institution shall provide direct access to source data/documents for study-related monitoring, audits, IEC/Institutional Review Board (IRB) review and regulatory inspection.

13 QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Conduct of the Study

Albireo/[redacted] shall implement and maintain quality control and quality assurance procedures with written standard operating procedures to ensure that the study is conducted and data are generated, documented, and reported in compliance with the protocol, ICH GCP and applicable regulatory requirements.

This study shall be conducted in accordance with the provisions of the Declaration of Helsinki (October 1996) and all revisions thereof, and in accordance with United States Food and Drug Administration (FDA) regulations (Code of Federal Regulations, Sections 312.50 and 312.56) and with ICH GCP (CPMP 135/95).

The investigator may not deviate from the protocol without a formal protocol amendment having been established and approved by an appropriate competent authority and IEC/IRB, except when necessary to eliminate immediate hazards to the patient or when the change(s) involve(s) only logistical or administrative aspects of the study. Any deviations may result in the patient having to be withdrawn from the study and render that patient non-evaluable.

13.2 Study Monitoring

Before study initiation, at a site initiation visit or at an investigator's meeting, an Albireo representative or designee will review the protocol and eCRF with the investigators and the investigative staff. During the study, the clinical monitor (clinical research associate [CRA]) will visit the site regularly to check the completeness of patient records, the accuracy of entries on the eCRFs, the adherence to the protocol and to GCP, the progress of enrollment, and to ensure that study drug is being stored, dispensed, and accounted for according to specifications.

The investigator must ensure that eCRFs are completed within a timely period of patient visits, as per individual site agreements, and must allow the CRA and Albireo representative or designee periodic access to patient records and all study-related materials, including relevant hospital or clinical records, to confirm their consistency with the eCRF entries. No information in these records about the identity of the patients will leave the study center. Albireo monitoring standards require full verification for the presence of the signed ICF, adherence to the inclusion/exclusion criteria, documentation of SAEs, and recording of primary efficacy and safety variables. The CRA will review source data compared with the eCRFs and will verify source data according to the study-specific monitoring plan. The design of the study, the frequency of patient visits, and the site enrollment rate will determine the frequency of monitoring visits. Upon study completion, the CRA will visit the site to conduct a study termination visit, which will include collection of any outstanding documentation.

It is recommended that the investigator/investigative staff log into the eCRF system every working day or at minimum twice weekly in order to provide a prompt response to queries.

The investigator/investigative staff should respond to queries and make any relevant changes to the study data within 3 working days.

14 ETHICS

14.1 Independent Ethics Committee/Institutional Review Board

Prior to the start of the study, the investigator is responsible for ensuring that the protocol and consent form have been reviewed and approved by a relevant IEC/IRB. The IEC/IRB shall be appropriately constituted and perform its functions in accordance with FDA ICH GCP and local requirements as applicable.

The IEC/IRB shall approve all protocol amendments (except for logistical or administrative changes), written informed consent documents and document updates, patient recruitment procedures (e.g., advertisements), written information to be provided to the patients and caregivers, IB, available safety information, information about payment and compensation available to patients, the investigator's curriculum vitae and/or other evidence of qualifications and any other documents requested by the IEC/IRB and regulatory authority (competent authority) as applicable.

14.2 Written Informed Consents and Assents

The investigator (physician) or investigative staff, in accordance with local regulations, will explain to each patient (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, alternative treatment, the potential risks and benefits involved, and any discomfort that may occur. Each caregiver/patient will be informed that participation in the study is voluntary and that he/she or their child may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

Caregivers/patients will be informed that they/their children may experience side-effects or be at risk for symptoms, illnesses, or complications that cannot be foreseen by Albireo. As with other medications, people treated with A4250 may be at risk of developing allergic reactions or anaphylaxis. Caregivers/patients will be advised that study procedures include regular blood sampling for measurement of safety parameters and biological markers and that some minor risks are associated with these procedures.

This informed consent should be given by means of a signed ICF, written in non-technical language in accordance with ICH GCP, the Declaration of Helsinki, and regulatory authorities. The caregiver(s)/patient should read and consider the statements before signing and dating them and should be given a copy of each signed document. If written consent is not possible, oral consent can be obtained if witnessed and followed by a signed statement from one or more persons not involved in the study, indicating why the patient was unable to sign the form. No patient can enter the study before his/her or caregiver(s) informed consent has been obtained, as required by country regulations.

The ICF is part of the protocol and must be submitted by the investigator/investigative staff with the protocol for IEC approval. Albireo will supply an ICF which complies with regulatory requirements and country laws, and is considered appropriate for the study. Any

changes to the ICF suggested by the investigator must be agreed to by Albireo before submission to the IEC and a copy of the approved version must be provided to the clinical monitor after IEC approval.

15 DATA HANDLING AND RECORD KEEPING

15.1 Case Report Forms/Source Data Handling

The investigator shall be provided with standardized eCRFs and shall ensure that all data from patient visits are promptly entered into the eCRFs in accordance with the specific instructions given. The investigator must sign each eCRF to verify the integrity of the data recorded.

A list of the normal ranges for all laboratory tests to be undertaken forms part of the documentation to be collated prior to study start. As a central laboratory has been selected to conduct any or all tests, it is essential that all samples be analyzed at that laboratory.

The investigator must maintain source documents such as laboratory reports, consultation reports, and complete medical history and physical examination reports.

As no ethnicity data can be collected in the source document for study sites in France, the eCRF will be the Source Document for “origines ethniques”,

15.2 Retention of Essential Documents

Essential documents, as defined by ICH GCP, include: the signed protocol and any amendment(s); copies of the completed eCRFs (for site archiving, compact discs of eCRF data for specific patients will be provided); signed ICFs; hospital records and other source documents; IEC approvals and all related correspondence including approved documents; drug accountability records; study correspondence; and a list of patients’ names and addresses.

The investigator/investigative staff must retain copies of these essential documents for the period specified by ICH GCP and by applicable regulatory requirements. The investigator/investigative staff will inform Albireo of the location where the essential documents are stored and must contact Albireo for approval before disposing of any essential documents. The investigator/investigative staff should take measures to prevent accidental or premature destruction of these documents.

16 FINANCING AND INSURANCE

Financing and insurance are addressed in a separate agreement.

17 PUBLICATION POLICY

Albireo will retain the ownership of all data. When the study is complete, Albireo shall arrange the analysis, tabulation of data and preparation of a clinical study report. Albireo may also use the data for publication, presentation at scientific meetings and submission to regulatory authorities. All proposed publications based on this study must be subject to the sponsor's approval requirements.

18 REFERENCE LIST

1. A4250 Investigator's Brochure.
2. Alissa FT, Jaffe R, Shneider BL. Update on progressive familial intrahepatic cholestasis. J Pediatr Gastroenterol Nutr. 2008;46(3):241-52.
3. Davit-Spraul A, Gonzales E, Baussan C, et al. Progressive familial intrahepatic cholestasis. Orphanet J Rare Disease 2009;4:1-12.
4. Hofmann AF. The enterohepatic circulation of bile acids in mammals: form and functions. Front Biosci (Landmark Ed). 2009 Jan 1;14:2584-98.
5. Hori T, Justin H, Nguyen JH and Uemoto S. Progressive familial intrahepatic cholestasis. Hepatobiliary pancreat Dis Int. 2010; 9(6):570-8.
6. Jacquemin E. Progressive familial intrahepatic cholestasis. Genetic basis and treatment. Clin Liver Dis. 2000;4(4):753-63.
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9. Pawlikowska L, Strautnieks S, Jankowska I, et al. Differences in presentation and progression between severe FIC1 and BSEP deficiencies. J Hepatol. 2010;53(1):170-8.
10. Shneider BL, Magee JC, Bezerra JA, et al. Efficacy of fat-soluble vitamin supplementation in infants with biliary atresia. Pediatrics, 2012;130(3):e607-14.
11. Suchy FJ, Sokol RJ, Balistreri WF, eds. Liver Disease in Children. 3rd Edition. New York, NY: Cambridge University Press; 2007:310–4.
12. Venkat VL, Shneider BL, Magee JC, et al. Total Serum Bilirubin Predicts Fat-Soluble Vitamin Deficiency Better Than Serum Bile Acids in Infants with Biliary Atresia. J Pediatr Gastroenterol Nutr. 2014;59(6):702-7.
13. Whitington PF, Whitington GL. Partial external diversion of bile for the treatment of intractable pruritus associated with intrahepatic cholestasis. Gastroenterology. 1988;95(1):130-6.

19 APPENDICES

Appendix 1 Concomitant Medication Guidelines

Appendix 2 Diary Questions including Albireo Patient-Reported Outcomes/Observer-Reported Outcome Instrument

Appendix 3 Patient Global Impression of Change (PGIC) and Patient Global Impression of Symptoms (PGIS)

Appendix 4 Pediatric Quality of Life Inventory (PedQL) Questionnaire

Appendix 5 Guideline for Fat-Soluble Vitamin Supplementation

Appendix 6 Contraceptive Requirements

Appendix 7 Blood Volumes

CLINICAL STUDY PROTOCOL A4250-005

A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

IND Number:	130591
EudraCT Number	2017-002338-21
Test Product:	A4250
Indication:	Progressive Familial Intrahepatic Cholestasis (PFIC)
Sponsor:	Albireo AB
Development Phase:	Phase 3
Sponsor Signatory:	[redacted]
Sponsor Medical Monitor:	[redacted]
Principal Investigator:	[redacted]
Date of the Protocol:	15 March 2018
Version of the Protocol:	Final Amendment 1.3 Canada

Confidential

The information in this document contains trade secrets and commercial information that are privileged or confidential and may not be used, divulged, published, or otherwise disclosed without the written consent of Albireo AB. These restrictions on disclosure apply equally to all future information supplied to you or generated by you in connection with the study.

SPONSOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

Albireo AB

[redacted]

15-March-2018
Date (day/month/year)

CLINICAL RESEARCH ORGANIZATION SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

[redacted]

[redacted] Date (day/month/year)

[redacted] Date (day/month/year)

INVESTIGATOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

I have read this protocol and agree that it contains all necessary details for performing this study. I will conduct the study as outlined herein and will complete the study within the time designated, in accordance with all stipulations of the protocol and in accordance with Good Clinical Practice (GCP), local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study drug and the conduct of the study.

I will use only the informed consent approved by the Independent Ethics Committee (IEC) and will fulfil all responsibilities for submitting pertinent information to the IEC responsible for this study.

I agree that the sponsor (Albireo AB) shall have access to any source documents from which case report form information may have been generated.

I further agree not to originate or use the name of Albireo AB, A4250 in any publicity, news release, or other public announcement, written or oral, whether to the public, press, or otherwise, relating to this protocol, to any amendment to the protocol, or to the performance of this protocol without the prior written consent of Albireo AB.

Name of Investigator

Signature

Date (day/month/year)

1 ADMINISTRATIVE INFORMATION

A Double-blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Protocol No.:	A4250-005
Date of the initial Protocol:	20 September 2017
Date and Number of Amendment(s):	Amendment 01, 06 December 2017 Amendment 1.3 Canada, 15 March 2018
Sponsor:	Albireo AB Arvid Wallgrens backe 20 413 46 Göteborg Sweden
Clinical Research Organization:	[redacted]
Sponsor Signatory:	
Sponsor Medical Monitor:	
Principal Investigator:	

2 STUDY SYNOPSIS

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250

Title of Study:
A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Principal Investigator:
[redacted]

Study Centers:
Up to 50 sites will be initiated for this study in the United States (US), Canada, European Union (EU), Middle East, and Australia.

Publication(s):
None.

Planned Study Period:	**Development Phase:**
Q1 2018 to Q2 2019	Phase 3

Objectives:

Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with progressive familial intrahepatic cholestasis (PFIC) Types 1 and 2, as determined by the following:

- Proportion of patients experiencing at least a 70% reduction in fasting serum bile acid (s-BA) concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- Change from baseline to Week 24 in pruritus compared to placebo

Secondary Objectives

- To evaluate the effect of A4250 on serum alanine aminotransferase (ALT) concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

Methodology:

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of familial intrahepatic cholestasis-1 or bile salt export pump).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-up Period. After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo. Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008) in which all patients will receive active treatment.

There will be up to 10 clinic visits during the study and one scheduled telephone contact.

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250

Number of Patients:
Approximately 60 patients diagnosed with PFIC-1 or PFIC-2 will be randomized.

Diagnosis and Main Criteria for Inclusion:

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples 7 to 28 days apart (Visits 1 and 2) prior to randomization
4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the electronic diary (eDiary) average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients are expected to have a consistent caregiver(s) for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

Test Product, Dose and Mode of Administration:
A4250, 40 µg/kg/day or 120 µg/kg/day, orally administered

Reference Therapy, Dose and Duration of Administration:
Matching placebo, orally administered

Duration of Treatment:
24 weeks

Variables

Efficacy:

Primary Efficacy Endpoints

The primary efficacy endpoints are:

- **EU and rest of world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  Fasting s-BA baseline will be calculated as the average of the value at randomization and the previous visit (Clinic Visit 2). The end value is the average of the values at Weeks 22 and 24 after the start of double-blind treatment.
- **US**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 24 as measured by the worst scratch of the Albireo observer-reported outcome (ObsRO) instrument. Scores for the 14 days baseline and post-treatment intervals will be calculated by averaging the worst scratching scores from each day in the interval.

Secondary Efficacy Endpoints

Secondary efficacy endpoints include the following:

- **EU and RoW**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 24 as measured by the worst scratch of the Albireo ObsRO instrument. Scores for the 14 days baseline and post-treatment intervals will be calculated by averaging the worst scratching scores from each day in the interval.
- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  All Regions:

  All secondary endpoints will be compared to placebo.

Name of Sponsor/Company:	Name of Product:	Name of Active Ingredient:
Albireo	A4250	A4250

- Change from baseline to Week 24 in fasting s-BA
- Change from baseline to Week 24 in serum ALT concentration
- Change in growth from baseline to Week 24, defined as the linear deficit (weight for age and body mass index) compared to the national growth curves (Z-score, standard deviation from P50)
- Proportion of patients achieving meaningful reduction in caregiver-reported observed scratching at Week 24
- Change from baseline to Week 24 in sleep parameters measured with the Albireo patient-reported outcome (PRO) and ObsRO instruments
- Change from baseline to Week 24 in patient-reported itch severity as measured by the average daily score of the Albireo PRO instrument; only patients ≥8 years of age will complete the Albireo PRO instrument
- Number of patients undergoing biliary diversion surgery or being listed for liver transplantation

Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Proportion of patients achieving meaningful reduction in patient-reported itch severity at Week 24
- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the patient global impression of change (PGIC) items
- Change from baseline in fasting s-BA at Weeks 4 and 12
- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Weeks 4, 12, and 24 as measured by the worst scratch. Scores for the 7 worst scratching days of the last 14 days at baseline and post-treatment intervals will be selected
- Change from baseline in patient-reported itch severity and observer-reported scratching at Weeks 4 and 12
- Change from baseline to Week 24 in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline to Week 24 in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline to Week 24 in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in Pediatric Quality of Life Inventory (PedsQL)
- Change from baseline in serum ALT concentration at Weeks 4 and 12
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, plasma 7α-hydroxy-4-cholesten-3-one concentration [p-C4]) at Week 24
- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events.
- All-cause mortality

Safety:

Safety criteria are as follows:

- Occurrence of treatment-emergent adverse events including severity and relatedness to study drug at all visits
- The incidence of treatment-emergent serious adverse events (SAEs), based upon information from patient reports, including the description, incidence, and severity of an SAE
- Trends evaluated for the following assessments: physical examinations, concomitant medications, vital signs, laboratory test results (including clinical chemistry, hematology, and urinalysis), and imaging

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250
studies		

Statistical Methods:

The primary objective of this study is to show superiority in efficacy of A4250 (120 µg/kg/day and 40 µg/kg/day) compared to placebo in patients with PFIC Types 1 and 2.

In the EU and RoW, for the primary efficacy variable fasting bile acid responder endpoint (at least a 70% reduction in fasting s-BA concentration or reaching a level ≤70 µmol/L at the end of treatment), the null hypothesis will be tested against the alternative hypothesis (α=0.0125, 1-sided). Generalized linear mixed modelling will be used with a logistic link, to allow for repeated assessments over time. The model will include baseline value, treatment group, PFIC class, age class, region, visit and visit*treatment group interaction. The contrasts of primary interest will be the treatment difference at Week 24 but also the difference at Week 4, Week 8, Week 12, and Week 18 will be estimated and analyzed.

In the US, for the primary efficacy variable pruritus score endpoint (change from baseline to Week 24 in observer-reported scratching), the null hypothesis will be tested against the alternative hypothesis (α=0.0125, 1-sided). The primary analysis of the change from baseline in pruritus score (indexed by observer-reported scratching) will be analyzed using a mixed model repeated measures analysis. The contrasts of primary interest will be the treatment difference at Week 24 but also the difference at the other weeks will be estimated and analyzed.

To strictly control overall Type I error rate across the dose groups and also across the primary and the main secondary endpoint to a 1-sided 2.5% level, a Bonferroni-based parallel gate-keeping procedure will be used.

Other secondary and exploratory outcome variables shall provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple-dose comparisons when testing the other secondary and exploratory efficacy variables.

Date of the Protocol: 15 March 2018

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3.1 List of Tables

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4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADME	absorption, distribution, metabolism, and excretion
AE(s)	adverse event(s)
AFP	alfa-fetoprotein
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APRI	AST to platelet ratio index
ASBT	apical sodium-dependent bile transporter (also known as IBAT)
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	body mass index
BSEP	bile salt export pump
CPK	creatine phosphokinase
CRA	clinical research associate
CYP	cytochrome P450
DSMB	Data and Safety Monitoring Board
eCRF	electronic case report form
eDiary	electronic diary
EOT	End of Treatment
EU	European Union
EudraCT	European Union drug regulatory agency clinical trial
FAS	full analysis set
FDA	United States Food and Drug Administration
FIC1	familial intrahepatic cholestasis-1
GCP	Good Clinical Practice
GI	gastrointestinal
GLMM	generalized linear mixed modelling
IB	Investigator's Brochure
IBAT	ileal bile acid transporter (also known as ASBT)
ICF	informed consent form
ICH	International Council for Harmonisation
[redacted]	[redacted]
IEC	Independent Ethics Committee
INR	international normalized ratio
IRB	Institutional Review Board
IWRS	Interactive Web Response System

LDH	lactate dehydrogenase
LFT	liver function test
MDR3	multidrug-resistance protein 3
MedDRA	Medical Dictionary for Regulatory Activities
MELD	model for end-stage liver disease
MMRM	mixed model repeated measures
ObsRO	observer-reported outcome
PBC	primary biliary cholangitis
p-C4	plasma 7α-hydroxy-4-cholesten-3-one concentration
PCS	potentially clinically significant
PedsQL	Pediatric Quality of Life Inventory
PELD	pediatric end-stage liver disease
PFIC	progressive familial intrahepatic cholestasis
PGIC	patient global impression of change
PGIS	patient global impression of symptoms
PP	per protocol
PRO	patient-reported outcome
PT	prothrombin time
PVM	pharmacovigilance manager
QoL	quality of life
RoW	rest of world
RR	respiratory rate
SAE(s)	serious adverse event(s)
SAP	statistical analysis plan
s-BA	serum bile acid
SD	standard deviation
s-FGF19	serum fibroblast growth factor 19 concentration
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction
TEAE(s)	treatment-emergent adverse event(s)
ULN	upper limit of normal
US	United States
WHO	World Health Organization

5 INTRODUCTION

5.1 Investigational Medicinal Product

A4250 is a small molecule and a potent selective inhibitor of the ileal bile acid transporter (IBAT), also known as the apical sodium-dependent bile acid transporter (ASBT). IBAT is an integral brush border membrane glycoprotein that co-transports sodium and bile acids and appears to be a critical component in the regulation of the bile acid pool size in animals and man. This transporter, expressed in the distal ileum, is a key element in the enterohepatic circulation of bile acids since it facilitates the high-affinity, high-capacity reabsorption of bile acids. Indeed, 95% of secreted bile acids are reabsorbed via IBAT [Hofmann 2009; Miethke 2016]. A4250 is orally administered and acts locally in the gut where it binds reversibly to IBAT to decrease the reuptake of bile acids. A4250 has minimal systemic exposure at the predicted therapeutic dose ranges.

No safety signals were found with A4250 in preclinical safety and toxicity studies. In the first clinical study of A4250 in healthy volunteers, A4250 was given as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. A4250 was well tolerated and the only findings were gastrointestinal (GI) related effects, such as diarrhea, at higher doses.

In an exploratory Phase 2 study in children with cholestatic pruritus, A4250 was given as oral doses of 10 to 200 µg/kg/day for 4 weeks. A4250 reduced mean levels of serum bile acids (s-BA) in all cohorts. In 11 of 13 progressive familial intrahepatic cholestasis (PFIC) patients, substantial reductions (ranging from 43% to 98%) were observed. Patient-reported diary data documented improvement of pruritus during treatment. A4250 was well tolerated. No serious adverse events (SAEs) were deemed to be related to study drug, and most adverse events (AEs), including some increased transaminases, were considered unrelated to study drug and transient in nature.

5.2 Background

5.2.1 Progressive Familial Intrahepatic Cholestasis

PFIC is a rare autosomal recessive cholestatic liver disease estimated to affect between 1 in every 50,000 to 100,000 children born worldwide. PFIC represents 10% to 15% of causes of cholestasis in children and 10% to 15% of liver transplantation indications in children. All types of PFIC exist worldwide and both sexes appear to be equally affected.

The common underlying pathogenesis of PFIC is disruption of bile formation and bile transport through the liver [Jacquemin 2000]. The classification of PFIC has evolved over the years. The most commonly used sub-classifications are PFIC Types 1, 2, and 3, which are based on the associated affected gene and described in more detail below.

- PFIC, Type 1: also referred to as “Byler disease” or “familial intrahepatic cholestasis-1 (FIC1) protein deficiency.” FIC1 protein is located on the canalicular membrane of hepatocytes and facilitates movement of aminophospholipids from the outer

to inner leaflet of the plasma membrane of the hepatocyte. The *ATP8B1* gene encodes FIC1 protein. Biallelic pathologic variants in the *ATP8B1* gene are associated with FIC1 dysfunction and classified clinically as PFIC Type 1 disease.

- PFIC, Type 2: also referred to as "Byler syndrome" or "bile salt export pump (BSEP) deficiency." BSEP is a transporter protein that is expressed at the canalicular membrane of hepatocytes, and is the primary exporter of bile acids. The *ABCB11* gene encodes the BSEP protein. Biallelic pathologic variations in the *ABCB11* gene are associated with BSEP dysfunction and classified clinically as PFIC Type 2 disease.
- PFIC, Type 3: is caused by a deficiency of the multidrug-resistance protein 3 (MDR3) due to mutations in the *ABCB4* gene. MDR3 is a phospholipid translocase critical for phospholipid secretion.

Severe pruritus is common in children diagnosed with PFIC. Itching (and subsequent scratching) is a significant morbidity for these patients and their families [Suchy 2007]. A more severe degree of pruritus is experienced compared to patients with other forms of liver disease and to other pruritic conditions such as atopic dermatitis [Murray 2011]. In patients with PFIC, liver biopsy reveals canalicular cholestasis and, later, the appearance of portal fibrosis. Serum biochemistry indicates cholestasis with hyperbilirubinemia, elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The concentrations of bile acids in serum are very high, while serum gamma-glutamyl transferase activity (exception being MDR3 variants) and cholesterol are normal [Hori 2010]. Symptoms of portal hypertension and liver failure will develop during the course of the disease [Davit-Spraul 2009; Alissa 2008]. Symptoms develop early; median age at onset of symptoms is 2 months, and 78% of PFIC patients present with jaundice [Pawlikowska 2010]. The life-threatening and debilitating nature of PFIC is reflected by the fact that survival in those not resorting to surgery is 50% at 10 years of age and almost zero at 20 years of age. Approximately half of PFIC patients undergo liver transplantation [Davit-Spraul 2009] and treatment-resistant pruritus is the leading indication for the surgical procedure partial external biliary diversion, mostly in PFIC Type 1 and 2 patients.

PFIC is life-threatening and debilitating. Currently, the treatment for PFIC is palliative and there is no pharmaceutical treatment approved for use in PFIC. The therapeutic choices are restricted to non-specific therapy of the symptoms and signs of the disease such as nutritional support, preventing vitamin deficiencies, and treatment of extrahepatic features. Medical treatment options include off-label use of ursodeoxycholic acid, rifampin, antihistamines, and naltrexone. A minority of patients respond nominally and transiently to these interventions. Biliary diversion is used to decrease systemic bile acids through interruption of the enterohepatic circulation and so avoid transplantation. Liver transplantation is typically only viewed as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

5.2.2 Summary of Clinical and Nonclinical Studies

This is a summary of nonclinical and clinical studies. More detail is provided in the Investigator's Brochure ([IB] see A4250 IB).

A4250 is a potent, selective IBAT inhibitor with no safety signals seen in the safety pharmacological studies. In addition, no safety signals were seen in repeat-dose oral toxicity studies in rats with up to 26 weeks of daily dosing and in dogs with up to 39 weeks of daily dosing. No safety signals were identified when A4250 was administered daily to juvenile rats from age 14 days to 63 days.

A4250 is minimally absorbed with very low systemic exposure in both humans and other species. In blood, protein binding is high (>99.7%). Systemically absorbed A4250 is slowly metabolized in humans and in preclinical studies by hydroxylation only, a metabolite that retains IBAT activity. A4250 did not show any significant inhibition of cytochrome P450 (CYP) enzymes in the rat. A4250 inhibited human CYP3A4 and CYP2D6 (IC_{50}: 16 µmol/L for both enzymes), and CYP2C9 (IC_{50}:1.2 µmol/L).

To date, A4250 has been studied in 3 Albireo-sponsored clinical studies: a study in healthy volunteers evaluating single and multiple administration of A4250; a single-dose absorption, distribution, metabolism, and excretion (ADME) study; and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with primary biliary cholangitis (PBC). In total, 98 subjects have been exposed to A4250.

In the first clinical study (A4250-001), A4250 was given to healthy volunteers as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. Following a single oral dose of 10 mg, the median maximum drug concentration was 0.2 nmol/L and the area under the curve was 0.8 nmol × h/L (n=6). Pharmacodynamic evaluation of bile acid synthesis biomarkers (serum fibroblast growth factor 19 [s-FGF19] and plasma 7α Hydroxy 4-cholesten-3-one [p-C4]) identified the expected decrease in s-FGF19 and an increase in p-C4. In addition, total plasma bile acids decreased and total fecal bile acids increased in A4250-treated patients compared to placebo. A4250 was generally well tolerated. The only findings were GI-related effects, such as diarrhea at higher doses.

In the ADME study (A4250-007), healthy subjects were administered a single oral dose of 3 mg [^{14}C]-A4250 capsule. The main excretion route was determined to be fecal. There were no quantifiable concentrations of A4250 in plasma or total radioactivity in plasma or whole blood. A4250 was well tolerated. AEs were reported and most were mild and not related to A4250. The 3 AEs experienced by 2 subjects reported as related to study drug were GI in nature, including diarrhea (2 AEs; 2 subjects) and abdominal pain (1 AE; 1 subject).

In an exploratory Phase 2 study in children with cholestatic pruritus (A4250-003), A4250 was administered as oral daily doses of 0.01 to 0.2 mg/kg for 4 weeks. Twenty patients (8 females and 12 males) aged 1 to 17 years were enrolled in this open-label dose-escalation study. Four patients were re-enrolled into a second dose level. No

treatment-related SAEs were reported, and most AEs, including some increased transaminases, were transient. Four-week, once-daily treatment with A4250 reduced mean levels of s-BA in all cohorts. Substantial s-BA reductions in 11 of 13 PFIC patients (ranging from 43% to 98%) were observed. A4250 also reduced s-BA in the 11 non-PFIC patients, but to a lesser extent than in PFIC patients. Patient-reported diaries documented improvement of the visual analogue scale itch score in 17 of 24 patients during treatment. The dose with the greatest improvement showed a mean decrease (± standard error of the mean) of 2.9 ±1.1 from baseline. The results from this study form the basis of the current (A4250-005) study design.

5.3 Rationale

In this investigational study, A4250 is under development for treatment of PFIC and is also being considered for other orphan cholestatic liver diseases.

There is currently no medical treatment approved for PFIC. In PFIC patients with severe pruritus, biliary diversion surgery is used to decrease systemic bile acids through interruption of the enterohepatic circulation and thereby reducing pruritus [Whitington 1988]. Liver transplantation is typically used as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

By inhibiting IBAT with high selectivity and potency, A4250 has the potential to relieve cholestasis and improve liver function without surgical intervention in patients with PFIC. Since A4250 is minimally absorbed and achieves a substantial reduction of s-BA, it has the potential to be a safer and less invasive alternative to surgical biliary drainage. The rationale for using A4250 is to decrease s-BA levels, and to reduce the major morbidity of pruritus, improving the health and wellbeing of patients affected with PFIC. Ultimately, this noninvasive therapeutic approach has the potential to not only improve quality of life (QoL), but also reduce the need for biliary diversion and subsequent liver failure and liver transplantation.

The doses selected, 40 and 120 µg/kg/day, were based on the efficacy and safety data generated from patients with PFIC (n=10 + 3 re-entered) and supporting data from all patients (n=20 + 4 re-entered) in the five dose groups dosed in the A4250-003 study. Improvements in s-BA and pruritus were observed with doses from 30 µg/kg to 100 µg/kg, with a dose-related trend. The best dose response in all patients was at the 60 µg/kg/day for s-BA and 100 µg/kg/day for pruritus. The best dose response in the PFIC subgroup was at 30 µg/kg/day for both s-BA and pruritus. Patients with PFIC responded well to the doses given from 30 µg/kg to 200 µg/kg; however, a clear dose relationship could not be established in order to select a single dose for this Phase 3 study. As efficacy was observed at both low and high doses, 40 µg/kg and 120 µg/kg/day are considered to be the most optimal for this study.

This study will be conducted in compliance with the protocol and with the International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP).

Patients completing 24 weeks of double-blind treatment will be offered to enter Study A4250-008 and receive open-label treatment with A4250.

5.3.1 Risk/Benefit

A4250 has been evaluated in three Albireo-sponsored clinical studies: a double-blind placebo-controlled study in healthy volunteers, a single-dose ADME study, and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with PBC.

A total of 98 subjects/patients have been exposed to A4250. Healthy subjects have been exposed to up to 10 mg as a single dose and up to 3 mg daily as part of a multiple-dose evaluation. Children with cholestatic liver disease have been treated with up to 0.2 mg/kg/day (200 µg/kg/day) for 4 weeks. In total, 2 SAEs have been reported; both were judged by the physician to be not related to the study drug.

Patients with cholestatic liver diseases suffer from excess bile acids in the liver resulting in tissue damage. A commonly used treatment in these patients is bile diversion surgery, whereby approximately 50% to 100% of the enterohepatic circulation of bile acids is interrupted. Inhibition of IBAT with A4250, thereby interrupting the enterohepatic circulation of bile acids, is therefore a potential medical alternative to surgery which could be of benefit to these patients if shown to be effective and safe. Data from the Phase 2 study showed efficacy of A4250 in reducing s-BA concentrations and pruritus in such patients.

Based on the mode of action of an IBAT inhibitor, loose stools or diarrhea could be expected. However, in the pediatric Phase 2 study with 4 weeks daily treatment, only one patient had mild transient diarrhea after a single dose that did not recur on multiple dosing.

6 STUDY OBJECTIVES

6.1 Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with PFIC Types 1 and 2, as determined by the following:

- The proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- Change from baseline to Week 24 in pruritus compared to placebo

6.2 Secondary Objectives

- To evaluate the effect of A4250 on serum ALT concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

7 INVESTIGATIONAL PLAN

7.1 Overall Study Design and Plan

7.1.1 Description

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of FIC1 or BSEP).

Study data will be periodically reviewed by an unblinded Data Safety and Monitoring Board (DSMB).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-Up Period.

After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo.

Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008) in which all patients will receive active treatment. A patient who prematurely withdraws due to no improvement/intolerable symptoms, and has completed at least 12 weeks of the Treatment Period and End of Treatment (EOT) Visit, will also be offered the opportunity to enter Study A4250-008.

Visit windows applicable to each visit are presented in the schedule of assessments (Table 1). There will be up to 10 clinic visits during the study (Figure 1) and one scheduled telephone contact, as follows:

- Visit 1: Screening Period start. Patient is considered enrolled after signing the informed consent form (ICF) (Days -56 to -35)
- Visit 2: second Screening Visit (Days -28 to -7)
- Visit 3: Randomization Visit (Day 0)
- Telephone Contact 1: (Day 14)
- Visit 4: Clinic Visit (Day 28)
- Visit 5: Clinic Visit (Day 56)
- Visit 6: Clinic Visit (Day 84)
- Visit 7: Clinic Visit (Day 126)
- Visit 8: Clinic Visit (Day 154)
- Visit 9: Clinic Visit, EOT (Day 168)

- Visit 10: Follow-Up Visit, 28 days after last dose of study drug (Day 196). All patients continuing in Study A4250-008 will not have this Follow-up Visit.

Additional clinic visits may be required for patients who need direct site assistance, e.g., due to AE monitoring, to fulfil screening requirements, and/or for safety maintenance.

Screening Period

Informed consent must be obtained at Visit 1 prior to performing any study procedures. After signing the ICF, patients will be considered enrolled and evaluated for study eligibility.

Daily recording of pruritus using an electronic diary (eDiary) will be started to determine confirmation of the magnitude and the baseline pruritus symptoms before the study. After consultation with the Medical Monitor, patients failing eligibility criteria may be re-screened after 2 months. Patients not fulfilling inclusion/exclusion criteria after 3 attempts are not allowed to re-screen.

Caregivers/patients will be instructed on the daily use of the eDiary. The eDiary will include patient-reported outcome (PRO) and observer-reported outcome (ObsRO) items for evaluation of itching (PRO), scratching (ObsRO), and sleep disturbance (PRO and ObsRO) during the Screening Period (study Days -56 to 0) and throughout the 24-week Treatment Period (study Days 0 to 168).

Only the 14 most recent days before randomization will be used for eligibility calculation of the diary entries. Observer-reported outcomes in patients of all ages will be recorded by a caregiver (or caregiver's designee). If possible, the same caregiver will complete the Albireo ObsRO items throughout the study.

At a second Screening Visit (Visit 2) additional laboratory samples will be taken for eligibility assessments.

Treatment Period

Eligibility for randomization will be determined at Visit 3 using the pruritus (scratching reports from the Albireo ObsRO) data in the 2 weeks before Visit 3, genetic confirmation of diagnosis, and the liver biochemistry evaluations from the previous screening visits. Two weeks after Visit 3, there will be a telephone contact with the caregiver/patient to evaluate any AEs.

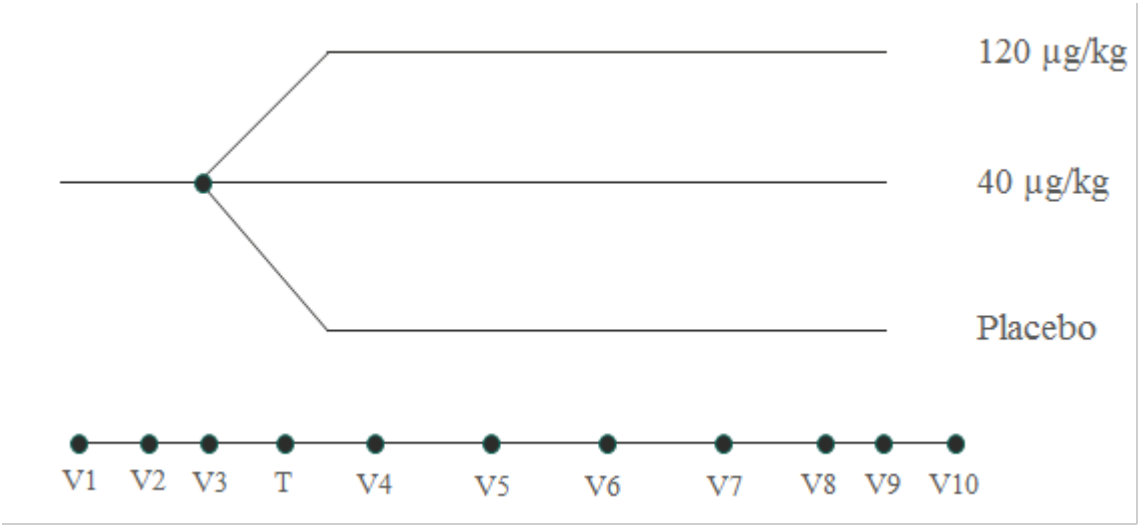
The patient will return to the clinic at Visit 4, Visit 5, Visit 6, Visit 7, Visit 8, and Visit 9 for assessments. If a new caregiver will begin to complete the Albireo ObsRO during the course of the trial, they will be provided with training during a clinic visit before they begin to use the eDiary. Additionally, caregivers/patients will be requested to use the diary to report the date and time each dose of study drug is administered during the Treatment Period.

The last dose of study drug will be administered the day before Visit 9. At study Visit 9, the patient has the option to enroll in an open-label extension study (A4250-008) should they meet the required entry criteria. If the patient is continuing into Study A4250-008 this will be

the last visit in this study and the first visit in Study A4250-008. Visit 9/EOT should also be performed at the time patients are prematurely withdrawn from the study.

Clinic Visit 10 will take place 28 days after Visit 9 for all patients not continuing into Study A4250-008.

Figure 1 Study Design



T: telephone contact; V: study visit

7.1.2 Schedule of Assessments

The schedule of assessments is presented in Table 1. For information on blood volumes please see Appendix 5.

Table 1 Schedule of Assessments

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Informed consent[c]	X										
Inclusion/exclusion criteria	X		X								
Demographics	X										
Concomitant medication[d]	X	X	X	X	X	X	X	X	X	X	X
Medical and surgical history	X		X								
Blood sampling for genetic testing[e]	X										
Physical examination including voluntary photography	X		X							X	
Skin examination	X		X		X		X			X	X
Vital signs[f]	X	X	X		X	X	X	X	X	X	X
eDiary: itching, scratching, and sleep scores[g]	Daily diary entry										
Clinical chemistry[h]	X		X		X	X	X	X		X	X
Hematology[h]			X		X	X	X	X		X	X
Urinalysis[h]		X					X			X	

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Serum bile acids[i]	X	X	X		X	X	X	X	X	X	X
INR		X			X		X		X		X
Autotaxin[j]			X		X					X	
p-C4[j]			X		X					X	
AFP			X							X	
Fat-soluble vitamins A & E		X								X	
Fat-soluble vitamin D			X							X	
Abdominal Ultrasound			X							X	
Fibroscan® (where available)			X							X	
QoL questionnaire (PedsQL)			X							X	
Patient/Caregiver/Clinician Global Impression of Change					X		X			X	
Patient/Caregiver/Clinician Global Impression of Symptoms			X		X		X			X	
Pregnancy test[k]	X[k]	X	X		X	X	X	X	X	X[k]	
Study drug dispensed[l]			X		X	X	X	X	X		

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Adverse events[m]	Continuous collection										
Study drug compliance				X	X	X	X	X	X	X	

AFP: alfa-fetoprotein; BMI: body mass index; eCRF: electronic case report form; eDiary: electronic diary; EOT: End of Treatment; ICF: informed consent form; INR: international normalized ratio; p-C4: plasma 7α Hydroxy 4-cholesten-3-one; PedsQL: Pediatric Quality of Life Inventory; QoL: quality of life; RR: respiratory rate; s-BA: serum bile acid.

a Must also be performed at the time a patient is prematurely withdrawn from the study. This visit is the same as Visit 1 in the extension protocol (Study A4250-008).
b Assessments must be performed 28 days following the final dose of study drug. This visit is omitted if patients are entering Study A4250-008.
c The ICF (and assent) must be signed before any study procedures are performed.
d Past (within 1 month prior to Visit 1) and current medication will be documented in the eCRF.
e Blood sample for genetic testing will be drawn for all patients. Genetic analysis will only be performed on a patient's extracted DNA if previous the clinical genetic result is equivocal, unavailable, or unobtainable.
f Includes blood pressure, pulse, RR, temperature, height (or length depending on age), and body weight (using a certified weight scale). BMI will be calculated.
g Includes training on use of the device at Screening (Visit 1) and review of patient daily entries during 14 days prior to Clinic Visit 3 to determine eligibility.
h See Table 3 for detailed parameters.
i Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA. Exceptions can be made for infants, less than 12 months of age, if unable to fast for the full 4 hours. All s-BA results during the treatment period and follow-up will be blinded.
j Samples will not be collected for patients ≤10 kg.
k For girls who have reached menarche. Serum test at Visit 1; urine test at other visits. A serum pregnancy test will also be conducted at Visit 9 for patients entering Study A4250-008. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy.
l Study drug to be taken once daily on Day 1 through Day 167 as described in Section 8.2.
m Adverse event information will be collected from the time of signing of the ICF to study discontinuation.

7.1.3 Study Procedures and Assessments

7.1.3.1 Screening Period (Days -56 to 0)

Day -56 to Day -35/Clinic Visit 1

Patients will undergo a Screening Visit up to 56 days prior to the planned first day of study treatment. Screening procedures and assessments are as follows:

- Obtain written informed consent
- Assess inclusion/exclusion criteria (Section 7.2)
- Record demographics (age, full date of birth, gender, race, and ethnicity)
- Document concomitant medications
- Medical and surgical history (date of diagnosis of PFIC Types 1 or 2, prior investigational medications for PFIC, historical liver function test [LFT] values, any surgery performed, any other diagnosis, and historical liver biopsy data)
- Blood sample for genetic testing
- Collect, if available, past confirmatory clinical genetic laboratory report for PFIC1/PFIC2 and send to central reader for review
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- eDiary training and compliance requirements; patients begin daily recording of pruritus using the eDiary (Appendix 2)
- Clinical chemistry (Section 10.2.1)
- s-BA (Section 9.2.1)
- Serum pregnancy test for girls of who have reached menarche
- AE monitoring

Day -28 to Day -7/Clinic Visit 2

A second Screening Visit will be performed. Screening procedures and assessments are as follows:

- Review concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for itching, scratching, and sleep scores and compliance
- Urinalysis (Section 10.2.1)

- s-BA (Section 9.2.1)
- International normalized ratio (INR)
- Fat-soluble vitamins A and E
- Urine pregnancy test for girls of who have reached menarche
- AE monitoring

7.1.3.2 Treatment Period (Day 0 to Day 168)

Study Day 0/Clinic Visit 3 (Randomization)

At the randomization visit, the following assessments will be conducted:

- Review inclusion/exclusion criteria (Section 7.2)
- Review concomitant medications, medical and surgical history
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance and entrance criteria requirement (see Inclusion Criteria 4, Section 7.2.1)
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin and p-C4 (samples will not be taken for patients ≤10 kg)
- Fat-soluble vitamin D
- Alfa-fetoprotein (AFP)
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)
- Administer QoL (Pediatric Quality of Life Inventory [PedsQL]) questionnaire
- Patient/caregiver/clinician complete the patient global impression of symptoms (PGIS)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Study drug is dispensed and patients are instructed to administer daily from Day 1

Study Day 14 (Telephone Contact)

A study nurse will contact the patient/caregiver by telephone for monitoring of the following:

- Review concomitant medications

- Albireo PRO/ObsRO eDiary review for compliance
- AEs
- Evaluation of study drug compliance

Study Day 28 and Day 84/Clinic Visits 4 and 6

The following procedures and assessments will be performed:

- Review of concomitant medications
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- Urinalysis (at Clinic Visit 6 only; Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- Autotaxin and p-C4 (at Clinic Visit 4 only; samples will not be taken for patients ≤10 kg)
- Patient/caregiver/clinician complete the patient global impression of change (PGIC) and PGIS
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 56 and Day 126/Clinic Visits 5 and 7

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance

- Study drug is dispensed

Study Day 154/Clinic Visit 8

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- s-BA (Section 9.2.1)
- INR
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 168/Clinic Visit 9/EOT

The last dose of study drug is taken in the morning the day before Clinic Visit 9, and the following procedures and assessments will be performed (also performed at the time a patient prematurely withdraws):

- Review of concomitant medications
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry, hematology, and urinalysis (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin and p-C4 (samples will not be taken for patients ≤10 kg)
- AFP
- Fat-soluble vitamins A, E, and D
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)
- Administer PedsQL questionnaire
- Patient/caregiver/clinician complete the PGIC and PGIS

- Urine pregnancy test for girls who have reached menarche, or serum pregnancy test for girls continuing to study A4250-008
- AE monitoring
- Evaluation of study drug compliance

7.1.3.3 Follow-Up Period

Study Day 196/Clinic Visit 10

All patients not continuing into Study A4250-008 will return to the study site 28 days after Clinic Visit 9 or the last dose of study drug for the following assessments:

- Review of concomitant medications
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- AE monitoring

7.2 Study Population

A total of 60 patients with clinical diagnosis of PFIC Type 1 or 2 that have been genetically confirmed will be randomized.

An attempt will be made to randomize a minimum of 10 patients from each age category (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years).

A maximum of 5 patients that were treated in Study A4250-003 will be allowed to participate in this study.

7.2.1 Inclusion Criteria

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples 7 to 28 days apart (Visits 1 and 2) prior to randomization

4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the eDiary average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients will be expected to have a consistent caregiver(s) for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

7.2.2 Exclusion Criteria

1. Patient with pathologic variations of the *ABCB11* gene that predict complete absence of the BSEP function
2. Patient with past medical history or ongoing presence of other types of liver disease including, but not limited to, the following:
   a) Biliary atresia of any kind
   b) Benign recurrent intrahepatic cholestasis, indicated by any history of normal s-BAs
   c) Suspected or proven liver cancer or metastasis to the liver on imaging studies
   d) Histopathology on liver biopsy is suggestive of alternate non-PFIC related etiology of cholestasis
3. Patient with a past medical history or ongoing presence of any other disease or condition known to interfere with the absorption, distribution, metabolism (specifically bile acid metabolism), or excretion of drugs in the intestine, including but not limited to, inflammatory bowel disease
4. Patient with past medical history or ongoing chronic (i.e., >3 months) diarrhea requiring intravenous fluid or nutritional intervention for treatment of the diarrhea and/or its sequelae
5. Patient has a confirmed past diagnosis of infection with human immunodeficiency virus or other present and active, clinically significant, acute, or chronic infection, or past medical history of any major episode of infection requiring hospitalization or treatment with parenteral anti-infective treatment within 4 weeks of treatment start (study Day 1) or completion of oral anti-infective treatment within 2 weeks prior to start of Screening Period
6. Any patient with suspected or confirmed cancers except for basal cell carcinoma, and non-liver cancers treated at least 5 years prior to Screening with no evidence of recurrence

7. Patient with a past medical history of chronic kidney disease with an impaired renal function and a glomerular filtration rate <70 mL/min/1.73 m^2
8. Patient with surgical history of disruption of the enterohepatic circulation excluding those who have undergone a successful reversal procedure that has permanently restored flow of bile acids from the liver to the duodenum
9. Patient has had a liver transplant or listed for liver transplant
10. Decompensated liver disease, coagulopathy, history or presence of clinically significant ascites, variceal hemorrhage, and/or encephalopathy
11. INR >1.4 (the patient may be treated with Vitamin K intravenously, and if INR is <1.4 at resampling the patient may be randomized)
12. Serum ALT >10 × upper limit of normal (ULN) at Screening
13. Serum ALT >15 × ULN at any time point during the last 6 months unless an alternate etiology was confirmed for the elevation
14. Total bilirubin >5 × ULN at Screening
15. Patient suffers from uncontrolled, recalcitrant pruritic condition other than PFIC. Examples include, but not limited to, refractory atopic dermatitis or other primary pruritic skin diseases
16. Any patient who is pregnant or lactating or who is planning to become pregnant within 24 weeks of randomization
17. Sexually active males and females who are not using a reliable contraceptive method with ≤1% failure rate (such as barrier protection, hormonal contraception, intrauterine device, or abstinence) throughout the duration of the study
18. Patient with a past medical history of alcohol or substance abuse will be excluded. Patient must agree to refrain from illicit drug and alcohol use during the study
19. Administration of bile acid or lipid binding resins and medications that slow GI motility (Refer to Appendix 1 – Concomitant Medications Prohibited during the study)
20. Patient has had investigational exposure to a drug, biologic agent, or medical device within 30 days prior to Screening, or 5 half-lives of the study agent, whichever is longer
21. Patient who has been previously treated with an IBAT inhibitor whose pruritus has not responded to treatment
22. Any other conditions or abnormalities which, in the opinion of the investigator or Medical Monitor, may compromise the safety of the patient, or interfere with the patient participating in or completing the study

7.2.3 Withdrawal of Patients

Patients/caregivers will be informed that they have the right to withdraw from the study at any time, without prejudice to their medical care, and that they are not obliged to state their reasons.

Any withdrawal, and reasons for withdrawal, must be fully documented in the electronic case report form (eCRF) and source documents and the patient followed by the investigator/investigative staff. Even after the study is completed at the Follow-up Visit, the investigator/investigative staff will, regardless of reason for withdrawal, record any patient data they receive concerning SAEs, and all drug-related non-serious AEs, and subsequently report these to Albireo. Withdrawn patients will not be replaced.

Patients will be withdrawn in the following circumstances:

- A patient's/caregiver's desire for withdrawal for any reason
- Lost to follow-up (every effort must be made to contact the patient/caregiver; a certified letter must be sent)
- An AE that, in the opinion of the investigator, necessitates withdrawal
- A worsening of the symptoms related to diarrhea occurs
- Death
- Patient/caregiver estimation of lack of improvement/intolerable symptoms
- A patient's/caregiver's substantial noncompliance (eDiary and study drug compliance) or protocol violation
- An investigator's opinion that continuing the patient in the study is not appropriate. The investigator may withdraw a patient at any time, if it is considered to be in the patient's best interest

A patient who prematurely withdraws due to no improvement/intolerable symptoms, and has completed at least 12 weeks treatment in the double-blind study, will be offered the opportunity to enter open-label Study A4250-008. A patient who withdraws from treatment prematurely and will not enter Study A4250-008 will be requested to complete all visits and safety assessments per the current protocol (A4250-005). As a minimum, patients will have EOT (Visit 9) assessments at the time of withdrawal and a follow-up assessment (Visit 10) 28 days following the last dose of study drug, and the patient will be recorded as withdrawn.

7.2.4 Study Termination by Sponsor

This study may be terminated at any time by Albireo if serious side effects should occur, if the investigator does not adhere to the protocol; or if, in the sponsor's judgment, there are no further benefits to be achieved from the study. In this event, Albireo/designee will inform the study investigators, institutions, and all regulatory authorities.

Albireo may temporarily or permanently discontinue the study at an investigative site at any time for safety, ethical, compliance, or other reasons. If this is necessary, Albireo will endeavor to provide advance notification to the site. If a site or the study is suspended or discontinued, the investigator/investigative staff will be responsible for promptly informing the Independent Ethics Committee (IEC). If required by local regulations, Albireo/designee will be responsible for informing the IEC and the regulatory authority of study or site discontinuation. In such an event, all study data and unused study drug must be returned to Albireo.

8 TREATMENT OF PATIENTS

8.1 Identity of Study Drug

A4250 and placebo will be supplied as capsules for oral administration. White opaque capsules filled with pellets containing A4250 or placebo will be provided. Two different capsule sizes will be available, as follows:

- Capsule size 0 that can be opened
- Capsule size 3 that can be opened or swallowed intact

The content of the A4250 and placebo capsules will be identical in appearance. Capsule filling weight will also be identical for high dose, low dose, and placebo. Bottles with 34 capsules will be given to the patient at each visit. A patient who requires 2 or more capsules per day will be given multiple bottles. Refer to the investigational product manual.

8.2 Administration of Study Drug

Patients will be dosed with A4250 at a dose of 40 µg/kg/day, 120 µg/kg/day, or placebo for 24 weeks. Study drug will be dispensed to the patient at Visits 3, 4, 5, 6, 7, and 8, together with instructions on how to store and take the drug. Study drug administration data, including whether each patient took each dose or partial doses of study drug, whether there were any delayed or missed doses, doses, and whether the capsule was opened or swallowed whole will be documented through the eDiary and transferred to the study database.

A4250 should be taken in the morning, together with food. On clinic visits days when laboratory assessments are conducted, study drug should be taken after the visit and after laboratory samples are taken. Patients should not crush or chew the capsule(s). When swallowing the capsule intact, the patient should administer the dose with a glass of water.

For a patient unable to swallow capsule intact, the capsule can be opened and the contents sprinkled and mixed in a small amount of soft room-temperature yogurt, apple sauce, or fruit purée. The amount of food should be less than what the patient/infant is expected to eat and should be followed by water.

1. Hold the capsule over the prepared food, gently twist open and allow the content to fall into food
2. Mix the entire capsule content with 1 or 2 tablespoons (15 to 30 mL) of soft food (apple sauce, fruit purée, or yogurt)
3. Administer the mixture immediately after mixing.

The number of capsules provided to the patient will be based on body weight at Visit 3. At Visit 6, if a patient's weight has shifted to another weight category (identified in Table 2), the number of capsules per day may be adjusted.

Table 2 Dosing and Capsule Strength

Body Weight (kg)	Capsule Size	Number of Capsules per Day	Capsule Strength, Low Dose (µg)	Total Dose (µg)	Capsule Strength, High Dose (µg)	Total Dose (µg)	Capsule Size, Placebo (µg)
5.0 to <7.5	0	1	200	200	600	600	0
7.5 to <12.5	0	2	200	400	600	1200	0
12.5 to <17.5	0	3	200	600	600	1800	0
17.5 to <19.5	0	4	200	800	600	2400	0
19.5 to <25.5	3	2	400	800	1200	2400	3
25.5 to <35.5	3	3	400	1200	1200	3600	3
35.5 to <45.5	3	4	400	1600	1200	4800	3
45.5 to 55.5	3	5	400	2000	1200	6000	3
>55.5	3	6	400	2400	1200	7200	3

8.3 Study Treatment Packaging and Labeling

8.3.1 Packaging and Labeling

The capsules will be packed in high-density polyethylene containers, with child-proof polypropylene caps. Study drug capsules containing A4250 in the strength specified in Table 2 above will be manufactured.

Packaging and labeling will be prepared to comply with applicable regulatory requirements.

8.3.2 Storage

Treatment packs containing A4250 capsules should be stored and dispensed in accordance with regulations in their original containers. The storage facility at the investigative site should be locked and the storage temperature should be between 15°C and 25°C.

Patients/caregivers should be informed of appropriate storage conditions (i.e., room temperature, between 15°C and 25°C).

Any deviations from the recommended storage conditions should be immediately reported to Albireo and the study drug should not be used until authorization has been given by Albireo.

8.3.3 Blinding and Randomization of Study Drug

A double-blind design is employed so that both the investigators and the patients will be unaware of the treatment assignment during the study.

After written informed consent is obtained from an eligible patient, an 8-digit patient identification number will be assigned. The first 2 digits will denote the country, followed by a 3-digit site number and a 3-digit patient sequence number. This number will be created and allocated by the Interactive Web Response System (IWRS), when the patient first enters the system at Screening. The randomization codes will be computer-generated by [redacted]

Biostatistics, and kept by a statistician independent from the project team. The randomization will be done in blocks and stratified according to PFIC class (Type 1 and 2) and age class (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) to ensure approximate balance between dose schemes (1:1:1). A separate randomization list will be prepared for the maximum 5 patients from Study A4250-003 regardless of stratification. Randomization codes will be assigned sequentially as patients become eligible for randomization.

Patients who are eligible for randomization will be assigned a unique 4-digit randomization number by IWRS. This randomization number identifies which treatment will be allocated to the patient. Patients who withdraw from the study after randomization visit are not to be replaced and their randomization number will not be reused.

To ensure blinding, the study drug and the matching placebo have the same shape and size. Patients will receive capsule(s) of A4250 according to their dose group or capsule(s) of matching placebo once a day during the double-blind Treatment Period. Labels on the study drug containers will not identify the treatment that a patient is randomized to. Traceability of the treatment is ensured by the study drug number.

The 5-digit study drug number will identify study drug packs and will be detailed on the study drug label. Dispensing of study drug will be coordinated by IWRS.

The system will assign a study drug number corresponding to the randomization arm. The randomization number will be used in the background only to ensure no unblinding and will not be displayed to end-users in the IWRS.

8.4 Procedure for Breaking the Randomization Code

Should a situation arise where unblinding is urgently required, i.e., only where knowledge of the study drug is required to adequately manage a life-threatening situation, the investigator at that study site may perform immediate unblinding through IWRS. The investigator should make every effort to discuss the rationale for emergency unblinding with the sponsor Medical Monitor or the [redacted] Medical Monitor prior to unblinding the individual patient.

The study site investigator and appropriate [redacted] project team members will be authorized to access the emergency unblinding functionality within the IWRS. Emergency unblinding takes place in the IWRS via a dedicated emergency unblinding module. The system will require the user to enter their emergency unblinding, which is typically provided at the same time login credentials are distributed. The exact description of the treatment assigned to the individual patient then will be accessible to the user. Once the randomization code is broken for a patient, he/she must be withdrawn from the study and all assessments and procedures at EOT should be performed. As a follow-up, Visit 10 will be performed according to the time schedule. Once a randomization code has been broken, the investigator must inform the [redacted] and sponsor Medical Monitor in writing within 24 hours.

8.5 Patient Compliance

The study nurse will monitor eDiary compliance by routine review of the CRF health website. If both diary entries on a day are missing during, the study nurse will call the patient/caregiver to remind them to complete all scheduled entries. Any noncompliance will be documented and explained in the source documents and in the eCRF.

Study drug compliance will also be checked through diary responses. Additionally, patients will return all unused study drug at Visits 4, 5, 6, 7, 8, and 9. The study site staff will count all returned drug, assess compliance, and record details in the eCRF.

Treatment Compliance = 100 × ((Number of study drug dispensed – number of study drug returned)/number of study drug that should be taken).

Treatment compliance between 80% and 120% will be acceptable.

8.6 Study Drug Accountability

Records shall be maintained of the delivery of study treatment(s) to the study site(s), of the inventory at the study site(s), of each use of the study treatment(s) for each patient, and of the return of unused study treatment(s) to Albireo.

These records shall include dates, quantities, batch numbers, expiry dates, and the unique code numbers assigned to the study drug and to the study patients.

The investigator will be responsible for ensuring that the records adequately document that the patients were provided the quantities specified in the protocol and that all study drug received from Albireo is reconciled.

8.7 Concomitant Therapy and Prohibited Medications

The investigator will note all ongoing medication and any medication recently stopped (within 1 month prior to Visit 1) in the eCRF. At Visits 2 through 10, all changes in medication (stopping or starting new medication or changes in dose) will be recorded in the eCRF. All medications (prescribed or over the counter) for pruritus and sleep will be recorded.

All medications taken by a patient within 3 months prior to the first intake of study drug are regarded as prior medication.

All medications taken by a patient on or after the first intake of study drug, and which continue to be taken during the study, are regarded as concomitant medication.

Prohibited medications are listed in Appendix 1.

9 ASSESSMENT OF EFFICACY

9.1 Efficacy Endpoints

9.1.1 Primary Efficacy Endpoint

The primary efficacy endpoints are as follows:

- **European Union (EU) and rest of the world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  Fasting s-BA baseline will be calculated as the average of the value at randomization and the previous visit (Clinic Visit 2). The end value is the average of the values at Weeks 22 and 24 after the start of double-blind treatment.

- **United States (US)**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 24 as measured by the worst scratch of the Albireo ObsRO instrument (Appendix 2). Scores for the 14 days baseline and post-treatment intervals will be calculated by averaging the worst scratching scores from each day in the interval.

9.1.2 Secondary Efficacy Endpoint

Secondary efficacy endpoints include the following:

- **EU and RoW**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 24 as measured by the worst scratch of the Albireo ObsRO instrument (Appendix 2). Scores for the 14 days baseline and post-treatment intervals will be calculated by averaging the worst scratching scores from each day in the interval.
- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.
- **All Regions:**
  - All secondary endpoints are compared to placebo.
    - Change from baseline to Week 24 in fasting s-BA
    - Change from baseline to Week 24 in serum ALT concentration
    - Change in growth from baseline to Week 24, defined as the linear deficit (weight for age, and body mass index [BMI]) compared to the national growth curves (Z-score, standard deviation [SD] from P50)
    - Proportion of patients achieving meaningful reduction in caregiver-reported observed scratching at Week 24

- Change from baseline to Week 24 in sleep parameters measured with the Albireo PRO and ObsRO instruments
- Change from baseline to Week 24 in patient-reported itch severity as measured by the average daily score of the Albireo PRO instrument (Appendix 2); only patients ≥8 years of age will complete the Albireo PRO instrument
- Number of patients undergoing biliary diversion surgery or being listed for liver transplantation

9.1.3 Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Proportion of patients achieving meaningful reduction in patient-reported itch severity at Week 24
- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the PGIC items (Appendix 3)
- Change from baseline in fasting s-BA at Weeks 4 and 12
- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Weeks 4, 12, and 24 as measured by the worst scratch. Scores for the 7 worst scratching days of the last 14 days at baseline and post-treatment intervals will be selected
- Change from baseline in patient-reported itch severity and observer-reported scratching at Weeks 4 and 12
- Change from baseline to Week 24 in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline to Week 24 in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline to Week 24 in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in PedsQL (Appendix 4)
- Change from baseline in serum ALT concentration at Weeks 4 and 12
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, p-C4) at Week 24

- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events
- All-cause mortality

9.2 Efficacy Assessments

9.2.1 Serum Bile Acids

Blood samples for analysis of fasting total s-BA will be drawn at all visits. Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA. Exceptions can be made for infants, <12 months of age, if unable to fast for the full 4 hours. Any visit at which a bile acid sample result is unreportable, an additional unscheduled visit for a repeat sample collection may be scheduled. All s-BA results during the treatment period and follow-up will be blinded. Samples will be processed and transported to a central laboratory per instructions in the laboratory manual.

9.2.2 Itching, Scratching and Sleep Score

Itching, observed scratching, and sleep disturbance will be recorded twice each day via eDiary. Patients and/or caregivers will be instructed to complete the eDiary every day in the morning after the patient wakes and in the evening just before the patient goes to sleep throughout the study. Patients and caregivers will receive a formal training session during Visit 1.

The eDiary includes Albireo ObsRO and PRO items. Patients <8 years of age will not be asked to complete the Albireo PRO items; only the Albireo ObsRO will be completed by caregivers of patients in this age group. Older patients, 8 to 17 years of age, will complete the Albireo PRO items and the caregiver will complete the Albireo ObsRO items. The Albireo PRO items assess severity of itch, aspects of sleep disturbance (morning diary only), and tiredness. For patients 8 to 12 years of age, the caregiver will read the Albireo PRO items along with the child and record the child's response. A guide will be provided to the caregivers that provides standardized explanations of the Albireo PRO items, in case the patient is confused or requires clarification about the meaning of a question. The Albireo ObsRO items assess severity of observed scratching, aspects of observed sleep disturbance (morning diary only), and observed signs of tiredness (evening diary only). The Albireo ObsRO and PRO scratching and itch severity items use 0 to 4 response scales, where each response is distinguished by a unique facial expression, verbal anchor, number, and color code (Appendix 2).

A daily score for the Albireo ObsRO scratching item will be calculated by selecting the maximum scratching severity score from the 2 ratings for each day. A daily score will be considered missing if both of the daily assessments are missing. A 14-days score will be calculated by averaging the daily scratching scores. A 14-days score will be considered missing if >4 out of 7 days a week of data are missing. The same approach will be used to calculate a patient-reported itch severity score.

9.2.3 Growth

Growth will be measured as height (velocity) and weight (Z-score) using a certified weight scale. BMI will be calculated by weight (kg) / height $(m)^2$. Change will be defined as linear growth deficit (weight and BMI for age) compared to national growth curves (Z-score, SD from P50).

9.2.4 Quality of Life Questionnaire (PedsQL)

Patients and caregivers will be asked to complete a QoL questionnaire (PedsQL) at Visit 3 and Visit 9. Details of the questions included on the questionnaire are provided in Appendix 4.

9.2.5 Biomarkers and Other Laboratory Samples

Blood samples for autotaxin and p-C4 will be drawn at Visits 3, 4, and 9 only for children with body weight ≥10 kg. Samples will be processed and transported to a laboratory per instructions in the laboratory manual.

9.2.6 PELD/MELD Score

At randomization and end of treatment, PELD score will be calculated for children up to 12 years of age. For children 13 years or older, the MELD score will be calculated.

PELD Score = 4.80 * ln(total bilirubin in mg/dL) + 18.57 * ln(INR) - 6.87 * ln(albumin in g/dL) + 4.36 (if patient <1 year: scores for patients listed for liver transplantation before the patient's first birthday continue to include the value assigned for age (<1 year) until the patient reaches the age of 24 months) + 6.67 (if the patient has growth failure [≤2 SD])

Laboratory values less than 1.0 will be set to 1.0 for the calculation of the PELD score.

MELD Score = 9.57 * ln(creatinine in mg/dL) + 3.78 * ln(total bilirubin in mg/dL) + 11.2 * ln(INR) + 6.43

Laboratory values less than 1.0 will be set to 1.0 and serum creatinine values greater than 4.0 mg/dL will be set to 4.0 for calculation of the MELD score.

9.2.7 Fibroscan®

Where available, Fibroscan® will be performed as per institution standard practice at Visits 3 and 9.

9.2.8 Markers of Fibrosis

APRI score and Fib-4 score will be calculated at randomization and at end of treatment.

APRI = [(AST in U/L) / (AST ULN in U/L)] / (Platelets in 10^9/L)

Fibrosis 4 Score = (Age*AST in U/L) / (Platelets in 10^9/L * √(ALT in U/L)).

9.2.9 Global Impression of Change and Global Impression of Symptom Measures

Patients (≥8 years of age), caregivers, and clinicians will complete the PGIC and the PGIS measures (Appendix 3) at randomization (Visit 3; PGIS only), Visits 4 and 6, and at EOT (Visit 9).

The PGIC items assess change in itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) since starting the study drug. The PGIS items assess itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) in the past week. Caregivers and clinicians will complete the PGIC and PGIS for all patients; those patients ≥8 years of age will complete the patient version.

10 ASSESSMENT OF SAFETY

The timing and frequency of safety assessments are described in Section 7.1.3 and in Table 1 above.

The primary safety analysis for this study will include the incidence of total treatment-emergent adverse events (TEAE) and TEAEs categorized by causality, severity, and seriousness assessments made by the investigator by comparing study drug exposure to placebo.

Trends in safety will also be evaluated for the following assessments:

- Physical examinations
- Concomitant medications
- Vital signs
- Laboratory test results (including clinical chemistry, hematology, urinalysis, AFP, and fat-soluble vitamins A, D, and E)
- Abdominal ultrasound
- Discontinuations due to AEs

10.1 Adverse Events

10.1.1 Definitions and Investigator Assessments

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 unfavorable and unintended sign, symptom, or disease that occurs once a patient is enrolled (ICF is signed) in the study until the patient is discharged from the study, whether or not related to the study drug.

10.1.1.1 Clinical Significance

Clinical significance is defined as any abnormal finding that results in further clinical investigation(s), treatment(s), or the diagnosis of new condition. Patient-reported events and protocol-mandated laboratory values, vital signs, and physical examination findings can be considered clinically significant (i.e., an AE) if there is a deterioration as compared to baseline. Examples of clinically significant worsening from baseline could include, but is not limited to, events causing withdrawal from the study and events requiring medical intervention outside of the study causing apparent clinical manifestations, or judged relevant by the investigator.

10.1.1.2 Serious Adverse Events

Serious criteria are applied by the investigator to each AE as specifically defined below. These criteria are used to determine whether an AE is serious or non-serious. The assessment is made independently of severity assessment (Section 10.1.1.3). For example, the

development of a severe rash that occurs after signing of informed consent may not meet serious criteria as defined below and therefore would be considered a severe, non-serious AE.

Any AE that meets any 1 of the following 6 criteria is considered an SAE:

- The outcome of the AE is **death**
- The AE is immediately **life-threatening**. Life-threatening means that the patient is, in the opinion of the investigator, at immediate risk of death from the reaction as it occurred. This does not include an AE that, if more severe, might have caused death
- The AE results in persistent or significant **disability/incapacity**. Disability means a substantial disruption of a person's ability to conduct normal life functions
- The AE requires or prolongs **hospitalization**
- The AE results in a **congenital anomaly/birth defect**
- **The AE is an important medical event**. Important medical events may meet serious criteria should the investigator assess that they may significantly jeopardize the patient, represent a significant hazard, or requires medical/surgical intervention to prevent one of the outcomes listed above. Examples of potential SAEs based on this criteria include, but are not limited to, allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions even if they do not result in inpatient hospitalization, or the development of drug dependency and drug abuse.

10.1.1.3 Severity Assessment

Severity assessments are based on the intensity of the event in relation to expectation. The investigator will assess the intensity of AEs based on the following definitions:

- Mild (awareness of sign or symptom, but easily tolerated)
- Moderate (discomfort sufficient to cause interference with normal activities)
- Severe (incapacitating, with inability to perform normal activities)

Severity is a measure of intensity where seriousness is defined by the criteria outlined in Section 10.1.1.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not meet serious criteria, and therefore would be assessed as a severe AE but not an SAE.

10.1.1.4 Causality Assessment

The investigator determines the causality of all AEs to the study drug using medical judgment and considering all relevant factors such as (but not limited to) the underlying study indication, coexisting disease, concomitant medication, relevant history, pattern of the AE, temporal relationship to the study drug, and de-challenge or re-challenge. The causality assessment of the AE/SAE is to be made as follows.

Related to study drug (possibly, probably, or definitely related)

Based on medical judgment, there is at least a reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event follows a reasonable temporal sequence from administration of study drug
- The event could not be reasonably attributed to the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient
- The event follows a known pattern of response to study drug
- The event disappears or decreases on cessation or reduction in dose of the study drug. (It should be noted that in some situations an AE will not disappear or decrease in intensity upon discontinuation of study drug despite other clear indications of relatedness)
- The event reappears or worsens when the study drug is re-administered

Unrelated to study drug (unlikely or unrelated)

Based on medical judgment there is no reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event does not follow a reasonable temporal sequence from administration of study drug
- The event could be reasonably attributed to the known characteristics of the patient's clinical state, concurrent illness, environment or toxic factors, or other modes of therapy administered to the patient
- The event does not follow a known pattern of response to study drug
- The event does not disappear or decrease on cessation or reduction in dose of the study drug, and it does not reappear or worsen when the study drug is re-administered

10.1.2 Recording of Adverse Events

It is the investigator's responsibility to assess whether each untoward event is a clinically significant worsening from baseline, thereby considered an AE. For all AEs, the severity, seriousness, and causality to study drug for each AE as outlined in Section 10.1.1 will be assessed and recorded in the eCRF.

All serious and non-serious AEs are collected once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10) or 28 days after the last dose of study drug.

Any AEs or SAEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator until resolution or stabilization up to the database lock and recorded in the eCRF. Albireo retains the right to request additional information for any patient with ongoing AE(s) or SAE(s) at the end of the study.

TEAEs are defined as any AE that occurs after randomization (Day 0). All AEs that occur in the Screening Period, i.e., after enrollment and prior to randomization, will be collected on the eCRF as non-treatment-emergent AEs.

If there is a clinically significant deterioration of a laboratory value/vital sign or other routine study assessment that is associated with a diagnosis, the clinical diagnosis will be reported as an AE and the associated signs and symptoms will be considered additional information unless the sign or symptom is more severe than expected given the diagnosis. For example, if an investigator diagnoses a study patient with hepatitis during the study period, hepatitis would be considered the AE and the concomitant signs and symptoms of abdominal pain, vomiting, and elevated ALT and AST would not be reported separately unless, in the opinion of the investigator, one of these signs or symptoms is more severe than expected and therefore a separate AE assessment is indicated.

10.1.3 Recording and Reporting of Serious Adverse Events

Every SAE (regardless of severity and causality) that occurs once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10), and 28 days after the final dose of study drug, should immediately and not later than within 24 hours of knowledge of the event, be reported by the investigator or delegate in the SAE report form.

Report of a SAE must include at least the following information:

- Patient identification information (study number, site number, initials, and date of birth [as per local country requirements for data protection])
- The last study drug administration date
- The diagnosis of the event with the description (or a brief description of signs/symptoms/clinical course if the diagnosis is not available) and the date of onset
- The action used to treat the event (i.e., treatment medications, temporary discontinuation)
- The reason(s) for considering the event serious
- The relationship of the event to the study drug or to the study procedure (e.g., the investigator's assessment of causality)
- A brief narrative of the SAE

Follow-up reports including all new information obtained of the subsequent course of the SAE must be prepared and the information collected in the SAE submitted to [redacted] (Pharmacovigilance and Safety Services Department) by e-mail within 24 hours of knowledge of the event.

The pharmacovigilance manager (PVM) may contact the investigator to obtain further information on a reported SAE. The investigator/investigative staff must respond to any request for follow-up information or answers to questions regarding the SAE within the same timelines as for initial reports.

The PVM reports the occurrence of the SAE and follow-up to the Albireo medical monitor for medical assessment of the case.

Should an outcome of death occur within the study period or within 28 days after the last administration of study drug, an SAE form should be completed including the main and contributory causes of death.

All SAE reports must be e-mailed to the following e-mail address within **24 hours**:

[redacted]

If email is unavailable, SAEs may be transmitted via fax to the following number:

[redacted]

In a study-related medical emergency situation, when the assigned medical monitors for the study cannot be reached by a caller, an on call physician can be reached 24 hours per day, 7 days per week, via the [redacted] call center. The following number is a chargeable telephone number allowing a global reach from both landlines and mobile phones and the internet page indicated below contains a list of country-specific toll-free telephone numbers that is provided. It should be noted that not all countries globally have access to toll-free numbers as indicated on the "24/7 Medical Help desk" index. Countries without toll-free numbers need to dial the chargeable number as indicated below. Furthermore, toll-free numbers might not be available from mobile phones.

[redacted]

10.1.4 Reporting of Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reaction (SUSAR) is an SAE that occurs in a patient, the nature or severity of which is not expected per the applicable product information (e.g., the IB for an unauthorized investigational product or summary of product characteristics for an authorized product).

Reporting and tracking of SUSARs will be in accordance to all applicable competent authority regulations. The Ethics Committee and all investigators involved in this study will be informed according to national and international requirements.

10.2 Laboratory Values/Vital Signs/Physical Examinations and Other Safety Assessments

10.2.1 Laboratory Assessments

Samples will be collected for clinical chemistry, hematology, and urinalysis testing at the time points specified in Table 1. The parameters assessed are presented in Table 3.

Blood for AFP will be drawn at Visits 3 and 9 and for fat-soluble vitamins at Visits 2, 3, and 9.

A serum pregnancy test will be performed at Visit 1 and Visit 9 if the patient is entering the A4250-008 study. A urine pregnancy test will be collected at all other visits for girls who have reached menarche. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy. Study drug administration will be held pending the confirmatory results. If the serum pregnancy test is negative, the patient can resume dosing. If the serum pregnancy test is positive, the patient should be discontinued (see Section 10.2.9).

All samples will be processed and transported to a laboratory per instructions in the laboratory manual.

The observed values will be recorded and assessed as "normal" or "abnormal not clinically significant" or "abnormal clinically significant".

Additional safety blood samples may be needed due to follow-up of an abnormal value or analysis failure. The blood samples collected for safety laboratory analysis will be destroyed after the analyses have been completed.

Table 3 Routine Laboratory Parameters

Clinical Chemistry	Hematology	Urinalysis
• Albumin • ALT • Alkaline phosphatase • AST • Bilirubin – total and direct • Calcium • Chloride • Creatinine • Creatine kinase • Gamma-glutamyl transferase • Potassium • Sodium	• Hematocrit • Hemoglobin • Platelet count • Red blood cell count • White blood cell count	• Blood • Glucose • Ketones • Leukocytes • Nitrites • pH • Protein

10.2.2 Individual Patient Safety Monitoring

10.2.2.1 Liver Monitoring

Strategies to monitor markers of liver disease throughout the study are outlined below where the ULN will be based on central laboratory reference values for age and gender.

If isolated transaminase elevations are observed, defined as:

1. Normal bilirubin AND absence of clinical hepatitis symptoms AND

- ALT or AST ≥5 × ULN (if normal at baseline) or an absolute threshold of 800 U/L, whichever comes first
- OR ALT or AST ≥3 × baseline (if abnormal at baseline) or an absolute threshold of 800 U/L, whichever comes first

Then:

a) Repeat liver profile (AST, ALT, bilirubin, and prothrombin time [PT] or INR) within 2 to 3 days

b) Evaluate creatine phosphokinase (CPK) and lactate dehydrogenase (LDH)

c) As needed (for example, persistent ALT/total bilirubin elevation or worsening of liver function), consider evaluation for alternative etiologies

Monitor the patient using close observation found in Section 10.2.2.2

If any ONE of the following criteria are met:

1. Transaminases (ALT or AST ≥3 × baseline or 800 U/L, whichever comes first) AND bilirubin (total bilirubin > 2 × ULN) elevations
2. Transaminase elevations alone (ALT or AST >10 × ULN or 5 × baseline or absolute threshold of 800 U/L, whichever comes first) in presence of normal LDH and CPK
3. Cholestatic marker elevations (alkaline phosphatase [ALP] or gamma-glutamyl transferase >2 × baseline) without alternative explanation
4. Total bilirubin increased, unrelated to hemolysis (elevated reticulocyte count) or established genetic diseases, such as Gilbert's syndrome
   a. Doubling if total bilirubin was <3 mg/dL at baseline
   b. OR Increase by >3 mg/dL if total bilirubin was ≥3 mg/dL at baseline
5. INR increase refractory to vitamin K administration
   a. Increase by >1.5 if INR was normal at baseline
   b. OR Increase by >0.4 if INR was abnormal at baseline
6. Any increase in total bilirubin and transaminases if accompanied by EITHER a symptom of clinical hepatitis (e.g., vomiting, nausea, right upper quadrant pain) OR immunological reaction (rash or >5% eosinophilia)

Then:

a) Interrupt study medication

b) Initiate drug-induced liver injury work-up for alternative etiologies

c) Repeat liver profile (AST, ALT, total bilirubin, direct bilirubin, ALP) and PT or INR within 48 to 72 hours

d) Monitor the patient using close observation found in Section 10.2.2.2

e) If a patient lives in a remote area, they may be tested locally and the results communicated to the investigator site promptly

10.2.2.2 Close Observation

- Repeat liver enzyme and serum bilirubin tests two or three times weekly. Frequency of re-testing can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the subject is asymptomatic
- Obtain a more detailed history of symptoms and prior or concurrent diseases
- Obtain a history of concomitant drug use (including non-prescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets
- Rule out alternate etiology including acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; non-alcoholic steatohepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease
- Obtain a history of exposure to environmental chemical agents.
- Obtain additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin)
- Consider gastroenterology or hepatology consultations.

10.2.2.3 De-challenge/Re-challenge for Liver and Clinical Hepatitis Monitoring

1. Re-challenge is not recommended:
   a. If a patient has had possible/probable drug-induced liver injury
   b. If a decompensation event has occurred (i.e., variceal hemorrhage, ascites, hepatic encephalopathy, etc.).
2. If due to underlying cholestatic liver disease variability or another alternative etiology is identified AND liver tests returned to baseline, re-challenge may be considered after consultation with the sponsor Medical Monitor.
3. If the ALT/total bilirubin elevations are observed after re-challenge, then repeat re-challenge is discouraged.

If patient is permanently discontinued, monitoring should be continued as outlined in Section 10.2.2.5.

10.2.2.4 Diarrhea

Study drug should be discontinued if a patient develops diarrhea with at least 1 of the following concomitant signs or symptoms: grossly bloody stools, vomiting, dehydration

requiring treatment with oral or intravenous rehydration and/or electrolyte imbalances, fever (≥38 C) and/or the diarrhea persists for 7 or more days.

If there is gross blood in stool, INR and platelets should be measured.

Study drug will be reintroduced (re-challenge) when the symptoms have resolved. If the diarrhea re-occurs within 1 week after the re-challenge with no alternate etiology, the patient will be permanently discontinued and monitored as outlined in Section 10.2.2.5.

10.2.2.5 Monitoring after Permanent Discontinuation of Study Drug Due to Safety

Once study drug is permanently discontinued for liver, hepatitis, diarrhea, or other severe AE related to study drug, the patient will be monitored weekly until the laboratory and clinical parameters have normalized/stabilized.

10.2.3 Demographics/Medical and Surgical History

Demographic information per country regulations (age, full date of birth, gender, race, and ethnicity), along with medical and surgical history, will be obtained and recorded in the eCRF at Visit 1.

Medical and surgical history will be entered in the eCRF at Visit 1. This includes date of diagnosis of PFIC, prior investigational medications for PFIC, historical (3 months prior to Screening) LFT values (AST, ALT, and total bilirubin), ongoing medication, any surgery performed, any other diagnosis, and historical biopsy data.

If a liver biopsy has been performed within 1 year prior to Screening, or during the study, the results will be recorded in the eCRF.

10.2.4 Clinical Genetic Testing

If confirmatory clinical genetic testing for PFIC Type 1 or 2 has been performed prior to Screening Visit 1, the confirmatory clinical genetic testing laboratory report will be verified by a central reader to determine eligibility.

Regardless of previous genetic testing, a blood sample will be drawn at Visit 1 for all patients for DNA extraction. Genetic testing will only be performed on a patient's extracted DNA if the previous clinical genetic result is equivocal, unavailable, or unobtainable. In such cases, screening for pathologic biallelic variations in the *ATP8B1* or *ABCB11* genes will be performed if patient meets remaining inclusion and exclusion criteria and only after sponsor case review. No other diagnostic genetic testing will be offered.

10.2.5 Physical Examination

A physician or suitably trained qualified assistant will perform a complete physical examination, including voluntary photography, at Visits 1, 3, and 9. A skin examination will be conducted at Visits 1, 3, 4, 6, 9, and 10 (see Table 1).

A complete physical examination will include assessment of general appearance, eyes, ears, nose, throat, head/neck/thyroid, lymph nodes, cardiovascular, lungs/chest, abdomen, genitourinary, extremities, skin, musculoskeletal, neurologic, and other.

Skin will be examined thoroughly and excoriations/scratch marks recorded.

10.2.6 Vital Signs

Evaluation of vital signs will be performed at all visits. This includes blood pressure (systolic and diastolic), pulse, respiratory rate, temperature, height (or length depending on age), and body weight (using a certified weight scale) at clinic visits. BMI will be calculated.

10.2.7 Abdominal Ultrasound

Ultrasound of the liver and spleen will be performed at randomization (Visit 3) and at EOT (Visit 9). Liver size, echogenicity, and presence of masses/nodules as well as spleen size will be recorded.

10.2.8 Overdose

A4250 is minimally absorbed and has a very low systemic availability. Based on toxicology data, for study purposes any dose exceeding a total of >3 mg/kg body weight of A4250 taken as a single dose or as a cumulative dose within 24 hours is defined as an overdose.

The no-adverse-effect dose level in the most sensitive species (defined as 20 mg/kg/day) in the rat 1 month toxicity study gives a human equivalent dose of approximately 194 mg/day in a 60 kg person. This dose is approximately 10-fold higher than the maximum possible dose predicted in human studies (20 mg).

The investigator/investigative staff should immediately and not later than within 24 hours of knowledge of the overdose, be reported by the investigator or delegate on the SAE report form. In the event of an A4250 overdose, the patient should be monitored closely.

10.2.9 Pregnancy

If a pregnancy is discovered in a female patient enrolled in the study before the end of dosing, the patient will be immediately discontinued from the study and will attend the same visits as a prematurely withdrawn patient. If the pregnancy is discovered in a female patient enrolled in the study after the end of dosing, the patient will continue in the study per protocol. If a pregnancy occurs in a male patient's partner at any time during the study, the pregnancy should also be reported and followed.

Pregnancy is not considered to be an AE. However, if the patient has been dosed with the study drug, the pregnancy must be reported on the Paper Pregnancy Notification Form immediately within 24 hours after recognition to Albireo/Safety Manager (please refer to Section 10.1.3). Date of exposure and as far as possible, details of the period of gestation at the time of exposure must be given.

The pregnancy should be followed to determine outcome, including spontaneous termination, details of birth, and presence of any birth defects, congenital anomalies or newborn or maternal complication. The infant who was exposed in-utero will be followed for up to 2 years after delivery. Individual cases with an abnormal outcome in association with the study drug should be reported on an expedited basis, i.e., reported rapidly to a competent authority.

11 STATISTICAL EVALUATION

All personnel involved with the analysis of the study will remain blinded until database lock for the double-blind Treatment Period and until major protocol violations have been identified.

All statistical analyses will be performed using SAS version 9.3 or higher.

11.1 Sample Size and Power

For the EU and RoW, the primary analysis will relate to the bile acid responder endpoint (at the 1-sided 1.25% level). For the US, the primary analysis will relate to the pruritus endpoint (at the 1-sided 1.25% level). The aim is to enroll 60 to 70 patients in order to get at least 60 evaluable patients.

Assuming 60% responders (patients experiencing at least 70% reduction in fasting s-BA concentration or reaching ≤70 µmol/L) in the A4250 dose group and 10% responders in the placebo group, approximately 20 patients per group are required to achieve a power of 84% with a 1.25% 1-sided Type-1 error.

For pruritus, with approximately 20 patients per treatment group, the power is 83% given an assumed treatment effect difference of -1.0 in change from baseline (-1.6 for A4250 and -0.6 for placebo) on the Albireo ObsRO scratching score with a SD of 0.95 and a Type I error of 1.25% (1-sided).

11.2 Statistical Methods

11.2.1 Statistical Analysis Sets

Safety Analysis Set

The safety analysis set will consist of all patients who received at least 1 dose of study drug. Patients will be analyzed according to the treatment they actually received. The safety analysis set will be the primary analysis set for safety analyses.

Full Analysis Set

The full analysis set (FAS) will consist of all randomized patients who received at least 1 dose of assigned treatment. Patients will be analyzed in the treatment group they were randomized to even if they received incorrect study drug. The FAS will be the primary analysis set for efficacy analyses.

Per Protocol Analysis Set

The per protocol (PP) analysis set is a subset of the FAS, and will consist of all randomized patients for whom no major protocol violation (no violation which may affect the study efficacy outcome) is documented. The PP analysis set will provide supportive data for the efficacy analyses.

Allocation of patients to the PP analysis set will be performed before unblinding of the study.

11.2.2 Methods of Statistical Analyses

11.2.2.1 General Principles

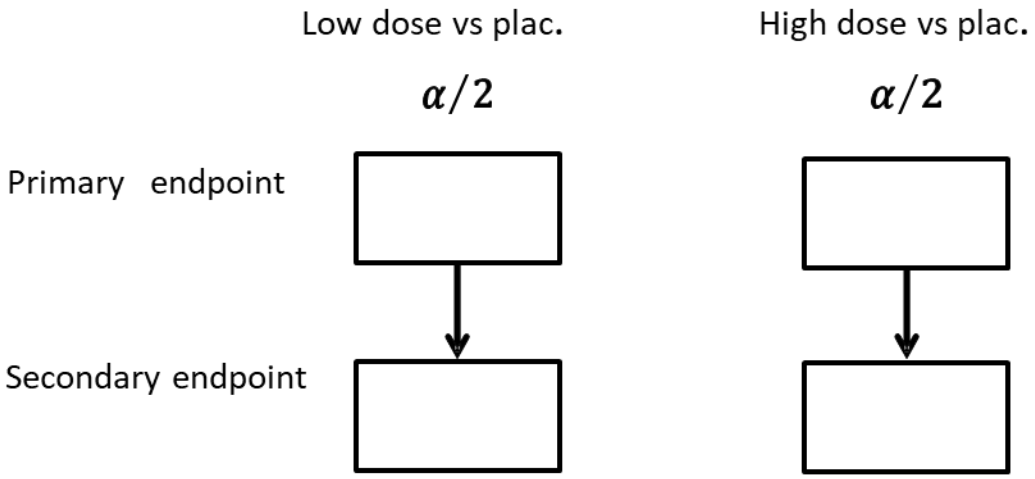
The primary objective of this study is to show superiority in efficacy of A4250 (120 µg/kg/day and 40 µg/kg/day) compared to placebo in patients with PFIC Types 1 and 2.

In the EU and RoW, for the primary efficacy variable fasting bile acid responder endpoint (at least a 70% reduction in fasting s-BA concentration or reaching a level ≤70 µmol/L at the end of treatment), the null hypothesis will be tested against the alternative hypothesis ($\alpha = 0.0125$, 1-sided).

The test will be performed at a Type I error rate of ≤0.0125 1-sided, as described above, based on the least squares means of the treatment difference in mean probability scale.

To strictly control overall Type I error rate across the dose groups and also across the primary and the main secondary endpoint to a 1-sided 2.5% level, a Bonferroni-based parallel gate-keeping procedure will be used. In this procedure the total significance level of 2.5% (1-sided) will be split equally between the dose groups. Only a dose group that is significant for the primary endpoint will be tested, at the nominal significance level of 1.25% (1-sided), for the main secondary endpoint (here the pruritus endpoint). If the previous step was not successful, the analysis of the following step will be considered descriptive. If the procedure reaches the secondary endpoint for both dose groups, these will be evaluated using the Bonferroni Holm procedure. The multiple comparison procedure is presented in Figure 2.

Figure 2 Bonferroni Holm Multiple Comparison Procedure



In the US, for the primary efficacy variable pruritus score endpoint (change from baseline to Week 24 in observer-reported scratching), the null hypothesis will be tested against the alternative hypothesis ($\alpha=0.0125$, 1-sided).

The test will be performed at a Type I error rate of ≤0.0125 1-sided, as described above, based on the least squares means of the treatment difference in mean change.

To strictly control overall Type I error rate across the dose groups and also across the primary and the main secondary endpoint to a one-sided 2.5% level, a Bonferroni-based parallel gate-keeping procedure will be used. In this procedure the total significance level of 2.5% (1-sided) will be split equally between the dose groups. Only a dose group that is significant for the primary endpoint will be tested, at the nominal significance level of 1.25% (1-sided), for the main secondary endpoint (here the bile acid responder endpoint). If the previous step was not successful, the analysis of the following step will be considered descriptive. If the procedure reaches the secondary endpoint for both dose groups, these will be evaluated using the Bonferroni Holm procedure.

Other secondary and exploratory outcome variables shall provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple dose comparisons when testing the other secondary and exploratory efficacy variables.

A more detailed statistical analysis plan (SAP) will be prepared and finalized before study data are locked and unblinded.

If not otherwise specified, the baseline value of a parameter is defined as the last assessment of that parameter on or prior to the first intake of study treatment date.

11.2.2.2 Missing Data

For efficacy variables, with repeated measures, a mixed model will be employed with no additional imputation for missing values, i.e., based on "observed cases", except where otherwise specified.

Follow-up and continued collection of efficacy data for patients who discontinue treatment will be made. These data will be included in the sensitivity analysis for primary endpoints estimating a treatment effect taking loss of effect after discontinuation into account. Because complete follow-up of all patients discontinuing treatment may not be possible, a method to replace missing data for patients who discontinue the study will be prespecified (in the SAP) taking into account loss of effect after discontinuation (for example multiple imputation).

No other replacement of missing values will be planned.

11.2.2.3 Demographic and Baseline Characteristics

Descriptive summaries of demographics and other baseline characteristics (including medical and surgical history) will be presented by treatment group and overall using the FAS.

Prior medication will be summarized by treatment group and overall using the safety analysis set.

11.2.2.4 Subject Disposition

The following will be summarized (by treatment group and overall where applicable):

- Patients enrolled (who signed the informed consent)
- Patients randomized
- Patients treated
- Patients completing the study/withdrawing early (including withdrawal reason)

Additionally, patients enrolled, included in the safety, in the FAS, and in the PP analysis set will be summarized by region.

11.2.2.5 Evaluation of Primary Efficacy Variables

The responder endpoint based on change in fasting bile acids will be analyzed in terms of the proportion of patients achieving the event of interest. Patients in need of rescue therapy, i.e., biliary diversion will be classified as non-responders after the biliary diversion.

Generalized linear mixed modelling (GLMM) will be used with a logistic link, to allow for repeated assessments over time. The model will include baseline value, treatment group, PFIC class, age class, region, visit, and visit*treatment group interaction. The treatment by visit interaction will remain in the model regardless of significance. Region will be included in the model as a random effect. The contrasts of primary interest will be the treatment difference at Week 24; also, the difference at Week 4, Week 8, Week 12, and Week 18 will be estimated and analyzed. An unstructured covariance matrix will be used to model the within-subject error. Another covariance structure may be chosen if the fit of this structure fails to converge. Model diagnostics checks will be made to ensure appropriate fit.

The primary analysis of the change from baseline in pruritus score (indexed by observer-reported scratching) will be analyzed using a mixed model repeated measures (MMRM) analysis. The contrast of primary interest will be treatment difference at Week 24 but also the differences at Week 4, Week 8, Week 12, and Week 18 will be estimated and analyzed. Baseline pruritus score will be included as covariate. The model will include baseline value, treatment group, PFIC class, age class, region, visit, and visit*treatment group interaction. The treatment by visit interaction will remain in the model regardless of significance. Region will be included in the model as a random effect and will be defined on country level. An unstructured covariance matrix will be used to model the within-subject error. Another covariance structure may be chosen if the fit of this structure fails to converge.

Non-parametric approach may be considered if more appropriate.

The primary analyses will be based on the FAS.

To assess the robustness of the primary efficacy analysis, the following analyses will be performed:

- Primary efficacy analysis using the PP set
- Analyses to investigate the influence of missing values, for example, by using pattern mixture models and tipping point analysis

Subgroup analyses will also be performed for each of the 3 age groups (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) and for patients who were not included in the previous Study A4250-003.

Several sensitivity analyses will be performed, such as the following:

- Cochran Mantel Haenzsel test stratified on PFIC class to compare the proportion in fasting bile acid responder endpoint at the end of treatment in the two A4250 dose groups to placebo group. Dropouts will be treated as non-responders
- Based on the primary analysis including the interaction age class*treatment
- Same as primary analysis in which the random factor region is considered as fixed factor. The region*treatment interaction will also be explored
- Same as primary analysis including the interaction PFIC*treatment

Other sensitivity analyses may be performed and will be detailed in the SAP.

11.2.2.6 Evaluation of Secondary and Exploratory Efficacy Variables

The change in the secondary and explorative endpoints such as s-BA, total bilirubin, ALT, growth and patient-reported itch severity will also be analyzed using the MMRM model as specified for the primary analysis. Nonparametric methods may be applied as an alternative approach for some endpoints, if appropriate. Change in growth (weight for age and BMI for age) will also be displayed using graphical presentations.

Proportions of responders (e.g., meaningful reduction in itch severity and scratching at Week 24) will be analyzed using the GLMM model as specified for the primary analysis.

Comparisons of the change from baseline at Week 24 in PedsQL between the treatment groups will be conducted using analysis of covariance. The model will include terms for baseline, PFIC class, age class, region, and treatment.

Undergoing biliary diversion surgery and/or listed for liver transplant, all-cause mortality, and liver decompensation events will mainly be analyzed by using descriptive statistics. Kaplan-Meier curves will be used when appropriate for time to event data.

Other exploratory variables (Global Symptom Relief, biochemical markers and measures of bile acid synthesis, Albireo PRO and ObsRO sleep parameters, PELD/MELD score, APRI score, and Fib-4 score) will be analyzed descriptively for categorical and continuous data, as applicable. For continuous data, the change from baseline will be analyzed in addition to the actual visit values. For categorical data, shift tables or frequency and percentages of patients will be presented as appropriate.

In addition, subgroup analyses will be performed for each of the 3 age classes (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years).

11.2.2.7 Evaluation of Safety Variables

Safety data will be analyzed using descriptive statistics and summaries by treatment group of SAEs, AEs, vital signs, clinical laboratory tests (hematology, clinical chemistry and urinalysis), and concomitant medication. Analyses will be performed using the safety analysis set.

Summaries of AEs (coded according to the Medical Dictionary for Regulatory Activities [MedDRA] system organ class [SOC] and MedDRA preferred term) will include the following:

- Overview of the incidence of TEAEs (TEAEs, drug-related TEAEs, TEAEs leading to study discontinuation, and treatment-emergent SAEs)
- TEAEs by SOC and preferred term
- Intensity of TEAEs by SOC and preferred term
- Drug-related TEAEs by SOC and preferred term
- TEAEs leading to study discontinuation by SOC and preferred term
- Treatment-emergent SAEs by SOC and preferred term

Concomitant medication use during the Treatment Period will be summarized by Anatomical Therapeutic Chemical (ATC) class and World Health Organization (WHO) preferred name.

Summaries of vital signs will be presented. For each visit, the actual results and the change from baseline, and the number and percentage of patients with potentially clinically significant (PCS) values observed post-baseline will be presented.

Summaries of clinical safety laboratory data will be presented. For each visit, the actual result and the change from baseline, and the number and percentage of patients with PCS values observed post-baseline will be presented.

Data listings will be provided for each patient for all safety parameters.

11.2.2.8 Compliance and Exposure

Exposure will be analyzed by calculating the number of days with exposure to study drug. Results will be presented by treatment group using the safety analysis set.

The percentage compliance will be described by treatment group and the number of patients with a compliance <80%, between 80% and 120%, and >120% will be presented based on the safety analysis set.

11.2.3 Blinded Analyses to Confirm Responder Definitions

A blinded analysis of Albireo ObsRO and PRO eDiary data will be performed after 50% or more of the planned sample has completed the Week 24 Follow-Up Period. The blinded analysis will be used to estimate a threshold of clinically meaningful change (i.e., responder definition) in Albireo ObsRO and PRO pruritus scores. The analysis will be performed by a group independent from both the study team and Albireo. Blinded data will be used for this analysis; data will be collapsed across treatment groups.

The analyses will include distribution- and anchor-based analyses to examine a responder definition for the Albireo PRO/ObsRO pruritus scores. Distribution-based analyses will include calculation of the SD and standard error of measurement for the baseline Albireo PRO and ObsRO scores. Anchor-based analyses will involve examining the degree of change on the Albireo PRO and ObsRO scores from baseline to Week 24 for patients who experience change in pruritus according to PGIC and symptom items. Details of this analysis will be included in a stand-alone SAP for assessing the measurement characteristics (reliability, validity, sensitivity to change, responder definition) of the Albireo PRO and ObsRO items.

11.2.4 Data Safety Monitoring Board

A DSMB consisting of sponsor-independent clinical and statistical experts will be established. The DSMB will periodically meet for the review of accumulating study data, including safety (AE and laboratory data) and liver values.

The DSMB will have access to unblinded data. The DSMB will make recommendations for the remaining part of the study (further details will be provided in the DSMB charter). The DSMB may recommend continuing with the study as planned or stopping the study early for safety reasons. The DSMB will submit its recommendations in writing to Albireo Medical/[redacted] who are responsible for responding to the recommendations of the DSMB and taking appropriate action. The investigators will only be informed by Albireo Medical/[redacted] if the study requires a protocol amendment or is stopped. The DSMB may choose to make additional evaluations at any time if they feel this is warranted from a safety point of view.

The DSMB will act according to its own written standard operating procedure described in a charter and will prepare written minutes of its meetings. The charter of the DSMB will be stored in the trial master file. The DSMB will maintain records of its meetings and these will become part of the study file when the study is complete. In order not to disseminate unblinded data and to ensure that all staff involved in the conduct and final analysis of the study remain blind to the data, only the members of the DSMB and the unblinded DSMB statistician will have access to these data.

12 DIRECT ACCESS TO SOURCE DATA/NOTES

The investigator/institution shall provide direct access to source data/documents for study-related monitoring, audits, IEC/Institutional Review Board (IRB) review and regulatory inspection.

13 QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Conduct of the Study

Albireo/[redacted] shall implement and maintain quality control and quality assurance procedures with written standard operating procedures to ensure that the study is conducted and data are generated, documented, and reported in compliance with the protocol, ICH GCP and applicable regulatory requirements.

This study shall be conducted in accordance with the provisions of the Declaration of Helsinki (October 1996) and all revisions thereof, and in accordance with United States Food and Drug Administration (FDA) regulations (Code of Federal Regulations, Sections 312.50 and 312.56) and with ICH GCP (CPMP 135/95).

The investigator may not deviate from the protocol without a formal protocol amendment having been established and approved by an appropriate IEC/IRB, except when necessary to eliminate immediate hazards to the patient or when the change(s) involve(s) only logistical or administrative aspects of the study. Any deviations may result in the patient having to be withdrawn from the study and render that patient non-evaluable.

13.2 Study Monitoring

Before study initiation, at a site initiation visit or at an investigator's meeting, an Albireo representative or designee will review the protocol and eCRF with the investigators and the investigative staff. During the study, the clinical monitor (clinical research associate [CRA]) will visit the site regularly to check the completeness of patient records, the accuracy of entries on the eCRFs, the adherence to the protocol and to GCP, the progress of enrollment, and to ensure that study drug is being stored, dispensed, and accounted for according to specifications.

The investigator must ensure that eCRFs are completed within a timely period of patient visits, as per individual site agreements, and must allow the CRA and Albireo representative or designee periodic access to patient records and all study-related materials, including relevant hospital or clinical records, to confirm their consistency with the eCRF entries. No information in these records about the identity of the patients will leave the study center. Albireo monitoring standards require full verification for the presence of the signed ICF, adherence to the inclusion/exclusion criteria, documentation of SAEs, and recording of primary efficacy and safety variables. The CRA will review source data compared with the eCRFs and will verify source data according to the study-specific monitoring plan. The design of the study, the frequency of patient visits, and the site enrollment rate will determine the frequency of monitoring visits. Upon study completion, the CRA will visit the site to conduct a study termination visit, which will include collection of any outstanding documentation.

It is recommended that the investigator/investigative staff log into the eCRF system every working day or at minimum twice weekly in order to provide a prompt response to queries.

The investigator/investigative staff should respond to queries and make any relevant changes to the study data within 3 working days.

14 ETHICS

14.1 Independent Ethics Committee/Institutional Review Board

Prior to the start of the study, the investigator is responsible for ensuring that the protocol and consent form have been reviewed and approved by a relevant IEC/IRB. The IEC/IRB shall be appropriately constituted and perform its functions in accordance with FDA ICH GCP and local requirements as applicable.

The IEC/IRB shall approve all protocol amendments (except for logistical or administrative changes), written informed consent documents and document updates, patient recruitment procedures (e.g., advertisements), written information to be provided to the patients and caregivers, IB, available safety information, information about payment and compensation available to patients, the investigator's curriculum vitae and/or other evidence of qualifications and any other documents requested by the IEC/IRB and regulatory authority (competent authority) as applicable.

14.2 Written Informed Consents and Assents

The investigator/investigative staff will explain to each patient (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, alternative treatment, the potential risks and benefits involved, and any discomfort that may occur. Each caregiver/patient will be informed that participation in the study is voluntary and that he/she or their child may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

Caregivers/patients will be informed that they/their children may experience side-effects or be at risk for symptoms, illnesses, or complications that cannot be foreseen by Albireo. As with other medications, people treated with A4250 may be at risk of developing allergic reactions or anaphylaxis. Caregivers/patients will be advised that study procedures include regular blood sampling for measurement of safety parameters and biological markers and that some minor risks are associated with these procedures.

This informed consent should be given by means of a signed ICF, written in non-technical language in accordance with ICH GCP, the Declaration of Helsinki, and regulatory authorities. The caregiver/patient should read and consider the statements before signing and dating them and should be given a copy of each signed document. If written consent is not possible, oral consent can be obtained if witnessed and followed by a signed statement from one or more persons not involved in the study, indicating why the patient was unable to sign the form. No patient can enter the study before his/her or caregiver informed consent has been obtained.

The ICF is part of the protocol and must be submitted by the investigator/investigative staff with the protocol for IEC approval. Albireo will supply an ICF which complies with regulatory requirements and is considered appropriate for the study. Any changes to the ICF

suggested by the investigator must be agreed to by Albireo before submission to the IEC and a copy of the approved version must be provided to the clinical monitor after IEC approval.

15 DATA HANDLING AND RECORD KEEPING

15.1 Case Report Forms/Source Data Handling

The investigator shall be provided with standardized eCRFs and shall ensure that all data from patient visits are promptly entered into the eCRFs in accordance with the specific instructions given. The investigator must sign each eCRF to verify the integrity of the data recorded.

A list of the normal ranges for all laboratory tests to be undertaken forms part of the documentation to be collated prior to study start. As a central laboratory has been selected to conduct any or all tests, it is essential that all samples be analyzed at that laboratory.

The investigator must maintain source documents such as laboratory reports, consultation reports, and complete medical history and physical examination reports.

As no ethnicity data can be collected in the source document for study sites in France, the eCRF will be the Source Document for “origines ethniques”,

15.2 Retention of Essential Documents

Essential documents, as defined by ICH GCP, include: the signed protocol and any amendment(s); copies of the completed eCRFs (for site archiving, compact discs of eCRF data for specific patients will be provided); signed ICFs; hospital records and other source documents; IEC approvals and all related correspondence including approved documents; drug accountability records; study correspondence; and a list of patients’ names and addresses.

The investigator/investigative staff must retain copies of these essential documents for the period specified by ICH GCP and by applicable regulatory requirements. The investigator/investigative staff will inform Albireo of the location where the essential documents are stored and must contact Albireo for approval before disposing of any essential documents. The investigator/investigative staff should take measures to prevent accidental or premature destruction of these documents.

16 FINANCING AND INSURANCE

Financing and insurance are addressed in a separate agreement.

17 PUBLICATION POLICY

Albireo will retain the ownership of all data. When the study is complete, Albireo shall arrange the analysis, tabulation of data and preparation of a clinical study report. Albireo may also use the data for publication, presentation at scientific meetings and submission to regulatory authorities. All proposed publications based on this study must be subject to the sponsor's approval requirements.

18 REFERENCE LIST

1. A4250 Investigator's Brochure.
2. Alissa FT, Jaffe R, Shneider BL. Update on progressive familial intrahepatic cholestasis. J Pediatr Gastroenterol Nutr. 2008;46(3):241-52.
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4. Hofmann AF. The enterohepatic circulation of bile acids in mammals: form and functions. Front Biosci (Landmark Ed). 2009 Jan 1;14:2584-98.
5. Hori T, Justin H, Nguyen JH and Uemoto S. Progressive familial intrahepatic cholestasis. Hepatobiliary pancreat Dis Int. 2010; 9(6):570-8.
6. Jacquemin E. Progressive familial intrahepatic cholestasis. Genetic basis and treatment. Clin Liver Dis. 2000;4(4):753-63.
7. Miethke AG, Zhang W, Simmons J, et al. Pharmacological inhibition of ASBT changes bile composition and blocks progression of sclerosing cholangitis in mdr2 knockout mice. Hepatology. 2016; 63(2):512–23.
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9. Pawlikowska L, Strautnieks S, Jankowska I, et al. Differences in presentation and progression between severe FIC1 and BSEP deficiencies. J Hepatol. 2010;53(1):170-8.
10. Suchy FJ, Sokol RJ, Balistreri WF, eds. Liver Disease in Children. 3rd Edition. New York, NY: Cambridge University Press; 2007:310–4.
11. Whitington PF, Whitington GL. Partial external diversion of bile for the treatment of intractable pruritus associated with intrahepatic cholestasis. Gastroenterology. 1988;95(1):130-6.

19 APPENDICES

Appendix 1 Concomitant Medications Prohibited During the Study

Appendix 2 Diary Questions including Albireo Patient-Reported Outcomes/Observer-Reported Outcome Instrument

Appendix 3 Patient Global Impression of Change (PGIC) and Patient Global Impression of Symptoms (PGIS)

Appendix 4 Pediatric Quality of Life Inventory (PedQL) Questionnaire

Appendix 5 Blood Volumes

CLINICAL STUDY PROTOCOL A4250-005

A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

IND Number:	130591
EudraCT Number	2017-002338-21
Test Product:	A4250
Indication:	Progressive Familial Intrahepatic Cholestasis (PFIC)
Sponsor:	Albireo AB
Development Phase:	Phase 3
Sponsor Signatory:	[redacted]
Sponsor Medical Monitor:	[redacted]
Principal Investigator:	[redacted]
Date of the Protocol:	15 March 2018
Version of the Protocol:	Final Amendment 1.2 France and UK

Confidential

The information in this document contains trade secrets and commercial information that are privileged or confidential and may not be used, divulged, published, or otherwise disclosed without the written consent of Albireo AB. These restrictions on disclosure apply equally to all future information supplied to you or generated by you in connection with the study.

SPONSOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

Albireo AB

[redacted]

15- March -2018
Date (day/month/year)

CLINICAL RESEARCH ORGANIZATION SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

[redacted]

[redacted] Date (day/month/year)

[redacted] Date (day/month/year)

INVESTIGATOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

I have read this protocol and agree that it contains all necessary details for performing this study. I will conduct the study as outlined herein and will complete the study within the time designated, in accordance with all stipulations of the protocol and in accordance with Good Clinical Practice (GCP), local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study drug and the conduct of the study.

I will use only the informed consent approved by the Independent Ethics Committee (IEC) and will fulfil all responsibilities for submitting pertinent information to the IEC responsible for this study.

I agree that the sponsor (Albireo AB) shall have access to any source documents from which case report form information may have been generated.

I further agree not to originate or use the name of Albireo AB, A4250 in any publicity, news release, or other public announcement, written or oral, whether to the public, press, or otherwise, relating to this protocol, to any amendment to the protocol, or to the performance of this protocol without the prior written consent of Albireo AB.

Name of Investigator

Signature

Date (day/month/year)

1 ADMINISTRATIVE INFORMATION

A Double-blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Protocol No.:	A4250-005
Date of the initial Protocol:	20 September 2017
Date and Number of Amendment(s):	Amendment 01,06 December 2017
Sponsor:	Amendment 1.2 Fr, UK, 15 March 2018 Albireo AB Arvid Wallgrens backe 20 413 46 Göteborg Sweden
Clinical Research Organization:	[redacted]
Sponsor Signatory:	
Sponsor Medical Monitor:	
Principal Investigator:	

2 STUDY SYNOPSIS

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250

Title of Study:
A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Principal Investigator:
[redacted]

Study Centers:
Up to 50 sites will be initiated for this study in the United States (US), Canada, European Union (EU), Middle East, and Australia.

Publication(s):
None.

Planned Study Period:	**Development Phase:**
Q1 2018 to Q2 2019	Phase 3

Objectives:

Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with progressive familial intrahepatic cholestasis (PFIC) Types 1 and 2, as determined by the following:

- Proportion of patients experiencing at least a 70% reduction in fasting serum bile acid (s-BA) concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- Change from baseline to Week 24 in pruritus compared to placebo

Secondary Objectives

- To evaluate the effect of A4250 on serum alanine aminotransferase (ALT) concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

Methodology:

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of familial intrahepatic cholestasis-1 or bile salt export pump).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-up Period. After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo. Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008) in which all patients will receive active treatment.

There will be up to 10 clinic visits during the study and one scheduled telephone contact.

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250

Number of Patients:
Approximately 60 patients diagnosed with PFIC-1 or PFIC-2 will be randomized.

Diagnosis and Main Criteria for Inclusion:

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples 7 to 28 days apart (Visits 1 and 2) prior to randomization
4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the electronic diary (eDiary) average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients are expected to have a consistent caregiver(s) for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

Test Product, Dose and Mode of Administration:
A4250, 40 µg/kg/day or 120 µg/kg/day, orally administered

Reference Therapy, Dose and Duration of Administration:
Matching placebo, orally administered

Duration of Treatment:
24 weeks

Variables

Efficacy:

Primary Efficacy Endpoints

The primary efficacy endpoints are:

- **EU and rest of world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  Fasting s-BA baseline will be calculated as the average of the value at randomization and the previous visit (Clinic Visit 2). The end value is the average of the values at Weeks 22 and 24 after the start of double-blind treatment.
- **US**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 24 as measured by the worst scratch of the Albireo observer-reported outcome (ObsRO) instrument. Scores for the 14 days baseline and post-treatment intervals will be calculated by averaging the worst scratching scores from each day in the interval.

Secondary Efficacy Endpoints

Secondary efficacy endpoints include the following:

- **EU and RoW**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 24 as measured by the worst scratch of the Albireo ObsRO instrument. Scores for the 14 days baseline and post-treatment intervals will be calculated by averaging the worst scratching scores from each day in the interval.
- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  All Regions:

  All secondary endpoints will be compared to placebo.

Name of Sponsor/Company:	Name of Product:	Name of Active Ingredient:
Albireo	A4250	A4250

- Change from baseline to Week 24 in fasting s-BA
- Change from baseline to Week 24 in serum ALT concentration
- Change in growth from baseline to Week 24, defined as the linear deficit (weight for age and body mass index) compared to the national growth curves (Z-score, standard deviation from P50)
- Proportion of patients achieving meaningful reduction in caregiver-reported observed scratching at Week 24
- Change from baseline to Week 24 in sleep parameters measured with the Albireo patient-reported outcome (PRO) and ObsRO instruments
- Change from baseline to Week 24 in patient-reported itch severity as measured by the average daily score of the Albireo PRO instrument; only patients ≥8 years of age will complete the Albireo PRO instrument
- Number of patients undergoing biliary diversion surgery or being listed for liver transplantation

Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Proportion of patients achieving meaningful reduction in patient-reported itch severity at Week 24
- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the patient global impression of change (PGIC) items
- Change from baseline in fasting s-BA at Weeks 4 and 12
- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Weeks 4, 12, and 24 as measured by the worst scratch. Scores for the 7 worst scratching days of the last 14 days at baseline and post-treatment intervals will be selected
- Change from baseline in patient-reported itch severity and observer-reported scratching at Weeks 4 and 12
- Change from baseline to Week 24 in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline to Week 24 in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline to Week 24 in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in Pediatric Quality of Life Inventory (PedsQL)
- Change from baseline in serum ALT concentration at Weeks 4 and 12
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, plasma 7α-hydroxy-4-cholesten-3-one concentration [p-C4]) at Week 24
- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events.
- All-cause mortality

Safety:

Safety criteria are as follows:

- Occurrence of treatment-emergent adverse events including severity and relatedness to study drug at all visits
- The incidence of treatment-emergent serious adverse events (SAEs), based upon information from patient reports, including the description, incidence, and severity of an SAE
- Trends evaluated for the following assessments: physical examinations, concomitant medications, vital signs, laboratory test results (including clinical chemistry, hematology, and urinalysis), and imaging

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250
studies		

Statistical Methods:

The primary objective of this study is to show superiority in efficacy of A4250 (120 µg/kg/day and 40 µg/kg/day) compared to placebo in patients with PFIC Types 1 and 2.

In the EU and RoW, for the primary efficacy variable fasting bile acid responder endpoint (at least a 70% reduction in fasting s-BA concentration or reaching a level ≤70 µmol/L at the end of treatment), the null hypothesis will be tested against the alternative hypothesis (α=0.0125, 1-sided). Generalized linear mixed modelling will be used with a logistic link, to allow for repeated assessments over time. The model will include baseline value, treatment group, PFIC class, age class, region, visit and visit*treatment group interaction. The contrasts of primary interest will be the treatment difference at Week 24 but also the difference at Week 4, Week 8, Week 12, and Week 18 will be estimated and analyzed.

In the US, for the primary efficacy variable pruritus score endpoint (change from baseline to Week 24 in observer-reported scratching), the null hypothesis will be tested against the alternative hypothesis (α=0.0125, 1-sided). The primary analysis of the change from baseline in pruritus score (indexed by observer-reported scratching) will be analyzed using a mixed model repeated measures analysis. The contrasts of primary interest will be the treatment difference at Week 24 but also the difference at the other weeks will be estimated and analyzed.

To strictly control overall Type I error rate across the dose groups and also across the primary and the main secondary endpoint to a 1-sided 2.5% level, a Bonferroni-based parallel gate-keeping procedure will be used.

Other secondary and exploratory outcome variables shall provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple-dose comparisons when testing the other secondary and exploratory efficacy variables.

Date of the Protocol: 15 March 2018

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4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADME	absorption, distribution, metabolism, and excretion
AE(s)	adverse event(s)
AFP	alfa-fetoprotein
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APRI	AST to platelet ratio index
ASBT	apical sodium-dependent bile transporter (also known as IBAT)
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	body mass index
BSEP	bile salt export pump
CPK	creatine phosphokinase
CRA	clinical research associate
CYP	cytochrome P450
DSMB	Data and Safety Monitoring Board
eCRF	electronic case report form
eDiary	electronic diary
EOT	End of Treatment
EU	European Union
EudraCT	European Union drug regulatory agency clinical trial
FAS	full analysis set
FDA	United States Food and Drug Administration
FIC1	familial intrahepatic cholestasis-1
GCP	Good Clinical Practice
GI	gastrointestinal
GLMM	generalized linear mixed modelling
IB	Investigator's Brochure
IBAT	ileal bile acid transporter (also known as ASBT)
ICF	informed consent form
ICH	International Council for Harmonisation
[redacted]	[redacted]
IEC	Independent Ethics Committee
INR	international normalized ratio
IRB	Institutional Review Board
IWRS	Interactive Web Response System

LDH	lactate dehydrogenase
LFT	liver function test
MDR3	multidrug-resistance protein 3
MedDRA	Medical Dictionary for Regulatory Activities
MELD	model for end-stage liver disease
MMRM	mixed model repeated measures
ObsRO	observer-reported outcome
PBC	primary biliary cholangitis
p-C4	plasma 7α-hydroxy-4-cholesten-3-one concentration
PCS	potentially clinically significant
PedsQL	Pediatric Quality of Life Inventory
PELD	pediatric end-stage liver disease
PFIC	progressive familial intrahepatic cholestasis
PGIC	patient global impression of change
PGIS	patient global impression of symptoms
PP	per protocol
PRO	patient-reported outcome
PT	prothrombin time
PVM	pharmacovigilance manager
QoL	quality of life
RoW	rest of world
RR	respiratory rate
SAE(s)	serious adverse event(s)
SAP	statistical analysis plan
s-BA	serum bile acid
SD	standard deviation
s-FGF19	serum fibroblast growth factor 19 concentration
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction
TEAE(s)	treatment-emergent adverse event(s)
ULN	upper limit of normal
US	United States
WHO	World Health Organization

5 INTRODUCTION

5.1 Investigational Medicinal Product

A4250 is a small molecule and a potent selective inhibitor of the ileal bile acid transporter (IBAT), also known as the apical sodium-dependent bile acid transporter (ASBT). IBAT is an integral brush border membrane glycoprotein that co-transports sodium and bile acids and appears to be a critical component in the regulation of the bile acid pool size in animals and man. This transporter, expressed in the distal ileum, is a key element in the enterohepatic circulation of bile acids since it facilitates the high-affinity, high-capacity reabsorption of bile acids. Indeed, 95% of secreted bile acids are reabsorbed via IBAT [Hofmann 2009; Miethke 2016]. A4250 is orally administered and acts locally in the gut where it binds reversibly to IBAT to decrease the reuptake of bile acids. A4250 has minimal systemic exposure at the predicted therapeutic dose ranges.

No safety signals were found with A4250 in preclinical safety and toxicity studies. In the first clinical study of A4250 in healthy volunteers, A4250 was given as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. A4250 was well tolerated and the only findings were gastrointestinal (GI) related effects, such as diarrhea, at higher doses.

In an exploratory Phase 2 study in children with cholestatic pruritus, A4250 was given as oral doses of 10 to 200 µg/kg/day for 4 weeks. A4250 reduced mean levels of serum bile acids (s-BA) in all cohorts. In 11 of 13 progressive familial intrahepatic cholestasis (PFIC) patients, substantial reductions (ranging from 43% to 98%) were observed. Patient-reported diary data documented improvement of pruritus during treatment. A4250 was well tolerated. No serious adverse events (SAEs) were deemed to be related to study drug, and most adverse events (AEs), including some increased transaminases, were considered unrelated to study drug and transient in nature.

5.2 Background

5.2.1 Progressive Familial Intrahepatic Cholestasis

PFIC is a rare autosomal recessive cholestatic liver disease estimated to affect between 1 in every 50,000 to 100,000 children born worldwide. PFIC represents 10% to 15% of causes of cholestasis in children and 10% to 15% of liver transplantation indications in children. All types of PFIC exist worldwide and both sexes appear to be equally affected.

The common underlying pathogenesis of PFIC is disruption of bile formation and bile transport through the liver [Jacquemin 2000]. The classification of PFIC has evolved over the years. The most commonly used sub-classifications are PFIC Types 1, 2, and 3, which are based on the associated affected gene and described in more detail below.

- PFIC, Type 1: also referred to as “Byler disease” or “familial intrahepatic cholestasis-1 (FIC1) protein deficiency.” FIC1 protein is located on the canalicular membrane of hepatocytes and facilitates movement of aminophospholipids from the outer

to inner leaflet of the plasma membrane of the hepatocyte. The *ATP8B1* gene encodes FIC1 protein. Biallelic pathologic variants in the *ATP8B1* gene are associated with FIC1 dysfunction and classified clinically as PFIC Type 1 disease.

- PFIC, Type 2: also referred to as "Byler syndrome" or "bile salt export pump (BSEP) deficiency." BSEP is a transporter protein that is expressed at the canalicular membrane of hepatocytes, and is the primary exporter of bile acids. The *ABCB11* gene encodes the BSEP protein. Biallelic pathologic variations in the *ABCB11* gene are associated with BSEP dysfunction and classified clinically as PFIC Type 2 disease.
- PFIC, Type 3: is caused by a deficiency of the multidrug-resistance protein 3 (MDR3) due to mutations in the *ABCB4* gene. MDR3 is a phospholipid translocase critical for phospholipid secretion.

Severe pruritus is common in children diagnosed with PFIC. Itching (and subsequent scratching) is a significant morbidity for these patients and their families [Suchy 2007]. A more severe degree of pruritus is experienced compared to patients with other forms of liver disease and to other pruritic conditions such as atopic dermatitis [Murray 2011]. In patients with PFIC, liver biopsy reveals canalicular cholestasis and, later, the appearance of portal fibrosis. Serum biochemistry indicates cholestasis with hyperbilirubinemia, elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The concentrations of bile acids in serum are very high, while serum gamma-glutamyl transferase activity (exception being MDR3 variants) and cholesterol are normal [Hori 2010]. Symptoms of portal hypertension and liver failure will develop during the course of the disease [Davit-Spraul 2009; Alissa 2008]. Symptoms develop early; median age at onset of symptoms is 2 months, and 78% of PFIC patients present with jaundice [Pawlikowska 2010]. The life-threatening and debilitating nature of PFIC is reflected by the fact that survival in those not resorting to surgery is 50% at 10 years of age and almost zero at 20 years of age. Approximately half of PFIC patients undergo liver transplantation [Davit-Spraul 2009] and treatment-resistant pruritus is the leading indication for the surgical procedure partial external biliary diversion, mostly in PFIC Type 1 and 2 patients.

PFIC is life-threatening and debilitating. Currently, the treatment for PFIC is palliative and there is no pharmaceutical treatment approved for use in PFIC. The therapeutic choices are restricted to non-specific therapy of the symptoms and signs of the disease such as nutritional support, preventing vitamin deficiencies, and treatment of extrahepatic features. Medical treatment options include off-label use of ursodeoxycholic acid, rifampin, antihistamines, and naltrexone. A minority of patients respond nominally and transiently to these interventions. Biliary diversion is used to decrease systemic bile acids through interruption of the enterohepatic circulation and so avoid transplantation. Liver transplantation is typically only viewed as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

5.2.2 Summary of Clinical and Nonclinical Studies

This is a summary of nonclinical and clinical studies. More detail is provided in the Investigator's Brochure ([IB] see A4250 IB).

A4250 is a potent, selective IBAT inhibitor with no safety signals seen in the safety pharmacological studies. In addition, no safety signals were seen in repeat-dose oral toxicity studies in rats with up to 26 weeks of daily dosing and in dogs with up to 39 weeks of daily dosing. No safety signals were identified when A4250 was administered daily to juvenile rats from age 14 days to 63 days.

A4250 is minimally absorbed with very low systemic exposure in both humans and other species. In blood, protein binding is high (>99.7%). Systemically absorbed A4250 is slowly metabolized in humans and in preclinical studies by hydroxylation only, a metabolite that retains IBAT activity. A4250 did not show any significant inhibition of cytochrome P450 (CYP) enzymes in the rat. A4250 inhibited human CYP3A4 and CYP2D6 (IC_{50}: 16 µmol/L for both enzymes), and CYP2C9 (IC_{50}:1.2 µmol/L).

To date, A4250 has been studied in 3 Albireo-sponsored clinical studies: a study in healthy volunteers evaluating single and multiple administration of A4250; a single-dose absorption, distribution, metabolism, and excretion (ADME) study; and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with primary biliary cholangitis (PBC). In total, 98 subjects have been exposed to A4250.

In the first clinical study (A4250-001), A4250 was given to healthy volunteers as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. Following a single oral dose of 10 mg, the median maximum drug concentration was 0.2 nmol/L and the area under the curve was 0.8 nmol × h/L (n=6). Pharmacodynamic evaluation of bile acid synthesis biomarkers (serum fibroblast growth factor 19 [s-FGF19] and plasma 7α Hydroxy 4-cholesten-3-one [p-C4]) identified the expected decrease in s-FGF19 and an increase in p-C4. In addition, total plasma bile acids decreased and total fecal bile acids increased in A4250-treated patients compared to placebo. A4250 was generally well tolerated. The only findings were GI-related effects, such as diarrhea at higher doses.

In the ADME study (A4250-007), healthy subjects were administered a single oral dose of 3 mg [^{14}C]-A4250 capsule. The main excretion route was determined to be fecal. There were no quantifiable concentrations of A4250 in plasma or total radioactivity in plasma or whole blood. A4250 was well tolerated. AEs were reported and most were mild and not related to A4250. The 3 AEs experienced by 2 subjects reported as related to study drug were GI in nature, including diarrhea (2 AEs; 2 subjects) and abdominal pain (1 AE; 1 subject).

In an exploratory Phase 2 study in children with cholestatic pruritus (A4250-003), A4250 was administered as oral daily doses of 0.01 to 0.2 mg/kg for 4 weeks. Twenty patients (8 females and 12 males) aged 1 to 17 years were enrolled in this open-label dose-escalation study. Four patients were re-enrolled into a second dose level. No

treatment-related SAEs were reported, and most AEs, including some increased transaminases, were transient. Four-week, once-daily treatment with A4250 reduced mean levels of s-BA in all cohorts. Substantial s-BA reductions in 11 of 13 PFIC patients (ranging from 43% to 98%) were observed. A4250 also reduced s-BA in the 11 non-PFIC patients, but to a lesser extent than in PFIC patients. Patient-reported diaries documented improvement of the visual analogue scale itch score in 17 of 24 patients during treatment. The dose with the greatest improvement showed a mean decrease (± standard error of the mean) of 2.9 ±1.1 from baseline. The results from this study form the basis of the current (A4250-005) study design.

5.3 Rationale

In this investigational study, A4250 is under development for treatment of PFIC and is also being considered for other orphan cholestatic liver diseases.

There is currently no medical treatment approved for PFIC. In PFIC patients with severe pruritus, biliary diversion surgery is used to decrease systemic bile acids through interruption of the enterohepatic circulation and thereby reducing pruritus [Whitington 1988]. Liver transplantation is typically used as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

By inhibiting IBAT with high selectivity and potency, A4250 has the potential to relieve cholestasis and improve liver function without surgical intervention in patients with PFIC. Since A4250 is minimally absorbed and achieves a substantial reduction of s-BA, it has the potential to be a safer and less invasive alternative to surgical biliary drainage. The rationale for using A4250 is to decrease s-BA levels, and to reduce the major morbidity of pruritus, improving the health and wellbeing of patients affected with PFIC. Ultimately, this noninvasive therapeutic approach has the potential to not only improve quality of life (QoL), but also reduce the need for biliary diversion and subsequent liver failure and liver transplantation.

The doses selected, 40 and 120 µg/kg/day, were based on the efficacy and safety data generated from patients with PFIC (n=10 + 3 re-entered) and supporting data from all patients (n=20 + 4 re-entered) in the five dose groups dosed in the A4250-003 study. Improvements in s-BA and pruritus were observed with doses from 30 µg/kg to 100 µg/kg, with a dose-related trend. The best dose response in all patients was at the 60 µg/kg/day for s-BA and 100 µg/kg/day for pruritus. The best dose response in the PFIC subgroup was at 30 µg/kg/day for both s-BA and pruritus. Patients with PFIC responded well to the doses given from 30 µg/kg to 200 µg/kg; however, a clear dose relationship could not be established in order to select a single dose for this Phase 3 study. As efficacy was observed at both low and high doses, 40 µg/kg and 120 µg/kg/day are considered to be the most optimal for this study.

This study will be conducted in compliance with the protocol and with the International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP).

Patients completing 24 weeks of double-blind treatment will be offered to enter Study A4250-008 and receive open-label treatment with A4250.

5.3.1 Risk/Benefit

A4250 has been evaluated in three Albireo-sponsored clinical studies: a double-blind placebo-controlled study in healthy volunteers, a single-dose ADME study, and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with PBC.

A total of 98 subjects/patients have been exposed to A4250. Healthy subjects have been exposed to up to 10 mg as a single dose and up to 3 mg daily as part of a multiple-dose evaluation. Children with cholestatic liver disease have been treated with up to 0.2 mg/kg/day (200 µg/kg/day) for 4 weeks. In total, 2 SAEs have been reported; both were judged by the physician to be not related to the study drug.

Patients with cholestatic liver diseases suffer from excess bile acids in the liver resulting in tissue damage. A commonly used treatment in these patients is bile diversion surgery, whereby approximately 50% to 100% of the enterohepatic circulation of bile acids is interrupted. Inhibition of IBAT with A4250, thereby interrupting the enterohepatic circulation of bile acids, is therefore a potential medical alternative to surgery which could be of benefit to these patients if shown to be effective and safe. Data from the Phase 2 study showed efficacy of A4250 in reducing s-BA concentrations and pruritus in such patients.

Based on the mode of action of an IBAT inhibitor, loose stools or diarrhea could be expected. However, in the pediatric Phase 2 study with 4 weeks daily treatment, only one patient had mild transient diarrhea after a single dose that did not recur on multiple dosing.

6 STUDY OBJECTIVES

6.1 Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with PFIC Types 1 and 2, as determined by the following:

- The proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- Change from baseline to Week 24 in pruritus compared to placebo

6.2 Secondary Objectives

- To evaluate the effect of A4250 on serum ALT concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

7 INVESTIGATIONAL PLAN

7.1 Overall Study Design and Plan

7.1.1 Description

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of FIC1 or BSEP).

Study data will be periodically reviewed by an unblinded Data Safety and Monitoring Board (DSMB).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-Up Period.

After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo.

Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008) in which all patients will receive active treatment. A patient who prematurely withdraws due to no improvement/intolerable symptoms, and has completed at least 12 weeks of the Treatment Period and End of Treatment (EOT) Visit, will also be offered the opportunity to enter Study A4250-008.

Visit windows applicable to each visit are presented in the schedule of assessments (Table 1). There will be up to 10 clinic visits during the study (Figure 1) and one scheduled telephone contact, as follows:

- Visit 1: Screening Period start. Patient is considered enrolled after signing the informed consent form (ICF) (Days -56 to -35)
- Visit 2: second Screening Visit (Days -28 to -7)
- Visit 3: Randomization Visit (Day 0)
- Telephone Contact 1: (Day 14)
- Visit 4: Clinic Visit (Day 28)
- Visit 5: Clinic Visit (Day 56)
- Visit 6: Clinic Visit (Day 84)
- Visit 7: Clinic Visit (Day 126)
- Visit 8: Clinic Visit (Day 154)
- Visit 9: Clinic Visit, EOT (Day 168)

- Visit 10: Follow-Up Visit, 28 days after last dose of study drug (Day 196). All patients continuing in Study A4250-008 will not have this Follow-up Visit.

Additional clinic visits may be required for patients who need direct site assistance, e.g., due to AE monitoring, to fulfil screening requirements, and/or for safety maintenance.

Screening Period

Informed consent must be obtained at Visit 1 prior to performing any study procedures. After signing the ICF, patients will be considered enrolled and evaluated for study eligibility.

Daily recording of pruritus using an electronic diary (eDiary) will be started to determine confirmation of the magnitude and the baseline pruritus symptoms before the study. After consultation with the Medical Monitor, patients failing eligibility criteria may be re-screened after 2 months. Patients not fulfilling inclusion/exclusion criteria after 3 attempts are not allowed to re-screen.

Caregivers/patients will be instructed on the daily use of the eDiary. The eDiary will include patient-reported outcome (PRO) and observer-reported outcome (ObsRO) items for evaluation of itching (PRO), scratching (ObsRO), and sleep disturbance (PRO and ObsRO) during the Screening Period (study Days -56 to 0) and throughout the 24-week Treatment Period (study Days 0 to 168).

Only the 14 most recent days before randomization will be used for eligibility calculation of the diary entries. Observer-reported outcomes in patients of all ages will be recorded by a caregiver (or caregiver's designee). If possible, the same caregiver will complete the Albireo ObsRO items throughout the study.

At a second Screening Visit (Visit 2) additional laboratory samples will be taken for eligibility assessments.

Treatment Period

Eligibility for randomization will be determined at Visit 3 using the pruritus (scratching reports from the Albireo ObsRO) data in the 2 weeks before Visit 3, genetic confirmation of diagnosis, and the liver biochemistry evaluations from the previous screening visits. Two weeks after Visit 3, there will be a telephone contact with the caregiver/patient to evaluate any AEs.

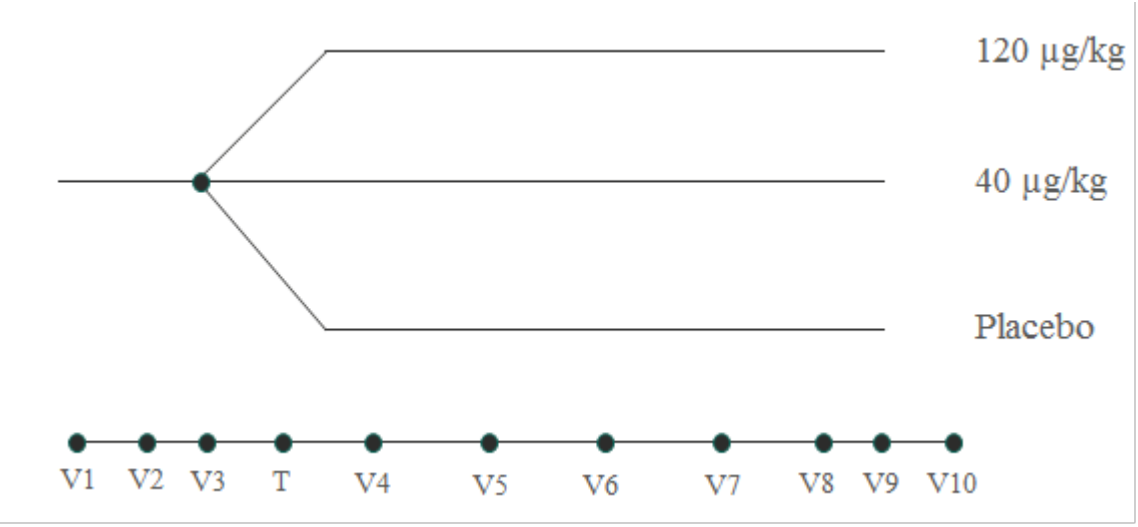
The patient will return to the clinic at Visit 4, Visit 5, Visit 6, Visit 7, Visit 8, and Visit 9 for assessments. If a new caregiver will begin to complete the Albireo ObsRO during the course of the trial, they will be provided with training during a clinic visit before they begin to use the eDiary. Additionally, caregivers/patients will be requested to use the diary to report the date and time each dose of study drug is administered during the Treatment Period.

The last dose of study drug will be administered the day before Visit 9. At study Visit 9, the patient has the option to enroll in an open-label extension study (A4250-008) should they meet the required entry criteria. If the patient is continuing into Study A4250-008 this will be

the last visit in this study and the first visit in Study A4250-008. Visit 9/EOT should also be performed at the time patients are prematurely withdrawn from the study.

Clinic Visit 10 will take place 28 days after Visit 9 for all patients not continuing into Study A4250-008.

Figure 1 Study Design



T: telephone contact; V: study visit

7.1.2 Schedule of Assessments

The schedule of assessments is presented in Table 1. For information on blood volumes please see Appendix 6.

Table 1 Schedule of Assessments

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Informed consent[c]	X										
Inclusion/exclusion criteria	X		X								
Demographics	X										
Concomitant medication[d]	X	X	X	X	X	X	X	X	X	X	X
Medical and surgical history	X		X								
Blood sampling for genetic testing[e]	X										
Physical examination including voluntary photography	X		X							X	
Skin examination	X		X		X		X			X	X
Vital signs[f]	X	X	X		X	X	X	X	X	X	X
eDiary: itching, scratching, and sleep scores[g]	Daily diary entry										
Clinical chemistry[h]	X		X		X	X	X	X		X	X
Hematology[h]			X		X	X	X	X		X	X
Urinalysis[h]		X					X			X	

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Serum bile acids[i]	X	X	X		X	X	X	X	X	X	X
INR		X			X		X		X		X
Autotaxin[j]			X		X					X	
p-C4[j]			X		X					X	
AFP			X							X	
Fat-soluble vitamins A & E		X								X	
Fat-soluble vitamin D			X							X	
Abdominal Ultrasound			X							X	
Fibroscan® (where available)			X							X	
QoL questionnaire (PedsQL)			X							X	
Patient/Caregiver/Clinician Global Impression of Change					X		X			X	
Patient/Caregiver/Clinician Global Impression of Symptoms			X		X		X			X	
Pregnancy test[k]	X[k]	X	X		X	X	X	X	X	X[k]	X
Study drug dispensed[l]			X		X	X	X	X	X		

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Adverse events[m]	Continuous collection										
Study drug compliance				X	X	X	X	X	X	X	

AFP: alfa-fetoprotein; BMI: body mass index; eCRF: electronic case report form; eDiary: electronic diary; EOT: End of Treatment; ICF: informed consent form; INR: international normalized ratio; p-C4: plasma 7α Hydroxy 4-cholesten-3-one; PedsQL: Pediatric Quality of Life Inventory; QoL: quality of life; RR: respiratory rate; s-BA: serum bile acid.

a Must also be performed at the time a patient is prematurely withdrawn from the study. This visit is the same as Visit 1 in the extension protocol (Study A4250-008).
b Assessments must be performed 28 days following the final dose of study drug. This visit is omitted if patients are entering Study A4250-008.
c The ICF (and assent) must be signed before any study procedures are performed.
d Past (within 1 month prior to Visit 1) and current medication will be documented in the eCRF.
e Blood sample for genetic testing will be drawn for all patients. Genetic analysis will only be performed on a patient's extracted DNA if previous the clinical genetic result is equivocal, unavailable, or unobtainable.
f Includes blood pressure, pulse, RR, temperature, height (or length depending on age), and body weight (using a certified weight scale). BMI will be calculated.
g Includes training on use of the device at Screening (Visit 1) and review of patient daily entries during 14 days prior to Clinic Visit 3 to determine eligibility.
h See Table 3 for detailed parameters.
i Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA. Exceptions can be made for infants, less than 12 months of age, if unable to fast for the full 4 hours. All s-BA results during the treatment period and follow-up will be blinded.
j Samples will not be collected for patients ≤10 kg.
k For girls who have reached menarche. Serum test at Visit 1; urine test at other visits. A serum pregnancy test will also be conducted at Visit 9 for patients entering Study A4250-008. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy.
l Study drug to be taken once daily on Day 1 through Day 167 as described in Section 8.2.
m Adverse event information will be collected from the time of signing of the ICF to study discontinuation.

7.1.3 Study Procedures and Assessments

7.1.3.1 Screening Period (Days -56 to 0)

Day -56 to Day -35/Clinic Visit 1

Patients will undergo a Screening Visit up to 56 days prior to the planned first day of study treatment. Screening procedures and assessments are as follows:

- Obtain written informed consent
- Assess inclusion/exclusion criteria (Section 7.2)
- Record demographics (age, full date of birth, gender, race, and ethnicity)
- Document concomitant medications
- Medical and surgical history (date of diagnosis of PFIC Types 1 or 2, prior investigational medications for PFIC, historical liver function test [LFT] values, any surgery performed, any other diagnosis, and historical liver biopsy data)
- Blood sample for genetic testing
- Collect, if available, past confirmatory clinical genetic laboratory report for PFIC1/PFIC2 and send to central reader for review
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- eDiary training and compliance requirements; patients begin daily recording of pruritus using the eDiary (Appendix 2)
- Clinical chemistry (Section 10.2.1)
- s-BA (Section 9.2.1)
- Serum pregnancy test for girls of who have reached menarche. Please see Appendix 5 for contraceptive advice.
- AE monitoring

Day -28 to Day -7/Clinic Visit 2

A second Screening Visit will be performed. Screening procedures and assessments are as follows:

- Review concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for itching, scratching, and sleep scores and compliance

- Urinalysis (Section 10.2.1)
- s-BA (Section 9.2.1)
- International normalized ratio (INR)
- Fat-soluble vitamins A and E
- Urine pregnancy test for girls of who have reached menarche
- AE monitoring

7.1.3.2 Treatment Period (Day 0 to Day 168)

Study Day 0/Clinic Visit 3 (Randomization)

At the randomization visit, the following assessments will be conducted:

- Review inclusion/exclusion criteria (Section 7.2)
- Review concomitant medications, medical and surgical history
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance and entrance criteria requirement (see Inclusion Criteria 4, Section 7.2.1)
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin and p-C4 (samples will not be taken for patients ≤10 kg)
- Fat-soluble vitamin D
- Alfa-fetoprotein (AFP)
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)
- Administer QoL (Pediatric Quality of Life Inventory [PedsQL]) questionnaire
- Patient/caregiver/clinician complete the patient global impression of symptoms (PGIS)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Study drug is dispensed and patients are instructed to administer daily from Day 1

Study Day 14 (Telephone Contact)

A study nurse will contact the patient/caregiver by telephone for monitoring of the following:

- Review concomitant medications
- Albireo PRO/ObsRO eDiary review for compliance
- AEs
- Evaluation of study drug compliance

Study Day 28 and Day 84/Clinic Visits 4 and 6

The following procedures and assessments will be performed:

- Review of concomitant medications
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- Urinalysis (at Clinic Visit 6 only; Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- Autotaxin and p-C4 (at Clinic Visit 4 only; samples will not be taken for patients ≤10 kg)
- Patient/caregiver/clinician complete the patient global impression of change (PGIC) and PGIS
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 56 and Day 126/Clinic Visits 5 and 7

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring

- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 154/Clinic Visit 8

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- s-BA (Section 9.2.1)
- INR
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 168/Clinic Visit 9/EOT

The last dose of study drug is taken in the morning the day before Clinic Visit 9, and the following procedures and assessments will be performed (also performed at the time a patient prematurely withdraws):

- Review of concomitant medications
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry, hematology, and urinalysis (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin and p-C4 (samples will not be taken for patients ≤10 kg)
- AFP
- Fat-soluble vitamins A, E, and D
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)
- Administer PedsQL questionnaire
- Patient/caregiver/clinician complete the PGIC and PGIS

- Urine pregnancy test for girls who have reached menarche, or serum pregnancy test for girls continuing to study A4250-008
- AE monitoring
- Evaluation of study drug compliance

7.1.3.3 Follow-Up Period

Study Day 196/Clinic Visit 10

All patients not continuing into Study A4250-008 will return to the study site 28 days after Clinic Visit 9 or the last dose of study drug for the following assessments:

- Review of concomitant medications
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- Urine pregnancy test for girls who have reached menarche

- AE monitoring

7.2 Study Population

A total of 60 patients with clinical diagnosis of PFIC Type 1 or 2 that have been genetically confirmed will be randomized.

An attempt will be made to randomize a minimum of 10 patients from each age category (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years).

A maximum of 5 patients that were treated in Study A4250-003 will be allowed to participate in this study.

7.2.1 Inclusion Criteria

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples 7 to 28 days apart (Visits 1 and 2) prior to randomization

4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the eDiary average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients will be expected to have a consistent caregiver(s) for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

7.2.2 Exclusion Criteria

1. Patient with pathologic variations of the *ABCB11* gene that predict complete absence of the BSEP function
2. Patient with past medical history or ongoing presence of other types of liver disease including, but not limited to, the following:
   a) Biliary atresia of any kind
   b) Benign recurrent intrahepatic cholestasis, indicated by any history of normal s-BAs
   c) Suspected or proven liver cancer or metastasis to the liver on imaging studies
   d) Histopathology on liver biopsy is suggestive of alternate non-PFIC related etiology of cholestasis
3. Patient with a past medical history or ongoing presence of any other disease or condition known to interfere with the absorption, distribution, metabolism (specifically bile acid metabolism), or excretion of drugs in the intestine, including but not limited to, inflammatory bowel disease
4. Patient with past medical history or ongoing chronic (i.e., >3 months) diarrhea requiring intravenous fluid or nutritional intervention for treatment of the diarrhea and/or its sequelae
5. Patient has a confirmed past diagnosis of infection with human immunodeficiency virus or other present and active, clinically significant, acute, or chronic infection, or past medical history of any major episode of infection requiring hospitalization or treatment with parenteral anti-infective treatment within 4 weeks of treatment start (study Day 1) or completion of oral anti-infective treatment within 2 weeks prior to start of Screening Period
6. Any patient with suspected or confirmed cancers except for basal cell carcinoma, and non-liver cancers treated at least 5 years prior to Screening with no evidence of recurrence

7. Patient with a past medical history of chronic kidney disease with an impaired renal function and a glomerular filtration rate <70 mL/min/1.73 m^2
8. Patient with surgical history of disruption of the enterohepatic circulation excluding those who have undergone a successful reversal procedure that has permanently restored flow of bile acids from the liver to the duodenum
9. Patient has had a liver transplant or listed for liver transplant
10. Decompensated liver disease, coagulopathy, history or presence of clinically significant ascites, variceal hemorrhage, and/or encephalopathy
11. INR >1.4 (the patient may be treated with Vitamin K intravenously, and if INR is <1.4 at resampling the patient may be randomized)
12. Serum ALT >10 × upper limit of normal (ULN) at Screening
13. Serum ALT >15 × ULN at any time point during the last 6 months unless an alternate etiology was confirmed for the elevation
14. Total bilirubin >5 × ULN at Screening
15. Patient suffers from uncontrolled, recalcitrant pruritic condition other than PFIC. Examples include, but not limited to, refractory atopic dermatitis or other primary pruritic skin diseases
16. Any patient who is pregnant or lactating or who is planning to become pregnant within 24 weeks of randomization
17. Sexually active males and females who are not using a reliable contraceptive method with ≤1% failure rate (such as barrier protection, hormonal contraception, intrauterine device, or complete abstinence) throughout the duration of the study and 90 days thereafter (from signed informed consent through 90 days after last day of treatment). See Appendix 5 for further details.
18. Patient with a past medical history of alcohol or substance abuse will be excluded. Patient must agree to refrain from illicit drug and alcohol use during the study
19. Administration of bile acid or lipid binding resins and medications that slow GI motility (Refer to Appendix 1 – Concomitant Medications Prohibited during the study)
20. Patient has had investigational exposure to a drug, biologic agent, or medical device within 30 days prior to Screening, or 5 half-lives of the study agent, whichever is longer
21. Patient who has been previously treated with an IBAT inhibitor whose pruritus has not responded to treatment
22. Any other conditions or abnormalities which, in the opinion of the investigator or Medical Monitor, may compromise the safety of the patient, or interfere with the patient participating in or completing the study

7.2.3 Withdrawal of Patients

Patients/caregivers will be informed that they have the right to withdraw from the study at any time, without prejudice to their medical care, and that they are not obliged to state their reasons.

Any withdrawal, and reasons for withdrawal, must be fully documented in the electronic case report form (eCRF) and source documents and the patient followed by the investigator/investigative staff. Even after the study is completed at the Follow-up Visit, the investigator/investigative staff will, regardless of reason for withdrawal, record any patient data they receive concerning SAEs, and all drug-related non-serious AEs, and subsequently report these to Albireo. Withdrawn patients will not be replaced.

Patients will be withdrawn in the following circumstances:

- A patient's/caregiver's desire for withdrawal for any reason
- Lost to follow-up (every effort must be made to contact the patient/caregiver; a certified letter must be sent)
- An AE that, in the opinion of the investigator, necessitates withdrawal
- A worsening of the symptoms related to diarrhea occurs
- Death
- Patient/caregiver estimation of lack of improvement/intolerable symptoms
- A patient's/caregiver's substantial noncompliance (eDiary and study drug compliance) or protocol violation
- An investigator's opinion that continuing the patient in the study is not appropriate. The investigator may withdraw a patient at any time, if it is considered to be in the patient's best interest

A patient who prematurely withdraws due to no improvement/intolerable symptoms, and has completed at least 12 weeks treatment in the double-blind study, will be offered the opportunity to enter open-label Study A4250-008. A patient who withdraws from treatment prematurely and will not enter Study A4250-008 will be requested to complete all visits and safety assessments per the current protocol (A4250-005). As a minimum, patients will have EOT (Visit 9) assessments at the time of withdrawal and a follow-up assessment (Visit 10) 28 days following the last dose of study drug, and the patient will be recorded as withdrawn.

7.2.4 Study Termination by Sponsor

This study may be terminated at any time by Albireo if serious side effects should occur, if the investigator does not adhere to the protocol; or if, in the sponsor's judgment, there are no further benefits to be achieved from the study. In this event, Albireo/designee will inform the study investigators, institutions, and all regulatory authorities.

Albireo may temporarily or permanently discontinue the study at an investigative site at any time for safety, ethical, compliance, or other reasons. If this is necessary, Albireo will endeavor to provide advance notification to the site. If a site or the study is suspended or discontinued, the investigator/investigative staff will be responsible for promptly informing the Independent Ethics Committee (IEC). If required by local regulations, Albireo/designee will be responsible for informing the IEC and the regulatory authority of study or site discontinuation. In such an event, all study data and unused study drug must be returned to Albireo.

8 TREATMENT OF PATIENTS

8.1 Identity of Study Drug

A4250 and placebo will be supplied as capsules for oral administration. White opaque capsules filled with pellets containing A4250 or placebo will be provided. Two different capsule sizes will be available, as follows:

- Capsule size 0 that can be opened
- Capsule size 3 that can be opened or swallowed intact

The content of the A4250 and placebo capsules will be identical in appearance. Capsule filling weight will also be identical for high dose, low dose, and placebo. Bottles with 34 capsules will be given to the patient at each visit. A patient who requires 2 or more capsules per day will be given multiple bottles. Refer to the investigational product manual.

8.2 Administration of Study Drug

Patients will be dosed with A4250 at a dose of 40 µg/kg/day, 120 µg/kg/day, or placebo for 24 weeks. Study drug will be dispensed to the patient at Visits 3, 4, 5, 6, 7, and 8, together with instructions on how to store and take the drug. Study drug administration data, including whether each patient took each dose or partial doses of study drug, whether there were any delayed or missed doses, doses, and whether the capsule was opened or swallowed whole will be documented through the eDiary and transferred to the study database.

A4250 should be taken in the morning, together with food. On clinic visits days when laboratory assessments are conducted, study drug should be taken after the visit and after laboratory samples are taken. Patients should not crush or chew the capsule(s). When swallowing the capsule intact, the patient should administer the dose with a glass of water.

For a patient unable to swallow capsule intact, the capsule can be opened and the contents sprinkled and mixed in a small amount of soft room-temperature yogurt, apple sauce, or fruit purée. The amount of food should be less than what the patient/infant is expected to eat and should be followed by water.

1. Hold the capsule over the prepared food, gently twist open and allow the content to fall into food
2. Mix the entire capsule content with 1 or 2 tablespoons (15 to 30 mL) of soft food (apple sauce, fruit purée, or yogurt)
3. Administer the mixture immediately after mixing.

The number of capsules provided to the patient will be based on body weight at Visit 3. At Visit 6, if a patient's weight has shifted to another weight category (identified in Table 2), the number of capsules per day may be adjusted.

Table 2 Dosing and Capsule Strength

Body Weight (kg)	Capsule Size	Number of Capsules per Day	Capsule Strength, Low Dose (µg)	Total Dose (µg)	Capsule Strength, High Dose (µg)	Total Dose (µg)	Capsule Size, Placebo (µg)
5.0 to <7.5	0	1	200	200	600	600	0
7.5 to <12.5	0	2	200	400	600	1200	0
12.5 to <17.5	0	3	200	600	600	1800	0
17.5 to <19.5	0	4	200	800	600	2400	0
19.5 to <25.5	3	2	400	800	1200	2400	3
25.5 to <35.5	3	3	400	1200	1200	3600	3
35.5 to <45.5	3	4	400	1600	1200	4800	3
45.5 to 55.5	3	5	400	2000	1200	6000	3
>55.5	3	6	400	2400	1200	7200	3

8.3 Study Treatment Packaging and Labeling

8.3.1 Packaging and Labeling

The capsules will be packed in high-density polyethylene containers, with child-proof polypropylene caps. Study drug capsules containing A4250 in the strength specified in Table 2 above will be manufactured.

Packaging and labeling will be prepared to comply with applicable regulatory requirements.

8.3.2 Storage

Treatment packs containing A4250 capsules should be stored and dispensed in accordance with regulations in their original containers. The storage facility at the investigative site should be locked and the storage temperature should be between 15°C and 25°C.

Patients/caregivers should be informed of appropriate storage conditions (i.e., room temperature, between 15°C and 25°C).

Any deviations from the recommended storage conditions should be immediately reported to Albireo and the study drug should not be used until authorization has been given by Albireo.

8.3.3 Blinding and Randomization of Study Drug

A double-blind design is employed so that both the investigators and the patients will be unaware of the treatment assignment during the study.

After written informed consent is obtained from an eligible patient, an 8-digit patient identification number will be assigned. The first 2 digits will denote the country, followed by a 3-digit site number and a 3-digit patient sequence number. This number will be created and allocated by the Interactive Web Response System (IWRS), when the patient first enters the system at Screening. The randomization codes will be computer-generated by [redacted]

Biostatistics, and kept by a statistician independent from the project team. The randomization will be done in blocks and stratified according to PFIC class (Type 1 and 2) and age class (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) to ensure approximate balance between dose schemes (1:1:1). A separate randomization list will be prepared for the maximum 5 patients from Study A4250-003 regardless of stratification. Randomization codes will be assigned sequentially as patients become eligible for randomization.

Patients who are eligible for randomization will be assigned a unique 4-digit randomization number by IWRS. This randomization number identifies which treatment will be allocated to the patient. Patients who withdraw from the study after randomization visit are not to be replaced and their randomization number will not be reused.

To ensure blinding, the study drug and the matching placebo have the same shape and size. Patients will receive capsule(s) of A4250 according to their dose group or capsule(s) of matching placebo once a day during the double-blind Treatment Period. Labels on the study drug containers will not identify the treatment that a patient is randomized to. Traceability of the treatment is ensured by the study drug number.

The 5-digit study drug number will identify study drug packs and will be detailed on the study drug label. Dispensing of study drug will be coordinated by IWRS.

The system will assign a study drug number corresponding to the randomization arm. The randomization number will be used in the background only to ensure no unblinding and will not be displayed to end-users in the IWRS.

8.4 Procedure for Breaking the Randomization Code

Should a situation arise where unblinding is urgently required, i.e., only where knowledge of the study drug is required to adequately manage a life-threatening situation, the investigator at that study site may perform immediate unblinding through IWRS. The responsibility to break the treatment code in emergency situations resides solely with the investigator. The investigator should make every effort to discuss the rationale (status and outcome) for emergency unblinding with the sponsor Medical Monitor or the [redacted] Medical Monitor as soon as possible to review the individual patient details.

The study site investigator and appropriate [redacted] project team members will be authorized to access the emergency unblinding functionality within the IWRS. Emergency unblinding takes place in the IWRS via a dedicated emergency unblinding module. The system will require the user to enter their emergency unblinding, which is typically provided at the same time login credentials are distributed. The exact description of the treatment assigned to the individual patient then will be accessible to the user. Once the randomization code is broken for a patient, he/she must be withdrawn from the study and all assessments and procedures at EOT should be performed. As a follow-up, Visit 10 will be performed according to the time schedule. Once a randomization code has been broken, the investigator must inform the [redacted] and sponsor Medical Monitor in writing within 24 hours.

8.5 Patient Compliance

The study nurse will monitor eDiary compliance by routine review of the CRF health website. If both diary entries on a day are missing during, the study nurse will call the patient/caregiver to remind them to complete all scheduled entries. Any noncompliance will be documented and explained in the source documents and in the eCRF.

Study drug compliance will also be checked through diary responses. Additionally, patients will return all unused study drug at Visits 4, 5, 6, 7, 8, and 9. The study site staff will count all returned drug, assess compliance, and record details in the eCRF.

Treatment Compliance = 100 × ((Number of study drug dispensed – number of study drug returned)/number of study drug that should be taken).

Treatment compliance between 80% and 120% will be acceptable.

8.6 Study Drug Accountability

Records shall be maintained of the delivery of study treatment(s) to the study site(s), of the inventory at the study site(s), of each use of the study treatment(s) for each patient, and of the return of unused study treatment(s) to Albireo.

These records shall include dates, quantities, batch numbers, expiry dates, and the unique code numbers assigned to the study drug and to the study patients.

The investigator will be responsible for ensuring that the records adequately document that the patients were provided the quantities specified in the protocol and that all study drug received from Albireo is reconciled.

8.7 Concomitant Therapy and Prohibited Medications

The investigator will note all ongoing medication and any medication recently stopped (within 1 month prior to Visit 1) in the eCRF. At Visits 2 through 10, all changes in medication (stopping or starting new medication or changes in dose) will be recorded in the eCRF. All medications (prescribed or over the counter) for pruritus and sleep will be recorded.

All medications taken by a patient within 3 months prior to the first intake of study drug are regarded as prior medication.

All medications taken by a patient on or after the first intake of study drug, and which continue to be taken during the study, are regarded as concomitant medication.

Prohibited medications are listed in Appendix 1.

9 ASSESSMENT OF EFFICACY

9.1 Efficacy Endpoints

9.1.1 Primary Efficacy Endpoint

The primary efficacy endpoints are as follows:

- **European Union (EU) and rest of the world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  Fasting s-BA baseline will be calculated as the average of the value at randomization and the previous visit (Clinic Visit 2). The end value is the average of the values at Weeks 22 and 24 after the start of double-blind treatment.

- **United States (US)**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 24 as measured by the worst scratch of the Albireo ObsRO instrument (Appendix 2). Scores for the 14 days baseline and post-treatment intervals will be calculated by averaging the worst scratching scores from each day in the interval.

9.1.2 Secondary Efficacy Endpoint

Secondary efficacy endpoints include the following:

- **EU and RoW**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 24 as measured by the worst scratch of the Albireo ObsRO instrument (Appendix 2). Scores for the 14 days baseline and post-treatment intervals will be calculated by averaging the worst scratching scores from each day in the interval.
- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.
- **All Regions:**
  - All secondary endpoints are compared to placebo.
    - Change from baseline to Week 24 in fasting s-BA
    - Change from baseline to Week 24 in serum ALT concentration
    - Change in growth from baseline to Week 24, defined as the linear deficit (weight for age, and body mass index [BMI]) compared to the national growth curves (Z-score, standard deviation [SD] from P50)
    - Proportion of patients achieving meaningful reduction in caregiver-reported observed scratching at Week 24

- Change from baseline to Week 24 in sleep parameters measured with the Albireo PRO and ObsRO instruments
- Change from baseline to Week 24 in patient-reported itch severity as measured by the average daily score of the Albireo PRO instrument (Appendix 2); only patients ≥8 years of age will complete the Albireo PRO instrument
- Number of patients undergoing biliary diversion surgery or being listed for liver transplantation

9.1.3 Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Proportion of patients achieving meaningful reduction in patient-reported itch severity at Week 24
- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the PGIC items (Appendix 3)
- Change from baseline in fasting s-BA at Weeks 4 and 12
- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Weeks 4, 12, and 24 as measured by the worst scratch. Scores for the 7 worst scratching days of the last 14 days at baseline and post-treatment intervals will be selected
- Change from baseline in patient-reported itch severity and observer-reported scratching at Weeks 4 and 12
- Change from baseline to Week 24 in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline to Week 24 in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline to Week 24 in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in PedsQL (Appendix 4)
- Change from baseline in serum ALT concentration at Weeks 4 and 12
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, p-C4) at Week 24

- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events
- All-cause mortality

9.2 Efficacy Assessments

9.2.1 Serum Bile Acids

Blood samples for analysis of fasting total s-BA will be drawn at all visits. Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA. Exceptions can be made for infants, <12 months of age, if unable to fast for the full 4 hours. Any visit at which a bile acid sample result is unreportable, an additional unscheduled visit for a repeat sample collection may be scheduled. All s-BA results during the treatment period and follow-up will be blinded. Samples will be processed and transported to a central laboratory per instructions in the laboratory manual.

9.2.2 Itching, Scratching and Sleep Score

Itching, observed scratching, and sleep disturbance will be recorded twice each day via eDiary. Patients and/or caregivers will be instructed to complete the eDiary every day in the morning after the patient wakes and in the evening just before the patient goes to sleep throughout the study. Patients and caregivers will receive a formal training session during Visit 1.

The eDiary includes Albireo ObsRO and PRO items. Patients <8 years of age will not be asked to complete the Albireo PRO items; only the Albireo ObsRO will be completed by caregivers of patients in this age group. Older patients, 8 to 17 years of age, will complete the Albireo PRO items and the caregiver will complete the Albireo ObsRO items. The Albireo PRO items assess severity of itch, aspects of sleep disturbance (morning diary only), and tiredness. For patients 8 to 12 years of age, the caregiver will read the Albireo PRO items along with the child and record the child's response. A guide will be provided to the caregivers that provides standardized explanations of the Albireo PRO items, in case the patient is confused or requires clarification about the meaning of a question. The Albireo ObsRO items assess severity of observed scratching, aspects of observed sleep disturbance (morning diary only), and observed signs of tiredness (evening diary only). The Albireo ObsRO and PRO scratching and itch severity items use 0 to 4 response scales, where each response is distinguished by a unique facial expression, verbal anchor, number, and color code (Appendix 2).

A daily score for the Albireo ObsRO scratching item will be calculated by selecting the maximum scratching severity score from the 2 ratings for each day. A daily score will be considered missing if both of the daily assessments are missing. A 14-days score will be calculated by averaging the daily scratching scores. A 14-days score will be considered missing if >4 out of 7 days a week of data are missing. The same approach will be used to calculate a patient-reported itch severity score.

9.2.3 Growth

Growth will be measured as height (velocity) and weight (Z-score) using a certified weight scale. BMI will be calculated by weight (kg) / height $(m)^2$. Change will be defined as linear growth deficit (weight and BMI for age) compared to national growth curves (Z-score, SD from P50).

9.2.4 Quality of Life Questionnaire (PedsQL)

Patients and caregivers will be asked to complete a QoL questionnaire (PedsQL) at Visit 3 and Visit 9. Details of the questions included on the questionnaire are provided in Appendix 4.

9.2.5 Biomarkers and Other Laboratory Samples

Blood samples for autotaxin and p-C4 will be drawn at Visits 3, 4, and 9 only for children with body weight ≥10 kg. Samples will be processed and transported to a laboratory per instructions in the laboratory manual.

9.2.6 PELD/MELD Score

At randomization and end of treatment, PELD score will be calculated for children up to 12 years of age. For children 13 years or older, the MELD score will be calculated.

PELD Score = 4.80 * ln(total bilirubin in mg/dL) + 18.57 * ln(INR) - 6.87 * ln(albumin in g/dL) + 4.36 (if patient <1 year: scores for patients listed for liver transplantation before the patient's first birthday continue to include the value assigned for age (<1 year) until the patient reaches the age of 24 months) + 6.67 (if the patient has growth failure [≤2 SD])

Laboratory values less than 1.0 will be set to 1.0 for the calculation of the PELD score.

MELD Score = 9.57 * ln(creatinine in mg/dL) + 3.78 * ln(total bilirubin in mg/dL) + 11.2 * ln(INR) + 6.43

Laboratory values less than 1.0 will be set to 1.0 and serum creatinine values greater than 4.0 mg/dL will be set to 4.0 for calculation of the MELD score.

9.2.7 Fibroscan®

Where available, Fibroscan® will be performed as per institution standard practice at Visits 3 and 9.

9.2.8 Markers of Fibrosis

APRI score and Fib-4 score will be calculated at randomization and at end of treatment.

APRI = [(AST in U/L) / (AST ULN in U/L)] / (Platelets in 10^9/L)

Fibrosis 4 Score = (Age*AST in U/L) / (Platelets in 10^9/L * √(ALT in U/L)).

9.2.9 Global Impression of Change and Global Impression of Symptom Measures

Patients (≥8 years of age), caregivers, and clinicians will complete the PGIC and the PGIS measures (Appendix 3) at randomization (Visit 3; PGIS only), Visits 4 and 6, and at EOT (Visit 9).

The PGIC items assess change in itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) since starting the study drug. The PGIS items assess itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) in the past week. Caregivers and clinicians will complete the PGIC and PGIS for all patients; those patients ≥8 years of age will complete the patient version.

10 ASSESSMENT OF SAFETY

The timing and frequency of safety assessments are described in Section 7.1.3 and in Table 1 above.

The primary safety analysis for this study will include the incidence of total treatment-emergent adverse events (TEAE) and TEAEs categorized by causality, severity, and seriousness assessments made by the investigator by comparing study drug exposure to placebo.

Trends in safety will also be evaluated for the following assessments:

- Physical examinations
- Concomitant medications
- Vital signs
- Laboratory test results (including clinical chemistry, hematology, urinalysis, AFP, and fat-soluble vitamins A, D, and E)
- Abdominal ultrasound
- Discontinuations due to AEs

10.1 Adverse Events

10.1.1 Definitions and Investigator Assessments

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 unfavorable and unintended sign, symptom, or disease that occurs once a patient is enrolled (ICF is signed) in the study until the patient is discharged from the study, whether or not related to the study drug.

10.1.1.1 Clinical Significance

Clinical significance is defined as any abnormal finding that results in further clinical investigation(s), treatment(s), or the diagnosis of new condition. Patient-reported events and protocol-mandated laboratory values, vital signs, and physical examination findings can be considered clinically significant (i.e., an AE) if there is a deterioration as compared to baseline. Examples of clinically significant worsening from baseline could include, but is not limited to, events causing withdrawal from the study and events requiring medical intervention outside of the study causing apparent clinical manifestations, or judged relevant by the investigator.

10.1.1.2 Serious Adverse Events

Serious criteria are applied by the investigator to each AE as specifically defined below. These criteria are used to determine whether an AE is serious or non-serious. The assessment is made independently of severity assessment (Section 10.1.1.3). For example, the

development of a severe rash that occurs after signing of informed consent may not meet serious criteria as defined below and therefore would be considered a severe, non-serious AE.

Any AE that meets any 1 of the following 6 criteria is considered an SAE:

- The outcome of the AE is **death**
- The AE is immediately **life-threatening**. Life-threatening means that the patient is, in the opinion of the investigator, at immediate risk of death from the reaction as it occurred. This does not include an AE that, if more severe, might have caused death
- The AE results in persistent or significant **disability/incapacity**. Disability means a substantial disruption of a person's ability to conduct normal life functions
- The AE requires or prolongs **hospitalization**
- The AE results in a **congenital anomaly/birth defect**
- **The AE is an important medical event**. Important medical events may meet serious criteria should the investigator assess that they may significantly jeopardize the patient, represent a significant hazard, or requires medical/surgical intervention to prevent one of the outcomes listed above. Examples of potential SAEs based on this criteria include, but are not limited to, allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions even if they do not result in inpatient hospitalization, or the development of drug dependency and drug abuse.

10.1.1.3 Severity Assessment

Severity assessments are based on the intensity of the event in relation to expectation. The investigator will assess the intensity of AEs based on the following definitions:

- Mild (awareness of sign or symptom, but easily tolerated)
- Moderate (discomfort sufficient to cause interference with normal activities)
- Severe (incapacitating, with inability to perform normal activities)

Severity is a measure of intensity where seriousness is defined by the criteria outlined in Section 10.1.1.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not meet serious criteria, and therefore would be assessed as a severe AE but not an SAE.

10.1.1.4 Causality Assessment

The investigator determines the causality of all AEs to the study drug using medical judgment and considering all relevant factors such as (but not limited to) the underlying study indication, coexisting disease, concomitant medication, relevant history, pattern of the AE, temporal relationship to the study drug, and de-challenge or re-challenge. The causality assessment of the AE/SAE is to be made as follows.

Related to study drug (possibly, probably, or definitely related)

Based on medical judgment, there is at least a reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event follows a reasonable temporal sequence from administration of study drug
- The event could not be reasonably attributed to the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient
- The event follows a known pattern of response to study drug
- The event disappears or decreases on cessation or reduction in dose of the study drug. (It should be noted that in some situations an AE will not disappear or decrease in intensity upon discontinuation of study drug despite other clear indications of relatedness)
- The event reappears or worsens when the study drug is re-administered

Unrelated to study drug (unlikely or unrelated)

Based on medical judgment there is no reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event does not follow a reasonable temporal sequence from administration of study drug
- The event could be reasonably attributed to the known characteristics of the patient's clinical state, concurrent illness, environment or toxic factors, or other modes of therapy administered to the patient
- The event does not follow a known pattern of response to study drug
- The event does not disappear or decrease on cessation or reduction in dose of the study drug, and it does not reappear or worsen when the study drug is re-administered

10.1.2 Recording of Adverse Events

It is the investigator's responsibility to assess whether each untoward event is a clinically significant worsening from baseline, thereby considered an AE. For all AEs, the severity, seriousness, and causality to study drug for each AE as outlined in Section 10.1.1 will be assessed and recorded in the eCRF.

All serious and non-serious AEs are collected once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10) or 28 days after the last dose of study drug.

Any AEs or SAEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator until resolution or stabilization up to the database lock and recorded in the eCRF. Albireo retains the right to request additional information for any patient with ongoing AE(s) or SAE(s) at the end of the study.

TEAEs are defined as any AE that occurs after randomization (Day 0). All AEs that occur in the Screening Period, i.e., after enrollment and prior to randomization, will be collected on the eCRF as non-treatment-emergent AEs.

If there is a clinically significant deterioration of a laboratory value/vital sign or other routine study assessment that is associated with a diagnosis, the clinical diagnosis will be reported as an AE and the associated signs and symptoms will be considered additional information unless the sign or symptom is more severe than expected given the diagnosis. For example, if an investigator diagnoses a study patient with hepatitis during the study period, hepatitis would be considered the AE and the concomitant signs and symptoms of abdominal pain, vomiting, and elevated ALT and AST would not be reported separately unless, in the opinion of the investigator, one of these signs or symptoms is more severe than expected and therefore a separate AE assessment is indicated.

10.1.3 Recording and Reporting of Serious Adverse Events

Every SAE (regardless of severity and causality) that occurs once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10), and 28 days after the final dose of study drug, should immediately and not later than within 24 hours of knowledge of the event, be reported by the investigator or delegate in the SAE report form.

Report of a SAE must include at least the following information:

- Patient identification information (study number, site number, initials, and date of birth [as per local country requirements for data protection])
- The last study drug administration date
- The diagnosis of the event with the description (or a brief description of signs/symptoms/clinical course if the diagnosis is not available) and the date of onset
- The action used to treat the event (i.e., treatment medications, temporary discontinuation)
- The reason(s) for considering the event serious
- The relationship of the event to the study drug or to the study procedure (e.g., the investigator's assessment of causality)
- A brief narrative of the SAE

Follow-up reports including all new information obtained of the subsequent course of the SAE must be prepared and the information collected in the SAE submitted to [redacted] (Pharmacovigilance and Safety Services Department) by e-mail within 24 hours of knowledge of the event.

The pharmacovigilance manager (PVM) may contact the investigator to obtain further information on a reported SAE. The investigator/investigative staff must respond to any request for follow-up information or answers to questions regarding the SAE within the same timelines as for initial reports.

The PVM reports the occurrence of the SAE and follow-up to the Albireo medical monitor for medical assessment of the case.

Should an outcome of death occur within the study period or within 28 days after the last administration of study drug, an SAE form should be completed including the main and contributory causes of death.

All SAE reports must be e-mailed to the following e-mail address within **24 hours**:

[redacted]

If email is unavailable, SAEs may be transmitted via fax to the following number:

[redacted]

In a study-related medical emergency situation, when the assigned medical monitors for the study cannot be reached by a caller, an on call physician can be reached 24 hours per day, 7 days per week, via the [redacted] call center. The following number is a chargeable telephone number allowing a global reach from both landlines and mobile phones and the internet page indicated below contains a list of country-specific toll-free telephone numbers that is provided. It should be noted that not all countries globally have access to toll-free numbers as indicated on the "24/7 Medical Help desk" index. Countries without toll-free numbers need to dial the chargeable number as indicated below. Furthermore, toll-free numbers might not be available from mobile phones.

[redacted]

10.1.4 Reporting of Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reaction (SUSAR) is an SAE that occurs in a patient, the nature or severity of which is not expected per the applicable product information (e.g., the IB for an unauthorized investigational product or summary of product characteristics for an authorized product).

Reporting and tracking of SUSARs will be in accordance to all applicable competent authority regulations. The Ethics Committee and all investigators involved in this study will be informed according to national and international requirements.

10.2 Laboratory Values/Vital Signs/Physical Examinations and Other Safety Assessments

10.2.1 Laboratory Assessments

Samples will be collected for clinical chemistry, hematology, and urinalysis testing at the time points specified in Table 1. The parameters assessed are presented in Table 3.

Blood for AFP will be drawn at Visits 3 and 9 and for fat-soluble vitamins at Visits 2, 3, and 9.

A serum pregnancy test will be performed at Visit 1 and Visit 9 if the patient is entering the A4250-008 study. A urine pregnancy test will be collected at all other visits for girls who have reached menarche. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy. Study drug administration will be held pending the confirmatory results. If the serum pregnancy test is negative, the patient can resume dosing. If the serum pregnancy test is positive, the patient should be discontinued (see Section 10.2.9).

All samples will be processed and transported to a laboratory per instructions in the laboratory manual.

The observed values will be recorded and assessed as "normal" or "abnormal not clinically significant" or "abnormal clinically significant".

Additional safety blood samples may be needed due to follow-up of an abnormal value or analysis failure. The blood samples collected for safety laboratory analysis will be destroyed after the analyses have been completed.

Table 3 Routine Laboratory Parameters

Clinical Chemistry	Hematology	Urinalysis
• Albumin • ALT • Alkaline phosphatase • AST • Bilirubin – total and direct • Calcium • Chloride • Creatinine • Creatine kinase • Gamma-glutamyl transferase • Potassium • Sodium	• Hematocrit • Hemoglobin • Platelet count • Red blood cell count • White blood cell count	• Blood • Glucose • Ketones • Leukocytes • Nitrites • pH • Protein

10.2.2 Individual Patient Safety Monitoring

10.2.2.1 Liver Monitoring

Strategies to monitor markers of liver disease throughout the study are outlined below where the ULN will be based on central laboratory reference values for age and gender.

If isolated transaminase elevations are observed, defined as:

1. Normal bilirubin AND absence of clinical hepatitis symptoms AND

- ALT or AST ≥5 × ULN (if normal at baseline) or an absolute threshold of 800 U/L, whichever comes first
- OR ALT or AST ≥3 × baseline (if abnormal at baseline) or an absolute threshold of 800 U/L, whichever comes first

Then:

a) Repeat liver profile (AST, ALT, bilirubin, and prothrombin time [PT] or INR) within 2 to 3 days

b) Evaluate creatine phosphokinase (CPK) and lactate dehydrogenase (LDH)

c) As needed (for example, persistent ALT/total bilirubin elevation or worsening of liver function), consider evaluation for alternative etiologies

Monitor the patient using close observation found in Section 10.2.2.2

If any ONE of the following criteria are met:

1. Transaminases (ALT or AST ≥3 × baseline or 800 U/L, whichever comes first) AND bilirubin (total bilirubin > 2 × ULN) elevations
2. Transaminase elevations alone (ALT or AST >10 × ULN or 5 × baseline or absolute threshold of 800 U/L, whichever comes first) in presence of normal LDH and CPK
3. Cholestatic marker elevations (alkaline phosphatase [ALP] or gamma-glutamyl transferase >2 × baseline) without alternative explanation
4. Total bilirubin increased, unrelated to hemolysis (elevated reticulocyte count) or established genetic diseases, such as Gilbert's syndrome
   a. Doubling if total bilirubin was <3 mg/dL at baseline
   b. OR Increase by >3 mg/dL if total bilirubin was ≥3 mg/dL at baseline
5. INR increase refractory to vitamin K administration
   a. Increase by >1.5 if INR was normal at baseline
   b. OR Increase by >0.4 if INR was abnormal at baseline
6. Any increase in total bilirubin and transaminases if accompanied by EITHER a symptom of clinical hepatitis (e.g., vomiting, nausea, right upper quadrant pain) OR immunological reaction (rash or >5% eosinophilia)

Then:

a) Interrupt study medication

b) Initiate drug-induced liver injury work-up for alternative etiologies

c) Repeat liver profile (AST, ALT, total bilirubin, direct bilirubin, ALP) and PT or INR within 48 to 72 hours

d) Monitor the patient using close observation found in Section 10.2.2.2

e) If a patient lives in a remote area, they may be tested locally and the results communicated to the investigator site promptly

10.2.2.2 Close Observation

- Repeat liver enzyme and serum bilirubin tests two or three times weekly. Frequency of re-testing can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the subject is asymptomatic
- Obtain a more detailed history of symptoms and prior or concurrent diseases
- Obtain a history of concomitant drug use (including non-prescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets
- Rule out alternate etiology including acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; non-alcoholic steatohepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease
- Obtain a history of exposure to environmental chemical agents.
- Obtain additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin)
- Consider gastroenterology or hepatology consultations.

10.2.2.3 De-challenge/Re-challenge for Liver and Clinical Hepatitis Monitoring

1. Re-challenge is not recommended:
   a. If a patient has had possible/probable drug-induced liver injury
   b. If a decompensation event has occurred (i.e., variceal hemorrhage, ascites, hepatic encephalopathy, etc.).
2. If due to underlying cholestatic liver disease variability or another alternative etiology is identified AND liver tests returned to baseline, re-challenge may be considered after consultation with the sponsor Medical Monitor.
3. If the ALT/total bilirubin elevations are observed after re-challenge, then repeat re-challenge is discouraged.

If patient is permanently discontinued, monitoring should be continued as outlined in Section 10.2.2.5.

10.2.2.4 Diarrhea

Study drug should be discontinued if a patient develops diarrhea with at least 1 of the following concomitant signs or symptoms: grossly bloody stools, vomiting, dehydration

requiring treatment with oral or intravenous rehydration and/or electrolyte imbalances, fever (≥38 C) and/or the diarrhea persists for 7 or more days.

If there is gross blood in stool, INR and platelets should be measured.

Study drug will be reintroduced (re-challenge) when the symptoms have resolved. If the diarrhea re-occurs within 1 week after the re-challenge with no alternate etiology, the patient will be permanently discontinued and monitored as outlined in Section 10.2.2.5.

10.2.2.5 Monitoring after Permanent Discontinuation of Study Drug Due to Safety

Once study drug is permanently discontinued for liver, hepatitis, diarrhea, or other severe AE related to study drug, the patient will be monitored weekly until the laboratory and clinical parameters have normalized/stabilized.

10.2.3 Demographics/Medical and Surgical History

Demographic information per country regulations (age, full date of birth, gender, race, and ethnicity), along with medical and surgical history, will be obtained and recorded in the eCRF at Visit 1.

Medical and surgical history will be entered in the eCRF at Visit 1. This includes date of diagnosis of PFIC, prior investigational medications for PFIC, historical (3 months prior to Screening) LFT values (AST, ALT, and total bilirubin), ongoing medication, any surgery performed, any other diagnosis, and historical biopsy data.

If a liver biopsy has been performed within 1 year prior to Screening, or during the study, the results will be recorded in the eCRF.

10.2.4 Clinical Genetic Testing

If confirmatory clinical genetic testing for PFIC Type 1 or 2 has been performed prior to Screening Visit 1, the confirmatory clinical genetic testing laboratory report will be verified by a central reader to determine eligibility.

Regardless of previous genetic testing, a blood sample will be drawn at Visit 1 for all patients for DNA extraction. Genetic testing will only be performed on a patient's extracted DNA if the previous clinical genetic result is equivocal, unavailable, or unobtainable. In such cases, screening for pathologic biallelic variations in the *ATP8B1* or *ABCB11* genes will be performed if patient meets remaining inclusion and exclusion criteria and only after sponsor case review. No other diagnostic genetic testing will be offered.

10.2.5 Physical Examination

A physician or suitably trained qualified assistant will perform a complete physical examination, including voluntary photography, at Visits 1, 3, and 9. A skin examination will be conducted at Visits 1, 3, 4, 6, 9, and 10 (see Table 1).

A complete physical examination will include assessment of general appearance, eyes, ears, nose, throat, head/neck/thyroid, lymph nodes, cardiovascular, lungs/chest, abdomen, genitourinary, extremities, skin, musculoskeletal, neurologic, and other.

Skin will be examined thoroughly and excoriations/scratch marks recorded.

10.2.6 Vital Signs

Evaluation of vital signs will be performed at all visits. This includes blood pressure (systolic and diastolic), pulse, respiratory rate, temperature, height (or length depending on age), and body weight (using a certified weight scale) at clinic visits. BMI will be calculated.

10.2.7 Abdominal Ultrasound

Ultrasound of the liver and spleen will be performed at randomization (Visit 3) and at EOT (Visit 9). Liver size, echogenicity, and presence of masses/nodules as well as spleen size will be recorded.

10.2.8 Overdose

A4250 is minimally absorbed and has a very low systemic availability. Based on toxicology data, for study purposes any dose exceeding a total of >3 mg/kg body weight of A4250 taken as a single dose or as a cumulative dose within 24 hours is defined as an overdose.

The no-adverse-effect dose level in the most sensitive species (defined as 20 mg/kg/day) in the rat 1 month toxicity study gives a human equivalent dose of approximately 194 mg/day in a 60 kg person. This dose is approximately 10-fold higher than the maximum possible dose predicted in human studies (20 mg).

The investigator/investigative staff should immediately and not later than within 24 hours of knowledge of the overdose, be reported by the investigator or delegate on the SAE report form. In the event of an A4250 overdose, the patient should be monitored closely.

10.2.9 Pregnancy

If a pregnancy is discovered in a female patient enrolled in the study before the end of dosing, the patient will be immediately discontinued from the study and will attend the same visits as a prematurely withdrawn patient. If the pregnancy is discovered in a female patient enrolled in the study after the end of dosing, the patient will continue in the study per protocol. If a pregnancy occurs in a male patient's partner at any time during the study, the pregnancy should also be reported and followed.

Pregnancy is not considered to be an AE. However, if the patient has been dosed with the study drug, the pregnancy must be reported on the Paper Pregnancy Notification Form immediately within 24 hours after recognition to Albireo/Safety Manager (please refer to Section 10.1.3). Date of exposure and as far as possible, details of the period of gestation at the time of exposure must be given.

The pregnancy should be followed to determine outcome, including spontaneous termination, details of birth, and presence of any birth defects, congenital anomalies or newborn or maternal complication. The infant who was exposed in-utero will be followed for up to 2 years after delivery. Individual cases with an abnormal outcome in association with the study drug should be reported on an expedited basis, i.e., reported rapidly to a competent authority.

11 STATISTICAL EVALUATION

All personnel involved with the analysis of the study will remain blinded until database lock for the double-blind Treatment Period and until major protocol violations have been identified.

All statistical analyses will be performed using SAS version 9.3 or higher.

11.1 Sample Size and Power

For the EU and RoW, the primary analysis will relate to the bile acid responder endpoint (at the 1-sided 1.25% level). For the US, the primary analysis will relate to the pruritus endpoint (at the 1-sided 1.25% level). The aim is to enroll 60 to 70 patients in order to get at least 60 evaluable patients.

Assuming 60% responders (patients experiencing at least 70% reduction in fasting s-BA concentration or reaching ≤70 µmol/L) in the A4250 dose group and 10% responders in the placebo group, approximately 20 patients per group are required to achieve a power of 84% with a 1.25% 1-sided Type-1 error.

For pruritus, with approximately 20 patients per treatment group, the power is 83% given an assumed treatment effect difference of -1.0 in change from baseline (-1.6 for A4250 and -0.6 for placebo) on the Albireo ObsRO scratching score with a SD of 0.95 and a Type I error of 1.25% (1-sided).

11.2 Statistical Methods

11.2.1 Statistical Analysis Sets

Safety Analysis Set

The safety analysis set will consist of all patients who received at least 1 dose of study drug. Patients will be analyzed according to the treatment they actually received. The safety analysis set will be the primary analysis set for safety analyses.

Full Analysis Set

The full analysis set (FAS) will consist of all randomized patients who received at least 1 dose of assigned treatment. Patients will be analyzed in the treatment group they were randomized to even if they received incorrect study drug. The FAS will be the primary analysis set for efficacy analyses.

Per Protocol Analysis Set

The per protocol (PP) analysis set is a subset of the FAS, and will consist of all randomized patients for whom no major protocol violation (no violation which may affect the study efficacy outcome) is documented. The PP analysis set will provide supportive data for the efficacy analyses.

Allocation of patients to the PP analysis set will be performed before unblinding of the study.

11.2.2 Methods of Statistical Analyses

11.2.2.1 General Principles

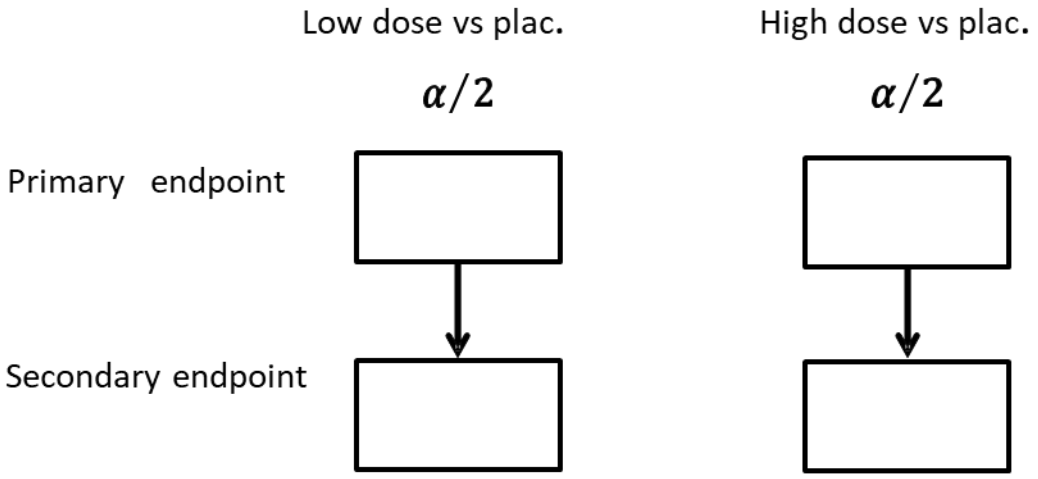
The primary objective of this study is to show superiority in efficacy of A4250 (120 µg/kg/day and 40 µg/kg/day) compared to placebo in patients with PFIC Types 1 and 2.

In the EU and RoW, for the primary efficacy variable fasting bile acid responder endpoint (at least a 70% reduction in fasting s-BA concentration or reaching a level ≤70 µmol/L at the end of treatment), the null hypothesis will be tested against the alternative hypothesis ($\alpha = 0.0125$, 1-sided).

The test will be performed at a Type I error rate of ≤0.0125 1-sided, as described above, based on the least squares means of the treatment difference in mean probability scale.

To strictly control overall Type I error rate across the dose groups and also across the primary and the main secondary endpoint to a 1-sided 2.5% level, a Bonferroni-based parallel gate-keeping procedure will be used. In this procedure the total significance level of 2.5% (1-sided) will be split equally between the dose groups. Only a dose group that is significant for the primary endpoint will be tested, at the nominal significance level of 1.25% (1-sided), for the main secondary endpoint (here the pruritus endpoint). If the previous step was not successful, the analysis of the following step will be considered descriptive. If the procedure reaches the secondary endpoint for both dose groups, these will be evaluated using the Bonferroni Holm procedure. The multiple comparison procedure is presented in Figure 2.

Figure 2 Bonferroni Holm Multiple Comparison Procedure



In the US, for the primary efficacy variable pruritus score endpoint (change from baseline to Week 24 in observer-reported scratching), the null hypothesis will be tested against the alternative hypothesis ($\alpha=0.0125$, 1-sided).

The test will be performed at a Type I error rate of ≤0.0125 1-sided, as described above, based on the least squares means of the treatment difference in mean change.

To strictly control overall Type I error rate across the dose groups and also across the primary and the main secondary endpoint to a one-sided 2.5% level, a Bonferroni-based parallel gate-keeping procedure will be used. In this procedure the total significance level of 2.5% (1-sided) will be split equally between the dose groups. Only a dose group that is significant for the primary endpoint will be tested, at the nominal significance level of 1.25% (1-sided), for the main secondary endpoint (here the bile acid responder endpoint). If the previous step was not successful, the analysis of the following step will be considered descriptive. If the procedure reaches the secondary endpoint for both dose groups, these will be evaluated using the Bonferroni Holm procedure.

Other secondary and exploratory outcome variables shall provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple dose comparisons when testing the other secondary and exploratory efficacy variables.

A more detailed statistical analysis plan (SAP) will be prepared and finalized before study data are locked and unblinded.

If not otherwise specified, the baseline value of a parameter is defined as the last assessment of that parameter on or prior to the first intake of study treatment date.

11.2.2.2 Missing Data

For efficacy variables, with repeated measures, a mixed model will be employed with no additional imputation for missing values, i.e., based on "observed cases", except where otherwise specified.

Follow-up and continued collection of efficacy data for patients who discontinue treatment will be made. These data will be included in the sensitivity analysis for primary endpoints estimating a treatment effect taking loss of effect after discontinuation into account. Because complete follow-up of all patients discontinuing treatment may not be possible, a method to replace missing data for patients who discontinue the study will be prespecified (in the SAP) taking into account loss of effect after discontinuation (for example multiple imputation).

No other replacement of missing values will be planned.

11.2.2.3 Demographic and Baseline Characteristics

Descriptive summaries of demographics and other baseline characteristics (including medical and surgical history) will be presented by treatment group and overall using the FAS.

Prior medication will be summarized by treatment group and overall using the safety analysis set.

11.2.2.4 Subject Disposition

The following will be summarized (by treatment group and overall where applicable):

- Patients enrolled (who signed the informed consent)
- Patients randomized
- Patients treated
- Patients completing the study/withdrawing early (including withdrawal reason)

Additionally, patients enrolled, included in the safety, in the FAS, and in the PP analysis set will be summarized by region.

11.2.2.5 Evaluation of Primary Efficacy Variables

The responder endpoint based on change in fasting bile acids will be analyzed in terms of the proportion of patients achieving the event of interest. Patients in need of rescue therapy, i.e., biliary diversion will be classified as non-responders after the biliary diversion.

Generalized linear mixed modelling (GLMM) will be used with a logistic link, to allow for repeated assessments over time. The model will include baseline value, treatment group, PFIC class, age class, region, visit, and visit*treatment group interaction. The treatment by visit interaction will remain in the model regardless of significance. Region will be included in the model as a random effect. The contrasts of primary interest will be the treatment difference at Week 24; also, the difference at Week 4, Week 8, Week 12, and Week 18 will be estimated and analyzed. An unstructured covariance matrix will be used to model the within-subject error. Another covariance structure may be chosen if the fit of this structure fails to converge. Model diagnostics checks will be made to ensure appropriate fit.

The primary analysis of the change from baseline in pruritus score (indexed by observer-reported scratching) will be analyzed using a mixed model repeated measures (MMRM) analysis. The contrast of primary interest will be treatment difference at Week 24 but also the differences at Week 4, Week 8, Week 12, and Week 18 will be estimated and analyzed. Baseline pruritus score will be included as covariate. The model will include baseline value, treatment group, PFIC class, age class, region, visit, and visit*treatment group interaction. The treatment by visit interaction will remain in the model regardless of significance. Region will be included in the model as a random effect and will be defined on country level. An unstructured covariance matrix will be used to model the within-subject error. Another covariance structure may be chosen if the fit of this structure fails to converge.

Non-parametric approach may be considered if more appropriate.

The primary analyses will be based on the FAS.

To assess the robustness of the primary efficacy analysis, the following analyses will be performed:

- Primary efficacy analysis using the PP set
- Analyses to investigate the influence of missing values, for example, by using pattern mixture models and tipping point analysis

Subgroup analyses will also be performed for each of the 3 age groups (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) and for patients who were not included in the previous Study A4250-003.

Several sensitivity analyses will be performed, such as the following:

- Cochran Mantel Haenzsel test stratified on PFIC class to compare the proportion in fasting bile acid responder endpoint at the end of treatment in the two A4250 dose groups to placebo group. Dropouts will be treated as non-responders
- Based on the primary analysis including the interaction age class*treatment
- Same as primary analysis in which the random factor region is considered as fixed factor. The region*treatment interaction will also be explored
- Same as primary analysis including the interaction PFIC*treatment

Other sensitivity analyses may be performed and will be detailed in the SAP.

11.2.2.6 Evaluation of Secondary and Exploratory Efficacy Variables

The change in the secondary and explorative endpoints such as s-BA, total bilirubin, ALT, growth and patient-reported itch severity will also be analyzed using the MMRM model as specified for the primary analysis. Nonparametric methods may be applied as an alternative approach for some endpoints, if appropriate. Change in growth (weight for age and BMI for age) will also be displayed using graphical presentations.

Proportions of responders (e.g., meaningful reduction in itch severity and scratching at Week 24) will be analyzed using the GLMM model as specified for the primary analysis.

Comparisons of the change from baseline at Week 24 in PedsQL between the treatment groups will be conducted using analysis of covariance. The model will include terms for baseline, PFIC class, age class, region, and treatment.

Undergoing biliary diversion surgery and/or listed for liver transplant, all-cause mortality, and liver decompensation events will mainly be analyzed by using descriptive statistics. Kaplan-Meier curves will be used when appropriate for time to event data.

Other exploratory variables (Global Symptom Relief, biochemical markers and measures of bile acid synthesis, Albireo PRO and ObsRO sleep parameters, PELD/MELD score, APRI score, and Fib-4 score) will be analyzed descriptively for categorical and continuous data, as applicable. For continuous data, the change from baseline will be analyzed in addition to the actual visit values. For categorical data, shift tables or frequency and percentages of patients will be presented as appropriate.

In addition, subgroup analyses will be performed for each of the 3 age classes (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years).

11.2.2.7 Evaluation of Safety Variables

Safety data will be analyzed using descriptive statistics and summaries by treatment group of SAEs, AEs, vital signs, clinical laboratory tests (hematology, clinical chemistry and urinalysis), and concomitant medication. Analyses will be performed using the safety analysis set.

Summaries of AEs (coded according to the Medical Dictionary for Regulatory Activities [MedDRA] system organ class [SOC] and MedDRA preferred term) will include the following:

- Overview of the incidence of TEAEs (TEAEs, drug-related TEAEs, TEAEs leading to study discontinuation, and treatment-emergent SAEs)
- TEAEs by SOC and preferred term
- Intensity of TEAEs by SOC and preferred term
- Drug-related TEAEs by SOC and preferred term
- TEAEs leading to study discontinuation by SOC and preferred term
- Treatment-emergent SAEs by SOC and preferred term

Concomitant medication use during the Treatment Period will be summarized by Anatomical Therapeutic Chemical (ATC) class and World Health Organization (WHO) preferred name.

Summaries of vital signs will be presented. For each visit, the actual results and the change from baseline, and the number and percentage of patients with potentially clinically significant (PCS) values observed post-baseline will be presented.

Summaries of clinical safety laboratory data will be presented. For each visit, the actual result and the change from baseline, and the number and percentage of patients with PCS values observed post-baseline will be presented.

Data listings will be provided for each patient for all safety parameters.

11.2.2.8 Compliance and Exposure

Exposure will be analyzed by calculating the number of days with exposure to study drug. Results will be presented by treatment group using the safety analysis set.

The percentage compliance will be described by treatment group and the number of patients with a compliance <80%, between 80% and 120%, and >120% will be presented based on the safety analysis set.

11.2.3 Blinded Analyses to Confirm Responder Definitions

A blinded analysis of Albireo ObsRO and PRO eDiary data will be performed after 50% or more of the planned sample has completed the Week 24 Follow-Up Period. The blinded analysis will be used to estimate a threshold of clinically meaningful change (i.e., responder definition) in Albireo ObsRO and PRO pruritus scores. The analysis will be performed by a group independent from both the study team and Albireo. Blinded data will be used for this analysis; data will be collapsed across treatment groups.

The analyses will include distribution- and anchor-based analyses to examine a responder definition for the Albireo PRO/ObsRO pruritus scores. Distribution-based analyses will include calculation of the SD and standard error of measurement for the baseline Albireo PRO and ObsRO scores. Anchor-based analyses will involve examining the degree of change on the Albireo PRO and ObsRO scores from baseline to Week 24 for patients who experience change in pruritus according to PGIC and symptom items. Details of this analysis will be included in a stand-alone SAP for assessing the measurement characteristics (reliability, validity, sensitivity to change, responder definition) of the Albireo PRO and ObsRO items.

11.2.4 Data Safety Monitoring Board

A DSMB consisting of sponsor-independent clinical and statistical experts will be established. The DSMB will periodically meet for the review of accumulating study data, including safety (AE and laboratory data) and liver values.

The DSMB will have access to unblinded data. The DSMB will make recommendations for the remaining part of the study (further details will be provided in the DSMB charter). The DSMB may recommend continuing with the study as planned or stopping the study early for safety reasons. The DSMB will submit its recommendations in writing to Albireo Medical/[redacted] who are responsible for responding to the recommendations of the DSMB and taking appropriate action. The investigators will only be informed by Albireo Medical/[redacted] if the study requires a protocol amendment or is stopped. The DSMB may choose to make additional evaluations at any time if they feel this is warranted from a safety point of view.

The DSMB will act according to its own written standard operating procedure described in a charter and will prepare written minutes of its meetings. The charter of the DSMB will be stored in the trial master file. The DSMB will maintain records of its meetings and these will become part of the study file when the study is complete. In order not to disseminate unblinded data and to ensure that all staff involved in the conduct and final analysis of the study remain blind to the data, only the members of the DSMB and the unblinded DSMB statistician will have access to these data.

12 DIRECT ACCESS TO SOURCE DATA/NOTES

The investigator/institution shall provide direct access to source data/documents for study-related monitoring, audits, IEC/Institutional Review Board (IRB) review and regulatory inspection.

13 QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Conduct of the Study

Albireo/[redacted] shall implement and maintain quality control and quality assurance procedures with written standard operating procedures to ensure that the study is conducted and data are generated, documented, and reported in compliance with the protocol, ICH GCP and applicable regulatory requirements.

This study shall be conducted in accordance with the provisions of the Declaration of Helsinki (October 1996) and all revisions thereof, and in accordance with United States Food and Drug Administration (FDA) regulations (Code of Federal Regulations, Sections 312.50 and 312.56) and with ICH GCP (CPMP 135/95).

The investigator may not deviate from the protocol without a formal protocol amendment having been established and approved by an appropriate IEC/IRB, except when necessary to eliminate immediate hazards to the patient or when the change(s) involve(s) only logistical or administrative aspects of the study. Any deviations may result in the patient having to be withdrawn from the study and render that patient non-evaluable.

13.2 Study Monitoring

Before study initiation, at a site initiation visit or at an investigator's meeting, an Albireo representative or designee will review the protocol and eCRF with the investigators and the investigative staff. During the study, the clinical monitor (clinical research associate [CRA]) will visit the site regularly to check the completeness of patient records, the accuracy of entries on the eCRFs, the adherence to the protocol and to GCP, the progress of enrollment, and to ensure that study drug is being stored, dispensed, and accounted for according to specifications.

The investigator must ensure that eCRFs are completed within a timely period of patient visits, as per individual site agreements, and must allow the CRA and Albireo representative or designee periodic access to patient records and all study-related materials, including relevant hospital or clinical records, to confirm their consistency with the eCRF entries. No information in these records about the identity of the patients will leave the study center. Albireo monitoring standards require full verification for the presence of the signed ICF, adherence to the inclusion/exclusion criteria, documentation of SAEs, and recording of primary efficacy and safety variables. The CRA will review source data compared with the eCRFs and will verify source data according to the study-specific monitoring plan. The design of the study, the frequency of patient visits, and the site enrollment rate will determine the frequency of monitoring visits. Upon study completion, the CRA will visit the site to conduct a study termination visit, which will include collection of any outstanding documentation.

It is recommended that the investigator/investigative staff log into the eCRF system every working day or at minimum twice weekly in order to provide a prompt response to queries.

The investigator/investigative staff should respond to queries and make any relevant changes to the study data within 3 working days.

14 ETHICS

14.1 Independent Ethics Committee/Institutional Review Board

Prior to the start of the study, the investigator is responsible for ensuring that the protocol and consent form have been reviewed and approved by a relevant IEC/IRB. The IEC/IRB shall be appropriately constituted and perform its functions in accordance with FDA ICH GCP and local requirements as applicable.

The IEC/IRB shall approve all protocol amendments (except for logistical or administrative changes), written informed consent documents and document updates, patient recruitment procedures (e.g., advertisements), written information to be provided to the patients and caregivers, IB, available safety information, information about payment and compensation available to patients, the investigator's curriculum vitae and/or other evidence of qualifications and any other documents requested by the IEC/IRB and regulatory authority (competent authority) as applicable.

14.2 Written Informed Consents and Assents

The investigator/investigative staff will explain to each patient (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, alternative treatment, the potential risks and benefits involved, and any discomfort that may occur. Each caregiver/patient will be informed that participation in the study is voluntary and that he/she or their child may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

Caregivers/patients will be informed that they/their children may experience side-effects or be at risk for symptoms, illnesses, or complications that cannot be foreseen by Albireo. As with other medications, people treated with A4250 may be at risk of developing allergic reactions or anaphylaxis. Caregivers/patients will be advised that study procedures include regular blood sampling for measurement of safety parameters and biological markers and that some minor risks are associated with these procedures.

This informed consent should be given by means of a signed ICF, written in non-technical language in accordance with ICH GCP, the Declaration of Helsinki, and regulatory authorities. The caregiver/patient should read and consider the statements before signing and dating them and should be given a copy of each signed document. If written consent is not possible, oral consent can be obtained if witnessed and followed by a signed statement from one or more persons not involved in the study, indicating why the patient was unable to sign the form. No patient can enter the study before his/her or caregiver informed consent has been obtained.

The ICF is part of the protocol and must be submitted by the investigator/investigative staff with the protocol for IEC approval. Albireo will supply an ICF which complies with regulatory requirements and is considered appropriate for the study. Any changes to the ICF

suggested by the investigator must be agreed to by Albireo before submission to the IEC and a copy of the approved version must be provided to the clinical monitor after IEC approval.

15 DATA HANDLING AND RECORD KEEPING

15.1 Case Report Forms/Source Data Handling

The investigator shall be provided with standardized eCRFs and shall ensure that all data from patient visits are promptly entered into the eCRFs in accordance with the specific instructions given. The investigator must sign each eCRF to verify the integrity of the data recorded.

A list of the normal ranges for all laboratory tests to be undertaken forms part of the documentation to be collated prior to study start. As a central laboratory has been selected to conduct any or all tests, it is essential that all samples be analyzed at that laboratory.

The investigator must maintain source documents such as laboratory reports, consultation reports, and complete medical history and physical examination reports.

As no ethnicity data can be collected in the source document for study sites in France, the eCRF will be the Source Document for “origines ethniques”,

15.2 Retention of Essential Documents

Essential documents, as defined by ICH GCP, include: the signed protocol and any amendment(s); copies of the completed eCRFs (for site archiving, compact discs of eCRF data for specific patients will be provided); signed ICFs; hospital records and other source documents; IEC approvals and all related correspondence including approved documents; drug accountability records; study correspondence; and a list of patients’ names and addresses.

The investigator/investigative staff must retain copies of these essential documents for the period specified by ICH GCP and by applicable regulatory requirements. The investigator/investigative staff will inform Albireo of the location where the essential documents are stored and must contact Albireo for approval before disposing of any essential documents. The investigator/investigative staff should take measures to prevent accidental or premature destruction of these documents.

16 FINANCING AND INSURANCE

Financing and insurance are addressed in a separate agreement.

17 PUBLICATION POLICY

Albireo will retain the ownership of all data. When the study is complete, Albireo shall arrange the analysis, tabulation of data and preparation of a clinical study report. Albireo may also use the data for publication, presentation at scientific meetings and submission to regulatory authorities. All proposed publications based on this study must be subject to the sponsor's approval requirements.

18 REFERENCE LIST

1. A4250 Investigator's Brochure.
2. Alissa FT, Jaffe R, Shneider BL. Update on progressive familial intrahepatic cholestasis. J Pediatr Gastroenterol Nutr. 2008;46(3):241-52.
3. Davit-Spraul A, Gonzales E, Baussan C, et al. Progressive familial intrahepatic cholestasis. Orphanet J Rare Disease 2009;4:1-12.
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5. Hori T, Justin H, Nguyen JH and Uemoto S. Progressive familial intrahepatic cholestasis. Hepatobiliary pancreat Dis Int. 2010; 9(6):570-8.
6. Jacquemin E. Progressive familial intrahepatic cholestasis. Genetic basis and treatment. Clin Liver Dis. 2000;4(4):753-63.
7. Miethke AG, Zhang W, Simmons J, et al. Pharmacological inhibition of ASBT changes bile composition and blocks progression of sclerosing cholangitis in mdr2 knockout mice. Hepatology. 2016; 63(2):512–23.
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9. Pawlikowska L, Strautnieks S, Jankowska I, et al. Differences in presentation and progression between severe FIC1 and BSEP deficiencies. J Hepatol. 2010;53(1):170-8.
10. Suchy FJ, Sokol RJ, Balistreri WF, eds. Liver Disease in Children. 3rd Edition. New York, NY: Cambridge University Press; 2007:310–4.
11. Whitington PF, Whitington GL. Partial external diversion of bile for the treatment of intractable pruritus associated with intrahepatic cholestasis. Gastroenterology. 1988;95(1):130-6.

19 APPENDICES

Appendix 1 Concomitant Medications Prohibited During the Study

Appendix 2 Diary Questions including Albireo Patient-Reported Outcomes/Observer-Reported Outcome Instrument

Appendix 3 Patient Global Impression of Change (PGIC) and Patient Global Impression of Symptoms (PGIS)

Appendix 4 Pediatric Quality of Life Inventory (PedQL) Questionnaires

Appendix 5 Contraceptive advice

Appendix 6 Blood volumes

CLINICAL STUDY PROTOCOL A4250-005

A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

IND Number:	130591
EudraCT Number	2017-002338-21
Test Product:	A4250
Indication:	Progressive Familial Intrahepatic Cholestasis (PFIC)
Sponsor:	Albireo AB
Development Phase:	Phase 3
Sponsor Signatory:	[redacted]
Sponsor Medical Monitor:	[redacted]
Principal Investigator:	[redacted]
Date of the Protocol:	01 March 2018
Version of the Protocol:	Final Amendment 1.1 Sweden

Confidential

The information in this document contains trade secrets and commercial information that are privileged or confidential and may not be used, divulged, published, or otherwise disclosed without the written consent of Albireo AB. These restrictions on disclosure apply equally to all future information supplied to you or generated by you in connection with the study.

SPONSOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

Albireo AB

[redacted]

01 March 2018

Date (day/month/year)

CLINICAL RESEARCH ORGANIZATION SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

[redacted]

[redacted] Date (day/month/year)

[redacted] Date (day/month/year)

INVESTIGATOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

I have read this protocol and agree that it contains all necessary details for performing this study. I will conduct the study as outlined herein and will complete the study within the time designated, in accordance with all stipulations of the protocol and in accordance with Good Clinical Practice (GCP), local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study drug and the conduct of the study.

I will use only the informed consent approved by the Independent Ethics Committee (IEC) and will fulfil all responsibilities for submitting pertinent information to the IEC responsible for this study.

I agree that the sponsor (Albireo AB) shall have access to any source documents from which case report form information may have been generated.

I further agree not to originate or use the name of Albireo AB, A4250 in any publicity, news release, or other public announcement, written or oral, whether to the public, press, or otherwise, relating to this protocol, to any amendment to the protocol, or to the performance of this protocol without the prior written consent of Albireo AB.

Name of Investigator

Signature

Date (day/month/year)

1 ADMINISTRATIVE INFORMATION

A Double-blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Protocol No.:	A4250-005
Date of the initial Protocol:	20 September 2017
Date and Number of Amendment(s):	Amendment 01, 06 December 2017
Sponsor:	Albireo AB Arvid Wallgrens backe 20 413 46 Göteborg Sweden
Clinical Research Organization:	[redacted]
Sponsor Signatory:	
Sponsor Medical Monitor:	
Principal Investigator:	

2 STUDY SYNOPSIS

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250
Title of Study: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)		
Principal Investigator: [redacted]		
Study Centers: Up to 50 sites will be initiated for this study in the United States (US), Canada, European Union (EU), Middle East, and Australia.		
Publication(s): None.		
Planned Study Period: Q1 2018 to Q2 2019	**Development Phase:** Phase 3	

Objectives:

Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with progressive familial intrahepatic cholestasis (PFIC) Types 1 and 2, as determined by the following:

- Proportion of patients experiencing at least a 70% reduction in fasting serum bile acid (s-BA) concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- Change from baseline to Week 24 in pruritus compared to placebo

Secondary Objectives

- To evaluate the effect of A4250 on serum alanine aminotransferase (ALT) concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

Methodology:

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of familial intrahepatic cholestasis-1 or bile salt export pump).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-up Period. After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo. Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008) in which all patients will receive active treatment.

There will be up to 10 clinic visits during the study and one scheduled telephone contact.

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250

Number of Patients:
Approximately 60 patients diagnosed with PFIC-1 or PFIC-2 will be randomized.

Diagnosis and Main Criteria for Inclusion:

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples 7 to 28 days apart (Visits 1 and 2) prior to randomization
4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the electronic diary (eDiary) average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients are expected to have a consistent caregiver(s) for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

Test Product, Dose and Mode of Administration:
A4250, 40 µg/kg/day or 120 µg/kg/day, orally administered

Reference Therapy, Dose and Duration of Administration:
Matching placebo, orally administered

Duration of Treatment:
24 weeks

Variables

Efficacy:

Primary Efficacy Endpoints

The primary efficacy endpoints are:

- **EU and rest of world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  Fasting s-BA baseline will be calculated as the average of the value at randomization and the previous visit (Clinic Visit 2). The end value is the average of the values at Weeks 22 and 24 after the start of double-blind treatment.
- **US**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 24 as measured by the worst scratch of the Albireo observer-reported outcome (ObsRO) instrument. Scores for the 14 days baseline and post-treatment intervals will be calculated by averaging the worst scratching scores from each day in the interval.

Secondary Efficacy Endpoints

Secondary efficacy endpoints include the following:

- **EU and RoW**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 24 as measured by the worst scratch of the Albireo ObsRO instrument. Scores for the 14 days baseline and post-treatment intervals will be calculated by averaging the worst scratching scores from each day in the interval.
- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  All Regions:

  All secondary endpoints will be compared to placebo.

Name of Sponsor/Company:	Name of Product:	Name of Active Ingredient:
Albireo	A4250	A4250

- Change from baseline to Week 24 in fasting s-BA
- Change from baseline to Week 24 in serum ALT concentration
- Change in growth from baseline to Week 24, defined as the linear deficit (weight for age and body mass index) compared to the national growth curves (Z-score, standard deviation from P50)
- Proportion of patients achieving meaningful reduction in caregiver-reported observed scratching at Week 24
- Change from baseline to Week 24 in sleep parameters measured with the Albireo patient-reported outcome (PRO) and ObsRO instruments
- Change from baseline to Week 24 in patient-reported itch severity as measured by the average daily score of the Albireo PRO instrument; only patients ≥8 years of age will complete the Albireo PRO instrument
- Number of patients undergoing biliary diversion surgery or being listed for liver transplantation

Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Proportion of patients achieving meaningful reduction in patient-reported itch severity at Week 24
- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the patient global impression of change (PGIC) items
- Change from baseline in fasting s-BA at Weeks 4 and 12
- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Weeks 4, 12, and 24 as measured by the worst scratch. Scores for the 7 worst scratching days of the last 14 days at baseline and post-treatment intervals will be selected
- Change from baseline in patient-reported itch severity and observer-reported scratching at Weeks 4 and 12
- Change from baseline to Week 24 in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline to Week 24 in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline to Week 24 in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in Pediatric Quality of Life Inventory (PedsQL)
- Change from baseline in serum ALT concentration at Weeks 4 and 12
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, plasma 7α-hydroxy-4-cholesten-3-one concentration [p-C4]) at Week 24
- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events.
- All-cause mortality

Safety:

Safety criteria are as follows:

- Occurrence of treatment-emergent adverse events including severity and relatedness to study drug at all visits
- The incidence of treatment-emergent serious adverse events (SAEs), based upon information from patient reports, including the description, incidence, and severity of an SAE
- Trends evaluated for the following assessments: physical examinations, concomitant medications, vital signs, laboratory test results (including clinical chemistry, hematology, and urinalysis), and imaging

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250
studies		

Statistical Methods:

The primary objective of this study is to show superiority in efficacy of A4250 (120 µg/kg/day and 40 µg/kg/day) compared to placebo in patients with PFIC Types 1 and 2.

In the EU and RoW, for the primary efficacy variable fasting bile acid responder endpoint (at least a 70% reduction in fasting s-BA concentration or reaching a level ≤70 µmol/L at the end of treatment), the null hypothesis will be tested against the alternative hypothesis (α=0.0125, 1-sided). Generalized linear mixed modelling will be used with a logistic link, to allow for repeated assessments over time. The model will include baseline value, treatment group, PFIC class, age class, region, visit and visit*treatment group interaction. The contrasts of primary interest will be the treatment difference at Week 24 but also the difference at Week 4, Week 8, Week 12, and Week 18 will be estimated and analyzed.

In the US, for the primary efficacy variable pruritus score endpoint (change from baseline to Week 24 in observer-reported scratching), the null hypothesis will be tested against the alternative hypothesis (α=0.0125, 1-sided). The primary analysis of the change from baseline in pruritus score (indexed by observer-reported scratching) will be analyzed using a mixed model repeated measures analysis. The contrasts of primary interest will be the treatment difference at Week 24 but also the difference at the other weeks will be estimated and analyzed.

To strictly control overall Type I error rate across the dose groups and also across the primary and the main secondary endpoint to a 1-sided 2.5% level, a Bonferroni-based parallel gate-keeping procedure will be used.

Other secondary and exploratory outcome variables shall provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple-dose comparisons when testing the other secondary and exploratory efficacy variables.

Date of the Protocol: 06 December 2017

3 TABLE OF CONTENTS

3.1 List of Tables

3.2 List of Figures

3.3 List of Appendices

4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADME	absorption, distribution, metabolism, and excretion
AE(s)	adverse event(s)
AFP	alfa-fetoprotein
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APRI	AST to platelet ratio index
ASBT	apical sodium-dependent bile transporter (also known as IBAT)
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	body mass index
BSEP	bile salt export pump
CPK	creatine phosphokinase
CRA	clinical research associate
CYP	cytochrome P450
DSMB	Data and Safety Monitoring Board
eCRF	electronic case report form
eDiary	electronic diary
EOT	End of Treatment
EU	European Union
EudraCT	European Union drug regulatory agency clinical trial
FAS	full analysis set
FDA	United States Food and Drug Administration
FIC1	familial intrahepatic cholestasis-1
GCP	Good Clinical Practice
GI	gastrointestinal
GLMM	generalized linear mixed modelling
IB	Investigator's Brochure
IBAT	ileal bile acid transporter (also known as ASBT)
ICF	informed consent form
ICH	International Council for Harmonisation
[redacted]	[redacted]
IEC	Independent Ethics Committee
INR	international normalized ratio
IRB	Institutional Review Board
IWRS	Interactive Web Response System

LDH	lactate dehydrogenase
LFT	liver function test
LPLV	last patient last visit
MDR3	multidrug-resistance protein 3
MedDRA	Medical Dictionary for Regulatory Activities
MELD	model for end-stage liver disease
MMRM	mixed model repeated measures
ObsRO	observer-reported outcome
PBC	primary biliary cholangitis
p-C4	plasma 7α-hydroxy-4-cholesten-3-one concentration
PCS	potentially clinically significant
PedsQL	Pediatric Quality of Life Inventory
PELD	pediatric end-stage liver disease
PFIC	progressive familial intrahepatic cholestasis
PGIC	patient global impression of change
PGIS	patient global impression of symptoms
PP	per protocol
PRO	patient-reported outcome
PT	prothrombin time
PVM	pharmacovigilance manager
QoL	quality of life
RoW	rest of world
RR	respiratory rate
SAE(s)	serious adverse event(s)
SAP	statistical analysis plan
s-BA	serum bile acid
SD	standard deviation
s-FGF19	serum fibroblast growth factor 19 concentration
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction
TEAE(s)	treatment-emergent adverse event(s)
ULN	upper limit of normal
US	United States
WHO	World Health Organization

5 INTRODUCTION

5.1 Investigational Medicinal Product

A4250 is a small molecule and a potent selective inhibitor of the ileal bile acid transporter (IBAT), also known as the apical sodium-dependent bile acid transporter (ASBT). IBAT is an integral brush border membrane glycoprotein that co-transports sodium and bile acids and appears to be a critical component in the regulation of the bile acid pool size in animals and man. This transporter, expressed in the distal ileum, is a key element in the enterohepatic circulation of bile acids since it facilitates the high-affinity, high-capacity reabsorption of bile acids. Indeed, 95% of secreted bile acids are reabsorbed via IBAT [Hofmann 2009; Miethke 2016]. A4250 is orally administered and acts locally in the gut where it binds reversibly to IBAT to decrease the reuptake of bile acids. A4250 has minimal systemic exposure at the predicted therapeutic dose ranges.

No safety signals were found with A4250 in preclinical safety and toxicity studies. In the first clinical study of A4250 in healthy volunteers, A4250 was given as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. A4250 was well tolerated and the only findings were gastrointestinal (GI) related effects, such as diarrhea, at higher doses.

In an exploratory Phase 2 study in children with cholestatic pruritus, A4250 was given as oral doses of 10 to 200 µg/kg/day for 4 weeks. A4250 reduced mean levels of serum bile acids (s-BA) in all cohorts. In 11 of 13 progressive familial intrahepatic cholestasis (PFIC) patients, substantial reductions (ranging from 43% to 98%) were observed. Patient-reported diary data documented improvement of pruritus during treatment. A4250 was well tolerated. No serious adverse events (SAEs) were deemed to be related to study drug, and most adverse events (AEs), including some increased transaminases, were considered unrelated to study drug and transient in nature.

5.2 Background

5.2.1 Progressive Familial Intrahepatic Cholestasis

PFIC is a rare autosomal recessive cholestatic liver disease estimated to affect between 1 in every 50,000 to 100,000 children born worldwide. PFIC represents 10% to 15% of causes of cholestasis in children and 10% to 15% of liver transplantation indications in children. All types of PFIC exist worldwide and both sexes appear to be equally affected.

The common underlying pathogenesis of PFIC is disruption of bile formation and bile transport through the liver [Jacquemin 2000]. The classification of PFIC has evolved over the years. The most commonly used sub-classifications are PFIC Types 1, 2, and 3, which are based on the associated affected gene and described in more detail below.

- PFIC, Type 1: also referred to as “Byler disease” or “familial intrahepatic cholestasis-1 (FIC1) protein deficiency.” FIC1 protein is located on the canalicular membrane of hepatocytes and facilitates movement of aminophospholipids from the outer

to inner leaflet of the plasma membrane of the hepatocyte. The *ATP8B1* gene encodes FIC1 protein. Biallelic pathologic variants in the *ATP8B1* gene are associated with FIC1 dysfunction and classified clinically as PFIC Type 1 disease.

- PFIC, Type 2: also referred to as "Byler syndrome" or "bile salt export pump (BSEP) deficiency." BSEP is a transporter protein that is expressed at the canalicular membrane of hepatocytes, and is the primary exporter of bile acids. The *ABCB11* gene encodes the BSEP protein. Biallelic pathologic variations in the *ABCB11* gene are associated with BSEP dysfunction and classified clinically as PFIC Type 2 disease.
- PFIC, Type 3: is caused by a deficiency of the multidrug-resistance protein 3 (MDR3) due to mutations in the *ABCB4* gene. MDR3 is a phospholipid translocase critical for phospholipid secretion.

Severe pruritus is common in children diagnosed with PFIC. Itching (and subsequent scratching) is a significant morbidity for these patients and their families [Suchy 2007]. A more severe degree of pruritus is experienced compared to patients with other forms of liver disease and to other pruritic conditions such as atopic dermatitis [Murray 2011]. In patients with PFIC, liver biopsy reveals canalicular cholestasis and, later, the appearance of portal fibrosis. Serum biochemistry indicates cholestasis with hyperbilirubinemia, elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The concentrations of bile acids in serum are very high, while serum gamma-glutamyl transferase activity (exception being MDR3 variants) and cholesterol are normal [Hori 2010]. Symptoms of portal hypertension and liver failure will develop during the course of the disease [Davit-Spraul 2009; Alissa 2008]. Symptoms develop early; median age at onset of symptoms is 2 months, and 78% of PFIC patients present with jaundice [Pawlikowska 2010]. The life-threatening and debilitating nature of PFIC is reflected by the fact that survival in those not resorting to surgery is 50% at 10 years of age and almost zero at 20 years of age. Approximately half of PFIC patients undergo liver transplantation [Davit-Spraul 2009] and treatment-resistant pruritus is the leading indication for the surgical procedure partial external biliary diversion, mostly in PFIC Type 1 and 2 patients.

PFIC is life-threatening and debilitating. Currently, the treatment for PFIC is palliative and there is no pharmaceutical treatment approved for use in PFIC. The therapeutic choices are restricted to non-specific therapy of the symptoms and signs of the disease such as nutritional support, preventing vitamin deficiencies, and treatment of extrahepatic features. Medical treatment options include off-label use of ursodeoxycholic acid, rifampin, antihistamines, and naltrexone. A minority of patients respond nominally and transiently to these interventions. Biliary diversion is used to decrease systemic bile acids through interruption of the enterohepatic circulation and so avoid transplantation. Liver transplantation is typically only viewed as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

5.2.2 Summary of Clinical and Nonclinical Studies

This is a summary of nonclinical and clinical studies. More detail is provided in the Investigator's Brochure ([IB] see A4250 IB).

A4250 is a potent, selective IBAT inhibitor with no safety signals seen in the safety pharmacological studies. In addition, no safety signals were seen in repeat-dose oral toxicity studies in rats with up to 26 weeks of daily dosing and in dogs with up to 39 weeks of daily dosing. No safety signals were identified when A4250 was administered daily to juvenile rats from age 14 days to 63 days.

A4250 is minimally absorbed with very low systemic exposure in both humans and other species. In blood, protein binding is high (>99.7%). Systemically absorbed A4250 is slowly metabolized in humans and in preclinical studies by hydroxylation only, a metabolite that retains IBAT activity. A4250 did not show any significant inhibition of cytochrome P450 (CYP) enzymes in the rat. A4250 inhibited human CYP3A4 and CYP2D6 (IC_{50}: 16 µmol/L for both enzymes), and CYP2C9 (IC_{50}:1.2 µmol/L).

To date, A4250 has been studied in 3 Albireo-sponsored clinical studies: a study in healthy volunteers evaluating single and multiple administration of A4250; a single-dose absorption, distribution, metabolism, and excretion (ADME) study; and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with primary biliary cholangitis (PBC). In total, 98 subjects have been exposed to A4250.

In the first clinical study (A4250-001), A4250 was given to healthy volunteers as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. Following a single oral dose of 10 mg, the median maximum drug concentration was 0.2 nmol/L and the area under the curve was 0.8 nmol × h/L (n=6). Pharmacodynamic evaluation of bile acid synthesis biomarkers (serum fibroblast growth factor 19 [s-FGF19] and plasma 7α Hydroxy 4-cholesten-3-one [p-C4]) identified the expected decrease in s-FGF19 and an increase in p-C4. In addition, total plasma bile acids decreased and total fecal bile acids increased in A4250-treated patients compared to placebo. A4250 was generally well tolerated. The only findings were GI-related effects, such as diarrhea at higher doses.

In the ADME study (A4250-007), healthy subjects were administered a single oral dose of 3 mg [^{14}C]-A4250 capsule. The main excretion route was determined to be fecal. There were no quantifiable concentrations of A4250 in plasma or total radioactivity in plasma or whole blood. A4250 was well tolerated. AEs were reported and most were mild and not related to A4250. The 3 AEs experienced by 2 subjects reported as related to study drug were GI in nature, including diarrhea (2 AEs; 2 subjects) and abdominal pain (1 AE; 1 subject).

In an exploratory Phase 2 study in children with cholestatic pruritus (A4250-003), A4250 was administered as oral daily doses of 0.01 to 0.2 mg/kg for 4 weeks. Twenty patients (8 females and 12 males) aged 1 to 17 years were enrolled in this open-label dose-escalation study. Four patients were re-enrolled into a second dose level. No

treatment-related SAEs were reported, and most AEs, including some increased transaminases, were transient. Four-week, once-daily treatment with A4250 reduced mean levels of s-BA in all cohorts. Substantial s-BA reductions in 11 of 13 PFIC patients (ranging from 43% to 98%) were observed. A4250 also reduced s-BA in the 11 non-PFIC patients, but to a lesser extent than in PFIC patients. Patient-reported diaries documented improvement of the visual analogue scale itch score in 17 of 24 patients during treatment. The dose with the greatest improvement showed a mean decrease (± standard error of the mean) of 2.9 ±1.1 from baseline. The results from this study form the basis of the current (A4250-005) study design.

5.3 Rationale

In this investigational study, A4250 is under development for treatment of PFIC and is also being considered for other orphan cholestatic liver diseases.

There is currently no medical treatment approved for PFIC. In PFIC patients with severe pruritus, biliary diversion surgery is used to decrease systemic bile acids through interruption of the enterohepatic circulation and thereby reducing pruritus [Whitington 1988]. Liver transplantation is typically used as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

By inhibiting IBAT with high selectivity and potency, A4250 has the potential to relieve cholestasis and improve liver function without surgical intervention in patients with PFIC. Since A4250 is minimally absorbed and achieves a substantial reduction of s-BA, it has the potential to be a safer and less invasive alternative to surgical biliary drainage. The rationale for using A4250 is to decrease s-BA levels, and to reduce the major morbidity of pruritus, improving the health and wellbeing of patients affected with PFIC. Ultimately, this noninvasive therapeutic approach has the potential to not only improve quality of life (QoL), but also reduce the need for biliary diversion and subsequent liver failure and liver transplantation.

The doses selected, 40 and 120 µg/kg/day, were based on the efficacy and safety data generated from patients with PFIC (n=10 + 3 re-entered) and supporting data from all patients (n=20 + 4 re-entered) in the five dose groups dosed in the A4250-003 study. Improvements in s-BA and pruritus were observed with doses from 30 µg/kg to 100 µg/kg, with a dose-related trend. The best dose response in all patients was at the 60 µg/kg/day for s-BA and 100 µg/kg/day for pruritus. The best dose response in the PFIC subgroup was at 30 µg/kg/day for both s-BA and pruritus. Patients with PFIC responded well to the doses given from 30 µg/kg to 200 µg/kg; however, a clear dose relationship could not be established in order to select a single dose for this Phase 3 study. As efficacy was observed at both low and high doses, 40 µg/kg and 120 µg/kg/day are considered to be the most optimal for this study.

This study will be conducted in compliance with the protocol and with the International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP).

Patients completing 24 weeks of double-blind treatment will be offered to enter Study A4250-008 and receive open-label treatment with A4250.

5.3.1 Risk/Benefit

A4250 has been evaluated in three Albireo-sponsored clinical studies: a double-blind placebo-controlled study in healthy volunteers, a single-dose ADME study, and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with PBC.

A total of 98 subjects/patients have been exposed to A4250. Healthy subjects have been exposed to up to 10 mg as a single dose and up to 3 mg daily as part of a multiple-dose evaluation. Children with cholestatic liver disease have been treated with up to 0.2 mg/kg/day (200 µg/kg/day) for 4 weeks. In total, 2 SAEs have been reported; both were judged by the physician to be not related to the study drug.

Patients with cholestatic liver diseases suffer from excess bile acids in the liver resulting in tissue damage. A commonly used treatment in these patients is bile diversion surgery, whereby approximately 50% to 100% of the enterohepatic circulation of bile acids is interrupted. Inhibition of IBAT with A4250, thereby interrupting the enterohepatic circulation of bile acids, is therefore a potential medical alternative to surgery which could be of benefit to these patients if shown to be effective and safe. Data from the Phase 2 study showed efficacy of A4250 in reducing s-BA concentrations and pruritus in such patients.

Based on the mode of action of an IBAT inhibitor, loose stools or diarrhea could be expected. However, in the pediatric Phase 2 study with 4 weeks daily treatment, only one patient had mild transient diarrhea after a single dose that did not recur on multiple dosing.

6 STUDY OBJECTIVES

6.1 Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with PFIC Types 1 and 2, as determined by the following:

- The proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- Change from baseline to Week 24 in pruritus compared to placebo

6.2 Secondary Objectives

- To evaluate the effect of A4250 on serum ALT concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

7 INVESTIGATIONAL PLAN

7.1 Overall Study Design and Plan

7.1.1 Description

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of FIC1 or BSEP).

Study data will be periodically reviewed by an unblinded Data Safety and Monitoring Board (DSMB).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-Up Period.

After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo.

Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008, EudraCT Number 017-002325-38, subject to a separate study approval) in which all patients will receive active treatment. A patient who prematurely withdraws due to no improvement/intolerable symptoms, and has completed at least 12 weeks of the Treatment Period and End of Treatment (EOT) Visit, will also be offered the opportunity to enter Study A4250-008.

Visit windows applicable to each visit are presented in the schedule of assessments (Table 1). There will be up to 10 clinic visits during the study (Figure 1) and one scheduled telephone contact, as follows:

- Visit 1: Screening Period start. Patient is considered enrolled after signing the informed consent form (ICF) (Days -56 to -35)
- Visit 2: second Screening Visit (Days -28 to -7)
- Visit 3: Randomization Visit (Day 0)
- Telephone Contact 1: (Day 14)
- Visit 4: Clinic Visit (Day 28)
- Visit 5: Clinic Visit (Day 56)
- Visit 6: Clinic Visit (Day 84)
- Visit 7: Clinic Visit (Day 126)
- Visit 8: Clinic Visit (Day 154)
- Visit 9: Clinic Visit, EOT (Day 168)

- Visit 10: Follow-Up Visit, 28 days after last dose of study drug (Day 196). All patients continuing in Study A4250-008 will not have this Follow-up Visit.

Additional clinic visits may be required for patients who need direct site assistance, e.g., due to AE monitoring, to fulfil screening requirements, and/or for safety maintenance.

Screening Period

Informed consent must be obtained at Visit 1 prior to performing any study procedures. After signing the ICF, patients will be considered enrolled and evaluated for study eligibility.

Daily recording of pruritus using an electronic diary (eDiary) will be started to determine confirmation of the magnitude and the baseline pruritus symptoms before the study. After consultation with the Medical Monitor, patients failing eligibility criteria may be re-screened after 2 months. Patients not fulfilling inclusion/exclusion criteria after 3 attempts are not allowed to re-screen.

Caregivers/patients will be instructed on the daily use of the eDiary. The eDiary will include patient-reported outcome (PRO) and observer-reported outcome (ObsRO) items for evaluation of itching (PRO), scratching (ObsRO), and sleep disturbance (PRO and ObsRO) during the Screening Period (study Days -56 to 0) and throughout the 24-week Treatment Period (study Days 0 to 168).

Only the 14 most recent days before randomization will be used for eligibility calculation of the diary entries. Observer-reported outcomes in patients of all ages will be recorded by a caregiver (or caregiver's designee). If possible, the same caregiver will complete the Albireo ObsRO items throughout the study.

At a second Screening Visit (Visit 2) additional laboratory samples will be taken for eligibility assessments.

Treatment Period

Eligibility for randomization will be determined at Visit 3 using the pruritus (scratching reports from the Albireo ObsRO) data in the 2 weeks before Visit 3, genetic confirmation of diagnosis, and the liver biochemistry evaluations from the previous screening visits. Two weeks after Visit 3, there will be a telephone contact with the caregiver/patient to evaluate any AEs.

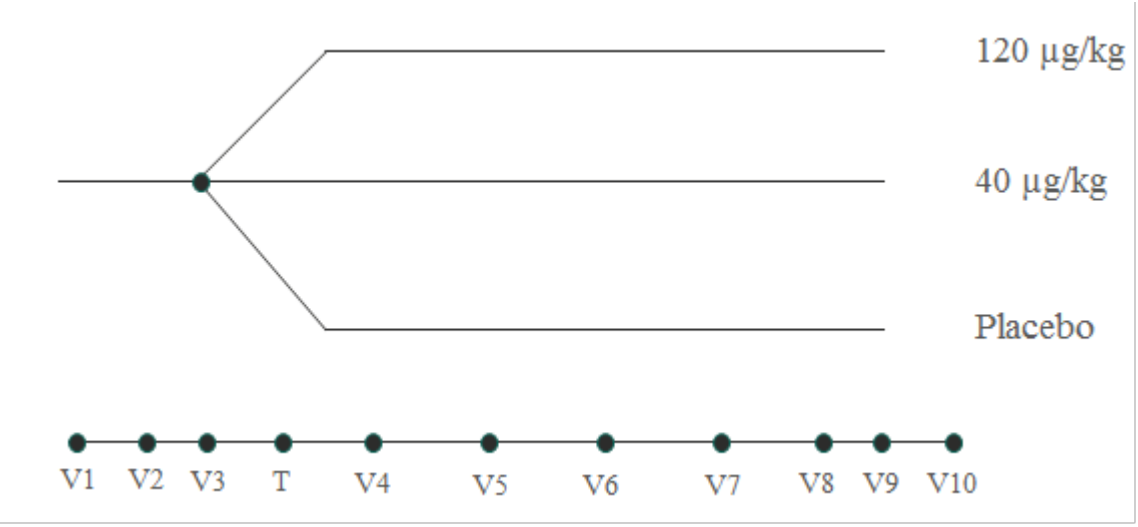
The patient will return to the clinic at Visit 4, Visit 5, Visit 6, Visit 7, Visit 8, and Visit 9 for assessments. If a new caregiver will begin to complete the Albireo ObsRO during the course of the trial, they will be provided with training during a clinic visit before they begin to use the eDiary. Additionally, caregivers/patients will be requested to use the diary to report the date and time each dose of study drug is administered during the Treatment Period.

The last dose of study drug will be administered the day before Visit 9. At study Visit 9, the patient has the option to enroll in an open-label extension study (A4250-008) should they meet the required entry criteria. If the patient is continuing into Study A4250-008 this will be

the last visit in this study and the first visit in Study A4250-008. Visit 9/EOT should also be performed at the time patients are prematurely withdrawn from the study.

Clinic Visit 10 will take place 28 days after Visit 9 for all patients not continuing into Study A4250-008.

Figure 1 Study Design



T: telephone contact; V: study visit

End of Study

The end of the study is defined as follows:

a) End of study in one country: last patient last visit (LPLV) and sites are closed.

b) End of study globally: LPLV globally and all sites closed.

7.1.2 Schedule of Assessments

The schedule of assessments is presented in Table 1.

Table 1 Schedule of Assessments

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Informed consent[c]	X										
Inclusion/exclusion criteria	X		X								
Demographics	X										
Concomitant medication[d]	X	X	X	X	X	X	X	X	X	X	X
Medical and surgical history	X		X								
Blood sampling for genetic testing[e]	X										
Physical examination including voluntary photography	X		X							X	
Skin examination	X		X		X		X			X	X
Vital signs[f]	X	X	X		X	X	X	X	X	X	X
eDiary: itching, scratching, and sleep scores[g]	Daily diary entry										
Clinical chemistry[h]	X		X		X	X	X	X		X	X
Hematology[h]			X		X	X	X	X		X	X
Urinalysis[h]		X					X			X	

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Serum bile acids[i]	X	X	X		X	X	X	X	X	X	X
INR		X			X		X		X		X
Autotaxin[j]			X		X					X	
p-C4[j]			X		X					X	
AFP			X							X	
Fat-soluble vitamins A & E		X								X	
Fat-soluble vitamin D			X							X	
Abdominal Ultrasound			X							X	
Fibroscan® (where available)			X							X	
QoL questionnaire (PedsQL)			X							X	
Patient/Caregiver/Clinician Global Impression of Change					X		X			X	
Patient/Caregiver/Clinician Global Impression of Symptoms			X		X		X			X	
Pregnancy test[k]	X[k]	X	X		X	X	X	X	X	X[k]	
Study drug dispensed[l]			X		X	X	X	X	X		

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Adverse events[m]	Continuous collection										
Study drug compliance				X	X	X	X	X	X	X	

AFP: alfa-fetoprotein; BMI: body mass index; eCRF: electronic case report form; eDiary: electronic diary; EOT: End of Treatment; ICF: informed consent form; INR: international normalized ratio; p-C4: plasma 7α Hydroxy 4-cholesten-3-one; PedsQL: Pediatric Quality of Life Inventory; QoL: quality of life; RR: respiratory rate; s-BA: serum bile acid.

a Must also be performed at the time a patient is prematurely withdrawn from the study. This visit is the same as Visit 1 in the extension protocol (Study A4250-008).
b Assessments must be performed 28 days following the final dose of study drug. This visit is omitted if patients are entering Study A4250-008.
c The ICF (and assent) must be signed before any study procedures are performed.
d Past (within 1 month prior to Visit 1) and current medication will be documented in the eCRF.
e Blood sample for genetic testing will be drawn for all patients. Genetic analysis will only be performed on a patient's extracted DNA if previous the clinical genetic result is equivocal, unavailable, or unobtainable.
f Includes blood pressure, pulse, RR, temperature, height (or length depending on age), and body weight (using a certified weight scale). BMI will be calculated.
g Includes training on use of the device at Screening (Visit 1) and review of patient daily entries during 14 days prior to Clinic Visit 3 to determine eligibility.
h See Table 3 for detailed parameters.
i Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA. Exceptions can be made for infants, less than 12 months of age, if unable to fast for the full 4 hours. All s-BA results during the treatment period and follow-up will be blinded.
j Samples will not be collected for patients ≤10 kg.
k For girls who have reached menarche. Serum test at Visit 1; urine test at other visits. A serum pregnancy test will also be conducted at Visit 9 for patients entering Study A4250-008. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy.
l Study drug to be taken once daily on Day 1 through Day 167 as described in Section 8.2.
m Adverse event information will be collected from the time of signing of the ICF to study discontinuation.

7.1.3 Study Procedures and Assessments

7.1.3.1 Screening Period (Days -56 to 0)

Day -56 to Day -35/Clinic Visit 1

Patients will undergo a Screening Visit up to 56 days prior to the planned first day of study treatment. Screening procedures and assessments are as follows:

- Obtain written informed consent
- Assess inclusion/exclusion criteria (Section 7.2)
- Record demographics (age, full date of birth, gender, race, and ethnicity)
- Document concomitant medications
- Medical and surgical history (date of diagnosis of PFIC Types 1 or 2, prior investigational medications for PFIC, historical liver function test [LFT] values, any surgery performed, any other diagnosis, and historical liver biopsy data)
- Blood sample for genetic testing
- Collect, if available, past confirmatory clinical genetic laboratory report for PFIC1/PFIC2 and send to central reader for review
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- eDiary training and compliance requirements; patients begin daily recording of pruritus using the eDiary (Appendix 2)
- Clinical chemistry (Section 10.2.1)
- s-BA (Section 9.2.1)
- Serum pregnancy test for girls of who have reached menarche
- AE monitoring

Day -28 to Day -7/Clinic Visit 2

A second Screening Visit will be performed. Screening procedures and assessments are as follows:

- Review concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for itching, scratching, and sleep scores and compliance
- Urinalysis (Section 10.2.1)

- s-BA (Section 9.2.1)
- International normalized ratio (INR)
- Fat-soluble vitamins A and E
- Urine pregnancy test for girls of who have reached menarche
- AE monitoring

7.1.3.2 Treatment Period (Day 0 to Day 168)

Study Day 0/Clinic Visit 3 (Randomization)

At the randomization visit, the following assessments will be conducted:

- Review inclusion/exclusion criteria (Section 7.2)
- Review concomitant medications, medical and surgical history
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance and entrance criteria requirement (see Inclusion Criteria 4, Section 7.2.1)
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin and p-C4 (samples will not be taken for patients ≤10 kg)
- Fat-soluble vitamin D
- Alfa-fetoprotein (AFP)
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)
- Administer QoL (Pediatric Quality of Life Inventory [PedsQL]) questionnaire
- Patient/caregiver/clinician complete the patient global impression of symptoms (PGIS)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Study drug is dispensed and patients are instructed to administer daily from Day 1

Study Day 14 (Telephone Contact)

A study nurse will contact the patient/caregiver by telephone for monitoring of the following:

- Review concomitant medications

- Albireo PRO/ObsRO eDiary review for compliance
- AEs
- Evaluation of study drug compliance

Study Day 28 and Day 84/Clinic Visits 4 and 6

The following procedures and assessments will be performed:

- Review of concomitant medications
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- Urinalysis (at Clinic Visit 6 only; Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- Autotaxin and p-C4 (at Clinic Visit 4 only; samples will not be taken for patients ≤10 kg)
- Patient/caregiver/clinician complete the patient global impression of change (PGIC) and PGIS
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 56 and Day 126/Clinic Visits 5 and 7

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance

- Study drug is dispensed

Study Day 154/Clinic Visit 8

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- s-BA (Section 9.2.1)
- INR
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 168/Clinic Visit 9/EOT

The last dose of study drug is taken in the morning the day before Clinic Visit 9, and the following procedures and assessments will be performed (also performed at the time a patient prematurely withdraws):

- Review of concomitant medications
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry, hematology, and urinalysis (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin and p-C4 (samples will not be taken for patients ≤10 kg)
- AFP
- Fat-soluble vitamins A, E, and D
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)
- Administer PedsQL questionnaire
- Patient/caregiver/clinician complete the PGIC and PGIS

- Urine pregnancy test for girls who have reached menarche, or serum pregnancy test for girls continuing to study A4250-008
- AE monitoring
- Evaluation of study drug compliance

7.1.3.3 Follow-Up Period

Study Day 196/Clinic Visit 10

All patients not continuing into Study A4250-008 will return to the study site 28 days after Clinic Visit 9 or the last dose of study drug for the following assessments:

- Review of concomitant medications
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- AE monitoring

7.2 Study Population

A total of 60 patients with clinical diagnosis of PFIC Type 1 or 2 that have been genetically confirmed will be randomized.

An attempt will be made to randomize a minimum of 10 patients from each age category (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years).

A maximum of 5 patients that were treated in Study A4250-003 will be allowed to participate in this study.

7.2.1 Inclusion Criteria

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples 7 to 28 days apart (Visits 1 and 2) prior to randomization

4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the eDiary average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients will be expected to have a consistent caregiver(s) for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

7.2.2 Exclusion Criteria

1. Patient with pathologic variations of the *ABCB11* gene that predict complete absence of the BSEP function
2. Patient with past medical history or ongoing presence of other types of liver disease including, but not limited to, the following:
   a) Biliary atresia of any kind
   b) Benign recurrent intrahepatic cholestasis, indicated by any history of normal s-BAs
   c) Suspected or proven liver cancer or metastasis to the liver on imaging studies
   d) Histopathology on liver biopsy is suggestive of alternate non-PFIC related etiology of cholestasis
3. Patient with a past medical history or ongoing presence of any other disease or condition known to interfere with the absorption, distribution, metabolism (specifically bile acid metabolism), or excretion of drugs in the intestine, including but not limited to, inflammatory bowel disease
4. Patient with past medical history or ongoing chronic (i.e., >3 months) diarrhea requiring intravenous fluid or nutritional intervention for treatment of the diarrhea and/or its sequelae
5. Patient has a confirmed past diagnosis of infection with human immunodeficiency virus or other present and active, clinically significant, acute, or chronic infection, or past medical history of any major episode of infection requiring hospitalization or treatment with parenteral anti-infective treatment within 4 weeks of treatment start (study Day 1) or completion of oral anti-infective treatment within 2 weeks prior to start of Screening Period
6. Any patient with suspected or confirmed cancers except for basal cell carcinoma, and non-liver cancers treated at least 5 years prior to Screening with no evidence of recurrence

7. Patient with a past medical history of chronic kidney disease with an impaired renal function and a glomerular filtration rate <70 mL/min/1.73 m^2
8. Patient with surgical history of disruption of the enterohepatic circulation excluding those who have undergone a successful reversal procedure that has permanently restored flow of bile acids from the liver to the duodenum
9. Patient has had a liver transplant or listed for liver transplant
10. Decompensated liver disease, coagulopathy, history or presence of clinically significant ascites, variceal hemorrhage, and/or encephalopathy
11. INR >1.4 (the patient may be treated with Vitamin K intravenously, and if INR is <1.4 at resampling the patient may be randomized)
12. Serum ALT >10 × upper limit of normal (ULN) at Screening
13. Serum ALT >15 × ULN at any time point during the last 6 months unless an alternate etiology was confirmed for the elevation
14. Total bilirubin >5 × ULN at Screening
15. Patient suffers from uncontrolled, recalcitrant pruritic condition other than PFIC. Examples include, but not limited to, refractory atopic dermatitis or other primary pruritic skin diseases
16. Any patient who is pregnant or lactating or who is planning to become pregnant within 24 weeks of randomization
17. Sexually active males and females who are not using a reliable contraceptive method with ≤1% failure rate (such as barrier protection, hormonal contraception, intrauterine device, or complete abstinence) throughout the duration of the study (from signed informed consent through Follow-up, Visit 10).
18. Patient with a past medical history of alcohol or substance abuse will be excluded. Patient must agree to refrain from illicit drug and alcohol use during the study
19. Administration of bile acid or lipid binding resins and medications that slow GI motility (Refer to Appendix 1 – Concomitant Medications Prohibited during the study)
20. Patient has had investigational exposure to a drug, biologic agent, or medical device within 30 days prior to Screening, or 5 half-lives of the study agent, whichever is longer
21. Patient who has been previously treated with an IBAT inhibitor whose pruritus has not responded to treatment
22. Any other conditions or abnormalities which, in the opinion of the investigator or Medical Monitor, may compromise the safety of the patient, or interfere with the patient participating in or completing the study

7.2.3 Withdrawal of Patients

Patients/caregivers will be informed that they have the right to withdraw from the study at any time, without prejudice to their medical care, and that they are not obliged to state their reasons.

Any withdrawal, and reasons for withdrawal, must be fully documented in the electronic case report form (eCRF) and source documents and the patient followed by the investigator/investigative staff. Even after the study is completed at the Follow-up Visit, the investigator/investigative staff will, regardless of reason for withdrawal, record any patient data they receive concerning SAEs, and all drug-related non-serious AEs, and subsequently report these to Albireo. Withdrawn patients will not be replaced.

Patients will be withdrawn in the following circumstances:

- A patient's/caregiver's desire for withdrawal for any reason
- Lost to follow-up (every effort must be made to contact the patient/caregiver; a certified letter must be sent)
- An AE that, in the opinion of the investigator, necessitates withdrawal
- A worsening of the symptoms related to diarrhea occurs
- Death
- Patient/caregiver estimation of lack of improvement/intolerable symptoms
- A patient's/caregiver's substantial noncompliance (eDiary and study drug compliance) or protocol violation
- An investigator's opinion that continuing the patient in the study is not appropriate. The investigator may withdraw a patient at any time, if it is considered to be in the patient's best interest

A patient who prematurely withdraws due to no improvement/intolerable symptoms, and has completed at least 12 weeks treatment in the double-blind study, will be offered the opportunity to enter open-label Study A4250-008. A patient who withdraws from treatment prematurely and will not enter Study A4250-008 will be requested to complete all visits and safety assessments per the current protocol (A4250-005). As a minimum, patients will have EOT (Visit 9) assessments at the time of withdrawal and a follow-up assessment (Visit 10) 28 days following the last dose of study drug, and the patient will be recorded as withdrawn.

7.2.4 Study Termination by Sponsor

This study may be terminated at any time by Albireo if serious side effects should occur, if the investigator does not adhere to the protocol; or if, in the sponsor's judgment, there are no further benefits to be achieved from the study. In this event, Albireo/designee will inform the study investigators, institutions, and all regulatory authorities.

Albireo may temporarily or permanently discontinue the study at an investigative site at any time for safety, ethical, compliance, or other reasons. If this is necessary, Albireo will endeavor to provide advance notification to the site. If a site or the study is suspended or discontinued, the investigator/investigative staff will be responsible for promptly informing the Independent Ethics Committee (IEC). If required by local regulations, Albireo/designee will be responsible for informing the IEC and the regulatory authority of study or site discontinuation. In such an event, all study data and unused study drug must be returned to Albireo.

8 TREATMENT OF PATIENTS

8.1 Identity of Study Drug

A4250 and placebo will be supplied as capsules for oral administration. White opaque capsules filled with pellets containing A4250 or placebo will be provided. Two different capsule sizes will be available, as follows:

- Capsule size 0 that can be opened
- Capsule size 3 that can be opened or swallowed intact

The content of the A4250 and placebo capsules will be identical in appearance. Capsule filling weight will also be identical for high dose, low dose, and placebo. Bottles with 34 capsules will be given to the patient at each visit. A patient who requires 2 or more capsules per day will be given multiple bottles. Refer to the investigational product manual.

8.2 Administration of Study Drug

Patients will be dosed with A4250 at a dose of 40 µg/kg/day, 120 µg/kg/day, or placebo for 24 weeks. Study drug will be dispensed to the patient at Visits 3, 4, 5, 6, 7, and 8, together with instructions on how to store and take the drug. Study drug administration data, including whether each patient took each dose or partial doses of study drug, whether there were any delayed or missed doses, doses, and whether the capsule was opened or swallowed whole will be documented through the eDiary and transferred to the study database.

A4250 should be taken in the morning, together with food. On clinic visits days when laboratory assessments are conducted, study drug should be taken after the visit and after laboratory samples are taken. Patients should not crush or chew the capsule(s). When swallowing the capsule intact, the patient should administer the dose with a glass of water.

For a patient unable to swallow capsule intact, the capsule can be opened and the contents sprinkled and mixed in a small amount of soft room-temperature yogurt, apple sauce, or fruit purée. The amount of food should be less than what the patient/infant is expected to eat and should be followed by water.

1. Hold the capsule over the prepared food, gently twist open and allow the content to fall into food
2. Mix the entire capsule content with 1 or 2 tablespoons (15 to 30 mL) of soft food (apple sauce, fruit purée, or yogurt)
3. Administer the mixture immediately after mixing.

The number of capsules provided to the patient will be based on body weight at Visit 3. At Visit 6, if a patient's weight has shifted to another weight category (identified in Table 2), the number of capsules per day may be adjusted.

Table 2 Dosing and Capsule Strength

Body Weight (kg)	Capsule Size	Number of Capsules per Day	Capsule Strength, Low Dose (µg)	Total Dose (µg)	Capsule Strength, High Dose (µg)	Total Dose (µg)	Capsule Size, Placebo (µg)
5.0 to <7.5	0	1	200	200	600	600	0
7.5 to <12.5	0	2	200	400	600	1200	0
12.5 to <17.5	0	3	200	600	600	1800	0
17.5 to <19.5	0	4	200	800	600	2400	0
19.5 to <25.5	3	2	400	800	1200	2400	3
25.5 to <35.5	3	3	400	1200	1200	3600	3
35.5 to <45.5	3	4	400	1600	1200	4800	3
45.5 to 55.5	3	5	400	2000	1200	6000	3
>55.5	3	6	400	2400	1200	7200	3

8.3 Study Treatment Packaging and Labeling

8.3.1 Packaging and Labeling

The capsules will be packed in high-density polyethylene containers, with child-proof polypropylene caps. Study drug capsules containing A4250 in the strength specified in Table 2 above will be manufactured.

Packaging and labeling will be prepared to comply with applicable regulatory requirements.

8.3.2 Storage

Treatment packs containing A4250 capsules should be stored and dispensed in accordance with regulations in their original containers. The storage facility at the investigative site should be locked and the storage temperature should be between 15°C and 25°C.

Patients/caregivers should be informed of appropriate storage conditions (i.e., room temperature, between 15°C and 25°C).

Any deviations from the recommended storage conditions should be immediately reported to Albireo and the study drug should not be used until authorization has been given by Albireo.

8.3.3 Blinding and Randomization of Study Drug

A double-blind design is employed so that both the investigators and the patients will be unaware of the treatment assignment during the study.

After written informed consent is obtained from an eligible patient, an 8-digit patient identification number will be assigned. The first 2 digits will denote the country, followed by a 3-digit site number and a 3-digit patient sequence number. This number will be created and allocated by the Interactive Web Response System (IWRS), when the patient first enters the system at Screening. The randomization codes will be computer-generated by [redacted]

[redacted], and kept by a statistician independent from the project team. The randomization will be done in blocks and stratified according to PFIC class (Type 1 and 2) and age class (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) to ensure approximate balance between dose schemes (1:1:1). A separate randomization list will be prepared for the maximum 5 patients from Study A4250-003 regardless of stratification. Randomization codes will be assigned sequentially as patients become eligible for randomization.

Patients who are eligible for randomization will be assigned a unique 4-digit randomization number by IWRS. This randomization number identifies which treatment will be allocated to the patient. Patients who withdraw from the study after randomization visit are not to be replaced and their randomization number will not be reused.

To ensure blinding, the study drug and the matching placebo have the same shape and size. Patients will receive capsule(s) of A4250 according to their dose group or capsule(s) of matching placebo once a day during the double-blind Treatment Period. Labels on the study drug containers will not identify the treatment that a patient is randomized to. Traceability of the treatment is ensured by the study drug number.

The 5-digit study drug number will identify study drug packs and will be detailed on the study drug label. Dispensing of study drug will be coordinated by IWRS.

The system will assign a study drug number corresponding to the randomization arm. The randomization number will be used in the background only to ensure no unblinding and will not be displayed to end-users in the IWRS.

8.4 Procedure for Breaking the Randomization Code

Should a situation arise where unblinding is urgently required, i.e., only where knowledge of the study drug is required to adequately manage a life-threatening situation, the investigator at that study site may perform immediate unblinding through IWRS. The investigator should make every effort to discuss the rationale for emergency unblinding with the sponsor Medical Monitor or the [redacted] Medical Monitor prior to unblinding the individual patient.

The study site investigator and appropriate [redacted] project team members will be authorized to access the emergency unblinding functionality within the IWRS. Emergency unblinding takes place in the IWRS via a dedicated emergency unblinding module. The system will require the user to enter their emergency unblinding, which is typically provided at the same time login credentials are distributed. The exact description of the treatment assigned to the individual patient then will be accessible to the user. Once the randomization code is broken for a patient, he/she must be withdrawn from the study and all assessments and procedures at EOT should be performed. As a follow-up, Visit 10 will be performed according to the time schedule. Once a randomization code has been broken, the investigator must inform the [redacted] and sponsor Medical Monitor in writing within 24 hours. In case of technical issues accessing the system online, please see the IWRS site user manual for country specific contact telephone numbers to the [redacted] HelpDesk 24/7 system support.

8.5 Patient Compliance

The study nurse will monitor eDiary compliance by routine review of the CRF health website. If both diary entries on a day are missing during, the study nurse will call the patient/caregiver to remind them to complete all scheduled entries. Any noncompliance will be documented and explained in the source documents and in the eCRF.

Study drug compliance will also be checked through diary responses. Additionally, patients will return all unused study drug at Visits 4, 5, 6, 7, 8, and 9. The study site staff will count all returned drug, assess compliance, and record details in the eCRF.

Treatment Compliance = 100 × ((Number of study drug dispensed – number of study drug returned)/number of study drug that should be taken).

Treatment compliance between 80% and 120% will be acceptable.

8.6 Study Drug Accountability

Records shall be maintained of the delivery of study treatment(s) to the study site(s), of the inventory at the study site(s), of each use of the study treatment(s) for each patient, and of the return of unused study treatment(s) to Albireo.

These records shall include dates, quantities, batch numbers, expiry dates, and the unique code numbers assigned to the study drug and to the study patients.

The investigator will be responsible for ensuring that the records adequately document that the patients were provided the quantities specified in the protocol and that all study drug received from Albireo is reconciled.

8.7 Concomitant Therapy and Prohibited Medications

The investigator will note all ongoing medication and any medication recently stopped (within 1 month prior to Visit 1) in the eCRF. At Visits 2 through 10, all changes in medication (stopping or starting new medication or changes in dose) will be recorded in the eCRF. All medications (prescribed or over the counter) for pruritus and sleep will be recorded.

All medications taken by a patient within 3 months prior to the first intake of study drug are regarded as prior medication.

All medications taken by a patient on or after the first intake of study drug, and which continue to be taken during the study, are regarded as concomitant medication.

Prohibited medications are listed in Appendix 1.

9 ASSESSMENT OF EFFICACY

9.1 Efficacy Endpoints

9.1.1 Primary Efficacy Endpoint

The primary efficacy endpoints are as follows:

- **European Union (EU) and rest of the world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  Fasting s-BA baseline will be calculated as the average of the value at randomization and the previous visit (Clinic Visit 2). The end value is the average of the values at Weeks 22 and 24 after the start of double-blind treatment.

- **United States (US)**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 24 as measured by the worst scratch of the Albireo ObsRO instrument (Appendix 2). Scores for the 14 days baseline and post-treatment intervals will be calculated by averaging the worst scratching scores from each day in the interval.

9.1.2 Secondary Efficacy Endpoint

Secondary efficacy endpoints include the following:

- **EU and RoW**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 24 as measured by the worst scratch of the Albireo ObsRO instrument (Appendix 2). Scores for the 14 days baseline and post-treatment intervals will be calculated by averaging the worst scratching scores from each day in the interval.
- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.
- **All Regions:**
  - All secondary endpoints are compared to placebo.
    - Change from baseline to Week 24 in fasting s-BA
    - Change from baseline to Week 24 in serum ALT concentration
    - Change in growth from baseline to Week 24, defined as the linear deficit (weight for age, and body mass index [BMI]) compared to the national growth curves (Z-score, standard deviation [SD] from P50)
    - Proportion of patients achieving meaningful reduction in caregiver-reported observed scratching at Week 24

- Change from baseline to Week 24 in sleep parameters measured with the Albireo PRO and ObsRO instruments
- Change from baseline to Week 24 in patient-reported itch severity as measured by the average daily score of the Albireo PRO instrument (Appendix 2); only patients ≥8 years of age will complete the Albireo PRO instrument
- Number of patients undergoing biliary diversion surgery or being listed for liver transplantation

9.1.3 Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Proportion of patients achieving meaningful reduction in patient-reported itch severity at Week 24
- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the PGIC items (Appendix 3)
- Change from baseline in fasting s-BA at Weeks 4 and 12
- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Weeks 4, 12, and 24 as measured by the worst scratch. Scores for the 7 worst scratching days of the last 14 days at baseline and post-treatment intervals will be selected
- Change from baseline in patient-reported itch severity and observer-reported scratching at Weeks 4 and 12
- Change from baseline to Week 24 in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline to Week 24 in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline to Week 24 in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in PedsQL (Appendix 4)
- Change from baseline in serum ALT concentration at Weeks 4 and 12
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, p-C4) at Week 24

- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events
- All-cause mortality

9.2 Efficacy Assessments

9.2.1 Serum Bile Acids

Blood samples for analysis of fasting total s-BA will be drawn at all visits. Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA. Exceptions can be made for infants, <12 months of age, if unable to fast for the full 4 hours. Any visit at which a bile acid sample result is unreportable, an additional unscheduled visit for a repeat sample collection may be scheduled. All s-BA results during the treatment period and follow-up will be blinded. Samples will be processed and transported to a central laboratory per instructions in the laboratory manual.

9.2.2 Itching, Scratching and Sleep Score

Itching, observed scratching, and sleep disturbance will be recorded twice each day via eDiary. Patients and/or caregivers will be instructed to complete the eDiary every day in the morning after the patient wakes and in the evening just before the patient goes to sleep throughout the study. Patients and caregivers will receive a formal training session during Visit 1.

The eDiary includes Albireo ObsRO and PRO items. Patients <8 years of age will not be asked to complete the Albireo PRO items; only the Albireo ObsRO will be completed by caregivers of patients in this age group. Older patients, 8 to 17 years of age, will complete the Albireo PRO items and the caregiver will complete the Albireo ObsRO items. The Albireo PRO items assess severity of itch, aspects of sleep disturbance (morning diary only), and tiredness. For patients 8 to 12 years of age, the caregiver will read the Albireo PRO items along with the child and record the child's response. A guide will be provided to the caregivers that provides standardized explanations of the Albireo PRO items, in case the patient is confused or requires clarification about the meaning of a question. The Albireo ObsRO items assess severity of observed scratching, aspects of observed sleep disturbance (morning diary only), and observed signs of tiredness (evening diary only). The Albireo ObsRO and PRO scratching and itch severity items use 0 to 4 response scales, where each response is distinguished by a unique facial expression, verbal anchor, number, and color code (Appendix 2).

A daily score for the Albireo ObsRO scratching item will be calculated by selecting the maximum scratching severity score from the 2 ratings for each day. A daily score will be considered missing if both of the daily assessments are missing. A 14-days score will be calculated by averaging the daily scratching scores. A 14-days score will be considered missing if >4 out of 7 days a week of data are missing. The same approach will be used to calculate a patient-reported itch severity score.

9.2.3 Growth

Growth will be measured as height (velocity) and weight (Z-score) using a certified weight scale. BMI will be calculated by weight (kg) / height $(m)^2$. Change will be defined as linear growth deficit (weight and BMI for age) compared to national growth curves (Z-score, SD from P50).

9.2.4 Quality of Life Questionnaire (PedsQL)

Patients and caregivers will be asked to complete a QoL questionnaire (PedsQL) at Visit 3 and Visit 9. Details of the questions included on the questionnaire are provided in Appendix 4.

9.2.5 Biomarkers and Other Laboratory Samples

Blood samples for autotaxin and p-C4 will be drawn at Visits 3, 4, and 9 only for children with body weight ≥10 kg. Samples will be processed and transported to a laboratory per instructions in the laboratory manual.

9.2.6 PELD/MELD Score

At randomization and end of treatment, PELD score will be calculated for children up to 12 years of age. For children 13 years or older, the MELD score will be calculated.

PELD Score = 4.80 * ln(total bilirubin in mg/dL) + 18.57 * ln(INR) - 6.87 * ln(albumin in g/dL) + 4.36 (if patient <1 year: scores for patients listed for liver transplantation before the patient's first birthday continue to include the value assigned for age (<1 year) until the patient reaches the age of 24 months) + 6.67 (if the patient has growth failure [≤2 SD])

Laboratory values less than 1.0 will be set to 1.0 for the calculation of the PELD score.

MELD Score = 9.57 * ln(creatinine in mg/dL) + 3.78 * ln(total bilirubin in mg/dL) + 11.2 * ln(INR) + 6.43

Laboratory values less than 1.0 will be set to 1.0 and serum creatinine values greater than 4.0 mg/dL will be set to 4.0 for calculation of the MELD score.

9.2.7 Fibroscan®

Where available, Fibroscan® will be performed as per institution standard practice at Visits 3 and 9.

9.2.8 Markers of Fibrosis

APRI score and Fib-4 score will be calculated at randomization and at end of treatment.

APRI = [(AST in U/L) / (AST ULN in U/L)] / (Platelets in 10^9/L)

Fibrosis 4 Score = (Age*AST in U/L) / (Platelets in 10^9/L * √(ALT in U/L)).

9.2.9 Global Impression of Change and Global Impression of Symptom Measures

Patients (≥8 years of age), caregivers, and clinicians will complete the PGIC and the PGIS measures (Appendix 3) at randomization (Visit 3; PGIS only), Visits 4 and 6, and at EOT (Visit 9).

The PGIC items assess change in itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) since starting the study drug. The PGIS items assess itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) in the past week. Caregivers and clinicians will complete the PGIC and PGIS for all patients; those patients ≥8 years of age will complete the patient version.

10 ASSESSMENT OF SAFETY

The timing and frequency of safety assessments are described in Section 7.1.3 and in Table 1 above.

The primary safety analysis for this study will include the incidence of total treatment-emergent adverse events (TEAE) and TEAEs categorized by causality, severity, and seriousness assessments made by the investigator by comparing study drug exposure to placebo.

Trends in safety will also be evaluated for the following assessments:

- Physical examinations
- Concomitant medications
- Vital signs
- Laboratory test results (including clinical chemistry, hematology, urinalysis, AFP, and fat-soluble vitamins A, D, and E)
- Abdominal ultrasound
- Discontinuations due to AEs

10.1 Adverse Events

10.1.1 Definitions and Investigator Assessments

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 unfavorable and unintended sign, symptom, or disease that occurs once a patient is enrolled (ICF is signed) in the study until the patient is discharged from the study, whether or not related to the study drug.

10.1.1.1 Clinical Significance

Clinical significance is defined as any abnormal finding that results in further clinical investigation(s), treatment(s), or the diagnosis of new condition. Patient-reported events and protocol-mandated laboratory values, vital signs, and physical examination findings can be considered clinically significant (i.e., an AE) if there is a deterioration as compared to baseline. Examples of clinically significant worsening from baseline could include, but is not limited to, events causing withdrawal from the study and events requiring medical intervention outside of the study causing apparent clinical manifestations, or judged relevant by the investigator.

10.1.1.2 Serious Adverse Events

Serious criteria are applied by the investigator to each AE as specifically defined below. These criteria are used to determine whether an AE is serious or non-serious. The assessment is made independently of severity assessment (Section 10.1.1.3). For example, the

development of a severe rash that occurs after signing of informed consent may not meet serious criteria as defined below and therefore would be considered a severe, non-serious AE.

Any AE that meets any 1 of the following 6 criteria is considered an SAE:

- The outcome of the AE is **death**
- The AE is immediately **life-threatening**. Life-threatening means that the patient is, in the opinion of the investigator, at immediate risk of death from the reaction as it occurred. This does not include an AE that, if more severe, might have caused death
- The AE results in persistent or significant **disability/incapacity**. Disability means a substantial disruption of a person's ability to conduct normal life functions
- The AE requires or prolongs **hospitalization**
- The AE results in a **congenital anomaly/birth defect**
- **The AE is an important medical event**. Important medical events may meet serious criteria should the investigator assess that they may significantly jeopardize the patient, represent a significant hazard, or requires medical/surgical intervention to prevent one of the outcomes listed above. Examples of potential SAEs based on this criteria include, but are not limited to, allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions even if they do not result in inpatient hospitalization, or the development of drug dependency and drug abuse.

10.1.1.3 Severity Assessment

Severity assessments are based on the intensity of the event in relation to expectation. The investigator will assess the intensity of AEs based on the following definitions:

- Mild (awareness of sign or symptom, but easily tolerated)
- Moderate (discomfort sufficient to cause interference with normal activities)
- Severe (incapacitating, with inability to perform normal activities)

Severity is a measure of intensity where seriousness is defined by the criteria outlined in Section 10.1.1.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not meet serious criteria, and therefore would be assessed as a severe AE but not an SAE.

10.1.1.4 Causality Assessment

The investigator determines the causality of all AEs to the study drug using medical judgment and considering all relevant factors such as (but not limited to) the underlying study indication, coexisting disease, concomitant medication, relevant history, pattern of the AE, temporal relationship to the study drug, and de-challenge or re-challenge. The causality assessment of the AE/SAE is to be made as follows.

Related to study drug (possibly, probably, or definitely related)

Based on medical judgment, there is at least a reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event follows a reasonable temporal sequence from administration of study drug
- The event could not be reasonably attributed to the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient
- The event follows a known pattern of response to study drug
- The event disappears or decreases on cessation or reduction in dose of the study drug. (It should be noted that in some situations an AE will not disappear or decrease in intensity upon discontinuation of study drug despite other clear indications of relatedness)
- The event reappears or worsens when the study drug is re-administered

Unrelated to study drug (unlikely or unrelated)

Based on medical judgment there is no reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event does not follow a reasonable temporal sequence from administration of study drug
- The event could be reasonably attributed to the known characteristics of the patient's clinical state, concurrent illness, environment or toxic factors, or other modes of therapy administered to the patient
- The event does not follow a known pattern of response to study drug
- The event does not disappear or decrease on cessation or reduction in dose of the study drug, and it does not reappear or worsen when the study drug is re-administered

10.1.2 Recording of Adverse Events

It is the investigator's responsibility to assess whether each untoward event is a clinically significant worsening from baseline, thereby considered an AE. For all AEs, the severity, seriousness, and causality to study drug for each AE as outlined in Section 10.1.1 will be assessed and recorded in the eCRF.

All serious and non-serious AEs are collected once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10) or 28 days after the last dose of study drug.

Any AEs or SAEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator until resolution or stabilization up to the database lock and recorded in the eCRF. Albireo retains the right to request additional information for any patient with ongoing AE(s) or SAE(s) at the end of the study.

TEAEs are defined as any AE that occurs after randomization (Day 0). All AEs that occur in the Screening Period, i.e., after enrollment and prior to randomization, will be collected on the eCRF as non-treatment-emergent AEs.

If there is a clinically significant deterioration of a laboratory value/vital sign or other routine study assessment that is associated with a diagnosis, the clinical diagnosis will be reported as an AE and the associated signs and symptoms will be considered additional information unless the sign or symptom is more severe than expected given the diagnosis. For example, if an investigator diagnoses a study patient with hepatitis during the study period, hepatitis would be considered the AE and the concomitant signs and symptoms of abdominal pain, vomiting, and elevated ALT and AST would not be reported separately unless, in the opinion of the investigator, one of these signs or symptoms is more severe than expected and therefore a separate AE assessment is indicated.

10.1.3 Recording and Reporting of Serious Adverse Events

Every SAE (regardless of severity and causality) that occurs once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10), and 28 days after the final dose of study drug, should immediately and not later than within 24 hours of knowledge of the event, be reported by the investigator or delegate in the SAE report form.

Report of a SAE must include at least the following information:

- Patient identification information (study number, site number, initials, and date of birth [as per local country requirements for data protection])
- The last study drug administration date
- The diagnosis of the event with the description (or a brief description of signs/symptoms/clinical course if the diagnosis is not available) and the date of onset
- The action used to treat the event (i.e., treatment medications, temporary discontinuation)
- The reason(s) for considering the event serious
- The relationship of the event to the study drug or to the study procedure (e.g., the investigator's assessment of causality)
- A brief narrative of the SAE

Follow-up reports including all new information obtained of the subsequent course of the SAE must be prepared and the information collected in the SAE submitted to [redacted] (Pharmacovigilance and Safety Services Department) by e-mail within 24 hours of knowledge of the event.

The pharmacovigilance manager (PVM) may contact the investigator to obtain further information on a reported SAE. The investigator/investigative staff must respond to any request for follow-up information or answers to questions regarding the SAE within the same timelines as for initial reports.

The PVM reports the occurrence of the SAE and follow-up to the Albireo medical monitor for medical assessment of the case.

Should an outcome of death occur within the study period or within 28 days after the last administration of study drug, an SAE form should be completed including the main and contributory causes of death.

All SAE reports must be e-mailed to the following e-mail address within **24 hours**:

[redacted]

If email is unavailable, SAEs may be transmitted via fax to the following number:

[redacted]

In a study-related medical emergency situation, when the assigned medical monitors for the study cannot be reached by a caller, an on call physician can be reached 24 hours per day, 7 days per week, via the [redacted] call center. The following number is a chargeable telephone number allowing a global reach from both landlines and mobile phones and the internet page indicated below contains a list of country-specific toll-free telephone numbers that is provided. It should be noted that not all countries globally have access to toll-free numbers as indicated on the "24/7 Medical Help desk" index. Countries without toll-free numbers need to dial the chargeable number as indicated below. Furthermore, toll-free numbers might not be available from mobile phones.

[redacted]

10.1.4 Reporting of Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reaction (SUSAR) is an SAE that occurs in a patient, the nature or severity of which is not expected per the applicable product information (e.g., the IB for an unauthorized investigational product or summary of product characteristics for an authorized product).

Reporting and tracking of SUSARs will be in accordance to all applicable competent authority regulations. The Ethics Committee and all investigators involved in this study will be informed according to national and international requirements.

10.2 Laboratory Values/Vital Signs/Physical Examinations and Other Safety Assessments

10.2.1 Laboratory Assessments

Samples will be collected for clinical chemistry, hematology, and urinalysis testing at the time points specified in Table 1. The parameters assessed are presented in Table 3.

Blood for AFP will be drawn at Visits 3 and 9 and for fat-soluble vitamins at Visits 2, 3, and 9.

A serum pregnancy test will be performed at Visit 1 and Visit 9 if the patient is entering the A4250-008 study. A urine pregnancy test will be collected at all other visits for girls who have reached menarche. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy. Study drug administration will be held pending the confirmatory results. If the serum pregnancy test is negative, the patient can resume dosing. If the serum pregnancy test is positive, the patient should be discontinued (see Section 10.2.9).

All samples will be processed and transported to a laboratory per instructions in the laboratory manual.

The observed values will be recorded and assessed as "normal" or "abnormal not clinically significant" or "abnormal clinically significant".

Additional safety blood samples may be needed due to follow-up of an abnormal value or analysis failure. The blood samples collected for safety laboratory analysis will be destroyed after the analyses have been completed.

Table 3 Routine Laboratory Parameters

Clinical Chemistry	Hematology	Urinalysis
• Albumin • ALT • Alkaline phosphatase • AST • Bilirubin – total and direct • Calcium • Chloride • Creatinine • Creatine kinase • Gamma-glutamyl transferase • Potassium • Sodium	• Hematocrit • Hemoglobin • Platelet count • Red blood cell count • White blood cell count	• Blood • Glucose • Ketones • Leukocytes • Nitrites • pH • Protein

10.2.2 Individual Patient Safety Monitoring

10.2.2.1 Liver Monitoring

Strategies to monitor markers of liver disease throughout the study are outlined below where the ULN will be based on central laboratory reference values for age and gender.

If isolated transaminase elevations are observed, defined as:

1. Normal bilirubin AND absence of clinical hepatitis symptoms AND

- ALT or AST ≥5 × ULN (if normal at baseline) or an absolute threshold of 800 U/L, whichever comes first
- OR ALT or AST ≥3 × baseline (if abnormal at baseline) or an absolute threshold of 800 U/L, whichever comes first

Then:

a) Repeat liver profile (AST, ALT, bilirubin, and prothrombin time [PT] or INR) within 2 to 3 days

b) Evaluate creatine phosphokinase (CPK) and lactate dehydrogenase (LDH)

c) As needed (for example, persistent ALT/total bilirubin elevation or worsening of liver function), consider evaluation for alternative etiologies

Monitor the patient using close observation found in Section 10.2.2.2

If any ONE of the following criteria are met:

1. Transaminases (ALT or AST ≥3 × baseline or 800 U/L, whichever comes first) AND bilirubin (total bilirubin > 2 × ULN) elevations
2. Transaminase elevations alone (ALT or AST >10 × ULN or 5 × baseline or absolute threshold of 800 U/L, whichever comes first) in presence of normal LDH and CPK
3. Cholestatic marker elevations (alkaline phosphatase [ALP] or gamma-glutamyl transferase >2 × baseline) without alternative explanation
4. Total bilirubin increased, unrelated to hemolysis (elevated reticulocyte count) or established genetic diseases, such as Gilbert's syndrome
   a. Doubling if total bilirubin was <3 mg/dL at baseline
   b. OR Increase by >3 mg/dL if total bilirubin was ≥3 mg/dL at baseline
5. INR increase refractory to vitamin K administration
   a. Increase by >1.5 if INR was normal at baseline
   b. OR Increase by >0.4 if INR was abnormal at baseline
6. Any increase in total bilirubin and transaminases if accompanied by EITHER a symptom of clinical hepatitis (e.g., vomiting, nausea, right upper quadrant pain) OR immunological reaction (rash or >5% eosinophilia)

Then:

a) Interrupt study medication

b) Initiate drug-induced liver injury work-up for alternative etiologies

c) Repeat liver profile (AST, ALT, total bilirubin, direct bilirubin, ALP) and PT or INR within 48 to 72 hours

d) Monitor the patient using close observation found in Section 10.2.2.2

e) If a patient lives in a remote area, they may be tested locally and the results communicated to the investigator site promptly

10.2.2.2 Close Observation

- Repeat liver enzyme and serum bilirubin tests two or three times weekly. Frequency of re-testing can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the subject is asymptomatic
- Obtain a more detailed history of symptoms and prior or concurrent diseases
- Obtain a history of concomitant drug use (including non-prescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets
- Rule out alternate etiology including acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; non-alcoholic steatohepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease
- Obtain a history of exposure to environmental chemical agents.
- Obtain additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin)
- Consider gastroenterology or hepatology consultations.

10.2.2.3 De-challenge/Re-challenge for Liver and Clinical Hepatitis Monitoring

1. Re-challenge is not recommended:
   a. If a patient has had possible/probable drug-induced liver injury
   b. If a decompensation event has occurred (i.e., variceal hemorrhage, ascites, hepatic encephalopathy, etc.).
2. If due to underlying cholestatic liver disease variability or another alternative etiology is identified AND liver tests returned to baseline, re-challenge may be considered after consultation with the sponsor Medical Monitor.
3. If the ALT/total bilirubin elevations are observed after re-challenge, then repeat re-challenge is discouraged.

If patient is permanently discontinued, monitoring should be continued as outlined in Section 10.2.2.5.

10.2.2.4 Diarrhea

Study drug should be discontinued if a patient develops diarrhea with at least 1 of the following concomitant signs or symptoms: grossly bloody stools, vomiting, dehydration

requiring treatment with oral or intravenous rehydration and/or electrolyte imbalances, fever (≥38 C) and/or the diarrhea persists for 7 or more days.

If there is gross blood in stool, INR and platelets should be measured.

Study drug will be reintroduced (re-challenge) when the symptoms have resolved. If the diarrhea re-occurs within 1 week after the re-challenge with no alternate etiology, the patient will be permanently discontinued and monitored as outlined in Section 10.2.2.5.

10.2.2.5 Monitoring after Permanent Discontinuation of Study Drug Due to Safety

Once study drug is permanently discontinued for liver, hepatitis, diarrhea, or other severe AE related to study drug, the patient will be monitored weekly until the laboratory and clinical parameters have normalized/stabilized.

10.2.3 Demographics/Medical and Surgical History

Demographic information per country regulations (age, full date of birth, gender, race, and ethnicity), along with medical and surgical history, will be obtained and recorded in the eCRF at Visit 1.

Medical and surgical history will be entered in the eCRF at Visit 1. This includes date of diagnosis of PFIC, prior investigational medications for PFIC, historical (3 months prior to Screening) LFT values (AST, ALT, and total bilirubin), ongoing medication, any surgery performed, any other diagnosis, and historical biopsy data.

If a liver biopsy has been performed within 1 year prior to Screening, or during the study, the results will be recorded in the eCRF.

10.2.4 Clinical Genetic Testing

If confirmatory clinical genetic testing for PFIC Type 1 or 2 has been performed prior to Screening Visit 1, the confirmatory clinical genetic testing laboratory report will be verified by a central reader to determine eligibility.

Regardless of previous genetic testing, a blood sample will be drawn at Visit 1 for all patients for DNA extraction. Genetic testing will only be performed on a patient's extracted DNA if the previous clinical genetic result is equivocal, unavailable, or unobtainable. In such cases, screening for pathologic biallelic variations in the *ATP8B1* or *ABCB11* genes will be performed if patient meets remaining inclusion and exclusion criteria and only after sponsor case review. No other diagnostic genetic testing will be offered.

10.2.5 Physical Examination

A physician or suitably trained qualified assistant will perform a complete physical examination, including voluntary photography, at Visits 1, 3, and 9. A skin examination will be conducted at Visits 1, 3, 4, 6, 9, and 10 (see Table 1).

A complete physical examination will include assessment of general appearance, eyes, ears, nose, throat, head/neck/thyroid, lymph nodes, cardiovascular, lungs/chest, abdomen, genitourinary, extremities, skin, musculoskeletal, neurologic, and other.

Skin will be examined thoroughly and excoriations/scratch marks recorded.

10.2.6 Vital Signs

Evaluation of vital signs will be performed at all visits. This includes blood pressure (systolic and diastolic), pulse, respiratory rate, temperature, height (or length depending on age), and body weight (using a certified weight scale) at clinic visits. BMI will be calculated.

10.2.7 Abdominal Ultrasound

Ultrasound of the liver and spleen will be performed at randomization (Visit 3) and at EOT (Visit 9). Liver size, echogenicity, and presence of masses/nodules as well as spleen size will be recorded.

10.2.8 Overdose

A4250 is minimally absorbed and has a very low systemic availability. Based on toxicology data, for study purposes any dose exceeding a total of >3 mg/kg body weight of A4250 taken as a single dose or as a cumulative dose within 24 hours is defined as an overdose.

The no-adverse-effect dose level in the most sensitive species (defined as 20 mg/kg/day) in the rat 1 month toxicity study gives a human equivalent dose of approximately 194 mg/day in a 60 kg person. This dose is approximately 10-fold higher than the maximum possible dose predicted in human studies (20 mg).

The investigator/investigative staff should immediately and not later than within 24 hours of knowledge of the overdose, be reported by the investigator or delegate on the SAE report form. In the event of an A4250 overdose, the patient should be monitored closely.

10.2.9 Pregnancy

If a pregnancy is discovered in a female patient enrolled in the study before the end of dosing, the patient will be immediately discontinued from the study and will attend the same visits as a prematurely withdrawn patient. If the pregnancy is discovered in a female patient enrolled in the study after the end of dosing, the patient will continue in the study per protocol. If a pregnancy occurs in a male patient's partner at any time during the study, the pregnancy should also be reported and followed.

Pregnancy is not considered to be an AE. However, if the patient has been dosed with the study drug, the pregnancy must be reported on the Paper Pregnancy Notification Form immediately within 24 hours after recognition to Albireo/Safety Manager (please refer to Section 10.1.3). Date of exposure and as far as possible, details of the period of gestation at the time of exposure must be given.

The pregnancy should be followed to determine outcome, including spontaneous termination, details of birth, and presence of any birth defects, congenital anomalies or newborn or maternal complication. The infant who was exposed in-utero will be followed for up to 2 years after delivery. Individual cases with an abnormal outcome in association with the study drug should be reported on an expedited basis, i.e., reported rapidly to a competent authority.

11 STATISTICAL EVALUATION

All personnel involved with the analysis of the study will remain blinded until database lock for the double-blind Treatment Period and until major protocol violations have been identified.

All statistical analyses will be performed using SAS version 9.3 or higher.

11.1 Sample Size and Power

For the EU and RoW, the primary analysis will relate to the bile acid responder endpoint (at the 1-sided 1.25% level). For the US, the primary analysis will relate to the pruritus endpoint (at the 1-sided 1.25% level). The aim is to enroll 60 to 70 patients in order to get at least 60 evaluable patients.

Assuming 60% responders (patients experiencing at least 70% reduction in fasting s-BA concentration or reaching ≤70 µmol/L) in the A4250 dose group and 10% responders in the placebo group, approximately 20 patients per group are required to achieve a power of 84% with a 1.25% 1-sided Type-1 error.

For pruritus, with approximately 20 patients per treatment group, the power is 83% given an assumed treatment effect difference of -1.0 in change from baseline (-1.6 for A4250 and -0.6 for placebo) on the Albireo ObsRO scratching score with a SD of 0.95 and a Type I error of 1.25% (1-sided).

11.2 Statistical Methods

11.2.1 Statistical Analysis Sets

Safety Analysis Set

The safety analysis set will consist of all patients who received at least 1 dose of study drug. Patients will be analyzed according to the treatment they actually received. The safety analysis set will be the primary analysis set for safety analyses.

Full Analysis Set

The full analysis set (FAS) will consist of all randomized patients who received at least 1 dose of assigned treatment. Patients will be analyzed in the treatment group they were randomized to even if they received incorrect study drug. The FAS will be the primary analysis set for efficacy analyses.

Per Protocol Analysis Set

The per protocol (PP) analysis set is a subset of the FAS, and will consist of all randomized patients for whom no major protocol violation (no violation which may affect the study efficacy outcome) is documented. The PP analysis set will provide supportive data for the efficacy analyses.

Allocation of patients to the PP analysis set will be performed before unblinding of the study.

11.2.2 Methods of Statistical Analyses

11.2.2.1 General Principles

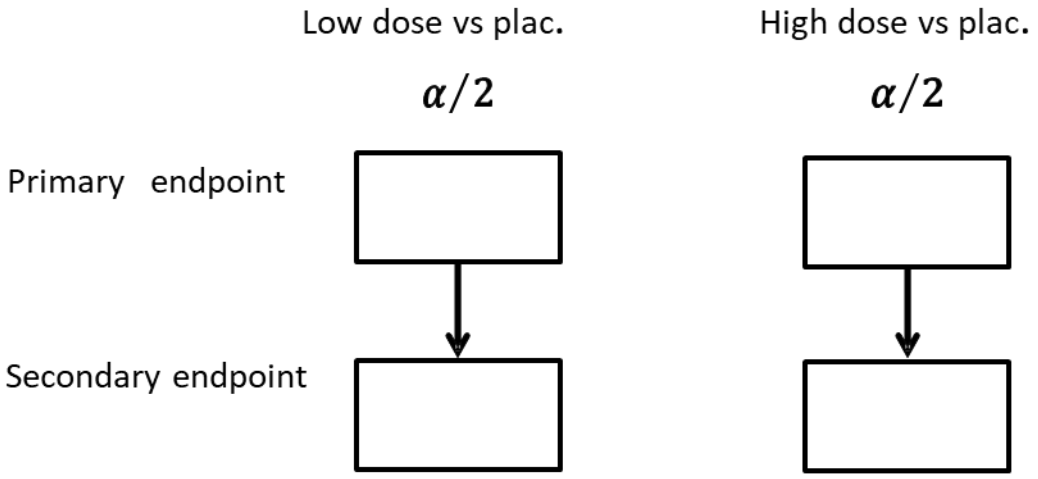
The primary objective of this study is to show superiority in efficacy of A4250 (120 µg/kg/day and 40 µg/kg/day) compared to placebo in patients with PFIC Types 1 and 2.

In the EU and RoW, for the primary efficacy variable fasting bile acid responder endpoint (at least a 70% reduction in fasting s-BA concentration or reaching a level ≤70 µmol/L at the end of treatment), the null hypothesis will be tested against the alternative hypothesis ($\alpha = 0.0125$, 1-sided).

The test will be performed at a Type I error rate of ≤0.0125 1-sided, as described above, based on the least squares means of the treatment difference in mean probability scale.

To strictly control overall Type I error rate across the dose groups and also across the primary and the main secondary endpoint to a 1-sided 2.5% level, a Bonferroni-based parallel gate-keeping procedure will be used. In this procedure the total significance level of 2.5% (1-sided) will be split equally between the dose groups. Only a dose group that is significant for the primary endpoint will be tested, at the nominal significance level of 1.25% (1-sided), for the main secondary endpoint (here the pruritus endpoint). If the previous step was not successful, the analysis of the following step will be considered descriptive. If the procedure reaches the secondary endpoint for both dose groups, these will be evaluated using the Bonferroni Holm procedure. The multiple comparison procedure is presented in Figure 2.

Figure 2 Bonferroni Holm Multiple Comparison Procedure



In the US, for the primary efficacy variable pruritus score endpoint (change from baseline to Week 24 in observer-reported scratching), the null hypothesis will be tested against the alternative hypothesis ($\alpha=0.0125$, 1-sided).

The test will be performed at a Type I error rate of ≤0.0125 1-sided, as described above, based on the least squares means of the treatment difference in mean change.

To strictly control overall Type I error rate across the dose groups and also across the primary and the main secondary endpoint to a one-sided 2.5% level, a Bonferroni-based parallel gate-keeping procedure will be used. In this procedure the total significance level of 2.5% (1-sided) will be split equally between the dose groups. Only a dose group that is significant for the primary endpoint will be tested, at the nominal significance level of 1.25% (1-sided), for the main secondary endpoint (here the bile acid responder endpoint). If the previous step was not successful, the analysis of the following step will be considered descriptive. If the procedure reaches the secondary endpoint for both dose groups, these will be evaluated using the Bonferroni Holm procedure.

Other secondary and exploratory outcome variables shall provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple dose comparisons when testing the other secondary and exploratory efficacy variables.

A more detailed statistical analysis plan (SAP) will be prepared and finalized before study data are locked and unblinded.

If not otherwise specified, the baseline value of a parameter is defined as the last assessment of that parameter on or prior to the first intake of study treatment date.

11.2.2.2 Missing Data

For efficacy variables, with repeated measures, a mixed model will be employed with no additional imputation for missing values, i.e., based on "observed cases", except where otherwise specified.

Follow-up and continued collection of efficacy data for patients who discontinue treatment will be made. These data will be included in the sensitivity analysis for primary endpoints estimating a treatment effect taking loss of effect after discontinuation into account. Because complete follow-up of all patients discontinuing treatment may not be possible, a method to replace missing data for patients who discontinue the study will be prespecified (in the SAP) taking into account loss of effect after discontinuation (for example multiple imputation).

No other replacement of missing values will be planned.

11.2.2.3 Demographic and Baseline Characteristics

Descriptive summaries of demographics and other baseline characteristics (including medical and surgical history) will be presented by treatment group and overall using the FAS.

Prior medication will be summarized by treatment group and overall using the safety analysis set.

11.2.2.4 Subject Disposition

The following will be summarized (by treatment group and overall where applicable):

- Patients enrolled (who signed the informed consent)
- Patients randomized
- Patients treated
- Patients completing the study/withdrawing early (including withdrawal reason)

Additionally, patients enrolled, included in the safety, in the FAS, and in the PP analysis set will be summarized by region.

11.2.2.5 Evaluation of Primary Efficacy Variables

The responder endpoint based on change in fasting bile acids will be analyzed in terms of the proportion of patients achieving the event of interest. Patients in need of rescue therapy, i.e., biliary diversion will be classified as non-responders after the biliary diversion.

Generalized linear mixed modelling (GLMM) will be used with a logistic link, to allow for repeated assessments over time. The model will include baseline value, treatment group, PFIC class, age class, region, visit, and visit*treatment group interaction. The treatment by visit interaction will remain in the model regardless of significance. Region will be included in the model as a random effect. The contrasts of primary interest will be the treatment difference at Week 24; also, the difference at Week 4, Week 8, Week 12, and Week 18 will be estimated and analyzed. An unstructured covariance matrix will be used to model the within-subject error. Another covariance structure may be chosen if the fit of this structure fails to converge. Model diagnostics checks will be made to ensure appropriate fit.

The primary analysis of the change from baseline in pruritus score (indexed by observer-reported scratching) will be analyzed using a mixed model repeated measures (MMRM) analysis. The contrast of primary interest will be treatment difference at Week 24 but also the differences at Week 4, Week 8, Week 12, and Week 18 will be estimated and analyzed. Baseline pruritus score will be included as covariate. The model will include baseline value, treatment group, PFIC class, age class, region, visit, and visit*treatment group interaction. The treatment by visit interaction will remain in the model regardless of significance. Region will be included in the model as a random effect and will be defined on country level. An unstructured covariance matrix will be used to model the within-subject error. Another covariance structure may be chosen if the fit of this structure fails to converge.

Non-parametric approach may be considered if more appropriate.

The primary analyses will be based on the FAS.

To assess the robustness of the primary efficacy analysis, the following analyses will be performed:

- Primary efficacy analysis using the PP set
- Analyses to investigate the influence of missing values, for example, by using pattern mixture models and tipping point analysis

Subgroup analyses will also be performed for each of the 3 age groups (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) and for patients who were not included in the previous Study A4250-003.

Several sensitivity analyses will be performed, such as the following:

- Cochran Mantel Haenzsel test stratified on PFIC class to compare the proportion in fasting bile acid responder endpoint at the end of treatment in the two A4250 dose groups to placebo group. Dropouts will be treated as non-responders
- Based on the primary analysis including the interaction age class*treatment
- Same as primary analysis in which the random factor region is considered as fixed factor. The region*treatment interaction will also be explored
- Same as primary analysis including the interaction PFIC*treatment

Other sensitivity analyses may be performed and will be detailed in the SAP.

11.2.2.6 Evaluation of Secondary and Exploratory Efficacy Variables

The change in the secondary and explorative endpoints such as s-BA, total bilirubin, ALT, growth and patient-reported itch severity will also be analyzed using the MMRM model as specified for the primary analysis. Nonparametric methods may be applied as an alternative approach for some endpoints, if appropriate. Change in growth (weight for age and BMI for age) will also be displayed using graphical presentations.

Proportions of responders (e.g., meaningful reduction in itch severity and scratching at Week 24) will be analyzed using the GLMM model as specified for the primary analysis.

Comparisons of the change from baseline at Week 24 in PedsQL between the treatment groups will be conducted using analysis of covariance. The model will include terms for baseline, PFIC class, age class, region, and treatment.

Undergoing biliary diversion surgery and/or listed for liver transplant, all-cause mortality, and liver decompensation events will mainly be analyzed by using descriptive statistics. Kaplan-Meier curves will be used when appropriate for time to event data.

Other exploratory variables (Global Symptom Relief, biochemical markers and measures of bile acid synthesis, Albireo PRO and ObsRO sleep parameters, PELD/MELD score, APRI score, and Fib-4 score) will be analyzed descriptively for categorical and continuous data, as applicable. For continuous data, the change from baseline will be analyzed in addition to the actual visit values. For categorical data, shift tables or frequency and percentages of patients will be presented as appropriate.

In addition, subgroup analyses will be performed for each of the 3 age classes (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years).

11.2.2.7 Evaluation of Safety Variables

Safety data will be analyzed using descriptive statistics and summaries by treatment group of SAEs, AEs, vital signs, clinical laboratory tests (hematology, clinical chemistry and urinalysis), and concomitant medication. Analyses will be performed using the safety analysis set.

Summaries of AEs (coded according to the Medical Dictionary for Regulatory Activities [MedDRA] system organ class [SOC] and MedDRA preferred term) will include the following:

- Overview of the incidence of TEAEs (TEAEs, drug-related TEAEs, TEAEs leading to study discontinuation, and treatment-emergent SAEs)
- TEAEs by SOC and preferred term
- Intensity of TEAEs by SOC and preferred term
- Drug-related TEAEs by SOC and preferred term
- TEAEs leading to study discontinuation by SOC and preferred term
- Treatment-emergent SAEs by SOC and preferred term

Concomitant medication use during the Treatment Period will be summarized by Anatomical Therapeutic Chemical (ATC) class and World Health Organization (WHO) preferred name.

Summaries of vital signs will be presented. For each visit, the actual results and the change from baseline, and the number and percentage of patients with potentially clinically significant (PCS) values observed post-baseline will be presented.

Summaries of clinical safety laboratory data will be presented. For each visit, the actual result and the change from baseline, and the number and percentage of patients with PCS values observed post-baseline will be presented.

Data listings will be provided for each patient for all safety parameters.

11.2.2.8 Compliance and Exposure

Exposure will be analyzed by calculating the number of days with exposure to study drug. Results will be presented by treatment group using the safety analysis set.

The percentage compliance will be described by treatment group and the number of patients with a compliance <80%, between 80% and 120%, and >120% will be presented based on the safety analysis set.

11.2.3 Blinded Analyses to Confirm Responder Definitions

A blinded analysis of Albireo ObsRO and PRO eDiary data will be performed after 50% or more of the planned sample has completed the Week 24 Follow-Up Period. The blinded analysis will be used to estimate a threshold of clinically meaningful change (i.e., responder definition) in Albireo ObsRO and PRO pruritus scores. The analysis will be performed by a group independent from both the study team and Albireo. Blinded data will be used for this analysis; data will be collapsed across treatment groups.

The analyses will include distribution- and anchor-based analyses to examine a responder definition for the Albireo PRO/ObsRO pruritus scores. Distribution-based analyses will include calculation of the SD and standard error of measurement for the baseline Albireo PRO and ObsRO scores. Anchor-based analyses will involve examining the degree of change on the Albireo PRO and ObsRO scores from baseline to Week 24 for patients who experience change in pruritus according to PGIC and symptom items. Details of this analysis will be included in a stand-alone SAP for assessing the measurement characteristics (reliability, validity, sensitivity to change, responder definition) of the Albireo PRO and ObsRO items.

11.2.4 Data Safety Monitoring Board

A DSMB consisting of sponsor-independent clinical and statistical experts will be established. The DSMB will periodically meet for the review of accumulating study data, including safety (AE and laboratory data) and liver values.

The DSMB will have access to unblinded data. The DSMB will make recommendations for the remaining part of the study (further details will be provided in the DSMB charter). The DSMB may recommend continuing with the study as planned or stopping the study early for safety reasons. The DSMB will submit its recommendations in writing to Albireo Medical/[redacted] who are responsible for responding to the recommendations of the DSMB and taking appropriate action. The investigators will only be informed by Albireo Medical/[redacted] if the study requires a protocol amendment or is stopped. The DSMB may choose to make additional evaluations at any time if they feel this is warranted from a safety point of view.

The DSMB will act according to its own written standard operating procedure described in a charter and will prepare written minutes of its meetings. The charter of the DSMB will be stored in the trial master file. The DSMB will maintain records of its meetings and these will become part of the study file when the study is complete. In order not to disseminate unblinded data and to ensure that all staff involved in the conduct and final analysis of the study remain blind to the data, only the members of the DSMB and the unblinded DSMB statistician will have access to these data.

12 DIRECT ACCESS TO SOURCE DATA/NOTES

The investigator/institution shall provide direct access to source data/documents for study-related monitoring, audits, IEC/Institutional Review Board (IRB) review and regulatory inspection.

13 QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Conduct of the Study

Albireo/[redacted] shall implement and maintain quality control and quality assurance procedures with written standard operating procedures to ensure that the study is conducted and data are generated, documented, and reported in compliance with the protocol, ICH GCP and applicable regulatory requirements.

This study shall be conducted in accordance with the provisions of the Declaration of Helsinki (October 1996) and all revisions thereof, and in accordance with United States Food and Drug Administration (FDA) regulations (Code of Federal Regulations, Sections 312.50 and 312.56) and with ICH GCP (CPMP 135/95).

The investigator may not deviate from the protocol without a formal protocol amendment having been established and approved by an appropriate competent authority and IEC/IRB, except when necessary to eliminate immediate hazards to the patient or when the change(s) involve(s) only logistical or administrative aspects of the study. Any deviations may result in the patient having to be withdrawn from the study and render that patient non-evaluable.

13.2 Study Monitoring

Before study initiation, at a site initiation visit or at an investigator's meeting, an Albireo representative or designee will review the protocol and eCRF with the investigators and the investigative staff. During the study, the clinical monitor (clinical research associate [CRA]) will visit the site regularly to check the completeness of patient records, the accuracy of entries on the eCRFs, the adherence to the protocol and to GCP, the progress of enrollment, and to ensure that study drug is being stored, dispensed, and accounted for according to specifications.

The investigator must ensure that eCRFs are completed within a timely period of patient visits, as per individual site agreements, and must allow the CRA and Albireo representative or designee periodic access to patient records and all study-related materials, including relevant hospital or clinical records, to confirm their consistency with the eCRF entries. No information in these records about the identity of the patients will leave the study center. Albireo monitoring standards require full verification for the presence of the signed ICF, adherence to the inclusion/exclusion criteria, documentation of SAEs, and recording of primary efficacy and safety variables. The CRA will review source data compared with the eCRFs and will verify source data according to the study-specific monitoring plan. The design of the study, the frequency of patient visits, and the site enrollment rate will determine the frequency of monitoring visits. Upon study completion, the CRA will visit the site to conduct a study termination visit, which will include collection of any outstanding documentation.

It is recommended that the investigator/investigative staff log into the eCRF system every working day or at minimum twice weekly in order to provide a prompt response to queries.

The investigator/investigative staff should respond to queries and make any relevant changes to the study data within 3 working days.

14 ETHICS

14.1 Independent Ethics Committee/Institutional Review Board

Prior to the start of the study, the investigator is responsible for ensuring that the protocol and consent form have been reviewed and approved by a relevant IEC/IRB. The IEC/IRB shall be appropriately constituted and perform its functions in accordance with FDA ICH GCP and local requirements as applicable.

The IEC/IRB shall approve all protocol amendments (except for logistical or administrative changes), written informed consent documents and document updates, patient recruitment procedures (e.g., advertisements), written information to be provided to the patients and caregivers, IB, available safety information, information about payment and compensation available to patients, the investigator's curriculum vitae and/or other evidence of qualifications and any other documents requested by the IEC/IRB and regulatory authority (competent authority) as applicable.

14.2 Written Informed Consents and Assents

The investigator (physician) or investigative staff, in accordance with local regulation, will explain to each patient (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, alternative treatment, the potential risks and benefits involved, and any discomfort that may occur. Each caregiver/patient will be informed that participation in the study is voluntary and that he/she or their child may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

Caregivers/patients will be informed that they/their children may experience side-effects or be at risk for symptoms, illnesses, or complications that cannot be foreseen by Albireo. As with other medications, people treated with A4250 may be at risk of developing allergic reactions or anaphylaxis. Caregivers/patients will be advised that study procedures include regular blood sampling for measurement of safety parameters and biological markers and that some minor risks are associated with these procedures.

This informed consent should be given by means of a signed ICF, written in non-technical language in accordance with ICH GCP, the Declaration of Helsinki, and regulatory authorities. The caregiver(s)/patient should read and consider the statements before signing and dating them and should be given a copy of each signed document. If written consent is not possible, oral consent can be obtained if witnessed and followed by a signed statement from one or more persons not involved in the study, indicating why the patient was unable to sign the form. No patient can enter the study before his/her or caregiver(s), as required by country regulation, informed consent has been obtained.

The ICF is part of the protocol and must be submitted by the investigator/investigative staff with the protocol for IEC approval. Albireo will supply an ICF which complies with regulatory requirements and country laws and is considered appropriate for the study. Any

changes to the ICF suggested by the investigator must be agreed to by Albireo before submission to the IEC and a copy of the approved version must be provided to the clinical monitor after IEC approval.

15 DATA HANDLING AND RECORD KEEPING

15.1 Case Report Forms/Source Data Handling

The investigator shall be provided with standardized eCRFs and shall ensure that all data from patient visits are promptly entered into the eCRFs in accordance with the specific instructions given. The investigator must sign each eCRF to verify the integrity of the data recorded.

A list of the normal ranges for all laboratory tests to be undertaken forms part of the documentation to be collated prior to study start. As a central laboratory has been selected to conduct any or all tests, it is essential that all samples be analyzed at that laboratory.

The investigator must maintain source documents such as laboratory reports, consultation reports, and complete medical history and physical examination reports.

As no ethnicity data can be collected in the source document for study sites in France, the eCRF will be the Source Document for “origines ethniques”,

15.2 Retention of Essential Documents

Essential documents, as defined by ICH GCP, include: the signed protocol and any amendment(s); copies of the completed eCRFs (for site archiving, compact discs of eCRF data for specific patients will be provided); signed ICFs; hospital records and other source documents; IEC approvals and all related correspondence including approved documents; drug accountability records; study correspondence; and a list of patients’ names and addresses.

The investigator/investigative staff must retain copies of these essential documents for the period specified by ICH GCP and by applicable regulatory requirements. The investigator/investigative staff will inform Albireo of the location where the essential documents are stored and must contact Albireo for approval before disposing of any essential documents. The investigator/investigative staff should take measures to prevent accidental or premature destruction of these documents.

16 FINANCING AND INSURANCE

Financing and insurance are addressed in a separate agreement.

17 PUBLICATION POLICY

Albireo will retain the ownership of all data. When the study is complete, Albireo shall arrange the analysis, tabulation of data and preparation of a clinical study report. Albireo may also use the data for publication, presentation at scientific meetings and submission to regulatory authorities. All proposed publications based on this study must be subject to the sponsor's approval requirements.

18 REFERENCE LIST

1. A4250 Investigator's Brochure.
2. Alissa FT, Jaffe R, Shneider BL. Update on progressive familial intrahepatic cholestasis. J Pediatr Gastroenterol Nutr. 2008;46(3):241-52.
3. Davit-Spraul A, Gonzales E, Baussan C, et al. Progressive familial intrahepatic cholestasis. Orphanet J Rare Disease 2009;4:1-12.
4. Hofmann AF. The enterohepatic circulation of bile acids in mammals: form and functions. Front Biosci (Landmark Ed). 2009 Jan 1;14:2584-98.
5. Hori T, Justin H, Nguyen JH and Uemoto S. Progressive familial intrahepatic cholestasis. Hepatobiliary pancreat Dis Int. 2010; 9(6):570-8.
6. Jacquemin E. Progressive familial intrahepatic cholestasis. Genetic basis and treatment. Clin Liver Dis. 2000;4(4):753-63.
7. Miethke AG, Zhang W, Simmons J, et al. Pharmacological inhibition of ASBT changes bile composition and blocks progression of sclerosing cholangitis in mdr2 knockout mice. Hepatology. 2016; 63(2):512–23.
8. Murray CS, Rees JL. Are subjective accounts of itch to be relied on? The lack of relation between visual analogue itch scores and actigraphic measures of scratch. Acta Derm Venereol. 2011;91(1):18-23.
9. Pawlikowska L, Strautnieks S, Jankowska I, et al. Differences in presentation and progression between severe FIC1 and BSEP deficiencies. J Hepatol. 2010;53(1):170-8.
10. Suchy FJ, Sokol RJ, Balistreri WF, eds. Liver Disease in Children. 3rd Edition. New York, NY: Cambridge University Press; 2007:310–4.
11. Whitington PF, Whitington GL. Partial external diversion of bile for the treatment of intractable pruritus associated with intrahepatic cholestasis. Gastroenterology. 1988;95(1):130-6.

19 APPENDICES

Appendix 1 Concomitant Medications Prohibited During the Study

Appendix 2 Diary Questions including Albireo Patient-Reported Outcomes/Observer-Reported Outcome Instrument

Appendix 3 Patient Global Impression of Change (PGIC) and Patient Global Impression of Symptoms (PGIS)

Appendix 4 Pediatric Quality of Life Inventory (PedQL) Questionnaire

CLINICAL STUDY PROTOCOL A4250-005

A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

IND Number:	130591
EudraCT Number	2017-002338-21
Test Product:	A4250
Indication:	Progressive Familial Intrahepatic Cholestasis (PFIC)
Sponsor:	Albireo AB
Development Phase:	Phase 3
Sponsor Signatory:	[redacted]
Sponsor Medical Monitor:	[redacted]
Principal Investigator:	[redacted]
Date of the Protocol:	06 December 2017
Version of the Protocol:	Final Amendment 01

Confidential

The information in this document contains trade secrets and commercial information that are privileged or confidential and may not be used, divulged, published, or otherwise disclosed without the written consent of Albireo AB. These restrictions on disclosure apply equally to all future information supplied to you or generated by you in connection with the study.

SPONSOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

Albireo AB

[redacted]

06 Dec. 2017

Date (day/month/year)

CLINICAL RESEARCH ORGANIZATION SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

[redacted]

[redacted]

7/12/2017
Date (day/month/year)

[redacted]

07-Dec-2017
Date (day/month/year)

INVESTIGATOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

I have read this protocol and agree that it contains all necessary details for performing this study. I will conduct the study as outlined herein and will complete the study within the time designated, in accordance with all stipulations of the protocol and in accordance with Good Clinical Practice (GCP), local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study drug and the conduct of the study.

I will use only the informed consent approved by the Independent Ethics Committee (IEC) and will fulfil all responsibilities for submitting pertinent information to the IEC responsible for this study.

I agree that the sponsor (Albireo AB) shall have access to any source documents from which case report form information may have been generated.

I further agree not to originate or use the name of Albireo AB, A4250 in any publicity, news release, or other public announcement, written or oral, whether to the public, press, or otherwise, relating to this protocol, to any amendment to the protocol, or to the performance of this protocol without the prior written consent of Albireo AB.

Name of Investigator

Signature

Date (day/month/year)

1 ADMINISTRATIVE INFORMATION

A Double-blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Protocol No.:	A4250-005
Date of the initial Protocol:	20 September 2017
Date and Number of Amendment(s):	Amendment 01, 06 December 2017
Sponsor:	Albireo AB Arvid Wallgrens backe 20 413 46 Göteborg Sweden
Clinical Research Organization:	[redacted]
Sponsor Signatory:	[redacted]
Sponsor Medical Monitor:	[redacted]
Principal Investigator:	[redacted]

2 STUDY SYNOPSIS

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250

Title of Study:
A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Principal Investigator:
[redacted]

Study Centers:
Up to 50 sites will be initiated for this study in the United States (US), Canada, European Union (EU), Middle East, and Australia.

Publication(s):
None.

Planned Study Period:	**Development Phase:**
Q1 2018 to Q2 2019	Phase 3

Objectives:

Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with progressive familial intrahepatic cholestasis (PFIC) Types 1 and 2, as determined by the following:

- Proportion of patients experiencing at least a 70% reduction in fasting serum bile acid (s-BA) concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- Change from baseline to Week 24 in pruritus compared to placebo

Secondary Objectives

- To evaluate the effect of A4250 on serum alanine aminotransferase (ALT) concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

Methodology:

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of familial intrahepatic cholestasis-1 or bile salt export pump).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-up Period. After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo. Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008) in which all patients will receive active treatment.

There will be up to 10 clinic visits during the study and one scheduled telephone contact.

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250

Number of Patients:
Approximately 60 patients diagnosed with PFIC-1 or PFIC-2 will be randomized.

Diagnosis and Main Criteria for Inclusion:

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples 7 to 28 days apart (Visits 1 and 2) prior to randomization
4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the electronic diary (eDiary) average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients are expected to have a consistent caregiver(s) for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

Test Product, Dose and Mode of Administration:
A4250, 40 µg/kg/day or 120 µg/kg/day, orally administered

Reference Therapy, Dose and Duration of Administration:
Matching placebo, orally administered

Duration of Treatment:
24 weeks

Variables

Efficacy:

Primary Efficacy Endpoints

The primary efficacy endpoints are:

- **EU and rest of world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  Fasting s-BA baseline will be calculated as the average of the value at randomization and the previous visit (Clinic Visit 2). The end value is the average of the values at Weeks 22 and 24 after the start of double-blind treatment.
- **US**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 24 as measured by the worst scratch of the Albireo observer-reported outcome (ObsRO) instrument. Scores for the 14 days baseline and post-treatment intervals will be calculated by averaging the worst scratching scores from each day in the interval.

Secondary Efficacy Endpoints

Secondary efficacy endpoints include the following:

- **EU and RoW**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 24 as measured by the worst scratch of the Albireo ObsRO instrument. Scores for the 14 days baseline and post-treatment intervals will be calculated by averaging the worst scratching scores from each day in the interval.
- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  All Regions:

  All secondary endpoints will be compared to placebo.

Name of Sponsor/Company:	Name of Product:	Name of Active Ingredient:
Albireo	A4250	A4250

- Change from baseline to Week 24 in fasting s-BA
- Change from baseline to Week 24 in serum ALT concentration
- Change in growth from baseline to Week 24, defined as the linear deficit (weight for age and body mass index) compared to the national growth curves (Z-score, standard deviation from P50)
- Proportion of patients achieving meaningful reduction in caregiver-reported observed scratching at Week 24
- Change from baseline to Week 24 in sleep parameters measured with the Albireo patient-reported outcome (PRO) and ObsRO instruments
- Change from baseline to Week 24 in patient-reported itch severity as measured by the average daily score of the Albireo PRO instrument; only patients ≥8 years of age will complete the Albireo PRO instrument
- Number of patients undergoing biliary diversion surgery or being listed for liver transplantation

Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Proportion of patients achieving meaningful reduction in patient-reported itch severity at Week 24
- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the patient global impression of change (PGIC) items
- Change from baseline in fasting s-BA at Weeks 4 and 12
- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Weeks 4, 12, and 24 as measured by the worst scratch. Scores for the 7 worst scratching days of the last 14 days at baseline and post-treatment intervals will be selected
- Change from baseline in patient-reported itch severity and observer-reported scratching at Weeks 4 and 12
- Change from baseline to Week 24 in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline to Week 24 in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline to Week 24 in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in Pediatric Quality of Life Inventory (PedsQL)
- Change from baseline in serum ALT concentration at Weeks 4 and 12
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, plasma 7α-hydroxy-4-cholesten-3-one concentration [p-C4]) at Week 24
- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events.
- All-cause mortality

Safety:

Safety criteria are as follows:

- Occurrence of treatment-emergent adverse events including severity and relatedness to study drug at all visits
- The incidence of treatment-emergent serious adverse events (SAEs), based upon information from patient reports, including the description, incidence, and severity of an SAE
- Trends evaluated for the following assessments: physical examinations, concomitant medications, vital signs, laboratory test results (including clinical chemistry, hematology, and urinalysis), and imaging

Name of Sponsor/Company: Albireo	**Name of Product:** A4250	**Name of Active Ingredient:** A4250
studies		
Statistical Methods: The primary objective of this study is to show superiority in efficacy of A4250 (120 µg/kg/day and 40 µg/kg/day) compared to placebo in patients with PFIC Types 1 and 2. In the EU and RoW, for the primary efficacy variable fasting bile acid responder endpoint (at least a 70% reduction in fasting s-BA concentration or reaching a level ≤70 µmol/L at the end of treatment), the null hypothesis will be tested against the alternative hypothesis (α=0.0125, 1-sided). Generalized linear mixed modelling will be used with a logistic link, to allow for repeated assessments over time. The model will include baseline value, treatment group, PFIC class, age class, region, visit and visit*treatment group interaction. The contrasts of primary interest will be the treatment difference at Week 24 but also the difference at Week 4, Week 8, Week 12, and Week 18 will be estimated and analyzed. In the US, for the primary efficacy variable pruritus score endpoint (change from baseline to Week 24 in observer-reported scratching), the null hypothesis will be tested against the alternative hypothesis (α=0.0125, 1-sided). The primary analysis of the change from baseline in pruritus score (indexed by observer-reported scratching) will be analyzed using a mixed model repeated measures analysis. The contrasts of primary interest will be the treatment difference at Week 24 but also the difference at the other weeks will be estimated and analyzed. To strictly control overall Type I error rate across the dose groups and also across the primary and the main secondary endpoint to a 1-sided 2.5% level, a Bonferroni-based parallel gate-keeping procedure will be used. Other secondary and exploratory outcome variables shall provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple-dose comparisons when testing the other secondary and exploratory efficacy variables.		
Date of the Protocol: 06 December 2017		

3 TABLE OF CONTENTS

3.1 List of Tables

3.2 List of Figures

3.3 List of Appendices

4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADME	absorption, distribution, metabolism, and excretion
AE(s)	adverse event(s)
AFP	alfa-fetoprotein
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APRI	AST to platelet ratio index
ASBT	apical sodium-dependent bile transporter (also known as IBAT)
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	body mass index
BSEP	bile salt export pump
CPK	creatine phosphokinase
CRA	clinical research associate
CYP	cytochrome P450
DSMB	Data and Safety Monitoring Board
eCRF	electronic case report form
eDiary	electronic diary
EOT	End of Treatment
EU	European Union
EudraCT	European Union drug regulatory agency clinical trial
FAS	full analysis set
FDA	United States Food and Drug Administration
FIC1	familial intrahepatic cholestasis-1
GCP	Good Clinical Practice
GI	gastrointestinal
GLMM	generalized linear mixed modelling
IB	Investigator's Brochure
IBAT	ileal bile acid transporter (also known as ASBT)
ICF	informed consent form
ICH	International Council for Harmonisation
[redacted]	[redacted]
IEC	Independent Ethics Committee
INR	international normalized ratio
IRB	Institutional Review Board
IWRS	Interactive Web Response System

LDH	lactate dehydrogenase
LFT	liver function test
MDR3	multidrug-resistance protein 3
MedDRA	Medical Dictionary for Regulatory Activities
MELD	model for end-stage liver disease
MMRM	mixed model repeated measures
ObsRO	observer-reported outcome
PBC	primary biliary cholangitis
p-C4	plasma 7α-hydroxy-4-cholesten-3-one concentration
PCS	potentially clinically significant
PedsQL	Pediatric Quality of Life Inventory
PELD	pediatric end-stage liver disease
PFIC	progressive familial intrahepatic cholestasis
PGIC	patient global impression of change
PGIS	patient global impression of symptoms
PP	per protocol
PRO	patient-reported outcome
PT	prothrombin time
PVM	pharmacovigilance manager
QoL	quality of life
RoW	rest of world
RR	respiratory rate
SAE(s)	serious adverse event(s)
SAP	statistical analysis plan
s-BA	serum bile acid
SD	standard deviation
s-FGF19	serum fibroblast growth factor 19 concentration
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction
TEAE(s)	treatment-emergent adverse event(s)
ULN	upper limit of normal
US	United States
WHO	World Health Organization

5 INTRODUCTION

5.1 Investigational Medicinal Product

A4250 is a small molecule and a potent selective inhibitor of the ileal bile acid transporter (IBAT), also known as the apical sodium-dependent bile acid transporter (ASBT). IBAT is an integral brush border membrane glycoprotein that co-transports sodium and bile acids and appears to be a critical component in the regulation of the bile acid pool size in animals and man. This transporter, expressed in the distal ileum, is a key element in the enterohepatic circulation of bile acids since it facilitates the high-affinity, high-capacity reabsorption of bile acids. Indeed, 95% of secreted bile acids are reabsorbed via IBAT [Hofmann 2009; Miethke 2016]. A4250 is orally administered and acts locally in the gut where it binds reversibly to IBAT to decrease the reuptake of bile acids. A4250 has minimal systemic exposure at the predicted therapeutic dose ranges.

No safety signals were found with A4250 in preclinical safety and toxicity studies. In the first clinical study of A4250 in healthy volunteers, A4250 was given as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. A4250 was well tolerated and the only findings were gastrointestinal (GI) related effects, such as diarrhea, at higher doses.

In an exploratory Phase 2 study in children with cholestatic pruritus, A4250 was given as oral doses of 10 to 200 µg/kg/day for 4 weeks. A4250 reduced mean levels of serum bile acids (s-BA) in all cohorts. In 11 of 13 progressive familial intrahepatic cholestasis (PFIC) patients, substantial reductions (ranging from 43% to 98%) were observed. Patient-reported diary data documented improvement of pruritus during treatment. A4250 was well tolerated. No serious adverse events (SAEs) were deemed to be related to study drug, and most adverse events (AEs), including some increased transaminases, were considered unrelated to study drug and transient in nature.

5.2 Background

5.2.1 Progressive Familial Intrahepatic Cholestasis

PFIC is a rare autosomal recessive cholestatic liver disease estimated to affect between 1 in every 50,000 to 100,000 children born worldwide. PFIC represents 10% to 15% of causes of cholestasis in children and 10% to 15% of liver transplantation indications in children. All types of PFIC exist worldwide and both sexes appear to be equally affected.

The common underlying pathogenesis of PFIC is disruption of bile formation and bile transport through the liver [Jacquemin 2000]. The classification of PFIC has evolved over the years. The most commonly used sub-classifications are PFIC Types 1, 2, and 3, which are based on the associated affected gene and described in more detail below.

- PFIC, Type 1: also referred to as “Byler disease” or “familial intrahepatic cholestasis-1 (FIC1) protein deficiency.” FIC1 protein is located on the canalicular membrane of hepatocytes and facilitates movement of aminophospholipids from the outer

to inner leaflet of the plasma membrane of the hepatocyte. The *ATP8B1* gene encodes FIC1 protein. Biallelic pathologic variants in the *ATP8B1* gene are associated with FIC1 dysfunction and classified clinically as PFIC Type 1 disease.

- PFIC, Type 2: also referred to as "Byler syndrome" or "bile salt export pump (BSEP) deficiency." BSEP is a transporter protein that is expressed at the canalicular membrane of hepatocytes, and is the primary exporter of bile acids. The *ABCB11* gene encodes the BSEP protein. Biallelic pathologic variations in the *ABCB11* gene are associated with BSEP dysfunction and classified clinically as PFIC Type 2 disease.
- PFIC, Type 3: is caused by a deficiency of the multidrug-resistance protein 3 (MDR3) due to mutations in the *ABCB4* gene. MDR3 is a phospholipid translocase critical for phospholipid secretion.

Severe pruritus is common in children diagnosed with PFIC. Itching (and subsequent scratching) is a significant morbidity for these patients and their families [Suchy 2007]. A more severe degree of pruritus is experienced compared to patients with other forms of liver disease and to other pruritic conditions such as atopic dermatitis [Murray 2011]. In patients with PFIC, liver biopsy reveals canalicular cholestasis and, later, the appearance of portal fibrosis. Serum biochemistry indicates cholestasis with hyperbilirubinemia, elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The concentrations of bile acids in serum are very high, while serum gamma-glutamyl transferase activity (exception being MDR3 variants) and cholesterol are normal [Hori 2010]. Symptoms of portal hypertension and liver failure will develop during the course of the disease [Davit-Spraul 2009; Alissa 2008]. Symptoms develop early; median age at onset of symptoms is 2 months, and 78% of PFIC patients present with jaundice [Pawlikowska 2010]. The life-threatening and debilitating nature of PFIC is reflected by the fact that survival in those not resorting to surgery is 50% at 10 years of age and almost zero at 20 years of age. Approximately half of PFIC patients undergo liver transplantation [Davit-Spraul 2009] and treatment-resistant pruritus is the leading indication for the surgical procedure partial external biliary diversion, mostly in PFIC Type 1 and 2 patients.

PFIC is life-threatening and debilitating. Currently, the treatment for PFIC is palliative and there is no pharmaceutical treatment approved for use in PFIC. The therapeutic choices are restricted to non-specific therapy of the symptoms and signs of the disease such as nutritional support, preventing vitamin deficiencies, and treatment of extrahepatic features. Medical treatment options include off-label use of ursodeoxycholic acid, rifampin, antihistamines, and naltrexone. A minority of patients respond nominally and transiently to these interventions. Biliary diversion is used to decrease systemic bile acids through interruption of the enterohepatic circulation and so avoid transplantation. Liver transplantation is typically only viewed as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

5.2.2 Summary of Clinical and Nonclinical Studies

This is a summary of nonclinical and clinical studies. More detail is provided in the Investigator's Brochure ([IB] see A4250 IB).

A4250 is a potent, selective IBAT inhibitor with no safety signals seen in the safety pharmacological studies. In addition, no safety signals were seen in repeat-dose oral toxicity studies in rats with up to 26 weeks of daily dosing and in dogs with up to 39 weeks of daily dosing. No safety signals were identified when A4250 was administered daily to juvenile rats from age 14 days to 63 days.

A4250 is minimally absorbed with very low systemic exposure in both humans and other species. In blood, protein binding is high (>99.7%). Systemically absorbed A4250 is slowly metabolized in humans and in preclinical studies by hydroxylation only, a metabolite that retains IBAT activity. A4250 did not show any significant inhibition of cytochrome P450 (CYP) enzymes in the rat. A4250 inhibited human CYP3A4 and CYP2D6 (IC_{50}: 16 µmol/L for both enzymes), and CYP2C9 (IC_{50}:1.2 µmol/L).

To date, A4250 has been studied in 3 Albireo-sponsored clinical studies: a study in healthy volunteers evaluating single and multiple administration of A4250; a single-dose absorption, distribution, metabolism, and excretion (ADME) study; and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with primary biliary cholangitis (PBC). In total, 98 subjects have been exposed to A4250.

In the first clinical study (A4250-001), A4250 was given to healthy volunteers as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. Following a single oral dose of 10 mg, the median maximum drug concentration was 0.2 nmol/L and the area under the curve was 0.8 nmol × h/L (n=6). Pharmacodynamic evaluation of bile acid synthesis biomarkers (serum fibroblast growth factor 19 [s-FGF19] and plasma 7α Hydroxy 4-cholesten-3-one [p-C4]) identified the expected decrease in s-FGF19 and an increase in p-C4. In addition, total plasma bile acids decreased and total fecal bile acids increased in A4250-treated patients compared to placebo. A4250 was generally well tolerated. The only findings were GI-related effects, such as diarrhea at higher doses.

In the ADME study (A4250-007), healthy subjects were administered a single oral dose of 3 mg [^{14}C]-A4250 capsule. The main excretion route was determined to be fecal. There were no quantifiable concentrations of A4250 in plasma or total radioactivity in plasma or whole blood. A4250 was well tolerated. AEs were reported and most were mild and not related to A4250. The 3 AEs experienced by 2 subjects reported as related to study drug were GI in nature, including diarrhea (2 AEs; 2 subjects) and abdominal pain (1 AE; 1 subject).

In an exploratory Phase 2 study in children with cholestatic pruritus (A4250-003), A4250 was administered as oral daily doses of 0.01 to 0.2 mg/kg for 4 weeks. Twenty patients (8 females and 12 males) aged 1 to 17 years were enrolled in this open-label dose-escalation study. Four patients were re-enrolled into a second dose level. No

treatment-related SAEs were reported, and most AEs, including some increased transaminases, were transient. Four-week, once-daily treatment with A4250 reduced mean levels of s-BA in all cohorts. Substantial s-BA reductions in 11 of 13 PFIC patients (ranging from 43% to 98%) were observed. A4250 also reduced s-BA in the 11 non-PFIC patients, but to a lesser extent than in PFIC patients. Patient-reported diaries documented improvement of the visual analogue scale itch score in 17 of 24 patients during treatment. The dose with the greatest improvement showed a mean decrease (± standard error of the mean) of 2.9 ±1.1 from baseline. The results from this study form the basis of the current (A4250-005) study design.

5.3 Rationale

In this investigational study, A4250 is under development for treatment of PFIC and is also being considered for other orphan cholestatic liver diseases.

There is currently no medical treatment approved for PFIC. In PFIC patients with severe pruritus, biliary diversion surgery is used to decrease systemic bile acids through interruption of the enterohepatic circulation and thereby reducing pruritus [Whitington 1988]. Liver transplantation is typically used as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

By inhibiting IBAT with high selectivity and potency, A4250 has the potential to relieve cholestasis and improve liver function without surgical intervention in patients with PFIC. Since A4250 is minimally absorbed and achieves a substantial reduction of s-BA, it has the potential to be a safer and less invasive alternative to surgical biliary drainage. The rationale for using A4250 is to decrease s-BA levels, and to reduce the major morbidity of pruritus, improving the health and wellbeing of patients affected with PFIC. Ultimately, this noninvasive therapeutic approach has the potential to not only improve quality of life (QoL), but also reduce the need for biliary diversion and subsequent liver failure and liver transplantation.

The doses selected, 40 and 120 µg/kg/day, were based on the efficacy and safety data generated from patients with PFIC (n=10 + 3 re-entered) and supporting data from all patients (n=20 + 4 re-entered) in the five dose groups dosed in the A4250-003 study. Improvements in s-BA and pruritus were observed with doses from 30 µg/kg to 100 µg/kg, with a dose-related trend. The best dose response in all patients was at the 60 µg/kg/day for s-BA and 100 µg/kg/day for pruritus. The best dose response in the PFIC subgroup was at 30 µg/kg/day for both s-BA and pruritus. Patients with PFIC responded well to the doses given from 30 µg/kg to 200 µg/kg; however, a clear dose relationship could not be established in order to select a single dose for this Phase 3 study. As efficacy was observed at both low and high doses, 40 µg/kg and 120 µg/kg/day are considered to be the most optimal for this study.

This study will be conducted in compliance with the protocol and with the International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP).

Patients completing 24 weeks of double-blind treatment will be offered to enter Study A4250-008 and receive open-label treatment with A4250.

5.3.1 Risk/Benefit

A4250 has been evaluated in three Albireo-sponsored clinical studies: a double-blind placebo-controlled study in healthy volunteers, a single-dose ADME study, and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with PBC.

A total of 98 subjects/patients have been exposed to A4250. Healthy subjects have been exposed to up to 10 mg as a single dose and up to 3 mg daily as part of a multiple-dose evaluation. Children with cholestatic liver disease have been treated with up to 0.2 mg/kg/day (200 µg/kg/day) for 4 weeks. In total, 2 SAEs have been reported; both were judged by the physician to be not related to the study drug.

Patients with cholestatic liver diseases suffer from excess bile acids in the liver resulting in tissue damage. A commonly used treatment in these patients is bile diversion surgery, whereby approximately 50% to 100% of the enterohepatic circulation of bile acids is interrupted. Inhibition of IBAT with A4250, thereby interrupting the enterohepatic circulation of bile acids, is therefore a potential medical alternative to surgery which could be of benefit to these patients if shown to be effective and safe. Data from the Phase 2 study showed efficacy of A4250 in reducing s-BA concentrations and pruritus in such patients.

Based on the mode of action of an IBAT inhibitor, loose stools or diarrhea could be expected. However, in the pediatric Phase 2 study with 4 weeks daily treatment, only one patient had mild transient diarrhea after a single dose that did not recur on multiple dosing.

6 STUDY OBJECTIVES

6.1 Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with PFIC Types 1 and 2, as determined by the following:

- The proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- Change from baseline to Week 24 in pruritus compared to placebo

6.2 Secondary Objectives

- To evaluate the effect of A4250 on serum ALT concentration
- To evaluate the effect of A4250 on growth
- To evaluate the effect of A4250 on sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion or liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

7 INVESTIGATIONAL PLAN

7.1 Overall Study Design and Plan

7.1.1 Description

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of FIC1 or BSEP).

Study data will be periodically reviewed by an unblinded Data Safety and Monitoring Board (DSMB).

The study includes a 35- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-Up Period.

After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo.

Patients who complete the Treatment Period and Visit 9 (Day 168) in this study will be invited to participate in a 72-week open-label extension study (A4250-008) in which all patients will receive active treatment. A patient who prematurely withdraws due to no improvement/intolerable symptoms, and has completed at least 12 weeks of the Treatment Period and End of Treatment (EOT) Visit, will also be offered the opportunity to enter Study A4250-008.

Visit windows applicable to each visit are presented in the schedule of assessments (Table 1). There will be up to 10 clinic visits during the study (Figure 1) and one scheduled telephone contact, as follows:

- Visit 1: Screening Period start. Patient is considered enrolled after signing the informed consent form (ICF) (Days -56 to -35)
- Visit 2: second Screening Visit (Days -28 to -7)
- Visit 3: Randomization Visit (Day 0)
- Telephone Contact 1: (Day 14)
- Visit 4: Clinic Visit (Day 28)
- Visit 5: Clinic Visit (Day 56)
- Visit 6: Clinic Visit (Day 84)
- Visit 7: Clinic Visit (Day 126)
- Visit 8: Clinic Visit (Day 154)
- Visit 9: Clinic Visit, EOT (Day 168)

- Visit 10: Follow-Up Visit, 28 days after last dose of study drug (Day 196). All patients continuing in Study A4250-008 will not have this Follow-up Visit.

Additional clinic visits may be required for patients who need direct site assistance, e.g., due to AE monitoring, to fulfil screening requirements, and/or for safety maintenance.

Screening Period

Informed consent must be obtained at Visit 1 prior to performing any study procedures. After signing the ICF, patients will be considered enrolled and evaluated for study eligibility.

Daily recording of pruritus using an electronic diary (eDiary) will be started to determine confirmation of the magnitude and the baseline pruritus symptoms before the study. After consultation with the Medical Monitor, patients failing eligibility criteria may be re-screened after 2 months. Patients not fulfilling inclusion/exclusion criteria after 3 attempts are not allowed to re-screen.

Caregivers/patients will be instructed on the daily use of the eDiary. The eDiary will include patient-reported outcome (PRO) and observer-reported outcome (ObsRO) items for evaluation of itching (PRO), scratching (ObsRO), and sleep disturbance (PRO and ObsRO) during the Screening Period (study Days -56 to 0) and throughout the 24-week Treatment Period (study Days 0 to 168).

Only the 14 most recent days before randomization will be used for eligibility calculation of the diary entries. Observer-reported outcomes in patients of all ages will be recorded by a caregiver (or caregiver's designee). If possible, the same caregiver will complete the Albireo ObsRO items throughout the study.

At a second Screening Visit (Visit 2) additional laboratory samples will be taken for eligibility assessments.

Treatment Period

Eligibility for randomization will be determined at Visit 3 using the pruritus (scratching reports from the Albireo ObsRO) data in the 2 weeks before Visit 3, genetic confirmation of diagnosis, and the liver biochemistry evaluations from the previous screening visits. Two weeks after Visit 3, there will be a telephone contact with the caregiver/patient to evaluate any AEs.

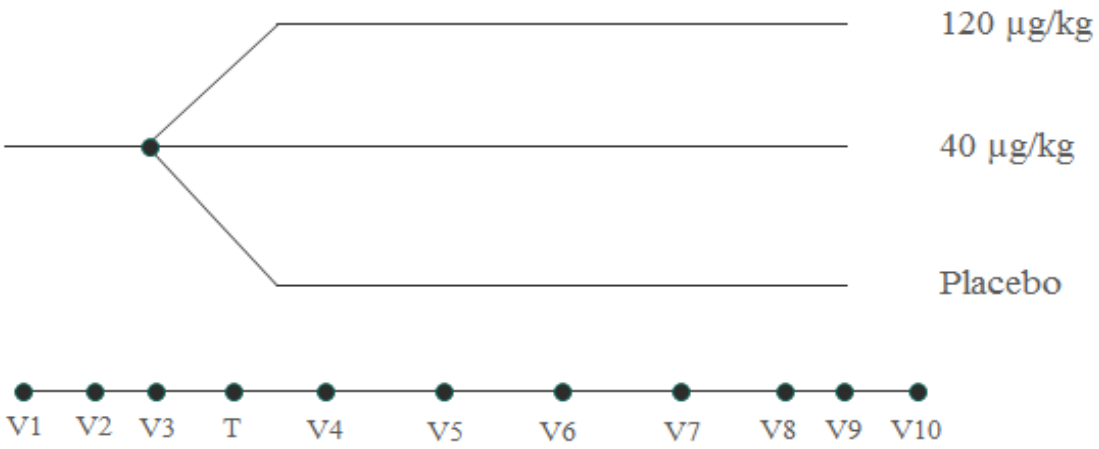
The patient will return to the clinic at Visit 4, Visit 5, Visit 6, Visit 7, Visit 8, and Visit 9 for assessments. If a new caregiver will begin to complete the Albireo ObsRO during the course of the trial, they will be provided with training during a clinic visit before they begin to use the eDiary. Additionally, caregivers/patients will be requested to use the diary to report the date and time each dose of study drug is administered during the Treatment Period.

The last dose of study drug will be administered the day before Visit 9. At study Visit 9, the patient has the option to enroll in an open-label extension study (A4250-008) should they meet the required entry criteria. If the patient is continuing into Study A4250-008 this will be

the last visit in this study and the first visit in Study A4250-008. Visit 9/EOT should also be performed at the time patients are prematurely withdrawn from the study.

Clinic Visit 10 will take place 28 days after Visit 9 for all patients not continuing into Study A4250-008.

Figure 1 Study Design



T: telephone contact; V: study visit

7.1.2 Schedule of Assessments

The schedule of assessments is presented in Table 1.

Table 1 Schedule of Assessments

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Informed consent [c]	X										
Inclusion/exclusion criteria	X		X								
Demographics	X										
Concomitant medication[d]	X	X	X	X	X	X	X	X	X	X	X
Medical and surgical history	X		X								
Blood sampling for genetic testing[e]	X										
Physical examination including voluntary photography	X		X							X	
Skin examination	X		X		X		X			X	X
Vital signs[f]	X	X	X		X	X	X	X	X	X	X
eDiary: itching, scratching, and sleep scores[g]	Daily diary entry										
Clinical chemistry[h]	X		X		X	X	X	X		X	X
Hematology[h]			X		X	X	X	X		X	X
Urinalysis[h]		X					X			X	

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Serum bile acids[i]	X	X	X		X	X	X	X	X	X	X
INR		X			X		X		X		X
Autotaxin[j]			X		X					X	
p-C4[j]			X		X					X	
AFP			X							X	
Fat-soluble vitamins A & E		X								X	
Fat-soluble vitamin D			X							X	
Abdominal Ultrasound			X							X	
Fibroscan® (where available)			X							X	
QoL questionnaire (PedsQL)			X							X	
Patient/Caregiver/Clinician Global Impression of Change					X		X			X	
Patient/Caregiver/Clinician Global Impression of Symptoms			X		X		X			X	
Pregnancy test[k]	X[k]	X	X		X	X	X	X	X	X[k]	
Study drug dispensed[l]			X		X	X	X	X	X		

Study Activity	Screening Period		Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-35) ±2**	**-28 – (-7) ±2**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic Visit 5**	**Clinic Visit 6**	**Clinic Visit 7**	**Clinic Visit 8**	**Clinic Visit 9/EOT[a]**	**Clinic Visit 10[b]**
Adverse events[m]	Continuous collection										
Study drug compliance				X	X	X	X	X	X	X	

AFP: alfa-fetoprotein; BMI: body mass index; eCRF: electronic case report form; eDiary: electronic diary; EOT: End of Treatment; ICF: informed consent form; INR: international normalized ratio; p-C4: plasma 7α Hydroxy 4-cholesten-3-one; PedsQL: Pediatric Quality of Life Inventory; QoL: quality of life; RR: respiratory rate; s-BA: serum bile acid.

a Must also be performed at the time a patient is prematurely withdrawn from the study. This visit is the same as Visit 1 in the extension protocol (Study A4250-008).

b Assessments must be performed 28 days following the final dose of study drug. This visit is omitted if patients are entering Study A4250-008.

c The ICF (and assent) must be signed before any study procedures are performed.

d Past (within 1 month prior to Visit 1) and current medication will be documented in the eCRF.

e Blood sample for genetic testing will be drawn for all patients. Genetic analysis will only be performed on a patient's extracted DNA if previous the clinical genetic result is equivocal, unavailable, or unobtainable.

f Includes blood pressure, pulse, RR, temperature, height (or length depending on age), and body weight (using a certified weight scale). BMI will be calculated.

g Includes training on use of the device at Screening (Visit 1) and review of patient daily entries during 14 days prior to Clinic Visit 3 to determine eligibility.

h See Table 3 for detailed parameters.

i Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA. Exceptions can be made for infants, less than 12 months of age, if unable to fast for the full 4 hours. All s-BA results during the treatment period and follow-up will be blinded.

j Samples will not be collected for patients ≤10 kg.

k For girls who have reached menarche. Serum test at Visit 1; urine test at other visits. A serum pregnancy test will also be conducted at Visit 9 for patients entering Study A4250-008. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy.

l Study drug to be taken once daily on Day 1 through Day 167 as described in Section 8.2.

m Adverse event information will be collected from the time of signing of the ICF to study discontinuation.

7.1.3 Study Procedures and Assessments

7.1.3.1 Screening Period (Days -56 to 0)

Day -56 to Day -35/Clinic Visit 1

Patients will undergo a Screening Visit up to 56 days prior to the planned first day of study treatment. Screening procedures and assessments are as follows:

- Obtain written informed consent
- Assess inclusion/exclusion criteria (Section 7.2)
- Record demographics (age, full date of birth, gender, race, and ethnicity)
- Document concomitant medications
- Medical and surgical history (date of diagnosis of PFIC Types 1 or 2, prior investigational medications for PFIC, historical liver function test [LFT] values, any surgery performed, any other diagnosis, and historical liver biopsy data)
- Blood sample for genetic testing
- Collect, if available, past confirmatory clinical genetic laboratory report for PFIC1/PFIC2 and send to central reader for review
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- eDiary training and compliance requirements; patients begin daily recording of pruritus using the eDiary (Appendix 2)
- Clinical chemistry (Section 10.2.1)
- s-BA (Section 9.2.1)
- Serum pregnancy test for girls of who have reached menarche
- AE monitoring

Day -28 to Day -7/Clinic Visit 2

A second Screening Visit will be performed. Screening procedures and assessments are as follows:

- Review concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for itching, scratching, and sleep scores and compliance
- Urinalysis (Section 10.2.1)

- s-BA (Section 9.2.1)
- International normalized ratio (INR)
- Fat-soluble vitamins A and E
- Urine pregnancy test for girls of who have reached menarche
- AE monitoring

7.1.3.2 Treatment Period (Day 0 to Day 168)

Study Day 0/Clinic Visit 3 (Randomization)

At the randomization visit, the following assessments will be conducted:

- Review inclusion/exclusion criteria (Section 7.2)
- Review concomitant medications, medical and surgical history
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance and entrance criteria requirement (see Inclusion Criteria 4, Section 7.2.1)
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin and p-C4 (samples will not be taken for patients ≤10 kg)
- Fat-soluble vitamin D
- Alfa-fetoprotein (AFP)
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)
- Administer QoL (Pediatric Quality of Life Inventory [PedsQL]) questionnaire
- Patient/caregiver/clinician complete the patient global impression of symptoms (PGIS)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Study drug is dispensed and patients are instructed to administer daily from Day 1

Study Day 14 (Telephone Contact)

A study nurse will contact the patient/caregiver by telephone for monitoring of the following:

- Review concomitant medications

- Albireo PRO/ObsRO eDiary review for compliance
- AEs
- Evaluation of study drug compliance

Study Day 28 and Day 84/Clinic Visits 4 and 6

The following procedures and assessments will be performed:

- Review of concomitant medications
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- Urinalysis (at Clinic Visit 6 only; Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- Autotaxin and p-C4 (at Clinic Visit 4 only; samples will not be taken for patients ≤10 kg)
- Patient/caregiver/clinician complete the patient global impression of change (PGIC) and PGIS
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 56 and Day 126/Clinic Visits 5 and 7

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance

- Study drug is dispensed

Study Day 154/Clinic Visit 8

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- s-BA (Section 9.2.1)
- INR
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 168/Clinic Visit 9/EOT

The last dose of study drug is taken in the morning the day before Clinic Visit 9, and the following procedures and assessments will be performed (also performed at the time a patient prematurely withdraws):

- Review of concomitant medications
- Physical examination, including voluntary photography (Section 10.2.5)
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry, hematology, and urinalysis (Section 10.2.1)
- s-BA (Section 9.2.1)
- Autotaxin and p-C4 (samples will not be taken for patients ≤10 kg)
- AFP
- Fat-soluble vitamins A, E, and D
- Abdominal ultrasound (Section 10.2.7)
- Fibroscan®, where available (Section 9.2.7)
- Administer PedsQL questionnaire
- Patient/caregiver/clinician complete the PGIC and PGIS

- Urine pregnancy test for girls who have reached menarche, or serum pregnancy test for girls continuing to study A4250-008
- AE monitoring
- Evaluation of study drug compliance

7.1.3.3 Follow-Up Period

Study Day 196/Clinic Visit 10

All patients not continuing into Study A4250-008 will return to the study site 28 days after Clinic Visit 9 or the last dose of study drug for the following assessments:

- Review of concomitant medications
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.6)
- Albireo PRO/ObsRO eDiary review for compliance
- Clinical chemistry and hematology (Section 10.2.1)
- s-BA (Section 9.2.1)
- INR
- AE monitoring

7.2 Study Population

A total of 60 patients with clinical diagnosis of PFIC Type 1 or 2 that have been genetically confirmed will be randomized.

An attempt will be made to randomize a minimum of 10 patients from each age category (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years).

A maximum of 5 patients that were treated in Study A4250-003 will be allowed to participate in this study.

7.2.1 Inclusion Criteria

1. A male or female patient, with clinical diagnosis of PFIC Type 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA concentration, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples 7 to 28 days apart (Visits 1 and 2) prior to randomization

4. Patient must have history of significant pruritus and a caregiver-reported observed scratching in the eDiary average of ≥2 (on 0 to 4 scale) in the 2 weeks prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients will be expected to have a consistent caregiver(s) for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

7.2.2 Exclusion Criteria

1. Patient with pathologic variations of the *ABCB11* gene that predict complete absence of the BSEP function
2. Patient with past medical history or ongoing presence of other types of liver disease including, but not limited to, the following:
   a) Biliary atresia of any kind
   b) Benign recurrent intrahepatic cholestasis, indicated by any history of normal s-BAs
   c) Suspected or proven liver cancer or metastasis to the liver on imaging studies
   d) Histopathology on liver biopsy is suggestive of alternate non-PFIC related etiology of cholestasis
3. Patient with a past medical history or ongoing presence of any other disease or condition known to interfere with the absorption, distribution, metabolism (specifically bile acid metabolism), or excretion of drugs in the intestine, including but not limited to, inflammatory bowel disease
4. Patient with past medical history or ongoing chronic (i.e., >3 months) diarrhea requiring intravenous fluid or nutritional intervention for treatment of the diarrhea and/or its sequelae
5. Patient has a confirmed past diagnosis of infection with human immunodeficiency virus or other present and active, clinically significant, acute, or chronic infection, or past medical history of any major episode of infection requiring hospitalization or treatment with parenteral anti-infective treatment within 4 weeks of treatment start (study Day 1) or completion of oral anti-infective treatment within 2 weeks prior to start of Screening Period
6. Any patient with suspected or confirmed cancers except for basal cell carcinoma, and non-liver cancers treated at least 5 years prior to Screening with no evidence of recurrence

7. Patient with a past medical history of chronic kidney disease with an impaired renal function and a glomerular filtration rate <70 mL/min/1.73 m^2
8. Patient with surgical history of disruption of the enterohepatic circulation excluding those who have undergone a successful reversal procedure that has permanently restored flow of bile acids from the liver to the duodenum
9. Patient has had a liver transplant or listed for liver transplant
10. Decompensated liver disease, coagulopathy, history or presence of clinically significant ascites, variceal hemorrhage, and/or encephalopathy
11. INR >1.4 (the patient may be treated with Vitamin K intravenously, and if INR is <1.4 at resampling the patient may be randomized)
12. Serum ALT >10 × upper limit of normal (ULN) at Screening
13. Serum ALT >15 × ULN at any time point during the last 6 months unless an alternate etiology was confirmed for the elevation
14. Total bilirubin >5 × ULN at Screening
15. Patient suffers from uncontrolled, recalcitrant pruritic condition other than PFIC. Examples include, but not limited to, refractory atopic dermatitis or other primary pruritic skin diseases
16. Any patient who is pregnant or lactating or who is planning to become pregnant within 24 weeks of randomization
17. Sexually active males and females who are not using a reliable contraceptive method with ≤1% failure rate (such as barrier protection, hormonal contraception, intrauterine device, or abstinence) throughout the duration of the study
18. Patient with a past medical history of alcohol or substance abuse will be excluded. Patient must agree to refrain from illicit drug and alcohol use during the study
19. Administration of bile acid or lipid binding resins and medications that slow GI motility (Refer to Appendix 1 – Concomitant Medications Prohibited during the study)
20. Patient has had investigational exposure to a drug, biologic agent, or medical device within 30 days prior to Screening, or 5 half-lives of the study agent, whichever is longer
21. Patient who has been previously treated with an IBAT inhibitor whose pruritus has not responded to treatment
22. Any other conditions or abnormalities which, in the opinion of the investigator or Medical Monitor, may compromise the safety of the patient, or interfere with the patient participating in or completing the study

7.2.3 Withdrawal of Patients

Patients/caregivers will be informed that they have the right to withdraw from the study at any time, without prejudice to their medical care, and that they are not obliged to state their reasons.

Any withdrawal, and reasons for withdrawal, must be fully documented in the electronic case report form (eCRF) and source documents and the patient followed by the investigator/investigative staff. Even after the study is completed at the Follow-up Visit, the investigator/investigative staff will, regardless of reason for withdrawal, record any patient data they receive concerning SAEs, and all drug-related non-serious AEs, and subsequently report these to Albireo. Withdrawn patients will not be replaced.

Patients will be withdrawn in the following circumstances:

- A patient's/caregiver's desire for withdrawal for any reason
- Lost to follow-up (every effort must be made to contact the patient/caregiver; a certified letter must be sent)
- An AE that, in the opinion of the investigator, necessitates withdrawal
- A worsening of the symptoms related to diarrhea occurs
- Death
- Patient/caregiver estimation of lack of improvement/intolerable symptoms
- A patient's/caregiver's substantial noncompliance (eDiary and study drug compliance) or protocol violation
- An investigator's opinion that continuing the patient in the study is not appropriate. The investigator may withdraw a patient at any time, if it is considered to be in the patient's best interest

A patient who prematurely withdraws due to no improvement/intolerable symptoms, and has completed at least 12 weeks treatment in the double-blind study, will be offered the opportunity to enter open-label Study A4250-008. A patient who withdraws from treatment prematurely and will not enter Study A4250-008 will be requested to complete all visits and safety assessments per the current protocol (A4250-005). As a minimum, patients will have EOT (Visit 9) assessments at the time of withdrawal and a follow-up assessment (Visit 10) 28 days following the last dose of study drug, and the patient will be recorded as withdrawn.

7.2.4 Study Termination by Sponsor

This study may be terminated at any time by Albireo if serious side effects should occur, if the investigator does not adhere to the protocol; or if, in the sponsor's judgment, there are no further benefits to be achieved from the study. In this event, Albireo/designee will inform the study investigators, institutions, and all regulatory authorities.

Albireo may temporarily or permanently discontinue the study at an investigative site at any time for safety, ethical, compliance, or other reasons. If this is necessary, Albireo will endeavor to provide advance notification to the site. If a site or the study is suspended or discontinued, the investigator/investigative staff will be responsible for promptly informing the Independent Ethics Committee (IEC). If required by local regulations, Albireo/designee will be responsible for informing the IEC and the regulatory authority of study or site discontinuation. In such an event, all study data and unused study drug must be returned to Albireo.

8 TREATMENT OF PATIENTS

8.1 Identity of Study Drug

A4250 and placebo will be supplied as capsules for oral administration. White opaque capsules filled with pellets containing A4250 or placebo will be provided. Two different capsule sizes will be available, as follows:

- Capsule size 0 that can be opened
- Capsule size 3 that can be opened or swallowed intact

The content of the A4250 and placebo capsules will be identical in appearance. Capsule filling weight will also be identical for high dose, low dose, and placebo. Bottles with 34 capsules will be given to the patient at each visit. A patient who requires 2 or more capsules per day will be given multiple bottles. Refer to the investigational product manual.

8.2 Administration of Study Drug

Patients will be dosed with A4250 at a dose of 40 µg/kg/day, 120 µg/kg/day, or placebo for 24 weeks. Study drug will be dispensed to the patient at Visits 3, 4, 5, 6, 7, and 8, together with instructions on how to store and take the drug. Study drug administration data, including whether each patient took each dose or partial doses of study drug, whether there were any delayed or missed doses, doses, and whether the capsule was opened or swallowed whole will be documented through the eDiary and transferred to the study database.

A4250 should be taken in the morning, together with food. On clinic visits days when laboratory assessments are conducted, study drug should be taken after the visit and after laboratory samples are taken. Patients should not crush or chew the capsule(s). When swallowing the capsule intact, the patient should administer the dose with a glass of water.

For a patient unable to swallow capsule intact, the capsule can be opened and the contents sprinkled and mixed in a small amount of soft room-temperature yogurt, apple sauce, or fruit purée. The amount of food should be less than what the patient/infant is expected to eat and should be followed by water.

1. Hold the capsule over the prepared food, gently twist open and allow the content to fall into food
2. Mix the entire capsule content with 1 or 2 tablespoons (15 to 30 mL) of soft food (apple sauce, fruit purée, or yogurt)
3. Administer the mixture immediately after mixing.

The number of capsules provided to the patient will be based on body weight at Visit 3. At Visit 6, if a patient's weight has shifted to another weight category (identified in Table 2), the number of capsules per day may be adjusted.

Table 2 Dosing and Capsule Strength

Body Weight (kg)	Capsule Size	Number of Capsules per Day	Capsule Strength, Low Dose (µg)	Total Dose (µg)	Capsule Strength, High Dose (µg)	Total Dose (µg)	Capsule Size, Placebo (µg)
5.0 to <7.5	0	1	200	200	600	600	0
7.5 to <12.5	0	2	200	400	600	1200	0
12.5 to <17.5	0	3	200	600	600	1800	0
17.5 to <19.5	0	4	200	800	600	2400	0
19.5 to <25.5	3	2	400	800	1200	2400	3
25.5 to <35.5	3	3	400	1200	1200	3600	3
35.5 to <45.5	3	4	400	1600	1200	4800	3
45.5 to 55.5	3	5	400	2000	1200	6000	3
>55.5	3	6	400	2400	1200	7200	3

8.3 Study Treatment Packaging and Labeling

8.3.1 Packaging and Labeling

The capsules will be packed in high-density polyethylene containers, with child-proof polypropylene caps. Study drug capsules containing A4250 in the strength specified in Table 2 above will be manufactured.

Packaging and labeling will be prepared to comply with applicable regulatory requirements.

8.3.2 Storage

Treatment packs containing A4250 capsules should be stored and dispensed in accordance with regulations in their original containers. The storage facility at the investigative site should be locked and the storage temperature should be between 15°C and 25°C.

Patients/caregivers should be informed of appropriate storage conditions (i.e., room temperature, between 15°C and 25°C).

Any deviations from the recommended storage conditions should be immediately reported to Albireo and the study drug should not be used until authorization has been given by Albireo.

8.3.3 Blinding and Randomization of Study Drug

A double-blind design is employed so that both the investigators and the patients will be unaware of the treatment assignment during the study.

After written informed consent is obtained from an eligible patient, an 8-digit patient identification number will be assigned. The first 2 digits will denote the country, followed by a 3-digit site number and a 3-digit patient sequence number. This number will be created and allocated by the Interactive Web Response System (IWRS), when the patient first enters the system at Screening. The randomization codes will be computer-generated by [redacted]

Biostatistics, and kept by a statistician independent from the project team. The randomization will be done in blocks and stratified according to PFIC class (Type 1 and 2) and age class (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) to ensure approximate balance between dose schemes (1:1:1). A separate randomization list will be prepared for the maximum 5 patients from Study A4250-003 regardless of stratification. Randomization codes will be assigned sequentially as patients become eligible for randomization.

Patients who are eligible for randomization will be assigned a unique 4-digit randomization number by IWRS. This randomization number identifies which treatment will be allocated to the patient. Patients who withdraw from the study after randomization visit are not to be replaced and their randomization number will not be reused.

To ensure blinding, the study drug and the matching placebo have the same shape and size. Patients will receive capsule(s) of A4250 according to their dose group or capsule(s) of matching placebo once a day during the double-blind Treatment Period. Labels on the study drug containers will not identify the treatment that a patient is randomized to. Traceability of the treatment is ensured by the study drug number.

The 5-digit study drug number will identify study drug packs and will be detailed on the study drug label. Dispensing of study drug will be coordinated by IWRS.

The system will assign a study drug number corresponding to the randomization arm. The randomization number will be used in the background only to ensure no unblinding and will not be displayed to end-users in the IWRS.

8.4 Procedure for Breaking the Randomization Code

Should a situation arise where unblinding is urgently required, i.e., only where knowledge of the study drug is required to adequately manage a life-threatening situation, the investigator at that study site may perform immediate unblinding through IWRS. The investigator should make every effort to discuss the rationale for emergency unblinding with the sponsor Medical Monitor or the [redacted] Medical Monitor prior to unblinding the individual patient.

The study site investigator and appropriate [redacted] project team members will be authorized to access the emergency unblinding functionality within the IWRS. Emergency unblinding takes place in the IWRS via a dedicated emergency unblinding module. The system will require the user to enter their emergency unblinding, which is typically provided at the same time login credentials are distributed. The exact description of the treatment assigned to the individual patient then will be accessible to the user. Once the randomization code is broken for a patient, he/she must be withdrawn from the study and all assessments and procedures at EOT should be performed. As a follow-up, Visit 10 will be performed according to the time schedule. Once a randomization code has been broken, the investigator must inform the [redacted] and sponsor Medical Monitor in writing within 24 hours.

8.5 Patient Compliance

The study nurse will monitor eDiary compliance by routine review of the CRF health website. If both diary entries on a day are missing during, the study nurse will call the patient/caregiver to remind them to complete all scheduled entries. Any noncompliance will be documented and explained in the source documents and in the eCRF.

Study drug compliance will also be checked through diary responses. Additionally, patients will return all unused study drug at Visits 4, 5, 6, 7, 8, and 9. The study site staff will count all returned drug, assess compliance, and record details in the eCRF.

Treatment Compliance = 100 × ((Number of study drug dispensed – number of study drug returned)/number of study drug that should be taken).

Treatment compliance between 80% and 120% will be acceptable.

8.6 Study Drug Accountability

Records shall be maintained of the delivery of study treatment(s) to the study site(s), of the inventory at the study site(s), of each use of the study treatment(s) for each patient, and of the return of unused study treatment(s) to Albireo.

These records shall include dates, quantities, batch numbers, expiry dates, and the unique code numbers assigned to the study drug and to the study patients.

The investigator will be responsible for ensuring that the records adequately document that the patients were provided the quantities specified in the protocol and that all study drug received from Albireo is reconciled.

8.7 Concomitant Therapy and Prohibited Medications

The investigator will note all ongoing medication and any medication recently stopped (within 1 month prior to Visit 1) in the eCRF. At Visits 2 through 10, all changes in medication (stopping or starting new medication or changes in dose) will be recorded in the eCRF. All medications (prescribed or over the counter) for pruritus and sleep will be recorded.

All medications taken by a patient within 3 months prior to the first intake of study drug are regarded as prior medication.

All medications taken by a patient on or after the first intake of study drug, and which continue to be taken during the study, are regarded as concomitant medication.

Prohibited medications are listed in Appendix 1.

9 ASSESSMENT OF EFFICACY

9.1 Efficacy Endpoints

9.1.1 Primary Efficacy Endpoint

The primary efficacy endpoints are as follows:

- **European Union (EU) and rest of the world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.

  Fasting s-BA baseline will be calculated as the average of the value at randomization and the previous visit (Clinic Visit 2). The end value is the average of the values at Weeks 22 and 24 after the start of double-blind treatment.

- **United States (US)**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 24 as measured by the worst scratch of the Albireo ObsRO instrument (Appendix 2). Scores for the 14 days baseline and post-treatment intervals will be calculated by averaging the worst scratching scores from each day in the interval.

9.1.2 Secondary Efficacy Endpoint

Secondary efficacy endpoints include the following:

- **EU and RoW**: Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Week 24 as measured by the worst scratch of the Albireo ObsRO instrument (Appendix 2). Scores for the 14 days baseline and post-treatment intervals will be calculated by averaging the worst scratching scores from each day in the interval.
- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA concentration from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment.
- **All Regions:**
  - All secondary endpoints are compared to placebo.
    - Change from baseline to Week 24 in fasting s-BA
    - Change from baseline to Week 24 in serum ALT concentration
    - Change in growth from baseline to Week 24, defined as the linear deficit (weight for age, and body mass index [BMI]) compared to the national growth curves (Z-score, standard deviation [SD] from P50)
    - Proportion of patients achieving meaningful reduction in caregiver-reported observed scratching at Week 24

- Change from baseline to Week 24 in sleep parameters measured with the Albireo PRO and ObsRO instruments
- Change from baseline to Week 24 in patient-reported itch severity as measured by the average daily score of the Albireo PRO instrument (Appendix 2); only patients ≥8 years of age will complete the Albireo PRO instrument
- Number of patients undergoing biliary diversion surgery or being listed for liver transplantation

9.1.3 Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Proportion of patients achieving meaningful reduction in patient-reported itch severity at Week 24
- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the PGIC items (Appendix 3)
- Change from baseline in fasting s-BA at Weeks 4 and 12
- Change in pruritus, as indexed by caregiver-reported observed scratching, from baseline to Weeks 4, 12, and 24 as measured by the worst scratch. Scores for the 7 worst scratching days of the last 14 days at baseline and post-treatment intervals will be selected
- Change from baseline in patient-reported itch severity and observer-reported scratching at Weeks 4 and 12
- Change from baseline to Week 24 in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline to Week 24 in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline to Week 24 in additional patient-reported and observer-reported sleep parameters (e.g., tiredness and number of awakenings)
- Change from baseline to Week 24 in PedsQL (Appendix 4)
- Change from baseline in serum ALT concentration at Weeks 4 and 12
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, p-C4) at Week 24

- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Change from baseline to Week 24 in stage of liver fibrosis as assessed by Fibroscan® (where available)
- Liver-related mortality and liver decompensation events
- All-cause mortality

9.2 Efficacy Assessments

9.2.1 Serum Bile Acids

Blood samples for analysis of fasting total s-BA will be drawn at all visits. Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for s-BA. Exceptions can be made for infants, <12 months of age, if unable to fast for the full 4 hours. Any visit at which a bile acid sample result is unreportable, an additional unscheduled visit for a repeat sample collection may be scheduled. All s-BA results during the treatment period and follow-up will be blinded. Samples will be processed and transported to a central laboratory per instructions in the laboratory manual.

9.2.2 Itching, Scratching and Sleep Score

Itching, observed scratching, and sleep disturbance will be recorded twice each day via eDiary. Patients and/or caregivers will be instructed to complete the eDiary every day in the morning after the patient wakes and in the evening just before the patient goes to sleep throughout the study. Patients and caregivers will receive a formal training session during Visit 1.

The eDiary includes Albireo ObsRO and PRO items. Patients <8 years of age will not be asked to complete the Albireo PRO items; only the Albireo ObsRO will be completed by caregivers of patients in this age group. Older patients, 8 to 17 years of age, will complete the Albireo PRO items and the caregiver will complete the Albireo ObsRO items. The Albireo PRO items assess severity of itch, aspects of sleep disturbance (morning diary only), and tiredness. For patients 8 to 12 years of age, the caregiver will read the Albireo PRO items along with the child and record the child's response. A guide will be provided to the caregivers that provides standardized explanations of the Albireo PRO items, in case the patient is confused or requires clarification about the meaning of a question. The Albireo ObsRO items assess severity of observed scratching, aspects of observed sleep disturbance (morning diary only), and observed signs of tiredness (evening diary only). The Albireo ObsRO and PRO scratching and itch severity items use 0 to 4 response scales, where each response is distinguished by a unique facial expression, verbal anchor, number, and color code (Appendix 2).

A daily score for the Albireo ObsRO scratching item will be calculated by selecting the maximum scratching severity score from the 2 ratings for each day. A daily score will be considered missing if both of the daily assessments are missing. A 14-days score will be calculated by averaging the daily scratching scores. A 14-days score will be considered missing if >4 out of 7 days a week of data are missing. The same approach will be used to calculate a patient-reported itch severity score.

9.2.3 Growth

Growth will be measured as height (velocity) and weight (Z-score) using a certified weight scale. BMI will be calculated by weight (kg) / height $(m)^2$. Change will be defined as linear growth deficit (weight and BMI for age) compared to national growth curves (Z-score, SD from P50).

9.2.4 Quality of Life Questionnaire (PedsQL)

Patients and caregivers will be asked to complete a QoL questionnaire (PedsQL) at Visit 3 and Visit 9. Details of the questions included on the questionnaire are provided in Appendix 4.

9.2.5 Biomarkers and Other Laboratory Samples

Blood samples for autotaxin and p-C4 will be drawn at Visits 3, 4, and 9 only for children with body weight ≥10 kg. Samples will be processed and transported to a laboratory per instructions in the laboratory manual.

9.2.6 PELD/MELD Score

At randomization and end of treatment, PELD score will be calculated for children up to 12 years of age. For children 13 years or older, the MELD score will be calculated.

PELD Score = 4.80 * ln(total bilirubin in mg/dL) + 18.57 * ln(INR) - 6.87 * ln(albumin in g/dL) + 4.36 (if patient <1 year: scores for patients listed for liver transplantation before the patient's first birthday continue to include the value assigned for age (<1 year) until the patient reaches the age of 24 months) + 6.67 (if the patient has growth failure [≤2 SD])

Laboratory values less than 1.0 will be set to 1.0 for the calculation of the PELD score.

MELD Score = 9.57 * ln(creatinine in mg/dL) + 3.78 * ln(total bilirubin in mg/dL) + 11.2 * ln(INR) + 6.43

Laboratory values less than 1.0 will be set to 1.0 and serum creatinine values greater than 4.0 mg/dL will be set to 4.0 for calculation of the MELD score.

9.2.7 Fibroscan®

Where available, Fibroscan® will be performed as per institution standard practice at Visits 3 and 9.

9.2.8 Markers of Fibrosis

APRI score and Fib-4 score will be calculated at randomization and at end of treatment.

APRI = [(AST in U/L) / (AST ULN in U/L)] / (Platelets in 10^9/L)

Fibrosis 4 Score = (Age*AST in U/L) / (Platelets in 10^9/L * √(ALT in U/L)).

9.2.9 Global Impression of Change and Global Impression of Symptom Measures

Patients (≥8 years of age), caregivers, and clinicians will complete the PGIC and the PGIS measures (Appendix 3) at randomization (Visit 3; PGIS only), Visits 4 and 6, and at EOT (Visit 9).

The PGIC items assess change in itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) since starting the study drug. The PGIS items assess itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) in the past week. Caregivers and clinicians will complete the PGIC and PGIS for all patients; those patients ≥8 years of age will complete the patient version.

10 ASSESSMENT OF SAFETY

The timing and frequency of safety assessments are described in Section 7.1.3 and in Table 1 above.

The primary safety analysis for this study will include the incidence of total treatment-emergent adverse events (TEAE) and TEAEs categorized by causality, severity, and seriousness assessments made by the investigator by comparing study drug exposure to placebo.

Trends in safety will also be evaluated for the following assessments:

- Physical examinations
- Concomitant medications
- Vital signs
- Laboratory test results (including clinical chemistry, hematology, urinalysis, AFP, and fat-soluble vitamins A, D, and E)
- Abdominal ultrasound
- Discontinuations due to AEs

10.1 Adverse Events

10.1.1 Definitions and Investigator Assessments

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 unfavorable and unintended sign, symptom, or disease that occurs once a patient is enrolled (ICF is signed) in the study until the patient is discharged from the study, whether or not related to the study drug.

10.1.1.1 Clinical Significance

Clinical significance is defined as any abnormal finding that results in further clinical investigation(s), treatment(s), or the diagnosis of new condition. Patient-reported events and protocol-mandated laboratory values, vital signs, and physical examination findings can be considered clinically significant (i.e., an AE) if there is a deterioration as compared to baseline. Examples of clinically significant worsening from baseline could include, but is not limited to, events causing withdrawal from the study and events requiring medical intervention outside of the study causing apparent clinical manifestations, or judged relevant by the investigator.

10.1.1.2 Serious Adverse Events

Serious criteria are applied by the investigator to each AE as specifically defined below. These criteria are used to determine whether an AE is serious or non-serious. The assessment is made independently of severity assessment (Section 10.1.1.3). For example, the

development of a severe rash that occurs after signing of informed consent may not meet serious criteria as defined below and therefore would be considered a severe, non-serious AE.

Any AE that meets any 1 of the following 6 criteria is considered an SAE:

- The outcome of the AE is **death**
- The AE is immediately **life-threatening**. Life-threatening means that the patient is, in the opinion of the investigator, at immediate risk of death from the reaction as it occurred. This does not include an AE that, if more severe, might have caused death
- The AE results in persistent or significant **disability/incapacity**. Disability means a substantial disruption of a person's ability to conduct normal life functions
- The AE requires or prolongs **hospitalization**
- The AE results in a **congenital anomaly/birth defect**
- **The AE is an important medical event**. Important medical events may meet serious criteria should the investigator assess that they may significantly jeopardize the patient, represent a significant hazard, or requires medical/surgical intervention to prevent one of the outcomes listed above. Examples of potential SAEs based on this criteria include, but are not limited to, allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions even if they do not result in inpatient hospitalization, or the development of drug dependency and drug abuse.

10.1.1.3 Severity Assessment

Severity assessments are based on the intensity of the event in relation to expectation. The investigator will assess the intensity of AEs based on the following definitions:

- Mild (awareness of sign or symptom, but easily tolerated)
- Moderate (discomfort sufficient to cause interference with normal activities)
- Severe (incapacitating, with inability to perform normal activities)

Severity is a measure of intensity where seriousness is defined by the criteria outlined in Section 10.1.1.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not meet serious criteria, and therefore would be assessed as a severe AE but not an SAE.

10.1.1.4 Causality Assessment

The investigator determines the causality of all AEs to the study drug using medical judgment and considering all relevant factors such as (but not limited to) the underlying study indication, coexisting disease, concomitant medication, relevant history, pattern of the AE, temporal relationship to the study drug, and de-challenge or re-challenge. The causality assessment of the AE/SAE is to be made as follows.

Related to study drug (possibly, probably, or definitely related)

Based on medical judgment, there is at least a reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event follows a reasonable temporal sequence from administration of study drug
- The event could not be reasonably attributed to the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient
- The event follows a known pattern of response to study drug
- The event disappears or decreases on cessation or reduction in dose of the study drug. (It should be noted that in some situations an AE will not disappear or decrease in intensity upon discontinuation of study drug despite other clear indications of relatedness)
- The event reappears or worsens when the study drug is re-administered

Unrelated to study drug (unlikely or unrelated)

Based on medical judgment there is no reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event does not follow a reasonable temporal sequence from administration of study drug
- The event could be reasonably attributed to the known characteristics of the patient's clinical state, concurrent illness, environment or toxic factors, or other modes of therapy administered to the patient
- The event does not follow a known pattern of response to study drug
- The event does not disappear or decrease on cessation or reduction in dose of the study drug, and it does not reappear or worsen when the study drug is re-administered

10.1.2 Recording of Adverse Events

It is the investigator's responsibility to assess whether each untoward event is a clinically significant worsening from baseline, thereby considered an AE. For all AEs, the severity, seriousness, and causality to study drug for each AE as outlined in Section 10.1.1 will be assessed and recorded in the eCRF.

All serious and non-serious AEs are collected once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10) or 28 days after the last dose of study drug.

Any AEs or SAEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator until resolution or stabilization up to the database lock and recorded in the eCRF. Albireo retains the right to request additional information for any patient with ongoing AE(s) or SAE(s) at the end of the study.

TEAEs are defined as any AE that occurs after randomization (Day 0). All AEs that occur in the Screening Period, i.e., after enrollment and prior to randomization, will be collected on the eCRF as non-treatment-emergent AEs.

If there is a clinically significant deterioration of a laboratory value/vital sign or other routine study assessment that is associated with a diagnosis, the clinical diagnosis will be reported as an AE and the associated signs and symptoms will be considered additional information unless the sign or symptom is more severe than expected given the diagnosis. For example, if an investigator diagnoses a study patient with hepatitis during the study period, hepatitis would be considered the AE and the concomitant signs and symptoms of abdominal pain, vomiting, and elevated ALT and AST would not be reported separately unless, in the opinion of the investigator, one of these signs or symptoms is more severe than expected and therefore a separate AE assessment is indicated.

10.1.3 Recording and Reporting of Serious Adverse Events

Every SAE (regardless of severity and causality) that occurs once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10), and 28 days after the final dose of study drug, should immediately and not later than within 24 hours of knowledge of the event, be reported by the investigator or delegate in the SAE report form.

Report of a SAE must include at least the following information:

- Patient identification information (study number, site number, initials, and date of birth [as per local country requirements for data protection])
- The last study drug administration date
- The diagnosis of the event with the description (or a brief description of signs/symptoms/clinical course if the diagnosis is not available) and the date of onset
- The action used to treat the event (i.e., treatment medications, temporary discontinuation)
- The reason(s) for considering the event serious
- The relationship of the event to the study drug or to the study procedure (e.g., the investigator's assessment of causality)
- A brief narrative of the SAE

Follow-up reports including all new information obtained of the subsequent course of the SAE must be prepared and the information collected in the SAE submitted to [redacted] (Pharmacovigilance and Safety Services Department) by e-mail within 24 hours of knowledge of the event.

The pharmacovigilance manager (PVM) may contact the investigator to obtain further information on a reported SAE. The investigator/investigative staff must respond to any request for follow-up information or answers to questions regarding the SAE within the same timelines as for initial reports.

The PVM reports the occurrence of the SAE and follow-up to the Albireo medical monitor for medical assessment of the case.

Should an outcome of death occur within the study period or within 28 days after the last administration of study drug, an SAE form should be completed including the main and contributory causes of death.

All SAE reports must be e-mailed to the following e-mail address within **24 hours**:

[redacted]

If email is unavailable, SAEs may be transmitted via fax to the following number:

[redacted]

In a study-related medical emergency situation, when the assigned medical monitors for the study cannot be reached by a caller, an on call physician can be reached 24 hours per day, 7 days per week, via the [redacted] call center. The following number is a chargeable telephone number allowing a global reach from both landlines and mobile phones and the internet page indicated below contains a list of country-specific toll-free telephone numbers that is provided. It should be noted that not all countries globally have access to toll-free numbers as indicated on the "24/7 Medical Help desk" index. Countries without toll-free numbers need to dial the chargeable number as indicated below. Furthermore, toll-free numbers might not be available from mobile phones.

[redacted]

10.1.4 Reporting of Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reaction (SUSAR) is an SAE that occurs in a patient, the nature or severity of which is not expected per the applicable product information (e.g., the IB for an unauthorized investigational product or summary of product characteristics for an authorized product).

Reporting and tracking of SUSARs will be in accordance to all applicable competent authority regulations. The Ethics Committee and all investigators involved in this study will be informed according to national and international requirements.

10.2 Laboratory Values/Vital Signs/Physical Examinations and Other Safety Assessments

10.2.1 Laboratory Assessments

Samples will be collected for clinical chemistry, hematology, and urinalysis testing at the time points specified in Table 1. The parameters assessed are presented in Table 3.

Blood for AFP will be drawn at Visits 3 and 9 and for fat-soluble vitamins at Visits 2, 3, and 9.

A serum pregnancy test will be performed at Visit 1 and Visit 9 if the patient is entering the A4250-008 study. A urine pregnancy test will be collected at all other visits for girls who have reached menarche. If a urine pregnancy test is positive, a serum pregnancy test should be performed to confirm the pregnancy. Study drug administration will be held pending the confirmatory results. If the serum pregnancy test is negative, the patient can resume dosing. If the serum pregnancy test is positive, the patient should be discontinued (see Section 10.2.9).

All samples will be processed and transported to a laboratory per instructions in the laboratory manual.

The observed values will be recorded and assessed as "normal" or "abnormal not clinically significant" or "abnormal clinically significant".

Additional safety blood samples may be needed due to follow-up of an abnormal value or analysis failure. The blood samples collected for safety laboratory analysis will be destroyed after the analyses have been completed.

Table 3 Routine Laboratory Parameters

Clinical Chemistry	Hematology	Urinalysis
• Albumin • ALT • Alkaline phosphatase • AST • Bilirubin – total and direct • Calcium • Chloride • Creatinine • Creatine kinase • Gamma-glutamyl transferase • Potassium • Sodium	• Hematocrit • Hemoglobin • Platelet count • Red blood cell count • White blood cell count	• Blood • Glucose • Ketones • Leukocytes • Nitrites • pH • Protein

10.2.2 Individual Patient Safety Monitoring

10.2.2.1 Liver Monitoring

Strategies to monitor markers of liver disease throughout the study are outlined below where the ULN will be based on central laboratory reference values for age and gender.

If isolated transaminase elevations are observed, defined as:

1. Normal bilirubin AND absence of clinical hepatitis symptoms AND

- ALT or AST ≥5 × ULN (if normal at baseline) or an absolute threshold of 800 U/L, whichever comes first
- OR ALT or AST ≥3 × baseline (if abnormal at baseline) or an absolute threshold of 800 U/L, whichever comes first

Then:

a) Repeat liver profile (AST, ALT, bilirubin, and prothrombin time [PT] or INR) within 2 to 3 days

b) Evaluate creatine phosphokinase (CPK) and lactate dehydrogenase (LDH)

c) As needed (for example, persistent ALT/total bilirubin elevation or worsening of liver function), consider evaluation for alternative etiologies

Monitor the patient using close observation found in Section 10.2.2.2

If any ONE of the following criteria are met:

1. Transaminases (ALT or AST ≥3 × baseline or 800 U/L, whichever comes first) AND bilirubin (total bilirubin > 2 × ULN) elevations
2. Transaminase elevations alone (ALT or AST >10 × ULN or 5 × baseline or absolute threshold of 800 U/L, whichever comes first) in presence of normal LDH and CPK
3. Cholestatic marker elevations (alkaline phosphatase [ALP] or gamma-glutamyl transferase >2 × baseline) without alternative explanation
4. Total bilirubin increased, unrelated to hemolysis (elevated reticulocyte count) or established genetic diseases, such as Gilbert's syndrome
   a. Doubling if total bilirubin was <3 mg/dL at baseline
   b. OR Increase by >3 mg/dL if total bilirubin was ≥3 mg/dL at baseline
5. INR increase refractory to vitamin K administration
   a. Increase by >1.5 if INR was normal at baseline
   b. OR Increase by >0.4 if INR was abnormal at baseline
6. Any increase in total bilirubin and transaminases if accompanied by EITHER a symptom of clinical hepatitis (e.g., vomiting, nausea, right upper quadrant pain) OR immunological reaction (rash or >5% eosinophilia)

Then:

a) Interrupt study medication

b) Initiate drug-induced liver injury work-up for alternative etiologies

c) Repeat liver profile (AST, ALT, total bilirubin, direct bilirubin, ALP) and PT or INR within 48 to 72 hours

d) Monitor the patient using close observation found in Section 10.2.2.2

e) If a patient lives in a remote area, they may be tested locally and the results communicated to the investigator site promptly

10.2.2.2 Close Observation

- Repeat liver enzyme and serum bilirubin tests two or three times weekly. Frequency of re-testing can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the subject is asymptomatic
- Obtain a more detailed history of symptoms and prior or concurrent diseases
- Obtain a history of concomitant drug use (including non-prescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets
- Rule out alternate etiology including acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; non-alcoholic steatohepatitis; hypoxic/ischemic hepatopathy; and biliary tract disease
- Obtain a history of exposure to environmental chemical agents.
- Obtain additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin)
- Consider gastroenterology or hepatology consultations.

10.2.2.3 De-challenge/Re-challenge for Liver and Clinical Hepatitis Monitoring

1. Re-challenge is not recommended:
   a. If a patient has had possible/probable drug-induced liver injury
   b. If a decompensation event has occurred (i.e., variceal hemorrhage, ascites, hepatic encephalopathy, etc.).
2. If due to underlying cholestatic liver disease variability or another alternative etiology is identified AND liver tests returned to baseline, re-challenge may be considered after consultation with the sponsor Medical Monitor.
3. If the ALT/total bilirubin elevations are observed after re-challenge, then repeat re-challenge is discouraged.

If patient is permanently discontinued, monitoring should be continued as outlined in Section 10.2.2.5.

10.2.2.4 Diarrhea

Study drug should be discontinued if a patient develops diarrhea with at least 1 of the following concomitant signs or symptoms: grossly bloody stools, vomiting, dehydration

requiring treatment with oral or intravenous rehydration and/or electrolyte imbalances, fever (≥38 C) and/or the diarrhea persists for 7 or more days.

If there is gross blood in stool, INR and platelets should be measured.

Study drug will be reintroduced (re-challenge) when the symptoms have resolved. If the diarrhea re-occurs within 1 week after the re-challenge with no alternate etiology, the patient will be permanently discontinued and monitored as outlined in Section 10.2.2.5.

10.2.2.5 Monitoring after Permanent Discontinuation of Study Drug Due to Safety

Once study drug is permanently discontinued for liver, hepatitis, diarrhea, or other severe AE related to study drug, the patient will be monitored weekly until the laboratory and clinical parameters have normalized/stabilized.

10.2.3 Demographics/Medical and Surgical History

Demographic information per country regulations (age, full date of birth, gender, race, and ethnicity), along with medical and surgical history, will be obtained and recorded in the eCRF at Visit 1.

Medical and surgical history will be entered in the eCRF at Visit 1. This includes date of diagnosis of PFIC, prior investigational medications for PFIC, historical (3 months prior to Screening) LFT values (AST, ALT, and total bilirubin), ongoing medication, any surgery performed, any other diagnosis, and historical biopsy data.

If a liver biopsy has been performed within 1 year prior to Screening, or during the study, the results will be recorded in the eCRF.

10.2.4 Clinical Genetic Testing

If confirmatory clinical genetic testing for PFIC Type 1 or 2 has been performed prior to Screening Visit 1, the confirmatory clinical genetic testing laboratory report will be verified by a central reader to determine eligibility.

Regardless of previous genetic testing, a blood sample will be drawn at Visit 1 for all patients for DNA extraction. Genetic testing will only be performed on a patient's extracted DNA if the previous clinical genetic result is equivocal, unavailable, or unobtainable. In such cases, screening for pathologic biallelic variations in the *ATP8B1* or *ABCB11* genes will be performed if patient meets remaining inclusion and exclusion criteria and only after sponsor case review. No other diagnostic genetic testing will be offered.

10.2.5 Physical Examination

A physician or suitably trained qualified assistant will perform a complete physical examination, including voluntary photography, at Visits 1, 3, and 9. A skin examination will be conducted at Visits 1, 3, 4, 6, 9, and 10 (see Table 1).

A complete physical examination will include assessment of general appearance, eyes, ears, nose, throat, head/neck/thyroid, lymph nodes, cardiovascular, lungs/chest, abdomen, genitourinary, extremities, skin, musculoskeletal, neurologic, and other.

Skin will be examined thoroughly and excoriations/scratch marks recorded.

10.2.6 Vital Signs

Evaluation of vital signs will be performed at all visits. This includes blood pressure (systolic and diastolic), pulse, respiratory rate, temperature, height (or length depending on age), and body weight (using a certified weight scale) at clinic visits. BMI will be calculated.

10.2.7 Abdominal Ultrasound

Ultrasound of the liver and spleen will be performed at randomization (Visit 3) and at EOT (Visit 9). Liver size, echogenicity, and presence of masses/nodules as well as spleen size will be recorded.

10.2.8 Overdose

A4250 is minimally absorbed and has a very low systemic availability. Based on toxicology data, for study purposes any dose exceeding a total of >3 mg/kg body weight of A4250 taken as a single dose or as a cumulative dose within 24 hours is defined as an overdose.

The no-adverse-effect dose level in the most sensitive species (defined as 20 mg/kg/day) in the rat 1 month toxicity study gives a human equivalent dose of approximately 194 mg/day in a 60 kg person. This dose is approximately 10-fold higher than the maximum possible dose predicted in human studies (20 mg).

The investigator/investigative staff should immediately and not later than within 24 hours of knowledge of the overdose, be reported by the investigator or delegate on the SAE report form. In the event of an A4250 overdose, the patient should be monitored closely.

10.2.9 Pregnancy

If a pregnancy is discovered in a female patient enrolled in the study before the end of dosing, the patient will be immediately discontinued from the study and will attend the same visits as a prematurely withdrawn patient. If the pregnancy is discovered in a female patient enrolled in the study after the end of dosing, the patient will continue in the study per protocol. If a pregnancy occurs in a male patient's partner at any time during the study, the pregnancy should also be reported and followed.

Pregnancy is not considered to be an AE. However, if the patient has been dosed with the study drug, the pregnancy must be reported on the Paper Pregnancy Notification Form immediately within 24 hours after recognition to Albireo/Safety Manager (please refer to Section 10.1.3). Date of exposure and as far as possible, details of the period of gestation at the time of exposure must be given.

The pregnancy should be followed to determine outcome, including spontaneous termination, details of birth, and presence of any birth defects, congenital anomalies or newborn or maternal complication. The infant who was exposed in-utero will be followed for up to 2 years after delivery. Individual cases with an abnormal outcome in association with the study drug should be reported on an expedited basis, i.e., reported rapidly to a competent authority.

11 STATISTICAL EVALUATION

All personnel involved with the analysis of the study will remain blinded until database lock for the double-blind Treatment Period and until major protocol violations have been identified.

All statistical analyses will be performed using SAS version 9.3 or higher.

11.1 Sample Size and Power

For the EU and RoW, the primary analysis will relate to the bile acid responder endpoint (at the 1-sided 1.25% level). For the US, the primary analysis will relate to the pruritus endpoint (at the 1-sided 1.25% level). The aim is to enroll 60 to 70 patients in order to get at least 60 evaluable patients.

Assuming 60% responders (patients experiencing at least 70% reduction in fasting s-BA concentration or reaching ≤70 µmol/L) in the A4250 dose group and 10% responders in the placebo group, approximately 20 patients per group are required to achieve a power of 84% with a 1.25% 1-sided Type-1 error.

For pruritus, with approximately 20 patients per treatment group, the power is 83% given an assumed treatment effect difference of -1.0 in change from baseline (-1.6 for A4250 and -0.6 for placebo) on the Albireo ObsRO scratching score with a SD of 0.95 and a Type I error of 1.25% (1-sided).

11.2 Statistical Methods

11.2.1 Statistical Analysis Sets

Safety Analysis Set

The safety analysis set will consist of all patients who received at least 1 dose of study drug. Patients will be analyzed according to the treatment they actually received. The safety analysis set will be the primary analysis set for safety analyses.

Full Analysis Set

The full analysis set (FAS) will consist of all randomized patients who received at least 1 dose of assigned treatment. Patients will be analyzed in the treatment group they were randomized to even if they received incorrect study drug. The FAS will be the primary analysis set for efficacy analyses.

Per Protocol Analysis Set

The per protocol (PP) analysis set is a subset of the FAS, and will consist of all randomized patients for whom no major protocol violation (no violation which may affect the study efficacy outcome) is documented. The PP analysis set will provide supportive data for the efficacy analyses.

Allocation of patients to the PP analysis set will be performed before unblinding of the study.

11.2.2 Methods of Statistical Analyses

11.2.2.1 General Principles

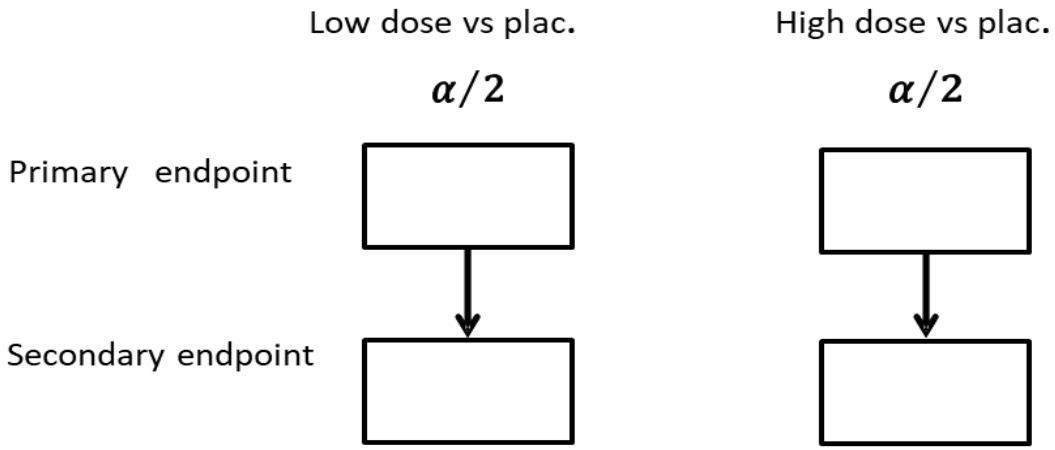
The primary objective of this study is to show superiority in efficacy of A4250 (120 µg/kg/day and 40 µg/kg/day) compared to placebo in patients with PFIC Types 1 and 2.

In the EU and RoW, for the primary efficacy variable fasting bile acid responder endpoint (at least a 70% reduction in fasting s-BA concentration or reaching a level ≤70 µmol/L at the end of treatment), the null hypothesis will be tested against the alternative hypothesis ($\alpha = 0.0125$, 1-sided).

The test will be performed at a Type I error rate of ≤0.0125 1-sided, as described above, based on the least squares means of the treatment difference in mean probability scale.

To strictly control overall Type I error rate across the dose groups and also across the primary and the main secondary endpoint to a 1-sided 2.5% level, a Bonferroni-based parallel gate-keeping procedure will be used. In this procedure the total significance level of 2.5% (1-sided) will be split equally between the dose groups. Only a dose group that is significant for the primary endpoint will be tested, at the nominal significance level of 1.25% (1-sided), for the main secondary endpoint (here the pruritus endpoint). If the previous step was not successful, the analysis of the following step will be considered descriptive. If the procedure reaches the secondary endpoint for both dose groups, these will be evaluated using the Bonferroni Holm procedure. The multiple comparison procedure is presented in Figure 2.

Figure 2 Bonferroni Holm Multiple Comparison Procedure



In the US, for the primary efficacy variable pruritus score endpoint (change from baseline to Week 24 in observer-reported scratching), the null hypothesis will be tested against the alternative hypothesis ($\alpha=0.0125$, 1-sided).

The test will be performed at a Type I error rate of ≤0.0125 1-sided, as described above, based on the least squares means of the treatment difference in mean change.

To strictly control overall Type I error rate across the dose groups and also across the primary and the main secondary endpoint to a one-sided 2.5% level, a Bonferroni-based parallel gate-keeping procedure will be used. In this procedure the total significance level of 2.5% (1-sided) will be split equally between the dose groups. Only a dose group that is significant for the primary endpoint will be tested, at the nominal significance level of 1.25% (1-sided), for the main secondary endpoint (here the bile acid responder endpoint). If the previous step was not successful, the analysis of the following step will be considered descriptive. If the procedure reaches the secondary endpoint for both dose groups, these will be evaluated using the Bonferroni Holm procedure.

Other secondary and exploratory outcome variables shall provide supportive efficacy and safety information regarding the differences between the treatment groups. No adjustments will be performed for multiple dose comparisons when testing the other secondary and exploratory efficacy variables.

A more detailed statistical analysis plan (SAP) will be prepared and finalized before study data are locked and unblinded.

If not otherwise specified, the baseline value of a parameter is defined as the last assessment of that parameter on or prior to the first intake of study treatment date.

11.2.2.2 Missing Data

For efficacy variables, with repeated measures, a mixed model will be employed with no additional imputation for missing values, i.e., based on "observed cases", except where otherwise specified.

Follow-up and continued collection of efficacy data for patients who discontinue treatment will be made. These data will be included in the sensitivity analysis for primary endpoints estimating a treatment effect taking loss of effect after discontinuation into account. Because complete follow-up of all patients discontinuing treatment may not be possible, a method to replace missing data for patients who discontinue the study will be prespecified (in the SAP) taking into account loss of effect after discontinuation (for example multiple imputation).

No other replacement of missing values will be planned.

11.2.2.3 Demographic and Baseline Characteristics

Descriptive summaries of demographics and other baseline characteristics (including medical and surgical history) will be presented by treatment group and overall using the FAS.

Prior medication will be summarized by treatment group and overall using the safety analysis set.

11.2.2.4 Subject Disposition

The following will be summarized (by treatment group and overall where applicable):

- Patients enrolled (who signed the informed consent)
- Patients randomized
- Patients treated
- Patients completing the study/withdrawing early (including withdrawal reason)

Additionally, patients enrolled, included in the safety, in the FAS, and in the PP analysis set will be summarized by region.

11.2.2.5 Evaluation of Primary Efficacy Variables

The responder endpoint based on change in fasting bile acids will be analyzed in terms of the proportion of patients achieving the event of interest. Patients in need of rescue therapy, i.e., biliary diversion will be classified as non-responders after the biliary diversion.

Generalized linear mixed modelling (GLMM) will be used with a logistic link, to allow for repeated assessments over time. The model will include baseline value, treatment group, PFIC class, age class, region, visit, and visit*treatment group interaction. The treatment by visit interaction will remain in the model regardless of significance. Region will be included in the model as a random effect. The contrasts of primary interest will be the treatment difference at Week 24; also, the difference at Week 4, Week 8, Week 12, and Week 18 will be estimated and analyzed. An unstructured covariance matrix will be used to model the within-subject error. Another covariance structure may be chosen if the fit of this structure fails to converge. Model diagnostics checks will be made to ensure appropriate fit.

The primary analysis of the change from baseline in pruritus score (indexed by observer-reported scratching) will be analyzed using a mixed model repeated measures (MMRM) analysis. The contrast of primary interest will be treatment difference at Week 24 but also the differences at Week 4, Week 8, Week 12, and Week 18 will be estimated and analyzed. Baseline pruritus score will be included as covariate. The model will include baseline value, treatment group, PFIC class, age class, region, visit, and visit*treatment group interaction. The treatment by visit interaction will remain in the model regardless of significance. Region will be included in the model as a random effect and will be defined on country level. An unstructured covariance matrix will be used to model the within-subject error. Another covariance structure may be chosen if the fit of this structure fails to converge.

Non-parametric approach may be considered if more appropriate.

The primary analyses will be based on the FAS.

To assess the robustness of the primary efficacy analysis, the following analyses will be performed:

- Primary efficacy analysis using the PP set
- Analyses to investigate the influence of missing values, for example, by using pattern mixture models and tipping point analysis

Subgroup analyses will also be performed for each of the 3 age groups (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) and for patients who were not included in the previous Study A4250-003.

Several sensitivity analyses will be performed, such as the following:

- Cochran Mantel Haenzsel test stratified on PFIC class to compare the proportion in fasting bile acid responder endpoint at the end of treatment in the two A4250 dose groups to placebo group. Dropouts will be treated as non-responders
- Based on the primary analysis including the interaction age class*treatment
- Same as primary analysis in which the random factor region is considered as fixed factor. The region*treatment interaction will also be explored
- Same as primary analysis including the interaction PFIC*treatment

Other sensitivity analyses may be performed and will be detailed in the SAP.

11.2.2.6 Evaluation of Secondary and Exploratory Efficacy Variables

The change in the secondary and explorative endpoints such as s-BA, total bilirubin, ALT, growth and patient-reported itch severity will also be analyzed using the MMRM model as specified for the primary analysis. Nonparametric methods may be applied as an alternative approach for some endpoints, if appropriate. Change in growth (weight for age and BMI for age) will also be displayed using graphical presentations.

Proportions of responders (e.g., meaningful reduction in itch severity and scratching at Week 24) will be analyzed using the GLMM model as specified for the primary analysis.

Comparisons of the change from baseline at Week 24 in PedsQL between the treatment groups will be conducted using analysis of covariance. The model will include terms for baseline, PFIC class, age class, region, and treatment.

Undergoing biliary diversion surgery and/or listed for liver transplant, all-cause mortality, and liver decompensation events will mainly be analyzed by using descriptive statistics. Kaplan-Meier curves will be used when appropriate for time to event data.

Other exploratory variables (Global Symptom Relief, biochemical markers and measures of bile acid synthesis, Albireo PRO and ObsRO sleep parameters, PELD/MELD score, APRI score, and Fib-4 score) will be analyzed descriptively for categorical and continuous data, as applicable. For continuous data, the change from baseline will be analyzed in addition to the actual visit values. For categorical data, shift tables or frequency and percentages of patients will be presented as appropriate.

In addition, subgroup analyses will be performed for each of the 3 age classes (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years).

11.2.2.7 Evaluation of Safety Variables

Safety data will be analyzed using descriptive statistics and summaries by treatment group of SAEs, AEs, vital signs, clinical laboratory tests (hematology, clinical chemistry and urinalysis), and concomitant medication. Analyses will be performed using the safety analysis set.

Summaries of AEs (coded according to the Medical Dictionary for Regulatory Activities [MedDRA] system organ class [SOC] and MedDRA preferred term) will include the following:

- Overview of the incidence of TEAEs (TEAEs, drug-related TEAEs, TEAEs leading to study discontinuation, and treatment-emergent SAEs)
- TEAEs by SOC and preferred term
- Intensity of TEAEs by SOC and preferred term
- Drug-related TEAEs by SOC and preferred term
- TEAEs leading to study discontinuation by SOC and preferred term
- Treatment-emergent SAEs by SOC and preferred term

Concomitant medication use during the Treatment Period will be summarized by Anatomical Therapeutic Chemical (ATC) class and World Health Organization (WHO) preferred name.

Summaries of vital signs will be presented. For each visit, the actual results and the change from baseline, and the number and percentage of patients with potentially clinically significant (PCS) values observed post-baseline will be presented.

Summaries of clinical safety laboratory data will be presented. For each visit, the actual result and the change from baseline, and the number and percentage of patients with PCS values observed post-baseline will be presented.

Data listings will be provided for each patient for all safety parameters.

11.2.2.8 Compliance and Exposure

Exposure will be analyzed by calculating the number of days with exposure to study drug. Results will be presented by treatment group using the safety analysis set.

The percentage compliance will be described by treatment group and the number of patients with a compliance <80%, between 80% and 120%, and >120% will be presented based on the safety analysis set.

11.2.3 Blinded Analyses to Confirm Responder Definitions

A blinded analysis of Albireo ObsRO and PRO eDiary data will be performed after 50% or more of the planned sample has completed the Week 24 Follow-Up Period. The blinded analysis will be used to estimate a threshold of clinically meaningful change (i.e., responder definition) in Albireo ObsRO and PRO pruritus scores. The analysis will be performed by a group independent from both the study team and Albireo. Blinded data will be used for this analysis; data will be collapsed across treatment groups.

The analyses will include distribution- and anchor-based analyses to examine a responder definition for the Albireo PRO/ObsRO pruritus scores. Distribution-based analyses will include calculation of the SD and standard error of measurement for the baseline Albireo PRO and ObsRO scores. Anchor-based analyses will involve examining the degree of change on the Albireo PRO and ObsRO scores from baseline to Week 24 for patients who experience change in pruritus according to PGIC and symptom items. Details of this analysis will be included in a stand-alone SAP for assessing the measurement characteristics (reliability, validity, sensitivity to change, responder definition) of the Albireo PRO and ObsRO items.

11.2.4 Data Safety Monitoring Board

A DSMB consisting of sponsor-independent clinical and statistical experts will be established. The DSMB will periodically meet for the review of accumulating study data, including safety (AE and laboratory data) and liver values.

The DSMB will have access to unblinded data. The DSMB will make recommendations for the remaining part of the study (further details will be provided in the DSMB charter). The DSMB may recommend continuing with the study as planned or stopping the study early for safety reasons. The DSMB will submit its recommendations in writing to Albireo Medical/[redacted] who are responsible for responding to the recommendations of the DSMB and taking appropriate action. The investigators will only be informed by Albireo Medical[redacted] if the study requires a protocol amendment or is stopped. The DSMB may choose to make additional evaluations at any time if they feel this is warranted from a safety point of view.

The DSMB will act according to its own written standard operating procedure described in a charter and will prepare written minutes of its meetings. The charter of the DSMB will be stored in the trial master file. The DSMB will maintain records of its meetings and these will become part of the study file when the study is complete. In order not to disseminate unblinded data and to ensure that all staff involved in the conduct and final analysis of the study remain blind to the data, only the members of the DSMB and the unblinded DSMB statistician will have access to these data.

12 DIRECT ACCESS TO SOURCE DATA/NOTES

The investigator/institution shall provide direct access to source data/documents for study-related monitoring, audits, IEC/Institutional Review Board (IRB) review and regulatory inspection.

13 QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Conduct of the Study

Albireo/[redacted] shall implement and maintain quality control and quality assurance procedures with written standard operating procedures to ensure that the study is conducted and data are generated, documented, and reported in compliance with the protocol, ICH GCP and applicable regulatory requirements.

This study shall be conducted in accordance with the provisions of the Declaration of Helsinki (October 1996) and all revisions thereof, and in accordance with United States Food and Drug Administration (FDA) regulations (Code of Federal Regulations, Sections 312.50 and 312.56) and with ICH GCP (CPMP 135/95).

The investigator may not deviate from the protocol without a formal protocol amendment having been established and approved by an appropriate IEC/IRB, except when necessary to eliminate immediate hazards to the patient or when the change(s) involve(s) only logistical or administrative aspects of the study. Any deviations may result in the patient having to be withdrawn from the study and render that patient non-evaluable.

13.2 Study Monitoring

Before study initiation, at a site initiation visit or at an investigator's meeting, an Albireo representative or designee will review the protocol and eCRF with the investigators and the investigative staff. During the study, the clinical monitor (clinical research associate [CRA]) will visit the site regularly to check the completeness of patient records, the accuracy of entries on the eCRFs, the adherence to the protocol and to GCP, the progress of enrollment, and to ensure that study drug is being stored, dispensed, and accounted for according to specifications.

The investigator must ensure that eCRFs are completed within a timely period of patient visits, as per individual site agreements, and must allow the CRA and Albireo representative or designee periodic access to patient records and all study-related materials, including relevant hospital or clinical records, to confirm their consistency with the eCRF entries. No information in these records about the identity of the patients will leave the study center. Albireo monitoring standards require full verification for the presence of the signed ICF, adherence to the inclusion/exclusion criteria, documentation of SAEs, and recording of primary efficacy and safety variables. The CRA will review source data compared with the eCRFs and will verify source data according to the study-specific monitoring plan. The design of the study, the frequency of patient visits, and the site enrollment rate will determine the frequency of monitoring visits. Upon study completion, the CRA will visit the site to conduct a study termination visit, which will include collection of any outstanding documentation.

It is recommended that the investigator/investigative staff log into the eCRF system every working day or at minimum twice weekly in order to provide a prompt response to queries.

The investigator/investigative staff should respond to queries and make any relevant changes to the study data within 3 working days.

14 ETHICS

14.1 Independent Ethics Committee/Institutional Review Board

Prior to the start of the study, the investigator is responsible for ensuring that the protocol and consent form have been reviewed and approved by a relevant IEC/IRB. The IEC/IRB shall be appropriately constituted and perform its functions in accordance with FDA ICH GCP and local requirements as applicable.

The IEC/IRB shall approve all protocol amendments (except for logistical or administrative changes), written informed consent documents and document updates, patient recruitment procedures (e.g., advertisements), written information to be provided to the patients and caregivers, IB, available safety information, information about payment and compensation available to patients, the investigator's curriculum vitae and/or other evidence of qualifications and any other documents requested by the IEC/IRB and regulatory authority (competent authority) as applicable.

14.2 Written Informed Consents and Assents

The investigator/investigative staff will explain to each patient (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, alternative treatment, the potential risks and benefits involved, and any discomfort that may occur. Each caregiver/patient will be informed that participation in the study is voluntary and that he/she or their child may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

Caregivers/patients will be informed that they/their children may experience side-effects or be at risk for symptoms, illnesses, or complications that cannot be foreseen by Albireo. As with other medications, people treated with A4250 may be at risk of developing allergic reactions or anaphylaxis. Caregivers/patients will be advised that study procedures include regular blood sampling for measurement of safety parameters and biological markers and that some minor risks are associated with these procedures.

This informed consent should be given by means of a signed ICF, written in non-technical language in accordance with ICH GCP, the Declaration of Helsinki, and regulatory authorities. The caregiver/patient should read and consider the statements before signing and dating them and should be given a copy of each signed document. If written consent is not possible, oral consent can be obtained if witnessed and followed by a signed statement from one or more persons not involved in the study, indicating why the patient was unable to sign the form. No patient can enter the study before his/her or caregiver informed consent has been obtained.

The ICF is part of the protocol and must be submitted by the investigator/investigative staff with the protocol for IEC approval. Albireo will supply an ICF which complies with regulatory requirements and is considered appropriate for the study. Any changes to the ICF

suggested by the investigator must be agreed to by Albireo before submission to the IEC and a copy of the approved version must be provided to the clinical monitor after IEC approval.

15 DATA HANDLING AND RECORD KEEPING

15.1 Case Report Forms/Source Data Handling

The investigator shall be provided with standardized eCRFs and shall ensure that all data from patient visits are promptly entered into the eCRFs in accordance with the specific instructions given. The investigator must sign each eCRF to verify the integrity of the data recorded.

A list of the normal ranges for all laboratory tests to be undertaken forms part of the documentation to be collated prior to study start. As a central laboratory has been selected to conduct any or all tests, it is essential that all samples be analyzed at that laboratory.

The investigator must maintain source documents such as laboratory reports, consultation reports, and complete medical history and physical examination reports.

As no ethnicity data can be collected in the source document for study sites in France, the eCRF will be the Source Document for “origines ethniques”,

15.2 Retention of Essential Documents

Essential documents, as defined by ICH GCP, include: the signed protocol and any amendment(s); copies of the completed eCRFs (for site archiving, compact discs of eCRF data for specific patients will be provided); signed ICFs; hospital records and other source documents; IEC approvals and all related correspondence including approved documents; drug accountability records; study correspondence; and a list of patients’ names and addresses.

The investigator/investigative staff must retain copies of these essential documents for the period specified by ICH GCP and by applicable regulatory requirements. The investigator/investigative staff will inform Albireo of the location where the essential documents are stored and must contact Albireo for approval before disposing of any essential documents. The investigator/investigative staff should take measures to prevent accidental or premature destruction of these documents.

16 FINANCING AND INSURANCE

Financing and insurance are addressed in a separate agreement.

17 PUBLICATION POLICY

Albireo will retain the ownership of all data. When the study is complete, Albireo shall arrange the analysis, tabulation of data and preparation of a clinical study report. Albireo may also use the data for publication, presentation at scientific meetings and submission to regulatory authorities. All proposed publications based on this study must be subject to the sponsor's approval requirements.

18 REFERENCE LIST

1. A4250 Investigator's Brochure.
2. Alissa FT, Jaffe R, Shneider BL. Update on progressive familial intrahepatic cholestasis. J Pediatr Gastroenterol Nutr. 2008;46(3):241-52.
3. Davit-Spraul A, Gonzales E, Baussan C, et al. Progressive familial intrahepatic cholestasis. Orphanet J Rare Disease 2009;4:1-12.
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5. Hori T, Justin H, Nguyen JH and Uemoto S. Progressive familial intrahepatic cholestasis. Hepatobiliary pancreat Dis Int. 2010; 9(6):570-8.
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7. Miethke AG, Zhang W, Simmons J, et al. Pharmacological inhibition of ASBT changes bile composition and blocks progression of sclerosing cholangitis in mdr2 knockout mice. Hepatology. 2016; 63(2):512–23.
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9. Pawlikowska L, Strautnieks S, Jankowska I, et al. Differences in presentation and progression between severe FIC1 and BSEP deficiencies. J Hepatol. 2010;53(1):170-8.
10. Suchy FJ, Sokol RJ, Balistreri WF, eds. Liver Disease in Children. 3rd Edition. New York, NY: Cambridge University Press; 2007:310–4.
11. Whitington PF, Whitington GL. Partial external diversion of bile for the treatment of intractable pruritus associated with intrahepatic cholestasis. Gastroenterology. 1988;95(1):130-6.

19 APPENDICES

Appendix 1 Concomitant Medications Prohibited During the Study

Appendix 2 Diary Questions including Albireo Patient-Reported Outcomes/Observer-Reported Outcome Instrument

Appendix 3 Patient Global Impression of Change (PGIC) and Patient Global Impression of Symptoms (PGIS)

Appendix 4 Pediatric Quality of Life Inventory (PedQL) Questionnaire

CLINICAL STUDY PROTOCOL A4250-005

A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

IND Number:	130591
Text Product:	A4250
Indication:	Progressive Familial Intrahepatic Cholestasis (PFIC)
Sponsor:	Albireo AB
Development Phase:	Phase 3
Sponsor Signatory:	[redacted]
Sponsor Medical Monitor:	[redacted]
Principal Investigator:	[redacted]
Date of the Protocol:	20 September 2017
Version of the Protocol:	Final

Confidential

The information in this document contains trade secrets and commercial information that are privileged or confidential and may not be used, divulged, published, or otherwise disclosed without the written consent of Albireo AB. These restrictions on disclosure apply equally to all future information supplied to you or generated by you in connection with the study.

SPONSOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

Albireo AB

[redacted] Date (day/month/year)

CLINICAL RESEARCH ORGANIZATION SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

[redacted]

[redacted] Date (day/month/year)

[redacted] Date (day/month/year)

INVESTIGATOR SIGNATURE PAGE

PROTOCOL TITLE: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

PROTOCOL NUMBER: A4250-005

I have read this protocol and agree that it contains all necessary details for performing this study. I will conduct the study as outlined herein and will complete the study within the time designated, in accordance with all stipulations of the protocol and in accordance with Good Clinical Practices (GCPs), local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study drug and the conduct of the study.

I will use only the informed consent approved by the Independent Ethics Committee (IEC) and will fulfil all responsibilities for submitting pertinent information to the IEC responsible for this study.

I agree that the sponsor (Albireo AB) shall have access to any source documents from which case report form information may have been generated.

I further agree not to originate or use the name of Albireo AB, A4250 in any publicity, news release, or other public announcement, written or oral, whether to the public, press, or otherwise, relating to this protocol, to any amendment to the protocol, or to the performance of this protocol without the prior written consent of Albireo AB.

[redacted]

Signature

Date (day/month/year)

1 ADMINISTRATIVE INFORMATION

A Double-blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Protocol No.:	A4250-005
Date of the Protocol:	20 September 2017
Date and Number of Amendment(s):	Not applicable
Sponsor:	Albireo AB Arvid Wallgrens backe 20 413 46 Göteborg, Sweden
Clinical Research Organization:	[redacted]
Sponsor Signatory:	[redacted]
Sponsor Medical Monitor:	[redacted]
Principal Investigator:	[redacted]
Clinical Laboratory:	[redacted]

2 STUDY SYNOPSIS

Name of Sponsor/Company: Albireo	**Individual Study Table Referring to Part of the Dossier:**	**(For National Authority Use Only)**
Name of Product: A4250		
Name of Active Ingredient: ((Insert name))	**Volume:** **Page:**	

Title of Study:
A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC 1)

Principal Investigator:
[redacted]

Study Center(s):
Up to 45 sites will be initiated for this study in the United States (US), Canada, Europe (EU), Middle East, and Australia.

Publication(s):
None.

Planned Study Period:	**Development Phase:**
Q4 2017 to Q2 2019	Phase 3

Objectives:

Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with PFIC Types 1 and 2, as determined by the following:

- Proportion of patients experiencing at least a 70% reduction in fasting s-BA levels or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- Change from baseline to Week 24 in pruritus compared to placebo

Secondary Objectives

- To evaluate the effect of A4250 based on alanine aminotransferase (ALT)
- To evaluate the effect of A4250 on insulin-like growth factor 1 (IGF-1) levels
- To evaluate the effect of A4250 on pruritus-related sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion and liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

Methodology:

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with progressive familial intrahepatic cholestasis (deficiencies of familial intrahepatic cholestasis -1 (FIC1) or bile salt export pump (BSEP).

The study includes a 14- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-up Period. After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo. All patients completing Day 168 (Visit 9) in this study will be invited to participate in a 72-week open-label extension study (A4250-008) in which all patients will receive active treatment.

There will be 10 visits during the study; 4 of them can be performed, where permitted by local regulations, by a study nurse visiting the patient at home, and 1 scheduled telephone contact.

Name of Sponsor/Company: Albireo	**Individual Study Table Referring to Part of the Dossier:**	**(For National Authority Use Only)**
Name of Product: A4250		
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Number of Patients:
Approximately 60 patients diagnosed with PFIC-1 or PFIC-2 will be enrolled.

Diagnosis and Main Criteria for Inclusion:

1. A male or female patient, with clinical diagnosis of PFIC 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg
2. Patient must have genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated serum bile acid (s-BA) levels, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples 7 to 28 days apart (Visits 1 and 2) prior to randomization
4. Patient must have history of significant pruritus as indexed by caregiver-reported observed scratching in the eDiary, average ≥2 (on 0 to 4 scale) in the week prior to randomization
5. Patient and/or legal guardian must sign informed consent (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients are expected to have a consistent caregiver(s) for the duration of the study
7. Caregivers and age-appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

Test Product, Dose and Mode of Administration:
A4250, 40 µg/kg/day or 120 µg/kg/day, orally administered

Reference Therapy, Dose and Duration of Administration:
Matching placebo, orally administered

Duration of Treatment:
24 weeks

Variables

Efficacy:

Primary Efficacy Endpoints

The primary efficacy endpoints are:

- **EU and rest of world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting serum bile acid (s-BA) levels from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo.

  Fasting s-BA baseline will be calculated as the average of the value at randomization and the previous screening (Visit 1). The end value is the average of the values at Weeks 22 and 24 after the start of double-blind treatment.
- **US**: Change in pruritus, indexed as caregiver-reported observed scratching score from baseline to Week 24 as measured by the average daily score of the ObsRO instrument. The average daily score will be calculated using the 7 days pre-treatment for baseline, and the last 7 days of treatment for Week 24 for the end value.

Secondary Efficacy Endpoints

Secondary efficacy endpoints include the following:

- **EU and RoW**: Change in pruritus, as indexed by caregiver-reported observed scratching score as measured by the average daily score of the Albireo ObsRO instrument compared to placebo. The average daily score will be calculated using the 7 days pretreatment for baseline, and the last 7 days of

Name of Sponsor/Company: Albireo	Individual Study Table Referring to Part of the Dossier:	(For National Authority Use Only)
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treatment for Week 24.

- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA levels or reaching a level ≤70 µmol/L from baseline to the end of treatment compared to placebo.

All Regions:

All secondary endpoints will be compared to placebo.

- Change from baseline to Week 24 in fasting s-BA
- Change from baseline to Week 24 in patient-reported itch severity as measured by the average daily score of the Albireo PRO instrument (only patients ≥8 years of age will complete the PRO instrument).
- Change from baseline to Week 24 in ALT
- Change from baseline to Week 24 in IGF-1
- Proportion of patients achieving meaningful reduction in caregiver-reported observed scratching at Week 24
- Change from baseline to Week 24 in sleep parameters measured with the Albireo PRO and ObsRO instruments
- Number of patients undergoing biliary diversion surgery or being listed for liver transplantation
- Change from baseline to Week 24 in PedsQL

Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Proportion of patients achieving meaningful reduction in patient-reported itch severity at Week 24
- Assessment of global symptom relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via the patient global impression of change (PGIC) items
- Change from baseline in fasting s-BA at Weeks 4 and 12
- Change from baseline in patient-reported itch severity and observer-reported scratching at Weeks 4 and 12
- Change from baseline to Week 24 in patient-reported and observer-reported night time itching and scratching severity scores, respectively
- Change from baseline to Week 24 in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline to Week 24 in additional patient-reported and observer-reported sleep parameters (e.g., tiredness, number of awakenings)
- Change from baseline in ALT at Weeks 4 and 12
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, p-C4, LDL-C) at Week 24
- Change in PELD/MELD score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Liver-related mortality and liver decompensation events.
- All-cause mortality

Safety:

Safety criteria are as follows:

- The incidence of treatment-emergent SAEs, based upon information from patient reports, including the

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description, incidence, and severity of an SAE

- Assessments based upon information in patients' diary reports as reviewed by the study nurse and on data collected at the study visits
- Occurrence of treatment-emergent AEs (TEAE) including severity and relatedness to study drug at all visits
- Physical examinations
- Concomitant medications at all visits
- Vital signs at all clinic visits
- Laboratory test results (including hematology, clinical chemistry, and urinalysis)

Statistical Methods:

The primary objective of this study is to show superiority in efficacy of A4250 (120 µg/kg/day and 40 µg/kg/day) compared to placebo in patients with PFIC Types 1 and 2.

In the EU and RoW, for the primary efficacy variable fasting bile acid responder endpoint (at least a 70% reduction in fasting s-BA levels or reaching a level ≤70 µmol/L at the end of treatment), the null hypothesis will be tested against the alternative hypothesis (α=0.025, 1-sided). Generalized linear mixed modelling (GLMM) will be used with a logistic link, to allow for repeated assessments over time. The model will include baseline value, treatment group, PFIC class, age class, region, visit and visit*treatment group interaction. The contrasts of primary interest will be the treatment difference at Week 24 but also the difference at Week 4, Week 8, Week 12, and Week 18 will be estimated and analyzed.

A fixed-sequence multiple testing approach will be used. In the first step, the high dose (120 µg/kg/day) versus placebo will be tested at 2.5% 1-sided significance level. If significant, the low dose (40 µg/kg/day) versus placebo will also be tested, in a second step, at 2.5% 1-sided significance level. The second step will only be considered confirmatory providing the previous step was successful.

In the US, for the primary efficacy variable pruritus score endpoint (change from baseline to Week 24 in observer-reported scratching), the null hypothesis will be tested against the alternative hypothesis (α=0.025, 1-sided). The primary analysis of the change from baseline in pruritus score (indexed by observer-reported scratching) will be analyzed using a mixed model repeated measures (MMRM) analysis. The contrasts of primary interest will be the treatment difference at Week 24 but also the difference at the other weeks will be estimated and analyzed.

In the first step, the high dose (120 µg/kg/day) versus placebo will be tested at 2.5% 1-sided significance level. If significant, the low dose (40 µg/kg/day) versus placebo will also be tested, in a second step, at 2.5% 1-sided significance level. The second step will only be considered confirmatory providing the previous step was successful.

Secondary and exploratory outcome variables shall provide supportive efficacy and safety information regarding the differences between the treatment groups. For secondary efficacy variables, supportive statistical tests will be made and *P*-values will be reported in an exploratory way (if not otherwise specified). No adjustments will be performed for multiple-dose comparisons when testing the secondary and exploratory efficacy variables.

Date of the Protocol: 20 September 2017

3 TABLE OF CONTENTS

3.1 List of Tables

3.2 List of Figures

3.3 List of Appendices

4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADME	absorption, distribution, metabolism, and excretion
AE(s)	adverse event(s)
AFP	alfa-fetoprotein
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
APRI	AST to platelet ratio index
ASBT	apical sodium-dependant bile transporter (also known as IBAT)
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
AUC	area under the curve
BMI	body mass index
BP	blood pressure
BRIC	benign recurrent intrahepatic cholestasis
BSEP	bile salt export pump
Cmax	maximum drug concentration
CRA	clinical research associate
CRF(s)	case report form(s)
CRO	clinical research organization
CRP	c-reactive protein
CYP450	cytochrome P450
DSMB	Data and Safety Monitoring Board
eCRF	electronic case report form
eDiary	electronic diary
EDTA	ethylenediaminetetraacetic acid
EOT	end of treatment
EU	European Union
EUDRACT	European Union drug regulatory agency clinical trial
EVCTM	Eudravigilance Clinical Trial Module
FAS	full analysis set
FDA	United States Food and Drug Administration
FIC1	familial intrahepatic cholestasis-1
GCP	Good Clinical Practice
GFR	glomerular filtration rate
GI	gastrointestinal
GLMM	generalized linear mixed modelling
HDPE	high-density polyethylene

HIV	human immunodeficiency virus
hsCRP	high sensitivity c-reactive protein
IB	Investigator's Brochure
IBAT	ileal bile acid transporter (also known as ASBT)
ICF	informed consent form
ICH	International Conference on Harmonisation
[redacted]	[redacted]
ICSR	individual case safety reports
IEC	Independent Ethics Committee
IGF-1	insulin-like growth factor 1
INR	international normalized ratio
IRB	Institutional Review Board
IWRS	Interactive Web Response System
LDL-C	low-density lipoprotein cholesterol
LFT(s)	liver function test(s)
MDR3	multidrug-resistant 3
MedDRA	Medical Dictionary for Regulatory Activities
MELD	model for end-stage liver disease
mFAS	modified full analysis set
MMRM	mixed model repeated measures
ObsRO	observer-reported outcome
PBC	primary biliary cholangitis
p-C4	serum 7α-hydroxy-4-cholesten-3-one concentration
PCS	potentially clinically significant
PD	pharmacodynamics(s)
PEBD	partial external biliary diversion
PedsQL	Pediatric Quality of Life Inventory
PELD	pediatric end-stage liver disease
PFIC	progressive familial intrahepatic cholestasis
PGIC	patient global impression of change
PGIS	patient global impression of symptoms
PP	per protocol
PRO	patient-reported outcome
PVM	pharmacovigilance manager
QoL	quality of life
RoW	rest of world
RR	respiratory rate

SAE(s)	serious adverse event(s)
SAP	statistical analysis plan
s-BA	serum bile acid
s-FGF19	serum fibroblast growth factor 19 concentration
s-IGF-1	serum insulin-like growth factor 1 concentration
SOC	system organ class
SST	serum separating tube
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment-emergent adverse events
ULN	upper limit of normal
US	United States
VAS	visual analogue scale
WHO	World Health Organization

5 INTRODUCTION

5.1 Investigational Medicinal Product

A4250 is a small molecule and a potent selective inhibitor of the ileal bile acid transporter (IBAT), also known as the apical sodium-dependent bile acid transporter (ASBT). IBAT is an integral brush border membrane glycoprotein that co-transports sodium and bile acids and appears to be a critical component in the regulation of the bile acid pool size in animals and man. This transporter, expressed in the distal ileum, is a key element in the enterohepatic circulation of bile acids since it facilitates the high-affinity, high-capacity reabsorption of bile acids. Indeed, 95% of secreted bile acids are reabsorbed via IBAT [Hofmann 2009; Miethke 2016]. A4250 is orally administered and acts locally in the gut where it binds reversibly to IBAT to decrease the reuptake of bile acids. A4250 has minimal systemic exposure at the predicted therapeutic dose ranges.

No safety signals were found with A4250 in preclinical safety and toxicity studies. In the first clinical study of A4250 in healthy volunteers, A4250 was given as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. A4250 was well tolerated and the only findings were gastrointestinal (GI) related effects, such as diarrhea, at higher doses.

In an exploratory Phase 2 study in children with cholestatic pruritus, A4250 was given as oral doses of 0.01 to 0.2 mg/kg/day for 4 weeks. A4250 reduced mean levels of serum bile acids (s-BA) in all cohorts. In 11 of 13 progressive familial intrahepatic cholestasis (PFIC) patients, substantial reductions (ranging from 43% to 98%) were observed. Patient reported diary data documented improvement of pruritus during treatment. A4250 was well tolerated. No serious adverse events (SAEs) were deemed to be related to study drug, and most adverse events (AEs), including some increased transaminases, were considered unrelated to study drug and transient in nature.

5.2 Background

5.2.1 Progressive Familial Intrahepatic Cholestasis

PFIC is a rare autosomal recessive cholestatic liver disease estimated to affect between 1 in every 50,000 to 100,000 children born worldwide. PFIC represents 10% to 15% of causes of cholestasis in children and 10% to 15% of liver transplantation indications in children. All types of PFIC exist worldwide and both sexes appear to be equally affected.

The common underlying pathogenesis of PFIC is disruption of bile formation and bile transport through the liver [Jacquemin 2000]. The classification of PFIC has evolved over the years. The most commonly used sub-classifications are PFIC Types 1, 2, and 3, which are based on the associated affected gene and described in more detail below.

- PFIC, Type 1: also referred to as “Byler disease” or “familial intrahepatic cholestasis-1 (FIC1) protein deficiency.” FIC1 protein is located on the canalicular membrane of hepatocytes and facilitates movement of aminophospholipids from the outer to inner leaflet of the plasma membrane of the hepatocyte. The *ATP8B1* gene encodes

FIC1 protein. Biallelic pathologic variants in the *ATP8B1* gene are associated with FIC1 dysfunction and classified clinically as PFIC Type 1 disease.

- PFIC, Type 2: also referred to as "Byler syndrome" or "bile salt export pump (BSEP) deficiency." BSEP is a transporter protein that is expressed at the canalicular membrane of hepatocytes, and is the primary exporter of bile acids. The *ABCB11* gene encodes the BSEP protein. Biallelic pathologic variations in the *ABCB11* gene are associated with BSEP dysfunction and classified clinically as PFIC Type 2 disease.
- PFIC, Type 3: is caused by a deficiency of the multidrug-resistant 3 protein (MDR3) due to mutations in the *ABCB4* gene. MDR3 is a phospholipid translocase critical for phospholipid secretion.

Severe pruritus is common in children diagnosed with PFIC. Itching (and subsequent scratching) is a significant morbidity for these patients and their families [Suchy 2007]. A more severe degree of pruritus is experienced compared to patients with other forms of liver disease and to other pruritic conditions such as atopic dermatitis [Murray 2011]. In patients with PFIC, liver biopsy reveals canalicular cholestasis and, later, the appearance of portal fibrosis. Serum biochemistry indicates cholestasis with hyperbilirubinemia, elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The concentrations of bile acids in serum are very high, while serum γ-GT activity (exception being MDR3 variants) and cholesterol are normal [Hori 2010]. Symptoms of portal hypertension and liver failure will develop during the course of the disease [Davit-Spraul 2009; Alissa 2008]. Symptoms develop early; median age at onset of symptoms is 2 months, and 78% of PFIC patients present with jaundice [Pawlikowska 2010]. The life-threatening and debilitating nature of PFIC is reflected by the fact that survival in those not resorting to surgery is 50% at 10 years of age and almost zero at 20 years of age. Approximately half of PFIC patients undergo liver transplantation [Davit-Spraul 2009] and treatment-resistant pruritus is the leading indication for the surgical procedure partial external biliary diversion (PEBD), mostly in PFIC Type 1 and 2 patients.

PFIC is life-threatening and debilitating. Currently, the treatment for PFIC is palliative and there is no pharmaceutical treatment approved for use in PFIC. The therapeutic choices are restricted to non-specific therapy of the symptoms and signs of the disease such as nutritional support, preventing vitamin deficiencies, and treatment of extrahepatic features. Medical treatment options include off-label use of ursodeoxycholic acid, rifampin, antihistamines, and naltrexone. A minority of patients respond nominally and transiently to these interventions. Biliary diversion is used to decrease systemic bile acids through interruption of the enterohepatic circulation and so avoid transplantation. Liver transplantation is typically only viewed as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

5.2.2 Summary of Clinical and Nonclinical Studies

This is a summary of nonclinical and clinical studies. More detail is provided in the Investigator's Brochure (IB; see A4250 IB).

A4250 is a potent, selective IBAT inhibitor with no safety signals seen in the safety pharmacological studies. In addition, no safety signals were seen in repeat-dose oral toxicity studies in rats with up to 26 weeks of daily dosing and in dogs with up to 39 weeks of daily dosing. No safety signals were identified when A4250 was administered daily to juvenile rats from age 14 days to 63 days.

A4250 is minimally absorbed with very low systemic exposure in both humans and other species. In blood, protein binding is high (>99.7%). Systemically absorbed A4250 is slowly metabolized in humans and in preclinical studies by hydroxylation only, a metabolite that retains IBAT activity. A4250 did not show any significant inhibition of cytochrome P450 (CYP450) enzymes in the rat. A4250 inhibited human CYP3A4 and CYP2D6 (IC50: 16 µmol/L for both enzymes), and CYP2C9 (IC50:1.2 µmol/L).

To date, A4250 has been studied in 3 Albireo-sponsored clinical studies: a study in healthy volunteers evaluating single and multiple administration of A4250; a single-dose absorption, distribution, metabolism, and excretion (ADME) study; and a Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with primary biliary cholangitis (PBC). In total, 98 subjects have been exposed to A4250.

In the first clinical study, A4250 was given to healthy volunteers as a single oral dose up to 10 mg or daily oral doses up to 3 mg/day for 7 days. Following a single oral dose of 10 mg, the median maximum drug concentration (C_{max}) was 0.2 nmol/L and the area under the curve (AUC) was 0.8 nmol × h/L (n=6). Pharmacodynamic (PD) evaluation of bile acid synthesis biomarkers (serum fibroblast growth factor 19 [s-FGF19] and plasma 7α Hydroxy 4-cholesten-3-one [p-C4]) identified the expected decrease in s-FGF19 and an increase in p-C4. In addition, total plasma bile acids decreased and total fecal bile acids increased in A4250-treated patients compared to placebo. A4250 was generally well tolerated. The only findings were GI-related effects, such as diarrhea at higher doses.

In the ADME study, healthy subjects were administered a single oral dose of 3 mg [^{14}C]-A4250 capsule. The main excretion route was determined to be fecal. There were no quantifiable concentrations of A4250 in plasma or total radioactivity in plasma or whole blood. A4250 was well tolerated. AEs were reported and most were mild and not related to A4250. The 3 AEs experienced by 2 subjects reported as related to study drug were GI in nature, including diarrhea (2 AEs; 2 subjects) and abdominal pain (1 AE; 1 subject).

In an exploratory Phase 2 study in children with cholestatic pruritus, A4250 was administered as oral daily doses of 0.01 to 0.2 mg/kg for 4 weeks. Twenty patients (8 females, 12 males) aged 1 to 17 years were enrolled in this open-label dose-escalation study. Four patients were re-enrolled into a second dose level. No treatment-related SAEs were reported, and most

AEs, including some increased transaminases, were transient. Four-week, once-daily treatment with A4250 reduced mean levels of s-BA in all cohorts. Substantial s-BA reductions in 11 of 13 PFIC patients (ranging from 43% to 98%) were observed. A4250 also reduced s-BA in the 11 non-PFIC patients, but to a lesser extent than in PFIC patients. Patient-reported diaries documented improvement of the visual analogue scale (VAS)-itch score in 17 of 24 patients during treatment. The dose with the greatest improvement showed a mean decrease (±SEM) of 2.9 ±1.1 from baseline. The results from this trial form the basis of the current (A4250-005) study design.

5.3 Rationale

In this investigational study, A4250 is under development for treatment of PFIC and is also being considered for other orphan cholestatic liver diseases.

There is currently no medical treatment approved for PFIC. In PFIC patients with severe pruritus, biliary diversion surgery is used to decrease systemic bile acids through interruption of the enterohepatic circulation and thereby reducing pruritus [Whitington 1988]. Liver transplantation is typically used as an option when patients have failed medical treatment and/or biliary diversion and have liver failure or persistent uncontrolled pruritus.

By inhibiting IBAT with high selectivity and potency, A4250 has the potential to relieve cholestasis and improve liver function without surgical intervention in patients with PFIC. Since A4250 is minimally absorbed and achieves a substantial reduction of s-BA, it has the potential to be a safer and less invasive alternative to surgical biliary drainage. The rationale for using A4250 is to decrease s-BA levels, and to reduce the major morbidity of pruritus, improving the health and wellbeing of patients affected with PFIC. Ultimately, this noninvasive therapeutic approach has the potential to not only improve quality of life (QoL), but also reduce the need for biliary diversion and subsequent liver failure and liver transplantation.

The doses selected for this study, 40 and 120 µg/kg/day, were chosen based on the efficacy (reduction of s-BA and pruritus) and safety data generated from patients with PFIC and other types of cholestasis dosed in the exploratory Phase 2 study (A4250-003).

This study will be conducted in compliance with the protocol and with the International Conference on Harmonisation (ICH) guidelines on Good Clinical Practice (GCP).

Patients completing 24 weeks of double-blind treatment will be offered to enter Study A4250-008 and receive open-label treatment with A4250.

6 STUDY OBJECTIVES

6.1 Primary Objectives

To demonstrate the efficacy of repeated daily doses of 40 µg/kg/day and 120 µg/kg/day A4250 in children with PFIC Types 1 and 2, as determined by the following:

- The proportion of patients experiencing at least a 70% reduction in fasting s-BA levels or reaching a level ≤70 µmol/L compared to placebo after 24 weeks of treatment
- A change from baseline to Week 24 in pruritus compared to placebo

6.2 Secondary Objectives

- To evaluate the effect of A4250 based on ALT
- To evaluate the effect of A4250 on insulin-like growth factor 1 (IGF-1) levels
- To evaluate the effect of A4250 on pruritus-related sleep disturbance
- To evaluate the effect of A4250 on the need for surgical treatment (biliary diversion and liver transplantation)
- To assess the safety and tolerability of repeated daily doses of A4250 for 24 weeks

7 INVESTIGATIONAL PLAN

7.1 Overall Study Design and Plan

7.1.1 Description

This is a double-blind, randomized, placebo-controlled, Phase 3 study to investigate the efficacy and safety of 40 µg/kg/day and 120 µg/kg/day of A4250 compared to placebo in children with PFIC (deficiencies of FIC1 or BSEP).

Study data will be periodically reviewed by an unblinded Data Safety and Monitoring Board (DSMB).

The study includes a 14- to 56-day Screening Period followed by a 24-week Treatment Period and a 4-week Follow-Up Period.

After completion of the Screening Period, eligible patients (20 per treatment group) will be randomized on Day 0 (Visit 3) in a 1:1:1 fashion to receive 40 µg/kg/day or 120 µg/kg/day of A4250, or a matching placebo.

All patients completing Day 168 (Visit 9) in this study will be invited to participate in a 72-week open-label extension study (A4250-008) in which all patients will receive active treatment.

There will be 10 visits during the study (Figure 1); 4 of them can be performed, where permitted by local regulations, by a study nurse visiting the patient at home, and 1 scheduled telephone contact, as follows:

- Visit 1: Screening Period start. Patient is considered enrolled after signing the informed consent form (ICF) (Days -56 to -14)
- Visit 2: Screening Visit 2 (Days -28 to -7)
- Visit 3: Randomization Visit (Day 0)
- Telephone Contact 1: (Day 14)
- Visit 4: Clinic Visit (Day 28)
- Visit 5: Clinic or Home Visit (Day 56)
- Visit 6: Clinic Visit (Day 84)
- Visit 7: Clinic or Home Visit (Day 126)
- Visit 8: Clinic or Home Visit (Day 154)
- Visit 9: Clinic Visit, End of Treatment (Day 168)
- Visit 10: Follow-Up Visit, 28 days after last dose of study drug, or if a patient discontinues prematurely (Day 196). Patients continuing in Study A4250-008 will not have this follow-up visit.

Additional clinical visits may be required for patients who need direct site assistance, e.g., due to AE monitoring, to fulfil screening requirements, and/or for safety maintenance.

Screening Period

As soon as a patient is considered for this study and prior to any other study procedures being conducted by the investigator/investigative staff, the caregiver/patient will have the nature of the study explained to him/her and be asked to sign an ICF. After signing the form, patients will be evaluated for study eligibility. Informed consent must be obtained prior to any study procedures that do not form a part of the patient's normal care.

Prior to entering the Screening Period, patients who complete the consenting process and require medication washout will have the appropriate washout period for prohibited medications (see Appendix 1) before the screening visit (Clinic Visit 1) is performed. This visit can be conducted immediately for patients who have signed the ICF and do not require drug washout.

If the patients have had a liver biopsy performed the results from the biopsy should be recorded in the electronic case report form (eCRF). Daily recording of pruritus using an electronic diary (eDiary) to determine confirmation of the magnitude and the baseline pruritus symptoms before the study will be started. Patients failing inclusion due to laboratory values or pruritus scores, as indexed by caregiver-reported observed scratching, can be rescreened after 2 months. Patients not fulfilling inclusion/exclusion criteria after 3 attempts are not allowed to rescreen.

Caregivers/patients will be instructed on the daily use of the eDiary (see Appendix 2). The eDiary will include patient-reported outcome (PRO) and observer-reported outcome (ObsRO) items for evaluation of itching (PRO), scratching (ObsRO), and sleep disturbance (PRO and ObsRO) during the Screening Period (Study Days -56 to 0) and throughout the 24-week Treatment Period (Study Days 0 to 168). Additionally, caregivers/patients will be requested to use the diary to report the date and time each dose of study drug is administered during the Treatment Period.

There will be a ±42 day window for the Screening Period; therefore, up to 56 days of diary entries are possible. However, only the most recent 7 days before randomization will be used for eligibility and baseline calculations. Observer-reported outcomes in patients of all ages will be recorded by a caregiver (or caregiver's designee). If possible, the same caregiver will complete the ObsRO items throughout the study. If there is more than one primary caregiver (e.g., if parents are divorced and share custody), multiple caregivers will be included in the initial training.

At a second screening visit (Visit 2) that can be performed at the clinic or in the patient's home, additional laboratory samples will be taken for eligibility assessments.

Treatment Period

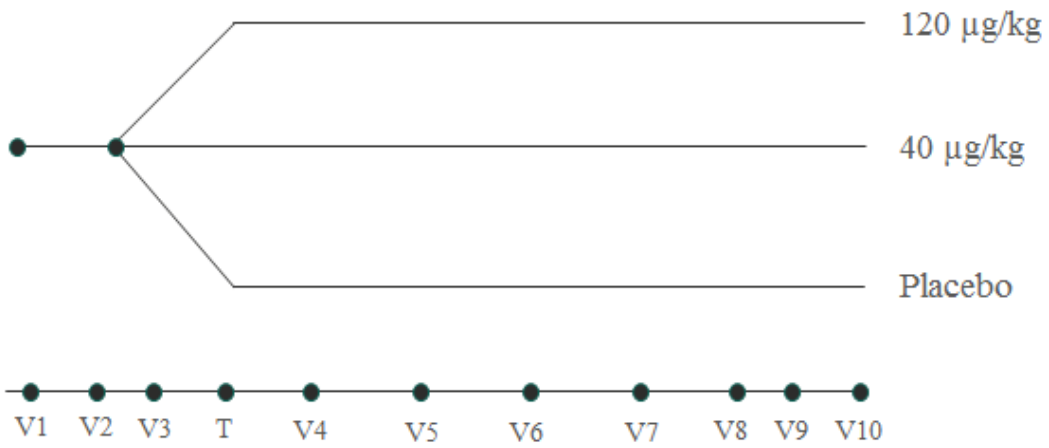
Eligibility for enrollment will be determined at Visit 3 using the pruritus (scratching reports from the ObsRO) data in the week before Visit 3 and the liver biochemistry evaluations from the 2 previous screening visits. Two weeks after Visit 3, there will be a telephone contact with the clinic to evaluate any AEs.

The patient will return to the clinic at Visit 4, Visit 5, Visit 6, Visit 7, Visit 8, and Visit 9 for assessments. Visit 5, Visit 7, and Visit 8 may, alternatively, be performed in the patient's home by a study nurse, if permitted by local regulations. If a new caregiver will begin to complete the ObsRO during the course of the trial, they will be provided with training during a clinic visit before they begin to use the eDiary.

The last dose of study drug will be administered the day before Visit 9. At Study Visit 9, the patient has the option to enroll in an open-label extension study (A4250-008). If the patient is continuing into Study A4250-008 this will be the last visit in this study and the first visit in Study A4250-008. This visit should also be performed if the patient is prematurely withdrawn from the study.

Clinic Visit 10 will take place 28 days after Visit 9 for patients not continuing into Study A4250-008. All patients prematurely withdrawn will have this visit 28 days after the last dose of study drug.

Figure 1 Study Design



T: telephone contact; V: study visit

7.1.2 Schedule of Assessments

The schedule of assessments is presented in Table 1.

7.1.3 Schedule of Blood Samples

The schedule and volumes of blood samples are presented in Table 2.

Table 1 Schedule of Assessments

Study Activity	Screening	Screening	Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-14) ±2[a]**	**-28 – (-7) ±2[a]**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic (or Home) Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic (or Home) Visit 5**	**Clinic Visit 6**	**Clinic (or Home) Visit 7**	**Clinic (or Home) Visit 8**	**Clinic Visit 9/EOT[b]**	**Clinic Visit 10[c]**
Informed consent[d]	X										
Inclusion/exclusion criteria	X		X								
Demographics	X										
Concomitant medication documented[e]	X	X	X	X	X	X	X	X	X	X	X
Medical and surgical history	X		X								
Blood sampling for genetic testing[f]	X										
Physical examination[g]	X		X		X		X			X	X
Vital signs[h]	X	X	X		X	X	X	X	X	X	X
eDiary: itching, scratching, and sleep scores[i]	X	X	X	X	X	X	X	X	X	X	X
Clinical chemistry and hematology tests[j]		X	X		X	X	X	X		X	X
Serum bile acids	X	X	X		X	X	X	X	X	X	X
INR		X			X		X		X		X
Growth marker (s-IGF-1)			X							X	

Study Activity	Screening	Screening	Treatment Period								Follow-Up
			Random-ization	Phone Contact	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	
Study Days	**-56 – (-14) ±2[a]**	**-28 – (-7) ±2[a]**	**0**	**14 ±3**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**
Visits	**Clinic Visit 1**	**Clinic (or Home) Visit 2**	**Clinic Visit 3**	**n/a**	**Clinic Visit 4**	**Clinic (or Home) Visit 5**	**Clinic Visit 6**	**Clinic (or Home) Visit 7**	**Clinic (or Home) Visit 8**	**Clinic Visit 9/EOT[b]**	**Clinic Visit 10[c]**
Biochemical markers[k]		X[l]	X[m]		X					X	
Liver ultrasonography			X							X	
QoL questionnaire (PedsQL)			X							X	
Patient/Caregiver/Clinician Global Impression of Change			X		X		X			X	
Patient/Caregiver/Clinician Global Impression of Symptoms			X		X		X			X	
Pregnancy test[n]	X	X	X		X	X	X	X		X	X
Study drug dispensed[o]			X		X	X	X	X	X		
AEs documented	X	X	X	X	X	X	X	X	X	X	X
Study drug compliance				X	X	X	X	X	X	X	

AE: adverse event; BMI: body mass index; BP: blood pressure; CRP: c-reactive protein; ICF: informed consent form; INR: international normalized ratio; LDL-C: low-density lipoprotein cholesterol; LFT: liver function test; PedsQL: Pediatric Quality of Life Inventory; RR: respiratory rate; s-IGF-1: serum insulin-like growth factor 1 concentration.

a Blood samples will be collected at both screening visits. A duration of 28 days is required between visits for small children <10 kg, 14 days for children 10 to 15 kg, and 7 days for children ≥15 kg. Thus, the total Screening Period will be 14 to 56 days.

b Must also be performed at the time a patient is prematurely withdrawn from the study. This visit is the same as Visit 1 in the extension protocol (Study A4250-008).

c Assessments must be performed 28 days following premature withdrawal (28 days following the final dose of study drug). This visit is omitted if patients are entering Study A4250-008.

d Adverse event information will be collected from the time of enrollment (signing of the informed consent) to study discontinuation. AEs that occur after enrollment and prior to randomization will be considered non-treatment emergent AEs. The ICF must be signed before any medication withdrawal required by the protocol is

performed. The consent may therefore be signed before Visit 1.

e Includes past (within 1 month prior to randomization) and current medication. Specific question regarding stopping or reducing anti-pruritus treatment.

f If unable to genetically confirm PFIC due to inaccessible or unavailable clinical genetic testing results, screening for pathologic variations of the *ATP8B1* and *ABCB11* genes will be performed at the sponsor's discretion if patient meets inclusion and exclusion criteria and after sponsor review.

g At Visits 1, 3 and 9, a complete physical examination will be performed. At Visits 4, 6 and 10, a skin exam (only) will be conducted.

h Includes BP (measured after the patient has been in a seated position for ≥3 minutes of rest), pulse, RR, and temperature. Height and body weight will be measured using a certified weight scale. BMI will be calculated.

i Includes training on use of the device at screening (Visit 1) and review of patient daily entries during 7 days prior to clinic Visit 3 to determine eligibility.

j Laboratory parameters include: CRP, hematology, clinical chemistry (including LFTs) and urinalysis assessments. Blood will be drawn predose from patients in a fasted state for ≥4 hours for assessments to be performed in the morning.

k Samples are taken for the analysis of alfa-fetoprotein and the pharmacodynamic biochemistry markers (autotaxin, p-C4, and LDL-C).

l Only alfa-fetoprotein and LDL-C.

m Only autotaxin and p-C4.

n For girls who have reached menarche. Serum test at Visit 2; urine test at other visits. A serum pregnancy test will also be conducted at Visit 9 for patients entering Study A4250-008.

o Study drug to be taken once daily on Day 1 through Day 168 as described in Section 8.2.

Table 2 Schedule and Volumes of Blood Sampling

	Screening	Screening	Randomization	4 weeks	8 weeks	12 weeks	18 weeks	22 weeks	24 weeks	FU	Total blood volume
Study Days	**-56 – (-14) ±2**	**-28 – (-7) ±2**	**0**	**28 ±3**	**56±3**	**84±3**	**126 ±3**	**154 ±3**	**168 ±3**	**196 ±3**	
Study Visits	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8**	**Visit 9/EOT**	**Visit 10**	
Hematology test (EDTA)		1	1	1	1	1	1		1	1	8
Clinical chemistry test (SST)[a]		2.5	2.5	2.5	2.5	2.5	2.5		2.5	2.5	20
Pregnancy		0									0
INR		2		2		2		2		2	10
Serum bile acid	2.5	0	0	0	0	0	0	2.5	0	0	5
Blood sample for genetic testing	3										3
Serum growth marker (IGF-1)			2						2		4
Autotaxin			1[a]	1[a]					1[a]		3
p-C4 (7α-hydroxy-4-cholesten-3-one)			2[a]	2[a]					2[a]		6
hsCRP		0	0	0	0	0	0		0	0	0
LDL-C		0		0					0		0
Alfa-fetoprotein (AFP)		0		0					0		0
Total amount of blood for children >5 kg	5.5	5.5	5.5	5.5	3.5	5.5	3.5	4.5	5.5	5.5	50
Total amount of blood for children >10 kg	5.5	5.5	8.5	8.5	3.5	5.5	3.5	4.5	8.5	5.5	59

Note: All blood volume presented in mL. All samples of 0 mL are included in the clinical chemistry test (SST).

CRP: c-reactive protein; EDTA: ethylenediaminetetraacetic acid; EOT: end of treatment; FU; Follow-up; INR: international normalized ratio; SST: serum-separating tube

a Not collected for children ≤ 10 kg.

7.1.4 Study Procedures and Assessments

7.1.4.1 Screening Period (Days -56 to 0)

Day -56 to Day -14/Clinic Visit 1

Patients will undergo a screening visit up to 56 days prior to the planned first day of study treatment. Screening procedures and assessments are as follows:

- Obtain written informed consent
- Assess inclusion/exclusion criteria (Section 7.3)
- Record demographics (age, gender, race)
- Document concomitant medications
- Medical and surgical history (date of diagnosis of PFIC Types 1 or 2, historical liver function test [LFT] values, any surgery performed, any other diagnosis, historical biopsy data, and date of menarche where applicable for female subjects)
- Blood sample for genetic testing
- Perform or collect past confirmatory clinical genetic laboratory report for PFIC1/PFIC2 and send to central reader for review. If clinical confirmatory genetic testing results are not attainable, contact sponsor for consideration
- Physical examination (Section 10.2.5) and vital signs (Section 10.2.6)
- eDiary training and compliance requirements; patients begin daily recording of pruritus using the eDiary (Appendix 2)
- s-BA (Section 9.2.1)
- Urine pregnancy test for girls of who have reached menarche
- AE monitoring

Day -28 to Day -7/Clinic or at-Home Visit 2

A second screening visit, which can take place either at the clinic or by a study nurse in the patient's home, will be performed. Since blood samples will be collected at both screening visits, a minimum duration of 28 days is required between visits for small children <10 kg, 14 days for children 10 to 15 kg, and 7 days for children ≥15 kg. Thus, the total Screening Period could range from 14 to 56 days. Screening procedures and assessments are as follows:

- Review concomitant medications
- PRO/ObsRO eDiary review for itching, scratching, and sleep scores and compliance
- Clinical chemistry and hematology tests (Table 4)
- s-BA (Section 9.2.1)
- International normalized ratio (INR)

- Biomarkers (alfa-fetoprotein [AFP] and LDL-C)
- Serum pregnancy test for girls of who have reached menarche
- AE monitoring

7.1.4.2 Treatment Period (Day 0 to Day 168)

Study Day 0/Clinic Visit 3 (Randomization)

At the randomization visit, the following assessments will be conducted:

- Review inclusion/exclusion criteria (Section 7.3)
- Review concomitant medications, medical and surgical history
- Physical examination (Section 10.2.5) and vital signs (Section 10.2.6)
- PRO/ObsRO eDiary review for compliance, entrance criteria requirement (see Inclusion Criteria 4, Section 7.3.1), and itching/scratching/sleep scores
- Clinical chemistry and hematology tests
- s-BA (Section 9.2.1)
- Serum IGF-1 growth marker
- Biomarkers (autotaxin and p-C4)
- Liver ultrasonography
- Administer QoL (Pediatric Quality of Life Inventory [PedsQL]) questionnaire
- Urine pregnancy test for girls who have reached menarche
- Patient/caregiver/clinician complete the patient global impression of change (PGIC) and the patient global impression of symptoms (PGIS)
- AE monitoring
- Study drug is dispensed and patients are instructed to administer daily from Day 1

Study Day 14 (Telephone Contact)

A study nurse will contact the patient/caregiver by telephone for monitoring of the following:

- Concomitant medications
- AEs
- Study drug compliance
- PRO/ObsRO eDiary review for itching, scratching, and sleep scores and compliance

Study Day 28 and Day 84/Clinic Visits 4 and 6

The following procedures and assessments will be performed:

- Review of concomitant medications

- Skin exam (Section 10.2.5) and vital signs (Section 10.2.6)
- PRO/ObsRO eDiary review for itching, scratching, and sleep scores and compliance
- Clinical chemistry and hematology tests
- s-BA (Section 9.2.1)
- INR
- Biomarkers (AFP and LDL-C; autotaxin and p-C4), at Visit 4 only
- Urine pregnancy test for girls who have reached menarche
- Patient/caregiver/clinician complete the PGIC and PGIS
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 56 and Day 126/Clinic or at-Home Visits 5 and 7

Visits 5 and 7 may be conducted in the patient's home by a study nurse. The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- PRO/ObsRO eDiary review for itching, scratching, and sleep scores and compliance
- Clinical chemistry and hematology tests
- s-BA (Section 9.2.1)
- Urine pregnancy test for girls who have reached menarche
- AE monitoring
- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 154/Clinic or at-Home Visit 8

The following procedures and assessments will be performed:

- Review of concomitant medications
- Vital signs (Section 10.2.6)
- PRO/ObsRO eDiary review for itching, scratching, and sleep scores and compliance
- s-BA (Section 9.2.1)
- INR
- AE monitoring

- Evaluation of study drug compliance
- Study drug is dispensed

Study Day 168/Clinic Visit 9

The last dose of study drug is taken in the morning the day before Visit 9, and the following procedures and assessments will be performed:

- Review of concomitant medications
- Physical examination (Section 10.2.5) and vital signs (Section 10.2.6)
- PRO/ObsRO eDiary review for itching, scratching, and sleep scores and compliance
- Clinical chemistry and hematology tests
- s-BA (Section 9.2.1)
- Serum IGF-1 growth marker
- Biomarkers (AFP and LDL-C; autotaxin and p-C4)
- Liver ultrasonography
- Administer PedsQL questionnaire
- Urine pregnancy test for girls who have reached menarche
- Patient/caregiver/clinician complete the PGIC and PGIS
- AE monitoring
- Evaluation of study drug compliance

7.1.4.3 Follow-Up Period

Study Day 196/Clinic Visit 10

Patients not continuing into Study A4250-008 and those patients who are prematurely withdrawn, will return to the study site 28 days after Visit 9 or the last dose of study drug for the following assessments:

- Review of concomitant medications
- Skin exam (Section 10.2.5) and vital signs (Section 10.2.6)
- PRO/ObsRO eDiary review for itching, scratching, and sleep scores and compliance
- Clinical chemistry and hematology tests
- s-BA (Section 9.2.1)
- INR
- Urine pregnancy text for girls who have reached menarche
- AE monitoring

7.2 Discussion of Study Design

The results from Study A4250-003 have informed the current (A4250-005) study design.

7.2.1 Risk/Benefit and Ethical Assessment

A4250 has been evaluated in 3 Albireo-sponsored clinical studies: 1 double-blind placebo-controlled study in healthy volunteers, 1 single-dose ADME study, and 1 Phase 2 study in children with cholestatic pruritus. In addition, an investigator-sponsored study has been conducted in patients with PBC.

A total of 98 subjects/patients have been exposed to A4250. Healthy subjects have been exposed to up to 10 mg as a single dose and up to 3 mg daily as part of a multiple-dose evaluation. Children with cholestatic liver disease have been treated with up to 0.2 mg/kg/day (200 µg/kg/day) for 4 weeks. In total, 2 SAEs have been reported; both were judged by the physician to be not related to the study drug.

Patients with cholestatic liver diseases suffer from excess bile acids in the liver resulting in tissue damage. A commonly used treatment in these patients is bile diversion surgery, whereby approximately 50% to 100% of the enterohepatic circulation of bile acids is interrupted. Inhibition of IBAT with A4250, thereby interrupting the enterohepatic circulation of bile acids, is therefore a potential medical alternative to surgery which could be of benefit to these patients if shown to be effective and safe.

Based on the mode of action of an IBAT inhibitor, loose stools or diarrhea could be expected. However, in the pediatric Phase 2 study with 4 weeks daily treatment, only 1 patient had mild transient diarrhea after a single dose that did not recur on multiple dosing.

7.2.2 Early Termination

This study may be terminated at any time by Albireo if serious side effects should occur, if the investigator does not adhere to the protocol; or if, in the sponsor's judgment, there are no further benefits to be achieved from the study. In this event, Albireo/designee will inform the study investigators, institutions, and all regulatory authorities.

Albireo may temporarily or permanently discontinue the study at an investigative site at any time for safety, ethical, compliance, or other reasons. If this is necessary, Albireo will endeavor to provide advance notification to the site. If a site or the study is suspended or discontinued, the investigator/investigative staff will be responsible for promptly informing the Independent Ethics Committee (IEC). If required by local regulations, Albireo/designee will be responsible for informing the IEC and the regulatory authority of study or site discontinuation. In such an event, all study data and unused study drug must be returned to Albireo.

7.3 Selection of Study Population

A total of 60 patients with clinical diagnosis of PFIC 1 or 2 that has been genetically confirmed will be randomized.

An attempt will be made to enroll a minimum of 10 patients from each age category (6 months to 5 years, 6 to 12, and 13 to ≤18 years).

A maximum of 5 patients that were treated in Study A4250-003 will be allowed to participate in this study.

7.3.1 Inclusion Criteria

1. A male or female patient, with clinical diagnosis of PFIC 1 or 2, between the ages of ≥6 months and ≤18 years at Visit 1 with a body weight above 5 kg.
2. Patient must have genetic confirmation of PFIC-1 or PFIC-2 through identification of biallelic pathogenic variants in either the *ATP8B1* or *ABCB11* genes
3. Patient must have elevated s-BA, specifically measured to be ≥100 µmol/L, taken as the average of 2 samples 7 to 28 days apart (Visits 1 and 2) prior to randomization
4. Patient must have history of significant pruritus as indexed by caregiver-reported observed scratching in the eDiary, average ≥2 (on 0-4 scale) in the week prior to randomization
5. Patient and/or legal guardian must sign ICF (and assent) as appropriate. Patients who turn 18 years of age (or legal age per country) during the study will be required to re-consent in order to remain in the study
6. Patients will be expected to have a consistent caregiver(s) for the duration of the study.
7. Caregivers and age appropriate patients (≥8 years of age) must be willing and able to use an eDiary device as required by the study

7.3.2 Exclusion Criteria

1. Patient with pathologic variations of the *ABCB11* gene that predict complete absence of the BSEP function
2. Patient with past medical history or ongoing presence of other types of liver disease including, but not limited to, the following:
   a) Biliary atresia of any kind
   b) Benign recurrent intrahepatic cholestasis (BRIC), indicated by a history of normal s-BAs
   c) Suspected or proven liver cancer or metastasis to the liver on imaging studies
   d) Histopathology on liver biopsy is suggestive of alternate non-PFIC related etiology of cholestasis
3. Patient with a past medical history or ongoing presence of any other disease or condition known to interfere with the absorption, distribution, metabolism (specifically bile acid metabolism), or excretion of drugs in the intestine, including but not limited to, inflammatory bowel disease

4. Patient with past medical history or ongoing chronic (i.e., >3 months) diarrhea requiring intravenous fluid or nutritional intervention for treatment of the diarrhea and/or its sequelae
5. Patient has a confirmed past diagnosis of infection with human immunodeficiency virus (HIV) or other present and active, clinically significant, acute, or chronic infection, or past medical history of any major episode of infection requiring hospitalization or treatment with parenteral anti-infective treatment within 4 weeks of treatment start (Study Day 1) or completion of oral anti-infective treatment within 2 weeks prior to start of Screening Period.
6. Any patient with suspected or confirmed cancers except for basal cell carcinoma, and non-liver cancers treated at least 5 years prior to Screening with no evidence of recurrence
7. Patient with a past medical history of chronic kidney disease with an impaired renal function and a glomerular filtration rate (GFR) <70 mL/min/1.73 m^2
8. Patient with surgical history of disruption of the enterohepatic circulation excluding those who have undergone a successful reversal procedure that has permanently restored flow of bile acids from the liver to the duodenum
9. Patient has had a liver transplant or listed for liver transplant
10. Decompensated liver disease, coagulopathy, history or presence of clinically significant ascites, variceal hemorrhage, and/or encephalopathy
11. INR >1.4 (the patient may be treated with Vitamin-K intravenously, and if INR is <1.4 at resampling the patient may be included)
12. ALT >15 × upper limit of normal (ULN)
13. ALT >20 × ULN at any time point during the last 6 months
14. Serum bilirubin >5 × ULN
15. Patient suffers from uncontrolled, recalcitrant pruritic condition other than PFIC. Examples include, but not limited to, refractory atopic dermatitis or other primary pruritic skin diseases
16. Any patient who is pregnant or lactating or who is planning to become pregnant within 24 weeks of randomization
17. Sexually active females who are not using a reliable contraceptive method with ≤1% failure rate, such as barrier protection, hormonal contraception, intrauterine device, or abstinence) throughout the duration of the study
18. Patient with a past medical history of alcohol or substance abuse will be excluded. Patient must agree to refrain from illicit drug and alcohol use during the study

19. Administration of bile acid or lipid binding resins within 30 days prior to baseline/Day 0 and throughout the trial
20. Patient has had investigational exposure to a drug, biologic agent, or medical device within 30 days prior to screening, or 5 half-lives of the study agent, whichever is longer
21. Any other conditions or abnormalities which, in the opinion of the investigator or medical monitor, may compromise the safety of the patient, or interfere with the patient participating in or completing the study

7.3.3 Withdrawal of Patients

Patients/caregivers will be informed that they have the right to withdraw from the study at any time, without prejudice to their medical care, and that they are not obliged to state their reasons.

Any withdrawal, and reasons for withdrawal, must be fully documented in the eCRF and source documents and the patient followed by the investigator/investigative staff. Even after the study is completed at the follow-up visit, the investigator/investigative staff will, regardless of reason for withdrawal, record any patient data they receive concerning SAEs, and all drug-related non-serious AEs, and subsequently report these to Albireo. Withdrawn patients will not be replaced.

Patients will be withdrawn in the following circumstances:

- A patient's/caregiver's desire for withdrawal for any reason
- Lost to follow-up (every effort must be made to contact the patient/caregiver; a certified letter must be sent)
- An AE that, in the opinion of the investigator, necessitates withdrawal
- A worsening of the symptoms related to diarrhea occurs
- Death
- Patient/caregiver estimation of lack of efficacy
- A patient's/caregiver's substantial noncompliance (eDiary and study drug compliance) or protocol violation
- An investigator's opinion that continuing the patient in the study is not appropriate. The investigator may withdraw a patient at any time, if it is considered to be in the patient's best interest

A patient who prematurely withdraws due to lack of efficacy, and has completed at least 12 weeks treatment in the double-blind study, will be offered the opportunity to enter open-label Study A4250-008. A patient who withdraws from treatment prematurely and will not enter Study A4250-008 will be requested to complete all visits and safety assessments per the current protocol (A4250-005). As a minimum, patients will have end of treatment

(Visit 9) assessments at the time of withdrawal and a follow-up assessment (Visit 10) 28 days following the last dose of study drug, and the patient will be recorded as withdrawn.

8 TREATMENT OF PATIENTS

8.1 Identity of Study Drug

A4250 and placebo will be supplied as capsules for oral administration. White opaque capsules filled with pellets containing A4250 or placebo will be provided. Two different capsule sizes will be available, as follows:

- Capsule size 0 that can be opened
- Capsule size 3 that can be opened or swallowed intact

The content of the A4250 and placebo capsules will be identical in appearance. Capsule filling weight will also be identical for high dose, low dose, and placebo. Bottles with 34 capsules will be given to the patient at each visit. A patient who requires 2 or more capsules per day will be given multiple bottles.

8.2 Administration of Study Drug

Patients will be dosed with A4250 at a dose of 40 µg/kg/day, 120 µg/kg/day, or placebo for 24 weeks. Study drug will be dispensed to the patient at Visits 3, 4, 5, 6, 7, and 8, together with instructions on how to store and take the drug. Study drug administration data, including whether each patient took each dose or partial doses of study drug, whether there were any delayed or missed doses, and whether the capsule was opened or swallowed whole will be documented through the eDiary and transferred to the study database.

A4250 should be taken in the morning, together with food. On clinic visits days when laboratory assessments are conducted (Visits 4, 5, 6, 7, and 9) study drug should be taken after the visit and after laboratory samples are taken. Patients should not crush or chew the capsule(s). When swallowing the capsule intact, the patient should administer the dose with a glass of water.

For a patient unable to swallow capsule intact, the capsule can be opened and the contents sprinkled and mixed in a small amount of soft room-temperature yogurt, apple sauce, or fruit purée. The amount of food should be less than what the patient/infant is expected to eat and should be followed by water.

1. Hold the capsule over the prepared food, gently twist open and allow the content to fall into food
2. Mix the entire capsule content with 1 or 2 tablespoons (15 to 30 mL) of soft food (apple sauce, fruit purée, or yogurt)
3. Administer the mixture immediately after mixing.

The number of capsules provided to the patient will be based on body weight at Visit 3. At Visit 6, if a patient's weight has shifted to another weight category (identified in Table 3), the number of capsules per day may be adjusted.

Table 3 Dosing and Capsule Strength

Body Weight (kg)	Capsule Size	Number of Capsules per Day	Capsule Strength, Low Dose (µg)	Total Dose (µg)	Capsule Strength, High Dose (µg)	Total Dose (µg)	Capsule Size, Placebo (µg)
5.0 to <7.5	0	1	200	200	600	600	0
7.5 to <12.5	0	2	200	400	600	1200	0
12.5 to <17.5	0	3	200	600	600	1800	0
17.5 to <19.5	0	4	200	800	600	2400	0
19.5 to <25.5	3	2	400	800	1200	2400	3
25.5 to <35.5	3	3	400	1200	1200	3600	3
35.5 to <45.5	3	4	400	1600	1200	4800	3
45.5 to 55.5	3	5	400	2000	1200	6000	3
>55.5	3	6	400	2400	1200	7200	3

8.3 Study Treatment Packaging and Labeling

8.3.1 Packaging and Labeling

The capsules will be packed in high-density polyethylene (HDPE) containers, with child-proof polypropylene caps. Study drug capsules containing A4250 in the strength specified in Table 3 above will be manufactured.

Packaging and labeling will be prepared to comply with applicable regulatory requirements.

8.3.2 Storage

Treatment packs containing A4250 capsules should be stored and dispensed in accordance with regulations in their original containers. The storage facility at the investigative site should be locked and the storage temperature should be between 15°C and 25°C.

Patients/caregivers should be informed of appropriate storage conditions (i.e., room temperature, between 15°C and 25°C).

Any deviations from the recommended storage conditions should be immediately reported to Albireo and the study drug should not be used until authorization has been given by Albireo.

8.3.3 Blinding and Randomization of Study Drug

A double-blind design is employed so that both the investigators and the patients will be unaware of the treatment assignment during the study.

After written informed consent is obtained from an eligible patient, an 8-digit patient identification number will be assigned. The first 2 digits will denote the country, followed by a 3-digit site number and a 3-digit patient sequence number. This number will be created and allocated by the Interactive Web Response System (IWRS), when the patient first enters the system at screening. The randomization codes will be computer-generated by [redacted] Biostatistics, and kept by a statistician independent from the project team. The

randomization will be done in blocks and stratified according to PFIC class (Type 1 and 2) and age class (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years) to ensure approximate balance between dose schemes (1:1:1). A separate randomization list will be prepared for the maximum 5 patients from Study A4250-003 regardless of stratification. Randomization codes will be assigned sequentially as patients become eligible for randomization.

Patients who are eligible for randomization will be assigned a unique 4-digit randomization number by IWRS. This randomization number identifies which treatment will be allocated to the patient. Patients who withdraw from the study after randomization visit are not to be replaced and their randomization number will not be reused.

To ensure blinding, the study drug and the matching placebo have the same shape and size. Patients will receive capsule(s) of A4250 according to their dose group or capsule(s) of matching placebo once a day during the double-blind Treatment Period. Labels on the study drug containers will not identify the treatment that a patient is randomized to. Traceability of the treatment is ensured by the study drug number.

The 5-digit study drug number will identify study drug packs and will be detailed on the study drug label. Dispensing of study drug will be coordinated by IWRS.

The system will assign a study drug number corresponding to the randomization arm. The randomization number will be used in the background only to ensure no unblinding and will not be displayed to end-users in the IWRS.

8.4 Procedure for Breaking the Randomization Code

Should a situation arise where unblinding is urgently required (i.e., knowledge of treatment codes is required to adequately manage a life-threatening situation), the investigator at that study site may perform immediate unblinding through IWRS without the need for communication with the [redacted] Medical Monitor.

The study site investigator and appropriate [redacted] project team members will be authorized to access the emergency unblinding functionality within the IWRS. The system will require the user to enter an authorization key number to complete the emergency unblinding transaction. The exact description of the treatment assigned to the individual patient then will be accessible. Emergency unblinding can thus be made for any patient without affecting the double-blind nature of the study. Patient treatment information may only be accessed in the event of an emergency and out of necessity to know the identity of the allocated study drug in order to institute appropriate therapeutic management. The investigator should make every effort to discuss the rationale for emergency unblinding with the [redacted] Medical Monitor prior to unblinding the individual patient. Once the randomization code is broken for a patient, he/she must be withdrawn from the study and all assessments and procedures at Visit 9 should be performed. As a follow-up, Visit 10 will be performed according to the time schedule. In the event that emergency unblinding is performed, the investigator can view and must print the unblinding confirmation document from IWRS. The investigator must record on the confirmation document printout the reason for the emergency unblinding,

and sign the document. The confirmation document must then be kept in a safe place until the end of the study. Once a randomization code has been broken, the investigator must inform the [redacted] Medical Monitor in writing within 24 hours.

8.5 Patient Compliance

The study nurse will monitor eDiary compliance by daily review of the CRF health website. If both diary entries on a day are missing during the baseline week or Week 24, the study nurse will call the patient/caregiver to remind them to complete all scheduled entries. Any noncompliance will be documented and explained in the source documents and in the eCRF.

Study drug compliance will also be checked through diary responses. Additionally, patients will return all unused study drug at Visits 4, 5, 6, 7, 8, and 9. The study site staff will count all returned drug, assess compliance, and record details in the eCRF.

Treatment Compliance = 100 × ((Number of study drug dispensed – number of study drug returned)/number of study drug that should be taken).

Treatment compliance between 80% and 120% will be acceptable.

8.6 Study Drug Accountability

Records shall be maintained of the delivery of study treatment(s) to the study site(s), of the inventory at the study site(s), of each use of the study treatment(s) for each patient, and of the return of unused study treatment(s) to Albireo.

These records shall include dates, quantities, batch numbers, expiry dates, and the unique code numbers assigned to the study drug and to the study patients.

The investigator will be responsible for ensuring that the records adequately document that the patients were provided the quantities specified in the protocol and that all study drug received from Albireo is reconciled.

8.7 Concomitant Therapy and Prohibited Medications

The investigator will note all ongoing medication and any medication recently (within 1 month) stopped in the eCRF at Visit 1. At Visits 2 through 10, all changes in medication (stopping or starting new medication and changes in dose) will be recorded in the eCRF. All treatment for pruritus (prescribed or not) and changes in the treatment will also be recorded.

All medications taken by a patient within 1 month prior to the first intake of study drug are regarded as prior medication.

All medications taken by a patient on or after the first intake of study drug, and which continue to be taken during the study, are regarded as concomitant medication.

Prohibited medications are listed in Appendix 1.

9 ASSESSMENT OF EFFICACY

9.1 Efficacy Endpoints

9.1.1 Primary Efficacy Endpoint

The primary efficacy endpoints are as follows:

- **Europe (EU) and rest of the world (RoW)**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA levels from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo

Fasting s-BA baseline will be calculated as the average of the value at randomization and the previous screening (Visit 2). The end value is the average of the values at Weeks 22 and 24 after the start of double-blind treatment.

- **United States (US)**: Change in pruritus, as indexed by caregiver-reported observed scratching score from baseline to Week 24 as measured by the average daily score of the Albireo ObsRO instrument (Appendix 2) compared to placebo. The average daily score will be calculated using the 7 days pretreatment for baseline, and the last 7 days of treatment for Week 24 for the end value

9.1.2 Secondary Efficacy Endpoint

Secondary efficacy endpoints include the following:

- **EU and RoW**: Change in pruritus, as indexed by caregiver-reported observed scratching score as measured by the average daily score of the Albireo ObsRO instrument (Appendix 2) compared to placebo. The average daily score will be calculated using the 7 days pretreatment for baseline, and the last 7 days of treatment for Week 24
- **US**: Proportion of patients experiencing at least a 70% reduction in fasting s-BA levels from baseline to the end of treatment or reaching a level ≤70 µmol/L compared to placebo
- **All Regions:**
  - All secondary endpoints are compared to placebo
    - Change from baseline to Week 24 in fasting s-BA
    - Change from baseline to Week 24 in patient-reported itch severity as measured by the average daily score of the Albireo PRO instrument (Appendix 2; only patients ≥8 years of age will complete the PRO instrument)
    - Change from baseline to Week 24 in ALT
    - Change from baseline to Week 24 in IGF-1
    - Proportion of patients achieving meaningful reduction in caregiver-reported observed scratching at Week 24

- Change from baseline to Week 24 in sleep parameters measured with the Albireo PRO and ObsRO instruments
- Number of patients undergoing biliary diversion surgery or being listed for liver transplantation
- Change from baseline to Week 24 in PedsQL (Appendix 4)

9.1.3 Exploratory Efficacy Endpoints

Exploratory efficacy endpoints are compared to placebo and include the following:

- Proportion of patients achieving meaningful reduction in patient-reported itch severity at Week 24
- Assessment of Global Symptom Relief at Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician via PGIC items (Appendix 3)
- Change from baseline in fasting s-BA at Weeks 4 and 12
- Change from baseline in patient-reported itch severity and observer-reported scratching at Weeks 4 and 12
- Change from baseline to Week 24 in patient-reported and observer-reported night time itching and scratching severity scores respectively
- Change from baseline to Week 24 in pooled pruritus score including observer-reported scratching for patients <8 years of age and patient-reported itch severity for patients ≥8 years of age
- Change from baseline to Week 24 in additional patient-reported and observer-reported sleep parameters (e.g., tiredness, number of awakenings)
- Change from baseline in ALT at Weeks 4 and 12
- Change from baseline in total bilirubin at Weeks 4, 12, and 24
- Change from baseline in AST at Weeks 4, 12, and 24
- Change from baseline for other biochemical markers and measures of bile acid synthesis (autotaxin, p-C4, LDL-C) at Week 24
- Change in pediatric end-stage liver disease/model for end-stage liver disease (PELD/MELD) score, AST to platelet ratio index (APRI) score, and Fib-4 score from baseline to Week 24
- Liver-related mortality and liver decompensation events
- All-cause mortality

9.2 Efficacy Assessments

9.2.1 Serum Bile Acids

Blood samples for analysis of fasting total s-BA will be drawn at all visits (in clinic and at patient's home). Patients will fast for at least 4 hours prior to the taking of samples for s-BA, except for water intake. However, less than a 4-hour fast prior to the blood draw will be accepted in infants who are unable to fast for 4 hours. Samples will be treated and transported to a central laboratory per instructions in the laboratory manual.

9.2.2 Itching and Sleep Score

Itching, observed scratching, and sleep disturbance will be assessed twice each day via eDiary. Patients and/or caregivers will be instructed to complete the eDiary every day in the morning after the patient wakes and in the evening just before the patient goes to sleep throughout the study. Patients and caregivers will receive a formal training session during Visit 1. Training materials describing the ObsRO and PRO items are included in Appendix 2.

The eDiary includes ObsRO and PRO items. Patients <8 years of age will not be asked to complete the PRO items; only the ObsRO will be completed by caregivers of patients in this age group. Older patients, 8 to 17 years of age, will complete the PRO items and the caregiver will complete the ObsRO items. The PRO items assess severity of itch, aspects of sleep disturbance (morning diary only), and tiredness. For patients 8 to 12 years of age, the caregiver will read the PRO items along with the child and record the child's response. A guide will be provided to the caregivers that provides standardized explanations of the PRO items, in case the patient is confused or requires clarification about the meaning of a question. The ObsRO items assess severity of observed scratching, aspects of observed sleep disturbance (morning diary only), and observed signs of tiredness (evening diary only). The ObsRO and PRO scratching and itch severity items use 0 to 4 response scales, where each response is distinguished by a unique facial expression, verbal anchor, number, and color code (Appendix 2).

A daily score for the ObsRO scratching item will be calculated by selecting the maximum scratching severity score from the 2 ratings for each day. A daily score will be considered missing if both of the daily assessments are missing. A weekly score will be calculated by averaging the daily scratching scores. A weekly score will be considered missing if >4 out of 7 days of data are missing. The same approach will be used to calculate a patient-reported itch severity score.

Additionally, patients/caregivers will be requested to report in the diary the date and time that each dose of study drug was administered during the Treatment Period. Details of the questions included in the diary, including the PRO/ObsRO tool used in the study, are located in Appendix 2.

9.2.3 Growth and IGF-1

Growth will be measured as height (velocity) and weight (z-score) using a certified weight scale (i.e., stadiometer).

Blood samples for IGF-1 will be drawn at randomization (Visit 3), and at end of treatment (Visit 9). Samples will be handled and transported to a central laboratory according to instructions in the laboratory manual.

9.2.4 Quality of Life (PedsQL) Questionnaire

Patients will be asked to fill out a QoL questionnaire (PedsQL) at Visit 3 and Visit 9. Details of the questions included on the questionnaire are provided in Appendix 4.

9.2.5 Biomarkers and Other Laboratory Samples

Blood samples for AFP and LDL-C will be drawn at Visits 2, 4, and 9. Blood samples for autotaxin and p-C4 will be drawn at Visits 3, 4, and 9 (only for children with body weight ≥10 kg). Samples will be handled and transported to a central laboratory per instructions in the laboratory manual.

9.2.6 PELD/MELD Score

At randomization and end of treatment, PELD score will be calculated for children up to 12 years of age. For children 13 years or older, the MELD score will be calculated.

PELD Score = 4.80 * ln(bilirubin in mg/dL) + 18.57 * ln(INR) - 6.87 * ln(albumin in g/dL) + 4.36 (if patient <1 year: scores for patients listed for liver transplantation before the patient's first birthday continue to include the value assigned for age (<1 year) until the patient reaches the age of 24 months) + 6.67 (if the patient has growth failure [≤2 standard deviation])

Laboratory values less than 1.0 will be set to 1.0 for the calculation of the PELD score.

MELD Score = 9.57 * ln(creatinine in mg/dL) + 3.78 * ln(bilirubin in mg/dL) + 11.2 * ln(INR) + 6.43

Laboratory values less than 1.0 will be set to 1.0 and serum creatinine values greater than 4.0 mg/dL will be set to 4.0 for calculation of the MELD score.

9.2.7 Liver Ultrasonography

Ultrasonography of liver and spleen will be performed at randomization (Visit 3) and at end of treatment (Visit 9). Liver and spleen size, masses, and nodules should be recorded.

9.2.8 Markers of Fibrosis

APRI score and Fib-4 score will be calculated at randomization and at end of treatment.

APRI = [(AST in U/L) / (AST ULN in U/L)] / (Platelets in 10^9/L)

Fibrosis 4 Score = (Age*AST in U/L) / (Platelets in 10^9/L * √(ALT in U/L).

9.2.9 Global Impression of Change and Global Impression of Symptom Measures

Patients, caregivers, and clinicians will complete the patient global impression of change (PGIC) and the patient global impression of symptom (PGIS) measures (Appendix 3) at randomization (Visit 3), Visits 4 and 6, and at end of treatment (Visit 9).

The PGIC items assess change in itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) since starting the study medication. The PGIS items assess itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) in the past week. Caregivers and clinicians will complete the PGIC and PGIS for all patients; those patients ≥8 years of age will complete the patient version.

10 ASSESSMENT OF SAFETY

The timing and frequency of safety assessments are described in Section 7.1.4 and in Table 1 above.

The primary safety analysis for this study will include the incidence of treatment-emergent adverse events (TEAE), study drug exposure compared to placebo, all safety assessments including AEs from patient reports, and the patient diaries. The analysis includes causality, severity, and seriousness assessments made by the investigator.

Trends in safety will also be assessed for the following assessments:

- Physical examinations
- Concomitant medications at all visits
- Vital signs at all clinic visits
- Laboratory test results (including hematology, clinical chemistry, liver monitoring, and urinalysis)
- SAEs including the description, incidence, severity, and causality from signing the ICF to completion of the study
- Discontinuations due to AEs

10.1 Adverse Events

10.1.1 Definitions and Investigator Assessments

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 unfavorable and unintended sign, symptom, or disease that occurs once a patient is enrolled (ICF is signed) in the study until the patient is discharged from the study, whether or not related to the study drug.

10.1.1.1 Clinical Significance

Clinical significance is defined as any abnormal finding that results in further clinical investigation(s), treatment(s), or the diagnosis of new condition. Patient-reported events and protocol-mandated laboratory values, vital signs, and physical examination findings can be considered clinically significant (i.e., an AE) if there is a deterioration as compared to baseline. Examples of clinically significant worsening from baseline could include, but is not limited to, events causing withdrawal from the study and events requiring medical intervention outside of the study causing apparent clinical manifestations, or judged relevant by the investigator.

10.1.1.2 Serious Adverse Events

Serious criteria are applied by the investigator to each AE as specifically defined below. These criteria are used to determine whether an AE is serious or non-serious. The assessment

is made independently of severity assessment (Section 10.1.1.3). For example, the development of a severe rash that occurs after signing of informed consent may not meet serious criteria as defined below and therefore would be considered a severe, non-serious AE.

Any AE that meets any 1 of the following 6 criteria is considered an SAE:

- The outcome of the AE is **death**
- The AE is immediately **life-threatening**. Life-threatening means that the patient is, in the opinion of the investigator, at immediate risk of death from the reaction as it occurred. This does not include an AE that, if more severe, might have caused death
- The AE results in persistent or significant **disability/incapacity**. Disability means a substantial disruption of a person's ability to conduct normal life functions
- The AE requires or prolongs **hospitalization**
- The AE results in a **congenital anomaly/birth defect**
- **The AE is an important medical event**. Important medical events may meet serious criteria should the investigator assess that they may significantly jeopardize the patient, represent a significant hazard, or requires medical/surgical intervention to prevent one of the outcomes listed above. Examples of potential SAEs based on this criteria include, but are not limited to, allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions even if they do not result in inpatient hospitalization, or the development of drug dependency and drug abuse.

10.1.1.3 Severity Assessment

Severity assessments are based on the intensity of the event in relation to expectation. The investigator will assess the intensity of AEs based on the following definitions:

- Mild (awareness of sign or symptom, but easily tolerated)
- Moderate (discomfort sufficient to cause interference with normal activities)
- Severe (incapacitating, with inability to perform normal activities)

Severity is a measure of intensity where seriousness is defined by the criteria outlined in Section 10.1.1.2. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but not meet serious criteria, and therefore would be assessed as a severe AE but not an SAE.

10.1.1.4 Causality Assessment

The investigator determines the causality of all AEs to the study drug using medical judgment and considering all relevant factors such as (but not limited to) the underlying study indication, coexisting disease, concomitant medication, relevant history, pattern of the AE, temporal relationship to the study medication, and de-challenge or re-challenge. The causality assessment of the AE/SAE is to be made as follows.

Related to study drug (i.e., possibly, probably, or definitely related)

Based on medical judgment, there is at least a reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event follows a reasonable temporal sequence from administration of study drug
- The event could not be reasonably attributed to the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient
- The event follows a known pattern of response to study drug
- The event disappears or decreases on cessation or reduction in dose of the study drug. (It should be noted that in some situations an AE will not disappear or decrease in intensity upon discontinuation of study drug despite other clear indications of relatedness)
- The event reappears or worsens when the study drug is re-administered

Unrelated to study drug (i.e., unlikely, probably not, or definitely not related)

Based on medical judgment there is no reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event does not follow a reasonable temporal sequence from administration of study drug
- The event could be reasonably attributed to the known characteristics of the patient's clinical state, concurrent illness, environment or toxic factors, or other modes of therapy administered to the patient
- The event does not follow a known pattern of response to study drug
- The event does not disappear or decrease on cessation or reduction in dose of the study drug, and it does not reappear or worsen when the study drug is re-administered

10.1.2 Recording of Adverse Events

It is the investigator's responsibility to assess whether each untoward event is a clinically significant worsening from baseline, thereby considered an AE. For all AEs, the severity, seriousness, and causality to study drug for each AE as outlined in Section 10.1.1 will be assessed and recorded in the eCRF.

All serious and non-serious AEs are collected once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10), 28 days after the final dose of study drug or up until premature discontinuation.

Any AEs or SAEs that are unresolved at the patient's last AE assessment in the study are followed up by the investigator for as long as medically indicated, but without further recording in the CRF. Albireo retains the right to request additional information for any patient with ongoing AE(s) or SAE(s) at the end of the study.

Investigators must follow patients with AEs and SAEs until the event has resolved or the condition has stabilized. If the patient dies, this should be captured as the outcome of the AE unless no link can be established, in which case death will be reported as a separate event.

If a patient is lost to follow-up, this should be captured accordingly within the AE eCRF and on a follow-up SAE report for tracking purposes.

TEAEs are defined as any AE that occurs after randomization (Day 0). All AEs that occur in the Screening Period, i.e., after enrollment and prior to randomization, will be collected on the eCRF as non-treatment emergent AEs.

If there is a clinically significant deterioration of a laboratory value/vital sign or other routine study assessment that is associated with a diagnosis, the clinical diagnosis will be reported as an AE and the associated signs and symptoms will be considered additional information unless the sign or symptom is more severe than expected given the diagnosis. For example, if an investigator diagnoses a study patient with hepatitis during the study period, hepatitis would be considered the AE and the concomitant signs and symptoms of abdominal pain, vomiting, and elevated ALT and AST would not be reported separately unless, in the opinion of the investigator, one of these signs or symptoms is more severe than expected and therefore a separate AE assessment is indicated.

10.1.3 Recording and Reporting of Serious Adverse Events

Every SAE (regardless of severity and causality) that occurs once the caregiver/patient has signed the ICF and until the post-treatment follow-up (Visit 10), and 28 days after the final dose of study drug, should immediately and not later than within 24 hours of knowledge of the event, be reported by the investigator or delegate in the eCRF SAE Report Form. The clinical research organization (CRO) pharmacovigilance manager (PVM) should at the same time be notified of the event by e-mail. A confirmation of receipt must be sent to the investigator within 1 working day.

Report of a SAE must include at least the following information:

- Patient identification information (study number, site number, initials, and date of birth)
- The last study drug administration date
- The diagnosis of the event with the description (or a brief description of signs/symptoms/clinical course if the diagnosis is not available) and the date of onset
- The action used to treat the event (i.e., treatment medications, temporary discontinuation)
- The reason(s) for considering the event serious
- The relationship of the event to the study drug or to the study procedure (e.g., the investigator's assessment of causality)
- A brief narrative of the SAE

Follow-up reports including all new information obtained of the subsequent course of the SAE must be prepared and the information collected in the SAE eCRF submitted to the CRO PVM by e-mail within 24 hours of knowledge of the event.

The PVM may contact the investigator to obtain further information on a reported SAE. The investigator/investigative staff must respond to any request for follow-up information or answers to questions regarding the SAE within the same timelines as for initial reports.

The PVM reports the occurrence of the SAE and follow-up to the Albireo medical monitor for medical assessment of the case.

Should an outcome of death occur within the study period or within 60 days after the last administration of study drug, an AE form and an SAE form should be completed, detailing the AE that resulted in the death. (Note: death is an outcome, not an event.)

The report should contain a comment regarding the co-involvement of progression of disease, if appropriate, and should assign main and contributory causes of death.

All SAE reports must be e-mailed to the following number within **24 hours**:

[redacted]

If email is unavailable, SAEs may be transmitted via fax to the following number:

[redacted]

In a study-related medical emergency situation, when the assigned medical monitors for the study cannot be reached by a caller, an on call physician can be reached 24 hours per day, 7 days per week, via the [redacted] call center. The following number is a chargeable telephone number allowing a global reach from both landlines and mobile phones and the internet page is a list of country-specific toll-free telephone numbers that is provided. It should be noted that not all countries globally have access to toll-free numbers as indicated on the "24/7 Medical Help desk" index. Countries without toll-free numbers need to dial the chargeable number as indicated above. Furthermore, toll-free numbers might not be available from mobile phones.

[redacted]

The [redacted] representative will work with the investigator to compile all the necessary information and ensure that the appropriate sponsor representative receives a report within 1 day (24 hours) for all fatal and life-threatening cases and within 5 days for all other SAEs.

10.1.4 Reporting of Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reaction (SUSAR) is an SAE that occurs in a patient due to exposure to an investigational product that may or may not be dose-related and is unexpected per sponsor assessment.

For this clinical study there is an established reporting system for SUSARs (definitions cf. Directive 2001/20/EC), which shall ensure that all relevant information about SUSARs is recorded and reported to the competent authority and the Ethics Committee according to the requirements of Article 17(1)(a), (b) and (d) of Directive 2001/20/EC, i.e., in the case of fatal or life-threatening SUSARs as soon as possible, and in any case no later than 7 days with relevant follow-up information within an additional 8 days, and all other SUSARs to be reported as soon as possible but within a maximum of 15 days of first knowledge by the sponsor. The investigator will be kept informed by Albireo without undue delay. The setup of the SUSAR reporting system is described in written standard operating procedures to ensure compliance with the necessary quality standards at every stage of reporting and follow-up, which is in line with the communication from the commission: detailed guidance on the collection, verification, and presentation of AE/reaction reports arising from clinical trials on medicinal products for human use (CT-3: O.J. 2011/C 172/01 of 11.6.2011).

It is the sponsor's responsibility to report SUSARs to appropriate regulatory authorities and ethics committees, which cumulatively have a reasonable possibility of a causal relationship to the investigational product, are serious, and are unexpected. The minimum information will include the valid European Union drug regulatory agency clinical trial (EUDRACT) number, study number, the identifiable coded patient, the identifiable reporter, the SUSAR, the investigational product (including active substance name and code), and the causality assessment.

Reporting of SUSARs as individual case safety reports (ICSR), including the required administrative information, will be performed through the Eudravigilance Clinical Trial Module (EVCTM) to the competent authorities in Europe and through the FDA Adverse Event Reporting System and ICH E2B(R3) message standard. The Ethics Committee and all investigators involved in this study will be informed according to national and international requirements. Albireo may delegate the SUSAR reporting to [redacted]

10.2 Laboratory Values/Vital Signs/Physical Examinations and Other Safety Assessments

10.2.1 Laboratory Assessments

Blood samples will be drawn for clinical chemistry, high sensitivity c-reactive protein (hsCRP), international normalized ratio (INR), and hematology analyses as shown in Table 1. Blood will be drawn with patient in a fasting state for ≥4 hours. Samples will be handled and transported to a central laboratory per instructions in the laboratory manual.

Routine laboratory parameters are listed in Table 4.

The observed values will be recorded and assessed as "normal" or "abnormal not clinically significant" or "abnormal clinically significant".

The total amount of blood drawn during the whole study period is estimated to be approximately 59 mL (11 mL for children with body weight <10 kg over a 4-week period; Table 2 above). Additional blood may also be drawn at unscheduled visits for bile acids if

needed due to analysis failure; if other safety blood samples are needed due to follow-up of an abnormal value or analysis failure, the blood volume will increase accordingly. The blood samples collected for safety laboratory analysis will be destroyed after the analyses have been completed.

Table 4 Routine Laboratory Parameters

Clinical Chemistry	Hematology	Urinalysis
• Albumin • ALT • Alkaline phosphatase • AST • Bilirubin – total and conjugated • Calcium • Chloride • Creatinine • Creatine kinase • Gamma-glutamyl transferase • Potassium • Sodium	• Hematocrit • Hemoglobin • Platelet count • Red blood cell count • White blood cell count	• Blood • Glucose • Ketones • Leukocytes • Nitrites • pH • Protein

A serum pregnancy test will be performed at Visit 2, and a urine pregnancy test will be collected at all other visits for girls who have reached menarche.

10.2.2 Liver Monitoring

Strategies to monitor markers of liver disease throughout the study are outlined below where the ULN will be based on central laboratory reference values for age and sex.

If ALT >3 × individual baseline or ALT >15 × ULN during treatment

AND

bilirubin >2 × individual baseline or bilirubin >2 × ULN (if baseline <ULN)

Weekly monitoring should be initiated

If ALT continues to increase AND ALT >5 × individual baseline

AND

bilirubin >3 × individual baseline OR bilirubin >3 × ULN (if baseline <ULN)

OR

ALT >20 × ULN irrespective of bilirubin

Study medication should be stopped and weekly monitoring continued.

If ALT and bilirubin returns to 1.5 × individual baseline or <2 × ULN study medication could be restarted.

ULN will be based on central laboratory reference values for age and sex.

10.2.3 Demographics/Medical and Surgical History

Demographic information (age, birth year, gender, race, and ethnicity), along with medical and surgical history, will be obtained and recorded in the eCRF at Visit 1.

Medical and surgical history will be entered in the CRF at Visit 1. This includes date of diagnosis of PFIC, historical (3 months prior to screening) LFT values (AST, ALT, bilirubin), ongoing medication, any surgery performed, any other diagnosis, and historical biopsy data. If a biopsy is performed during the study, this will be recorded in the eCRF at the visit closest after the biopsy. Patients with biopsy data not older than 2 years at study start will be given the opportunity to participate in a sub-study to evaluate the effect of A4250 on liver histology.

10.2.4 Clinical Genetic Testing

If clinical genetic testing for verification of PFIC diagnosis has been performed prior to enrollment of patients in the study, the results will be entered in the eCRF. Verification of the clinical laboratory report will be performed by a central reader to determine eligibility. Eligibility confirmation will be returned to the site within 7 days after receipt of the clinical genetic test result laboratory form.

Regardless of previous testing, a blood sample will be drawn at Visit 1 on all patients for DNA extract and freeze. Genetic testing will only be performed on this sample if clinical testing is equivocal, unavailable, or unobtainable. In such cases, screening for pathologic biallelic variations in the *ATP8B1* or *ABCB11* genes will be performed if patient meets remaining inclusion and exclusion criteria and only after sponsor case review. No other diagnostic genetic testing will be offered.

10.2.5 Physical Examination

A physician or suitably trained qualified assistant will perform a complete physical examination at Visits 1, 3, and 9. A skin exam (only) will be conducted at Visits 4, 6, and 10 (see Table 1).

A complete physical examination will include assessment of general appearance, eyes, ears, nose, throat, head/neck/thyroid, lymph nodes, cardiovascular, lungs/chest, abdomen, genitourinary, extremities, skin, musculoskeletal, neurologic, and other.

Skin will be examined thoroughly and excoriations/scratch marks recorded.

10.2.6 Vital Signs

Evaluation of vital signs will be performed at all visits. This includes blood pressure (systolic and diastolic, measured after the patient has been in a seated position for ≥3 minutes of rest), pulse, respiratory rate, temperature, height and body weight (using a certified weight scale and consistent site-specific guidelines; e.g., removal of outer coat, shoes) at clinic visits. Body mass index (BMI) will be calculated.

10.2.7 Overdose

A4250 is minimally absorbed and has a very low systemic availability. Based on toxicology data, for study purposes any dose exceeding a total of >3 mg/kg body weight of A4250 taken as a single dose or as a cumulative dose within 24 hours is defined as an overdose.

The no-adverse-effect dose level in the most sensitive species (defined as 20 mg/kg/day) in the rat 1 month toxicity study gives a human equivalent dose of approximately 194 mg/day in a 60 kg person. This dose is approximately 10-fold higher than the maximum possible dose predicted in human studies (20 mg).

The investigator/investigative staff should immediately and not later than within 24 hours of knowledge of the overdose, be reported by the investigator or delegate in the eCRF SAE report form. Any overdose, according to the definition above, must also be recorded in the study drug section of the patient's eCRF. In the event of an A4250 overdose, the patient should be monitored closely. Hospitalization should be considered as an alternative for observation and for appropriate supportive treatment.

If the investigator and/or Albireo consider the overdose event to be a medically important condition, then it should be reported as an SAE in the same expedited manner as an SAE (please refer to Section 10.1.3).

10.2.8 Pregnancy

If a pregnancy is discovered before the end of dosing the patient will be immediately discontinued from further dosing in the study. If the pregnancy is discovered after the end of dosing the patient will continue in the study per protocol.

Pregnancy is not considered to be an AE. However, if the patient has been dosed with the study drug, the pregnancy must be reported on the Paper Pregnancy Notification Form immediately within 24 hours after recognition to Albireo/Safety Manager (please refer to Section 10.1.3). Date of exposure and as far as possible, details of the period of gestation at the time of exposure must be given.

The pregnancy should be followed up to determine outcome, including spontaneous termination, details of birth, and presence of any birth defects, congenital anomalies or newborn or maternal complication. Individual cases with an abnormal outcome in association with the study drug should be reported on an expedited basis, i.e., reported rapidly to a competent authority.

11 STATISTICAL EVALUATION

All personnel involved with the analysis of the study will remain blinded until database lock for the double-blind Treatment Period and until major protocol violations have been identified.

All statistical analyses will be performed using SAS version 9.3 or higher.

11.1 Sample Size and Power

For the EU and RoW, the primary analysis will relate to the bile acid responder endpoint (at the 1-sided 2.5% level). For the US, the primary analysis will relate to the pruritus endpoint (at the 1-sided 2.5% level).

Assuming 60% responders (patients experiencing at least 70% reduction in fasting s-BA levels or reaching ≤70 µmol/L) in the A4250 dose group and 10% responders in the placebo group, 20 patients per group are needed to achieve a power of 90% with a 2.5% 1-sided Type-1 error.

For pruritus, with 20 patients per treatment group, the power is approximately 90% given an assumed treatment effect difference of -1.0 in change from baseline (-1.6 for A4250 and -0.6 for placebo) on the ObsRO scratching score with a standard deviation of 0.95 and a Type I error of 2.5% (1-sided).

This gives an overall power of approximately 80% for both endpoints.

11.2 Statistical Methods

11.2.1 Statistical Analysis Sets

Safety Analysis Set

The safety analysis set will consist of all patients who received at least 1 dose of study drug. Patients will be analyzed according to the treatment they actually received. The safety analysis set will be the primary analysis set for safety analyses.

Full Analysis Set

The full analysis set (FAS) will consist of all patients randomized with a valid baseline and at least 1 valid post-baseline measurement for at least 1 efficacy variable, such as fasting s-BA, pruritus score, ALT, IGF-1, sleep disturbance (PRO/ObsRO), and PedsQL. Patients will be analyzed in the treatment group they were randomized to even if they received incorrect study drug. The FAS will be the primary analysis set for efficacy analyses.

Modified Full Analysis Set

The modified full analysis set (mFAS) is a subset of the FAS and will consist of all randomized patients who were not included in Study A4250-003.

Per Protocol Analysis Set

The per protocol (PP) analysis set is a subset of the FAS, and will consist of all randomized patients for whom no major protocol violation (no violation which may affect the study efficacy outcome) is documented. The PP analysis set will provide supportive data for the efficacy analyses.

Allocation of patients to the PP analysis set will be performed before unblinding of the study.

11.2.2 Methods of Statistical Analyses

11.2.2.1 General Principles

The primary objective of this study is to show superiority in efficacy of A4250 (120 µg/kg/day and 40 µg/kg/day) compared to placebo in patients with PFIC Types 1 and 2.

In the EU and RoW, for the primary efficacy variable fasting bile acid responder endpoint (at least a 70% reduction in fasting s-BA levels or reaching a level ≤70 µmol/L at the end of treatment), the null hypothesis will be tested against the alternative hypothesis ($\alpha = 0.025$, 1-sided).

The test will be performed at a Type I error rate of ≤0.025 1-sided, as described above, based on the least squares means of the treatment difference in mean probability scale with corresponding 95% confidence intervals.

The Type I error will be protected by performing a hierarchical structure of analysis with closed tests of hypotheses.

A fixed-sequence multiple testing approach will be used. In the first step, the high dose (120 µg/kg/day) versus placebo will be tested at 2.5% 1-sided significance level. If significant, the low dose (40 µg/kg/day) versus placebo will also be tested, in a second step, at 2.5 % 1-sided significance level. The second step will only be considered confirmatory providing the previous step was successful. If the previous step was not successful, the analysis of the following step will be considered descriptive.

In the US, for the primary efficacy variable pruritus score endpoint (change from baseline to Week 24 in observer-reported scratching), the null hypothesis will be tested against the alternative hypothesis ($\alpha=0.025$, 1-sided).

The test will be performed at a Type I error rate of ≤0.025 1-sided, as described above, based on the least squares means of the treatment difference in mean change with corresponding 95% confidence intervals.

The Type I error will be protected by performing a hierarchical structure of analysis with closed tests of hypotheses using a fixed-sequence multiple testing approach. In the first step, the high dose (120 µg/kg/day) versus placebo will be tested at 2.5% 1-sided significance level. If significant, also the low dose (40 µg/kg/day) versus placebo will be tested, in a second step, at 2.5 % 1-sided significance level. The second step will only be considered

confirmatory providing the previous step was successful. If the previous step was not successful, the analysis of the following step will be considered descriptive.

Secondary and exploratory outcome variables shall provide supportive efficacy and safety information regarding the differences between the treatment groups. For secondary efficacy variables, supportive statistical tests will be made and P-values will be reported in exploratory way (if not otherwise specified). No adjustments will be performed for multiple dose comparisons when testing the secondary and exploratory efficacy variables.

A more detailed statistical analysis plan (SAP) will be prepared and finalized before study data are locked and unblinded.

If not otherwise specified, the baseline value of a parameter is defined as the last assessment of that parameter on or prior to the first intake of study treatment date.

11.2.2.2 Missing Data

For efficacy variables, with repeated measures, a mixed model will be employed with no additional imputation for missing values, i.e., based on "observed cases", except where otherwise specified.

Follow-up and continued collection of efficacy data for patients who discontinue treatment will be made. These data will be included in the sensitivity analysis for primary endpoints estimating a treatment effect taking loss of effect after discontinuation into account. Because complete follow-up of all patients discontinuing treatment may not be possible, a method to replace missing data for patients who discontinue the study will be prespecified (in the SAP) taking into account loss of effect after discontinuation (for example multiple imputation).

No other replacement of missing values will be planned.

11.2.2.3 Demographic and Baseline Characteristics

Descriptive summaries of demographics and other baseline characteristics (including medical and surgical history) will be presented by treatment group and overall using the FAS.

Prior medication will be summarized by treatment group and overall using the safety analysis set.

11.2.2.4 Subject Disposition

The following will be summarized (by treatment group and overall where applicable):

- Patients enrolled (who signed the informed consent)
- Patients randomized
- Patients treated
- Patients completing the study/withdrawing early (including withdrawal reason)

Additionally, patients enrolled, included in the safety, in the FAS, and in the PP analysis set will be summarized by region.

11.2.2.5 Evaluation of Primary Efficacy Variables

The responder endpoint based on change in fasting bile acids will be analyzed in terms of the proportion of patients achieving the event of interest. Patients in need of rescue therapy, i.e., biliary diversion will be classified as non-responders after the biliary diversion.

Generalized linear mixed modelling (GLMM) will be used with a logistic link, to allow for repeated assessments over time. The model will include baseline value, treatment group, PFIC class, age class, region, visit, and visit*treatment group interaction. The treatment by visit interaction will remain in the model regardless of significance. Region will be included in the model as a random effect. The contrasts of primary interest will be the treatment difference at Week 24; also, the difference at Week 4, Week 8, Week 12, and Week 18 will be estimated and analyzed. An unstructured covariance matrix will be used to model the within-subject error. Another covariance structure may be chosen if the fit of this structure fails to converge.

The primary analysis of the change from baseline in pruritus score (indexed by observer-reported scratching) will be analyzed using a mixed model repeated measures (MMRM) analysis. The contrast of primary interest will be treatment difference at Week 24 but also the differences at Week 4, Week 8, Week 12, and Week 18 will be estimated and analyzed. Baseline pruritus score will be included as covariate. The model will include baseline value, treatment group, PFIC class, age class, region, visit, and visit*treatment group interaction. The treatment by visit interaction will remain in the model regardless of significance. Region will be included in the model as a random effect and will be defined on country level. An unstructured covariance matrix will be used to model the within-subject error. Another covariance structure may be chosen if the fit of this structure fails to converge.

Non-parametric approach may be considered if more appropriate.

The primary analyses will be based on the FAS.

To assess the robustness of the primary efficacy analysis, the following analyses will be performed:

- Primary efficacy analysis using the PP set
- Primary efficacy analysis using the mFAS
- Analyses to investigate the influence of missing values, for example, by multiple imputation technique

Subgroup analyses will also be performed for each of the 3 age groups (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years).

Several sensitivity analyses will be performed, such as the following:

- Cochran Mantel Haenzsel test stratified on PFIC class to compare the proportion in fasting bile acid responder endpoint at the end of treatment in the 2 A4250 dose groups to placebo group
- Based on the primary analysis including the interaction age class*treatment
- Same as primary analysis in which the random factor region is considered as fixed factor. The region*treatment interaction will also be explored
- Same as primary analysis including the interaction PFIC*treatment

Other sensitivity analyses may be performed and will be detailed in the SAP.

11.2.2.6 Evaluation of Secondary and Exploratory Efficacy Variables

The change in the secondary and explorative endpoints such as s-BA, total bilirubin, ALT, IGF-1, and patient-reported itch severity will also be analyzed using the MMRM model as specified for the primary analysis. Nonparametric methods may be applied as an alternative approach for some endpoints, if appropriate.

Proportions of responders (e.g., meaningful reduction in itch severity and scratching at Week 24) will be analysed using the GLMM model as specified for the primary analysis.

Comparisons of the change from baseline at Week 24 in PedsQL between the treatment groups will be conducted using analysis of covariance (ANCOVA). The model will include terms for baseline, PFIC class, age class, region, and treatment.

Undergoing biliary diversion surgery and/or listed for liver transplant, all-cause mortality, and liver decompensation events will mainly be analyzed by using descriptive statistics. Kaplan-Meier curves will be used when appropriate for time to event data.

Other exploratory variables (global symptom relief, biochemical markers and measures of bile acid synthesis, PRO and ObsRO sleep parameters, PELD/MELD score, APRI score, and Fib-4 score) will be analyzed descriptively for categorical and continuous data, as applicable. For continuous data, the change from baseline will be analyzed in addition to the actual visit values. For categorical data, shift tables or frequency and percentages of patients will be presented as appropriate.

In addition, subgroup analyses will be performed for each of the 3 age classes (6 months to 5 years, 6 to 12 years, and 13 to ≤18 years).

11.2.2.7 Evaluation of Safety Variables

Safety data will be analyzed using descriptive statistics and summaries by treatment group of SAEs, AEs, vital signs, clinical laboratory tests (hematology, clinical chemistry and urinalysis), and concomitant medication. Analyses will be performed using the safety analysis set.

Summaries of AEs (coded according to the Medical Dictionary for Regulatory Activities [MedDRA] system organ class [SOC] and MedDRA preferred term) will include the following:

- Overview of the incidence of TEAEs (TEAEs, drug-related TEAEs, TEAEs leading to study discontinuation, and treatment-emergent SAEs)
- TEAEs by SOC and preferred term
- Intensity of TEAEs by SOC and preferred term
- Drug-related TEAEs by SOC and preferred term
- TEAEs leading to study discontinuation by SOC and preferred term
- Treatment-emergent SAEs by SOC and preferred term

Concomitant medication use during the Treatment Period will be summarized by Anatomical Therapeutic Chemical (ATC) class and World Health Organization (WHO) preferred name.

Summaries of vital signs will be presented. For each visit, the actual results and the change from baseline, and the number and percentage of patients with potentially clinically significant (PCS) values observed post-baseline will be presented.

Summaries of clinical safety laboratory data will be presented. For each visit, the actual result and the change from baseline, and the number and percentage of patients with PCS values observed post-baseline will be presented.

Data listings will be provided for each patient for all safety parameters.

11.2.2.8 Compliance and Exposure

Exposure will be analyzed by calculating the number of days with exposure to study drug. Results will be presented by treatment group using the safety analysis set.

The percentage compliance will be described by treatment group and the number of subjects with a compliance <80%, between 80% and 120%, and >120% will be presented based on the safety analysis set.

11.2.3 Blinded Analyses to Confirm Responder Definitions

A blinded analysis of ObsRO and PRO eDiary data will be performed after 50% or more of the planned sample has completed the Week 24 Follow-Up Period. The blinded analysis will be used to estimate a threshold of clinically meaningful change (i.e., responder definition) in ObsRO and PRO pruritus scores. The analysis will be performed by a group independent from both the study team and Albireo. Blinded data will be used for this analysis; data will be collapsed across treatment groups.

The analyses will include distribution- and anchor-based analyses to examine a responder definition for the PRO/ObsRO pruritus scores. Distribution-based analyses will include calculation of the standard deviation and standard error of measurement for the baseline PRO and ObsRO scores. Anchor-based analyses will involve examining the degree of change on

the PRO and ObsRO scores from baseline to Week 24 for patients who experience change in pruritus according to PGIC and symptom items. Details of this analysis will be included in a stand-alone SAP for assessing the measurement characteristics (reliability, validity, sensitivity to change, responder definition) of the PRO and ObsRO items.

11.2.4 Data Safety Monitoring Board

A DSMB consisting of sponsor-independent clinical and statistical experts will be established. The DSMB will periodically meet for the review of accumulating study data, including safety (AE and laboratory data) and liver values.

The DSMB will have access to unblinded data. The DSMB will make recommendations for the remaining part of the study (further details will be provided in the DSMB charter). The DSMB may recommend continuing with the study as planned or stopping the study early for safety reasons. The DSMB will submit its recommendations in writing to Albireo Medical/[redacted] who are responsible for responding to the recommendations of the DSMB and taking appropriate action. The investigators will only be informed by Albireo Medical/[redacted] if the study requires a protocol amendment or is stopped. The DSMB may choose to make additional evaluations at any time if they feel this is warranted from a safety point of view.

The DSMB will act according to its own written standard operating procedure described in a charter and will prepare written minutes of its meetings. The charter of the DSMB will be stored in the trial master file. The DSMB will maintain records of its meetings and these will become part of the study file when the study is complete. In order not to disseminate unblinded data and to ensure that all staff involved in the conduct and final analysis of the study remain blind to the data, only the members of the DSMB and the unblinded DSMB statistician will have access to these data.

12 DIRECT ACCESS TO SOURCE DATA/NOTES

The investigator/institution shall provide direct access to source data/documents for study-related monitoring, audits, IEC/IRB review and regulatory inspection.

13 QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Conduct of the Study

Albireo/[redacted] shall implement and maintain quality control and quality assurance procedures with written SOPs to ensure that the study is conducted and data are generated, documented, and reported in compliance with the protocol, ICH GCP and applicable regulatory requirements.

This study shall be conducted in accordance with the provisions of the Declaration of Helsinki (October 1996) and all revisions thereof, and in accordance with FDA regulations (CFR, Sections 312.50 and 312.56) and with ICH GCP (CPMP 135/95).

The investigator may not deviate from the protocol without a formal protocol amendment having been established and approved by an appropriate IEC/IRB, except when necessary to eliminate immediate hazards to the patient or when the change(s) involve(s) only logistical or administrative aspects of the study. Any deviations may result in the patient having to be withdrawn from the study and render that patient non-evaluable.

13.2 Study Monitoring

Before study initiation, at a site initiation visit or at an investigator's meeting, an Albireo representative will review the protocol and eCRF with the investigators and the investigative staff. During the study, the clinical monitor (clinical research associate [CRA]) will visit the site regularly to check the completeness of patient records, the accuracy of entries on the CRFs, the adherence to the protocol and to GCP, the progress of enrollment, and to ensure that study drug is being stored, dispensed, and accounted for according to specifications.

The investigator must ensure that CRFs are completed within 5 days of patient visits and must allow the Albireo medical monitor or CRA periodic access to patient records and all study-related materials, including relevant hospital or clinical records, to confirm their consistency with the CRF entries. No information in these records about the identity of the patients will leave the study center. Albireo monitoring standards require full verification for the presence of the signed ICF, adherence to the inclusion/exclusion criteria, documentation of SAEs, and recording of primary efficacy and safety variables. The CRA will review source data compared with the CRFs and will verify source data according to the study-specific monitoring plan. The design of the study, the frequency of patient visits, and the site enrollment rate will determine the frequency of monitoring visits. Upon study completion, the CRA will visit the site to conduct a study termination visit, which will include collection of any outstanding documentation.

It is recommended that the investigator/investigative staff log into the system every working day or at minimum twice weekly in order to provide a prompt response to queries. The investigator/investigative staff should log into the system the day after submitting an SAE report to answer any queries. This practice should continue until all questions regarding the SAE have been answered. The investigator/investigative staff should respond to queries and

make any relevant changes to the study date within 3 working days. The investigator/investigative staff should respond as quickly as possible to queries received concerning an SAE.

14 ETHICS

14.1 Independent Ethics Committee/Institutional Review Board

Prior to the start of the study, the investigator is responsible for ensuring that the protocol and consent form have been reviewed and approved by a relevant IEC/IRB. The IEC/IRB shall be appropriately constituted and perform its functions in accordance with FDA ICH GCP and local requirements as applicable.

The IEC/IRB shall approve all protocol amendments (except for logistical or administrative changes), written informed consent documents and document updates, patient recruitment procedures (e.g., advertisements), written information to be provided to the patients, IB, available safety information, information about payment and compensation available to patients, the investigator's curriculum vitae and/or other evidence of qualifications and any other documents requested by the IEC/IRB and regulatory authority (competent authority) as applicable.

14.2 Written Informed Consents and Assents

The investigator/investigative staff will explain to each patient (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, alternative treatment, the potential risks and benefits involved, and any discomfort that may occur. Each caregiver/patient will be informed that participation in the study is voluntary and that he/she or their child may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

Caregivers/patients will be informed that they/their children may experience side-effects or be at risk for symptoms, illnesses, or complications that cannot be foreseen by Albireo. As with other medications, people treated with A4250 may be at risk of developing allergic reactions or anaphylaxis. Caregivers/patients will be advised that study procedures include regular blood sampling for measurement of safety parameters and biological markers and that some minor risks are associated with these procedures.

This informed consent should be given by means of a signed ICF, written in non-technical language in accordance with ICH GCP, the Declaration of Helsinki, and regulatory authorities. The caregiver/patient should read and consider the statements before signing and dating them and should be given a copy of each signed document. If written consent is not possible, oral consent can be obtained if witnessed and followed by a signed statement from one or more persons not involved in the study, indicating why the patient was unable to sign the form. No patient can enter the study before his/her or caregiver informed consent has been obtained.

The ICF is part of the protocol and must be submitted by the investigator/investigative staff with the protocol for IEC approval. Albireo will supply an ICF which complies with regulatory requirements and is considered appropriate for the study. Any changes to the ICF

suggested by the investigator must be agreed to by Albireo before submission to the IEC and a copy of the approved version must be provided to the clinical monitor after IEC approval.

15 DATA HANDLING AND RECORD KEEPING

15.1 Case Report Forms/Source Data Handling

The investigator shall be provided with standardized CRFs and shall ensure that all data from patient visits are promptly entered into the CRFs in accordance with the specific instructions given. The investigator must sign each CRF to verify the integrity of the data recorded.

A list of the normal ranges for all laboratory tests to be undertaken forms part of the documentation to be collated prior to study start. If a central laboratory has been selected to conduct any or all tests, it is essential that all samples be analyzed at that laboratory.

The investigator must maintain source documents such as laboratory reports, consultation reports, and complete medical history and physical examination reports.

Data to be recorded directly on the CRFs (i.e., no prior written or electronic record of data) and considered to be source data must be identified in the protocol.

15.2 Retention of Essential Documents

Essential documents, as defined by ICH GCP, include: the signed protocol and any amendment(s); copies of the completed CRFs (for site archiving, CDs of CRF data for specific patients will be provided); signed ICFs; hospital records and other source documents; IEC approvals and all related correspondence including approved documents; drug accountability records; study correspondence; and a list of patients' names and addresses.

The investigator/investigative staff must retain copies of these essential documents for the period specified by ICH GCP and by applicable regulatory requirements. The investigator/investigative staff will inform Albireo of the location where the essential documents are stored and must contact Albireo for approval before disposing of any essential documents. The investigator/investigative staff should take measures to prevent accidental or premature destruction of these documents.

16 FINANCING AND INSURANCE

Financing and insurance are addressed in a separate agreement.

17 PUBLICATION POLICY

Albireo will retain the ownership of all data. When the study is complete, Albireo shall arrange the analysis, tabulation of data and preparation of a clinical study report. Albireo may also use the data for publication, presentation at scientific meetings and submission to regulatory authorities. All proposed publications based on this study must be subject to the sponsor's approval requirements.

18 REFERENCE LIST

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19 APPENDICES

Appendix 1 Concurrent Medications Prohibited During the Study

Appendix 2 Diary Questions including Albireo Patient-Reported Outcomes/Observer-Reported Outcome Instrument

Appendix 3 Patient Global Impression of Change (PGIC) and Patient Global Impression of Symptoms (PGIS)

Appendix 4 Pediatric Quality of Life Inventory (PedQL) Questionnaire