CLINICAL STUDY PROTOCOL A4250-012

A Phase 3 Double-blind, Randomized, Placebo-controlled Study of the Safety and Efficacy of Odevixibat (A4250) in Patients with Alagille Syndrome (ASSERT)

IND Number:	145988
EudraCT Number:	2020-004011-28
Test Product:	Odevixibat (A4250)
Sponsor:	Albireo AB
Development Phase:	Phase 3
Sponsor Signatory:	PPD
Date of the Protocol:	28 August 2020
Version of the Protocol:	2.0

Confidential

SPONSOR SIGNATURE PAGE

PROTOCOL TITLE: A Phase 3 Double-blind, Randomized, Placebo-controlled Study of the Safety and Efficacy of Odevixibat (A4250) in Patients with Alagille Syndrome (ASSERT)

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Albireo AB

PPD

INVESTIGATOR SIGNATURE PAGE

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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)/ Institutional Review Board (IRB) and will fulfil all responsibilities for submitting pertinent information to the IEC/IRB 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 or odevixibat 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 Phase 3 Double-blind, Randomized, Placebo-controlled Study of the Safety and Efficacy of Odevixibat (A4250) in Patients with Alagille Syndrome (ASSERT)

Protocol No.:	A4250-012
Date of the initial Protocol:	21 July 2020
Date and version of amended protocol:	28 August 2020; Protocol v2.0
Sponsor:	Albireo AB Arvid Wallgrens backe 20 413 46 Göteborg Sweden
Sponsor Chief Medical Officer:	PPD Albireo Pharma, Inc. 10 Post Office Square, Suite 1000 Boston, MA 02109 United States Tel PPD
Sponsor Medical Monitor:	PPD Albireo Pharma, Inc. 10 Post Office Square, Suite 1000 Boston, MA 02109 United States Tel PPD

2 STUDY SYNOPSIS

Name of Sponsor/Company: Albireo AB	**Name of Product:** Odevixibat	**Name of Active Ingredient:** Odevixibat
Title of Study: A Phase 3 Double-blind, Randomized, Placebo-controlled Study of the Safety and Efficacy of Odevixibat (A4250) in Patients with Alagille Syndrome (ASSERT)		
Study Centers: Approximately 35 sites will be initiated for this study North America, Europe, Middle East, and Asia Pacific.		
Publication(s): None.		
Planned Study Period: 2020 to 2021	**Development Phase:** Phase 3	
Primary Objective: To demonstrate the efficacy of repeated daily doses of 120 µg/kg/day odevixibat in relieving pruritus in patients with ALGS. **Secondary Objectives:** To assess the impact of odevixibat on serum bile acid levels in patients with ALGS. To evaluate the safety and tolerability of odevixibat in patients with ALGS.		
Methodology: This study includes a screening period of up to 56 days, followed by a 24-week treatment period, and a safety Follow-up Period. Screening Period: Two Screening visits will occur to establish eligibility and provide electronic diary (eDiary) instructions for recording the observer-reported outcomes (ObsRO) and patient reported outcomes (PRO) for pruritus. Treatment period: On Day 1, patients will be randomized 2:1 to receive odevixibat 120 µg/kg/day or placebo. The treatment period duration is 24 weeks. Safety Follow-up Period: A Safety Follow-up visit will take place 28 days (±7) following the Week 24/EOT visit or the date of last dose for patients who prematurely discontinue study drug. Patients who complete the Week 24/EOT visit in this study will be invited to participate in an open-label extension study in which all patients will receive active treatment. Patients who enroll in the open-label extension study will not participate in the Safety Follow-up Period.		
Number of Patients: Approximately 45 patients <18 years of age at randomization with a genetically confirmed diagnosis of ALGS will be enrolled. An additional exploratory cohort of patients ≥18 years of age with genetically confirmed diagnosis will be enrolled not to exceed 18 patients in total.		
Criteria for Inclusion and Exclusion: *Inclusion Criteria* 1. A male or female patient (of any age) with genetically confirmed diagnosis of ALGS. A patient may be randomized based on genetic testing results in the medical record. If genetic testing results are not available, testing will be performed at Screening Visit 1, and the patient may not be randomized until the genetic diagnosis is confirmed 2. Patient must have a history of significant pruritus and a caregiver reported observed scratching or a patient-reported pruritus score at an average of ≥2 (on 0 to 4 scale), as measured by the Albireo ObsRO instrument (for patients <18 years of age) or the PRO instrument (for patients ≥18 years of age) in the 14 days prior to randomization. For each AM and PM weekly assessment a minimum of 4 out of 7 expected scores must be recorded. The mean of the weekly AM and the mean of the weekly PM scores will be averaged to determine the pruritus score as measured by ObsRO or PRO, if the patient is ≥18 years of age.		

3. Patient must have an elevated baseline serum bile acid level. Each of the serum bile acid levels obtained at Screening Visit 1 and Screening Visit 2 must be greater than the upper limit of normal (>ULN)
4. 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
5. Caregivers must be willing and able to use an eDiary device as required by the study and patients ≥8 years of age must be willing to use an eDiary if able to do so
6. Sexually active males and females must agree to use 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)

Exclusion Criteria

1. Patient with past medical history or ongoing presence of other types of liver disease including, but not limited to, the following:
    a) Biliary atresia
    b) Progressive Familial Intrahepatic Cholestasis (PFIC)
    c) Benign recurrent intrahepatic cholestasis
    d) Suspected or proven liver cancer or metastasis to the liver on imaging studies
2. 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
3. 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
4. Patient has a confirmed past diagnosis of infection with human immunodeficiency virus or other present and active, clinically significant chronic infection
5. Recent infection requiring hospitalization or treatment with parenteral anti-infective within 4 weeks of randomization (Study Day 1) or completion of oral anti-infective treatment within 2 weeks prior to start of Screening Period
6. Cancer within the last 5 years except for basal cell carcinoma
7. Cancer >5 years prior to screening except for non-liver cancers with no evidence of recurrence
8. Chronic kidney disease with an impaired renal function and a glomerular filtration rate <70 mL/min/1.73 m^2
9. Patient with surgical history of disruption of the enterohepatic circulation (biliary diversion surgery) within 6 months prior to start of Screening Period
10. Patient has had a liver transplant or a liver transplant is planned within 6 months of randomization
11. Decompensated liver disease, history or presence of clinically significant ascites, variceal hemorrhage, and/or encephalopathy
12. International normalized ratio (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)
13. Serum alanine aminotransferase (ALT) >10 × ULN at Screening
14. Serum ALT >15 × ULN at any time point during the last 6 months unless an alternate etiology was confirmed for the elevation
15. Total bilirubin >15 × ULN at Screening
16. Patient suffers from uncontrolled, recalcitrant pruritic condition other than ALGS. Examples include, but not limited to, refractory atopic dermatitis or other primary pruritic skin diseases
17. Any patient who is pregnant or lactating or who is planning to become pregnant within 24 weeks of randomization
18. Patient with a past medical history of alcohol or substance abuse. 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 gastrointestinal motility

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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 may compromise the safety of the patient, or interfere with the patient participating in or completing the study

Test Product, Dose and Mode of Administration:
Odevixibat (A4250), 120 µg/kg/day once daily, orally administered

Reference Therapy, Dose and Duration of Administration:
Matching placebo, orally administered

Duration of Treatment:
24 weeks

Variables

Efficacy:

Primary Efficacy Endpoint

- Change from baseline in scratching to Month 6 (Weeks 21 to 24) as measured by the Albireo ObsRO caregiver instrument.

Key Secondary Efficacy Endpoint

- Change in serum bile acid levels from baseline to the average of Week 20 and Week 24

Secondary Efficacy Endpoints

- Change from baseline in pruritus to Month 6 (Weeks 21 to 24) as measured by the Albireo PRO instrument
- Percentage of patients achieving a clinically meaningful decrease in pruritus (pruritis responders) as measured by the Albireo ObsRO/PRO instruments
- Change from baseline through Week 24 in patient reported and observer reported itching and scratching severity scores, respectively, for the morning assessment, for the evening assessment. These endpoints will be assessed combining age groups, and by age group, 0 to <8, 8 to <12, 12 to <18, and 18 years and over
- Change from baseline to Week 24 in sleep parameters as measured with the Albireo ObsRO/PRO instruments (e.g. tiredness and number of awakenings)
- Change from baseline to Week 24 in Pediatric Quality of Life Inventory (PedsQL) subdomain scores
- Assessment of Global Symptom Relief to from baseline to Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician Global Impression of Symptoms (PGIS, CaGIS, CGIS) items
- Assessment of Global Symptom Relief as measured by patient, caregiver, and clinician Global Impression of Change (PGIC, CaGIC, CGIC) items at Weeks 4, 12, and 24
- Patient impression of treatment effect as recorded during exit interviews at Week 24
- Change from baseline to Week 24 in xanthomatosis as assessed by the Clinician Xanthoma Scale
- Change in serum bile acid levels from baseline through Week 24
- Change from baseline to Week 24 in serum ALT concentration
- Change from baseline to Week 24 in serum aspartate aminotransferase (AST) concentration
- Change from baseline to Week 24 in gamma-glutamyl transferase concentration
- Change from baseline to Week 24 in total bilirubin concentration
- Change from baseline in biochemical markers and measures of bile acid synthesis (autotaxin, plasma 7α-hydroxy-4cholesten-3one concentration [pC4])
- Change from baseline in total cholesterol concentration

Safety:

Safety criteria are as follows:

- Occurrence of treatment-emergent adverse events (TEAEs) including severity and relatedness to study drug at all visits

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Albireo AB	Odevixibat	Odevixibat

- 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)

Pharmacokinetic samples (pre-dose) will be collected at Week 4, Week 20, and at EOT when feasible.

Endpoints for the Exploratory Cohort of patients ≥18 years of age at randomization:

- All endpoints assessed for the primary analysis population will be assessed for the exploratory cohort and are considered exploratory. Of note, the PRO instead of the ObsRO will be assessed in all cases

Statistical Methods:

The primary analysis for the primary endpoint will be determined from a mixed-effect model repeated measures (MMRM) modelling change from baseline for each four-week average pruritis score, with baseline age, baseline pruritus, baseline conjugated bilirubin, treatment group, time (in months), and treatment-by-time interaction in the model. The contrast statement for Month 6 (Weeks 21 to 24) will be used to assess treatment effect.

The key secondary efficacy endpoint of change in serum bile acid will be assessed for statistical significance if and only if the success criterion for the primary endpoint is met and in the same population of patients <18 years of age. This hierarchical testing strategy prevents alpha inflation from the assessment of multiple endpoints. The MMRM utilized for the primary efficacy endpoint will also be used for assessment of the key secondary endpoint.

Secondary efficacy endpoints will provide supportive efficacy information regarding the differences between treatment with odevixibat and placebo. No adjustments will be performed for multiple comparisons when testing other efficacy endpoints. A summary of safety data will be presented.

Sample size justification:

Forty-five (45) patients <18 years of age will be randomized at an experimental to control allocation of 2 to 1 in order to obtain approximately 36 completers, assuming an approximate 20% drop-out rate. Subjects <18 years of age will be randomized according one age stratification factor, i.e. <10, and 10 to <18 years of age. At a 1-sided significance level of $alpha_{1\text{-sided}} = 0.025$, assuming a pooled standard deviation (SD) of 1.0, and a difference between the treatment groups of 1.2 in change of pruritus, favoring response, the power of the study is 0.909, using the exact method (Proc Power, SAS v.9.4, Cary, NC). During study conduct, the pooled SD will be estimated, and the sample size may be adapted, based on non-comparative blinded data for which no alpha adjustment is required. The key secondary endpoint is also powered for a standardized treatment effect (treatment effect/SD) of 1.2.

The exploratory cohort of up to 18 patients ≥18 years of age does not have a formal sample size calculation and will be used to evaluate if the PRO scratching data from patients 8 to <18 years is consistent with the PRO scratching in patients ≥18 years of age.

Date of the Protocol: 28 August 2020

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

AE(s)	adverse event(s)
AFP	alfa-fetoprotein
ALGS	Alagille syndrome
ALT	alanine aminotransferase
ASBT	apical sodium dependent bile acid transporter (also known as IBAT)
AST	aspartate aminotransferase
CaGIC	caregiver Global Impression of Change
CaGIS	caregiver Global Impression of Symptom
CGIC	clinical Global Impression of Change
CGIS	clinical Global Impression of Symptom
CRA	clinical research associate
CTCAE	Common Terminology Criteria for Adverse Events
DILI	Drug induced liver injury
DSMB	Data and Safety Monitoring Board
eCRF	electronic case report form
eDiary	electronic diary
ED_{XX}	dose required to produce XX% of the response
EOT	end of treatment
FAS	full analysis set
FDA	United States Food and Drug Administration
GCP	Good Clinical Practice
GIC	Global Impression of Change
GIS	Global Impression of Symptoms
HPE	hepatoportoenterostomy
IBAT	ileal bile acid transporter (also known as ASBT)
ICF	informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
INR	international normalized ratio
IRB	Institutional Review Board
IWRS	Interactive Web Response System
LFT	liver function test
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed-effect model for repeated measures
NAPPED	Natural Course and Prognosis of PFIC and Effect of Biliary Diversion

ObsRO	Observer reported outcome
p-C4	plasma 7α-hydroxy-4-cholesten-3-one concentration
PCS	potentially clinically significant
PedsQL	Pediatric Quality of Life Inventory
PFIC	progressive familial intrahepatic cholestasis
PGIC	patient Global Impression of Change
PGIS	patient Global Impression of Symptoms
PP	per protocol
PRO	Patient reported outcome
QoL	quality of life
SAE(s)	serious adverse event(s)
SD	standard deviation
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

5 INTRODUCTION

5.1 Investigational Medicinal Product

Odevixibat (A4250), a small molecule, is 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 humans. 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]. Odevixibat is orally administered and acts locally in the gut where it binds reversibly to IBAT to decrease the reuptake of bile acids. Odevixibat has minimal systemic exposure at the predicted therapeutic dose ranges. Because of its mechanism of action, odevixibat has the potential to ameliorate cholestatic liver disease.

5.2 Alagille Syndrome

Alagille syndrome (ALGS) is a rare multisystem disorder with a wide variety of clinical manifestations affecting the liver, heart, skeleton, eyes, central nervous system, kidneys, and facial features. It is an autosomal dominantly inherited disorder caused by defects in components of the NOTCH signaling pathway, most commonly due to mutations in JAG1, in about 90% of the patients [Oda 1997; Turnpenny 2012; Kamath 2018]. A small number of patients with ALGS have mutations in the gene for the NOTCH2 receptor [Singh 2018]. Approximately 60% of the cases represent *de novo* mutations. The majority of patients present early, often within the first 3 months of life, with jaundice or cardiac symptoms [Turnpenny 2012; Diaz-Frias 2019].

Due to the variable clinical presentations, the diagnosis of ALGS has traditionally been difficult; even the findings on histological review of liver biopsy materials may not be definitive [Turnpenny 2012]. With the advent of genetic testing, the clinical diagnosis of ALGS is confirmed or the diagnosis itself is made by finding a mutation within the sequence analysis of JAG1 or NOTCH2.

ALGS is characterized by one or more of a number of organ manifestations [Krantz 1997; Turnpenny 2012], as follows:

- Hepatic manifestations: cholestasis, bile duct paucity, pruritus, xanthomas, and cirrhosis that can lead to end-stage liver disease (approximately 95%)
- Cardiac defects: peripheral pulmonic stenosis, tetralogy of Fallot, ventricular septal defect, atrial septal defect, aortic stenosis, and coarctation of the aorta (approximately 90%)
- Dysmorphic face: prominent and broad forehead, deep-set eyes, prominent ears, triangular face with pointed chin, and broad nasal bridge (approximately 90%)

- Renal abnormalities: dysplastic kidneys, glomerular mesangiolipidosis, and renal tubular acidosis (74%)
- Skeletal malformations: butterfly vertebrae, hemivertebrae, and pathologic fractures of the long bones (70%)
- Vascular abnormalities: cerebral artery stenosis and aneurysms, Moyamoya syndrome, reno-vascular abnormalities, and middle aortic syndrome (up to 15%)
- Ophthalmologic manifestations: ocular xanthelasma and posterior embryotoxon (78% to 90%)

Other features associated with ALGS include failure to thrive, short stature, immunodeficiency with recurrent infections, pancreatic insufficiency, delayed puberty, and developmental delays [Diaz-Frias 2019]. The clinical presentation of ALGS is extremely variable and even patients from the same family with the same genetic mutation may have different presentations [Kamath 2003].

Approximately 95% of patients with ALGS present with chronic cholestasis, usually within the first 3 months of life [Emerick 2002]. Laboratory evaluation of these patients revealed elevated serum bile acids, elevated liver function tests (LFTs), and conjugated hyperbilirubinemia. Associated symptoms include xanthomas, growth failure, and pruritus.

The majority (45% to 88%) of patients with ALGS present with severe, intractable pruritus, which can be disabling. Patients with ALGS and their caregivers confirm that pruritus is the most bothersome symptom [Kamath 2018].

Currently, there is no approved medical therapy for the treatment of ALGS.

5.3 Rationale

In this investigational study, odevixibat is under development for treatment of pruritus in patients with ALGS.

Currently, there is no approved medical therapy for the treatment of pruritus in patients with ALGS. The majority of patients present with severe, intractable pruritus, which can be disabling. Attempts at managing pruritus are made by including ursodeoxycholic acid, cholestyramine, rifampin, ondansetron, or naltrexone in the patient's treatment regimen; these agents are at best partially effective [Singh 2018]. Biliary diversion surgery is occasionally used to treat intractable pruritus with some success [Emerick 2002; Mattei 2006]. Treatment of persistent cholestasis and progressive liver cirrhosis is supportive and usually includes a choleretic agent. Kasai hepatoportoenterostomy (HPE) has been attempted in an effort to increase biliary flow from the liver to the intestine, but unlike patients with biliary atresia, those with ALGS who undergo the procedure have a worse outcome [Sheflin-Findling 2012]. Approximately 15% to 25% of patients with ALGS will require a liver transplant during childhood. For patients with ALGS there is a positive response to transplant with about 90%

of patients showing improvement in liver parameters and some degree of catch-up growth. The 5-year survival post-transplant in this population is about 80% [Pawlowska 2010].

By inhibiting IBAT with high selectivity and potency, odevixibat has the potential to reduce the elevations in systemic bile acids that result from cholestasis and decrease pruritus, in patients with ALGS. The rationale for using odevixibat is to decrease serum bile acid levels, and to reduce the major morbidity of pruritus, improving the health and wellbeing of patients affected with ALGS. By reducing the elevations in systemic bile acids, odevixibat also has the potential to improve liver function and modify the progression of liver damage in patients with ALGS.

5.4 Dosing Rationale

Selection of the dose for this study was based on nonclinical and clinical data. In preclinical studies with odevixibat, the dose required to produce 50% of the response (ED_{50}) was found to be CCI µmol/kg CCI mg/kg) and the ED_{90} and ED_{95} values were calculated to be CCI mg/kg and CCI mg/kg, respectively. Using the regulatory guidance[1] on the conversion of animal dose to human effective dose, the doses observed in mice need to be divided by a factor of 12.3 yielding values of CC µg/kg, CCI µg/kg, and CCI µg/kg for the human ED_{50}, ED_{90}, and ED_{95} values, respectively.

CCI

[redacted]

[1] Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. US FDA; July 2005.

CCI [redacted]

In selecting a dose to take forward into the pivotal trial, consideration was given to the route of administration and the site of action of odevixibat. It is administered orally, has minimal systemic absorption, and acts locally within the terminal ileum by reversibly binding to and inhibiting IBAT. The magnitude and duration of IBAT inhibition is likely to be impacted by factors such as gastrointestinal transit time, presence of food, and volume of contents within the gastrointestinal tract. These factors are expected to show not only inter-individual variation, but intra-individual variation as well depending on diet, timing of meals, and intercurrent illness. In order to minimize the amount of time that a fixed dose of odevixibat produces subtherapeutic IBAT inhibition and to reduce the potential for adverse events (AEs) (i.e. maximize potential for clinical efficacy and minimize potential safety concerns), a dose (120 µg/kg/day) that is halfway between the 30 µg/kg/day dose and the 200 µg/kg/day dose was selected and is very close to the ED_{90} dose.

Using the doses and the weight range specified in the protocol, the doses that will be administered are between the calculated ED_{50} and ED_{95} values and are within the range that was demonstrated to be well tolerated in the Phase 2 study in children with pediatric cholestatic liver disease.

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

5.5 Risk/Benefit

In adult healthy volunteers and pediatric patients with cholestatic liver disease, odevixibat has been generally safe and well tolerated in all completed studies. Reported AEs have primarily been of mild intensity. Abdominal pain and diarrhea have been the most common AEs in adults, and only 1 AE of diarrhea was reported in the pediatric Phase 2 study. Based on the mode of action of odevixibat as an IBAT inhibitor, loose stools or diarrhea are expected.

Infants with ALGS have elevated serum bile acids. Serum bile acids are an indicator of elevated bile acids within the liver, which in turn are thought to play a contributory role in hepatic oxidative stress and fibrosis. It has been shown that serum bile acid levels can predict long-term outcomes in patients with biliary atresia; even in patients with successful Kasai HPE procedures (defined as serum total bilirubin levels <1.5 mg/dL at 6 months post Kasai HPE); elevated serum bile acids may persist and can predict continued loss of hepatic function [Harpavat 2018]. Likewise, data from the Natural Course and Prognosis of Progressive Familial Intrahepatic Cholestasis (PFIC) and Effect of Biliary Diversion (NAPPED) consortium have shown that serum bile acid levels can predict long-term outcomes in patients with PFIC Type 1 [Van Wessel 2019] and Type 2 [Van Wessel 2020]. These clinical

observations, along with preclinical data demonstrating the adverse impact of elevated bile acids on the liver, provide support for the hypothesis that lowering serum bile acids may be of benefit in the long-term outcome of patients with cholestatic liver diseases including ALGS. By reducing the bile acid load, odevixibat has the potential not only to reduce the pruritus associated with chronic cholestasis, but also to ameliorate or slow hepatic injury or fibrosis and improve the long-term hepatic outcomes in patients with ALGS. The risk/benefit profile of odevixibat in patients with ALGS is considered acceptable.

6 STUDY OBJECTIVES

6.1 Primary Objective

- To demonstrate the efficacy of repeated daily doses of 120 µg/kg/day odevixibat in relieving pruritus in patients with ALGS.

6.2 Secondary Objectives

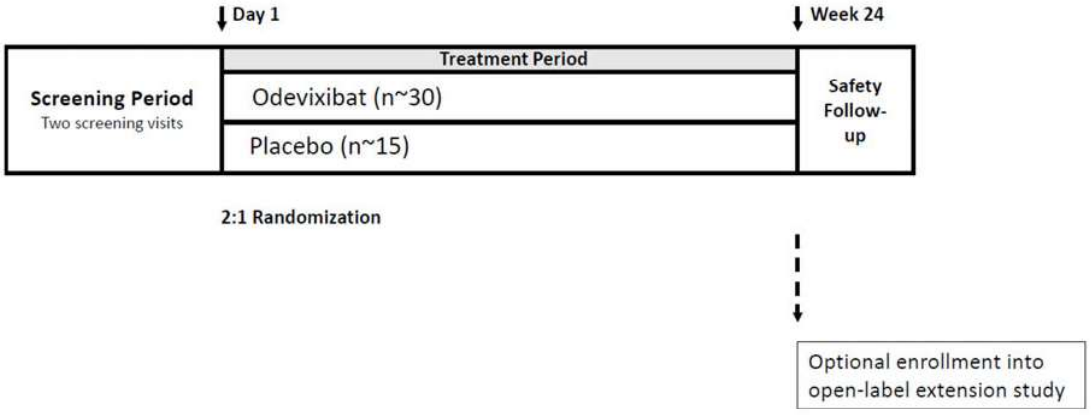
- To assess the impact of odevixibat on serum bile acid levels in patients with ALGS.
- To evaluate the safety and tolerability of odevixibat in patients with ALGS.

7 INVESTIGATIONAL PLAN

7.1 Overall Study Design and Plan

This is a Phase 3, double-blind, randomized, placebo-controlled study to investigate the efficacy and safety of 120 µg/kg/day odevixibat in patients with ALGS.

Figure 1 Study Design



Odevixibat = once daily treatment with 120 µg/kg/day odevixibat

7.1.1 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[a]	Screening Period[b]		Random-ization	Phone Call	Week 4	Week 8	Week 12	Week 16	Week 20	24 weeks/End of Treatment (EOT)[c]	Safety Follow-up[d]
Study Days	**-56 to -21**	**-49 to -14**	**1**	**14 ± 3**	**28 ± 3**	**56 ± 3**	**84 ± 3**	**112 ± 3**	**140 ± 3**	**168 ±3**	**196 ±7**
Clinic 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**	**Clinic Visit 10**
Phone Call				**X**							
Informed consent[e]	X										
Inclusion/exclusion criteria	X	X	X								
Demographics	X										
Prior and Concomitant medications[f]	X	X	X	X	X	X	X	X	X	X	X
Medical and surgical history	X	X	X								
Clinical Genetic Testing	X										
Full physical examination[g]	X		X								
Skin examination and symptom based physical examination[g]		X			X	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								
Clinical chemistry[j]	X	X	X		X	X	X	X	X	X	X

Study Activity[a]	Screening Period[b]		Random-ization	Phone Call	Week 4	Week 8	Week 12	Week 16	Week 20	24 weeks/End of Treatment (EOT)[c]	Safety Follow-up[d]
Study Days	**-56 to -21**	**-49 to -14**	**1**	**14 ± 3**	**28 ± 3**	**56 ± 3**	**84 ± 3**	**112 ± 3**	**140 ± 3**	**168 ±3**	**196 ±7**
Clinic 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**	**Clinic Visit 10**
Phone Call				**X**							
Hematology[j]	X		X		X		X			X	X
Urinalysis[j]	X						X			X	X
Serum bile acids (fasting[k])	X	X	X		X	X	X	X	X	X	X
INR	X		X		X	X	X	X	X	X	X
Autotaxin[l]			X				X			X	
p-C4[l]			X				X			X	
AFP			X							X	
Vitamins A (fasting[k]) & E	X		X				X			X	
25-hydroxy vitamin D	X		X				X			X	
Blood sample for odevixibat PK[l]					X				X	X (EOT only)	
Liver ultrasound and elastography (where available)			X							X	
QoL questionnaire (PedsQL)			X				X			X	
Patient/Caregiver/ Clinician Global Impression of Change					X		X			X	

Study Activity[a]	Screening Period[b]		Random-ization	Phone Call	Week 4	Week 8	Week 12	Week 16	Week 20	24 weeks/End of Treatment (EOT)[c]	Safety Follow-up[d]
Study Days	**-56 to -21**	**-49 to -14**	**1**	**14 ± 3**	**28 ± 3**	**56 ± 3**	**84 ± 3**	**112 ± 3**	**140 ± 3**	**168 ±3**	**196 ±7**
Clinic 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**	**Clinic Visit 10**
Phone Call				**X**							
Patient/Caregiver/ Clinician Global Impression of Symptoms			X		X		X			X	
Clinician assessment of xanthoma			X				X			X	
Pregnancy test[m]	X serum	X urine	X urine		X urine	X urine	X urine	X urine	X urine	X urine	X urine
Study drug dispensed[n]			X		X	X	X	X	X		
Adverse events[o]	SAE only		All Adverse Events								
FSV Deficiency Questionnaire	X	X	X	X	X	X	X	X	X	X	X
Study drug compliance					X	X	X	X	X	X	
eDiary compliance[i]		X	X	X	X	X	X	X	X	X	
Exit Interview										X	

AFP: alfa-fetoprotein; eCRF: electronic case report form; eDiary: electronic diary; EOT: end of treatment; FSV: fat soluble vitamin; 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; SAE: serious adverse event.

[a] All assessments will be performed before dosing unless otherwise noted.

[b] The two screening visits must be separated by at least 7 days, and Screening Visit 2 must occur at least 14 days prior to Study Day 1.

[c] An EOT visit must be performed as soon as possible if study drug is prematurely discontinued. The EOT visit requires the same assessments as the Week 24 visit.

[d] The Safety Follow-up visit is required 28 days (±7) following the Week 24/EOT visit or the date of last dose for patients who prematurely discontinued study treatment. See Section 7.1.3 and Section 7.1.4 for when a Safety Follow-up visit is not required.

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

[f] Past (within 28 days prior to Screening Visit 1) and current medication will be documented in the eCRF.

[g] A full physical exam including skin exam will occur at Screening Visit 1 and Day 1. At all other clinic visits a skin exam and symptom-based physical exam will occur. Findings from the symptom-based physical exam will be documented as adverse events if appropriate.

[h] Includes blood pressure, pulse, respiratory rate, temperature, height (or length depending on age), and body weight.

[i] Includes training on use of the device at Screening Visit 1, retraining (if applicable) at Screening Visit 2, and review of patient daily entries during 14 days prior to Day 1 to determine eligibility. eDiary compliance will be reviewed at all visits and the Day 14 phone call.

[j] See Table 4 for detailed parameters.

[k] Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for serum bile acid and vitamin A. Exceptions can be made for infants, less than 12 months of age, if unable to fast for the full 4 hours.

[l] Samples will not be collected for patients ≤10 kg. In the event of an SAE, samples are to be collected as close to the onset of the event as possible.

[m] For females who have reached menarche. If a urine pregnancy test is positive, a serum pregnancy test is to be performed to confirm the pregnancy.

[n] Study drug to be taken once daily from randomization (Study Day 1) through the Week 24/EOT visit as described in Section 7.1and 7.1.2.2.

[o] Serious adverse events will be collected from the time of signing of the ICF through study completion. Non-serious adverse events will be collected from the first dose of study drug through study completion. See section 10.1.1 for additional information.

7.1.2 Study Procedures and Assessments

When conditions make it challenging for a patient to attend a visit at the study center and where applicable country and local regulations and infrastructure allow, routine assessments may be performed outside of the study center (eg, the patient's home) by a trained healthcare professional at all timepoints following randomization (Day 1). These assessments can include the following: vital signs, height and weight, blood draws, urine collection, and urine pregnancy test. All laboratory samples should be sent to the central laboratory; an exception is for situations related to the COVID-19 pandemic if central laboratory assessments are not possible, then a local laboratory may be used. These local lab results must be sent to the site for review by the Investigator and entry into the clinical database as applicable. Collection of other visit requirements, eg concomitant medication and AE collection associated with visits outside of the clinic will be collected by qualified site staff through verbal contact with the patient.

For any study visit, the study physician (or delegate) must, at a minimum, verbally contact the patient within the expected window for each study visit to collect relevant safety information (including, but not limited to, AEs, concomitant medications, hospitalizations/procedures, and vital status). Any missed assessment due to a study visit being performed outside of the study center will be considered a protocol deviation.

7.1.2.1 Screening Period (Day -56 to Day -1)

7.1.2.1.1 Screening Visit 1 (Day -56 to Day -21)

Patients will undergo Screening Visit 1 up to 56 days prior to the planned first day of study treatment.

Informed consent/assent must be obtained at the Screening Visit prior to performing any study procedures. After signing the informed consent form (ICF), patients will be evaluated for study eligibility.

Caregivers/patients will be instructed on the daily use of the Albireo electronic diary (eDiary). The eDiary will include observer reported outcome (ObsRO)/patient reported outcome (PRO) items for evaluation of itching (PRO), scratching (ObsRO), and sleep disturbance (PRO and ObsRO).

Daily recording of pruritus using the eDiary will be started to confirm the magnitude of baseline pruritus symptoms before randomization. The 14 consecutive days prior to randomization will be used for eligibility calculation of the diary entries. ObsRO in patients <18 years of age will be recorded by a caregiver. If possible, the same caregiver will complete the Albireo ObsRO items throughout the study.

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, as local regulations permit)
- Record any medications the patient has taken starting from Day 28 days before Screening Visit 1
- Medical and surgical history (date of diagnosis of ALGS, prior investigational medications for ALGS, historical LFT values [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubin values obtained in the 2 years prior to screening if available should be entered into the electronic case report form (eCRF), any surgery performed including biliary diversion (if applicable), any other diagnosis, and historical liver biopsy data]. If a liver biopsy has been performed and the results are available, the results will be recorded in the eCRF. Record any symptoms of FSV deficiency. Medical and surgical history will be recorded through the timing of the first dose of study drug.
- Collect blood sample for ALGS genetic testing to determine eligibility if not available in the medical record. If a historical clinical genetic laboratory report for ALGS is available in the medical record, it is not required to collect a sample for genetic testing at this visit.
- Full physical examination (Section 10.2.5)
- Vital signs (Section 10.2.7)
- eDiary training and review of compliance requirements; caregivers and, if applicable, patients begin daily recording of pruritus using the eDiary (Appendix 2)
- Clinical chemistry, hematology, and urinalysis (Section 10.2.1)
- Serum bile acid (Section 9.2.2)
- International normalized ratio (INR)
- Vitamins A and E
- 25-hydroxy vitamin D
- Serum pregnancy test for all females who have reached menarche. Please see Appendix 6 for contraceptive requirements
- SAE monitoring

7.1.2.1.2 Screening Visit 2 (Day -49 to Day -14)

A second Screening Visit will be performed. This visit is to confirm eDiary compliance and provide retraining if required. The two screening visits must be separated by at least 7 days, and Screening Visit 2 must occur at least 14 days prior to Study Day 1.

Screening procedures and assessments are as follows:

- Assess inclusion/exclusion criteria (Section 7.2)
- Review concomitant medications
- Medical and surgical history will be recorded through the timing of the first dose of study drug
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.7)
- Albireo ObsRO/PRO eDiary review of scores and compliance
- Clinical chemistry (Section 10.2.1)
- Serum bile acid (Section 9.2.2)
- Urine pregnancy test for females of who have reached menarche
- SAE monitoring and FSV deficiency questionnaire

7.1.2.1.3 Repetition of Screening Assessments

Screening assessments may be repeated to establish eligibility if the original assessment did not yield an interpretable result (e.g., quantity insufficient).

7.1.2.1.4 Rescreening:

Patients may be rescreened a total of 2 times.

7.1.2.2 Treatment Period

7.1.2.2.1 Randomization (Study Day 1)

Eligibility for randomization will be determined at Study Day 1 using the pruritus or scratching reports from the Albireo ObsRO/PRO instruments data in the 14 days before Study Day 1, clinical genetic confirmation of diagnosis, and the liver biochemistry evaluations from the previous screening visits.

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

- Review inclusion/exclusion criteria (Section 7.2)
- Review concomitant medications
- Medical and surgical history will be recorded through the timing of the first dose of study drug.
- Full physical examination (Section 10.2.5)
- Vital signs (Section 10.2.7)
- Albireo ObsRO/PRO eDiary review for compliance and entrance criteria requirement (see Inclusion Criterion 2 and 5, Section 7.2.1)
- Clinical chemistry and hematology (Section 10.2.1)

- Serum bile acid (Section 9.2.2)
- INR
- Autotaxin and plasma 7α-hydroxy-4 cholesten-3 one concentration (p-C4) (samples will not be taken for patients ≤10 kg)
- Alfa-fetoprotein (AFP)
- Vitamins A and E
- 25-hydroxy vitamin D
- Liver ultrasound and elastography (where available) (Section 10.2.6)
- Quality of life (QoL) questionnaire (Pediatric Quality of Life Inventory [PedsQL])
- Patient/caregiver/clinician complete the Global Impression of Symptoms (GIS) measures (PGIS/CaGIS/CGIS)
- Clinician assessment of xanthoma
- Urine pregnancy test for females who have reached menarche
- Study drug is dispensed and patients are instructed to administer daily from randomization (Study Day 1)
- SAE monitoring and FSV deficiency questionnaire. Non-serious AE monitoring begins after the first dose of study drug is taken

7.1.2.2.2 Study Day 14 (Phone Call)

A study site staff member will contact the patient/caregiver by telephone for monitoring of the following:

- Review concomitant medications
- Albireo ObsRO/PRO eDiary review for compliance
- AE monitoring and FSV deficiency questionnaire

7.1.2.2.3 Week 4, 8, 16, and 20 Visits

The following procedures and assessments will be performed:

- Review of concomitant medications
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.7)
- Albireo ObsRO/PRO eDiary review for compliance
- Clinical chemistry (Section 10.2.1)
- Hematology (at Week 4 visit only) (Section 10.2.1)
- Serum bile acid (Section 9.2.2)

- INR
- Odevixibat PK sample (at Week 4 and 20 only; samples will not be taken for patients ≤10 kg)
- Patient/caregiver/clinician complete the patient Global Impression of Change (GIC) measures (PGIC/CaGIC/CGIC) and GIS measures (PGIS/CaGIS/CGIS) (Week 4 visit only)
- Urine pregnancy test for females who have reached menarche
- AE monitoring and FSV deficiency questionnaire
- Evaluation of study drug compliance
- Study drug is dispensed

7.1.2.2.4 Week 12 Visit

The following procedures and assessments will be performed:

- Review of concomitant medications
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.7)
- Albireo ObsRO/PRO eDiary review for compliance
- Clinical chemistry, hematology, and urinalysis (Section 10.2.1)
- Serum bile acid (Section 9.2.2)
- INR
- Autotaxin and p-C4 (samples will not be taken for patients ≤10 kg)
- Vitamins A and E, and 25-hydroxy vitamin D
- QoL questionnaire (PedsQL)
- Patient/caregiver/clinician complete the PGIC/CaGIC/CGIC and PGIS/CaGIS/CGIS
- Clinician assessment of xanthoma
- Urine pregnancy test for females who have reached menarche
- AE monitoring and FSV deficiency questionnaire
- Evaluation of study drug compliance
- Study drug is dispensed

7.1.2.2.5 Week 24/EOT Visit

For the Week 24/end of treatment (EOT), the patient has the option to continue in an open-label extension study, provided they meet eligibility criteria for that study.

For patients who permanently discontinue treatment early, during the treatment period, an EOT visit will be performed which includes the identical procedures that are performed at Week 24.

The following procedures and assessments will be performed:

- Review of concomitant medications
- Skin examination (Section 10.2.5)
- Vital signs (Section 10.2.7)
- Albireo ObsRO/PRO eDiary review for compliance
- Clinical chemistry, hematology, and urinalysis (Section 10.2.1)
- Serum bile acid (Section 9.2.2)
- INR
- Autotaxin and p-C4 (samples will not be taken for patients ≤10 kg)
- AFP
- Vitamins A and E, and 25-hydroxy vitamin D
- Odevixibat PK sample (samples will not be taken for patients ≤10 kg; EOT only)
- Liver ultrasound and elastography (where available) (Section 10.2.6)
- QoL questionnaire (PedsQL)
- Patient/caregiver/clinician complete the PGIC/CaGIC/CGIC and PGIS/CaGIS/CGIS
- Clinician assessment of xanthoma
- Urine pregnancy test for females who have reached menarche
- AE monitoring and FSV deficiency questionnaire
- Evaluation of study drug compliance
- Exit interview

7.1.3 Safety Follow-Up

The Safety Follow-up visit is required 28 days (±7) following the Week24/EOT visit. For patients prematurely discontinued study treatment, this visit is not required if the EOT visit occurs more than 3 weeks after the last dose of study drug (Section 7.1.4). This visit is not required for patients who enroll in the open-label extension study within 28 days of the Week 24 Visit.

The following procedures and assessments will be performed:

- Review of concomitant medications
- Skin examination (Section 10.2.5)

- Vital signs (Section 10.2.7)
- Clinical chemistry, hematology, and urinalysis (Section 10.2.1)
- Serum bile acid (Section 9.2.2)
- INR
- Urine pregnancy test for females who have reached menarche
- AE monitoring and FSV deficiency questionnaire

7.1.4 Early Discontinuation of Treatment During the Treatment Period

Patients who prematurely discontinue study drug treatment during the treatment period will have an EOT visit as soon as possible after the decision is made to permanently discontinue study drug, and no later than their next scheduled visit. All efforts will be made to have patients continue to complete all scheduled study visits for assessments following completion of the EOT visit, as detailed in Table 1. A safety follow-up visit is not required if patients continue to complete scheduled study visits after EOT. For patients who do not continue to attend scheduled study visits after EOT, a safety follow--up visit is not required if the EOT visit occurred more than 3 weeks after the last dose of study drug.

If study drug is permanently discontinued for any reason, the patient will be monitored weekly until the laboratory and clinical parameters have normalized/stabilized as clinically indicated. See Section 10.1.3 regarding monitoring AEs and serious AEs (SAEs) after the patient's last study visit.

Permanent discontinuation of study drug may occur for any of the following reasons:

- Death
- Patient/caregiver estimation of intolerable symptoms
- Safety, behavior, or noncompliance with study procedures as determined by the investigator or Sponsor
- Treatment un-blinding
- An investigator's opinion that continuing the patient in the study is not appropriate. The investigator may permanently discontinue study drug at any time, if it is considered to be in the patient's best interest

If a patient permanently discontinues study drug and also withdraws consent or assent, no further assessments will be performed. Albireo may retain and continue to use any data and samples collected before such withdrawal of consent or assent.

7.2 Study Population

A total of approximately 45 patients <18 years of age at randomization with a genetically confirmed diagnosis of ALGS will be enrolled. An additional exploratory cohort of patients ≥18 years of age with genetically confirmed diagnosis will be enrolled not to exceed

18 patients in total, who will also be randomized 2:1 favoring active treatment. A separate randomization scheme will be provided for this cohort.

7.2.1 Inclusion Criteria

1. A male or female patient (of any age) with genetically confirmed diagnosis of ALGS. A patient may be randomized based on genetic testing results in the medical record. If genetic testing results are not available, testing will be performed at Screening Visit 1, and the patient may not be randomized until the genetic diagnosis is confirmed
2. Patient must have a history of significant pruritus and a caregiver reported observed scratching or a patient-reported pruritus score at an average of ≥2 (on 0 to 4 scale), as measured by the Albireo ObsRO instrument (for patients <18 years of age) or the PRO instrument (for patients ≥18 years of age) in the 14 days prior to randomization. For each AM and PM weekly assessment a minimum of 4 out of 7 expected scores must be recorded. The mean of the weekly AM and the mean of the weekly PM scores will be averaged to determine the pruritus score as measured by ObsRO or PRO, if the patient is ≥18 years of age
3. Patient must have an elevated baseline serum bile acid level. Each of the serum bile acid levels obtained at Screening Visit 1 and Screening Visit 2 must be greater than the upper limit of normal (>ULN)
4. 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
5. Caregivers must be willing and able to use an eDiary device as required by the study and patients ≥8 years of age must be willing to use an eDiary if able to do so
6. Sexually active males and females must agree to use 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

7.2.2 Exclusion Criteria

1. 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) PFIC
    c) Benign recurrent intrahepatic cholestasis
    d) Suspected or proven liver cancer or metastasis to the liver on imaging studies

2. 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
3. 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
4. Patient has a confirmed past diagnosis of infection with human immunodeficiency virus or other present and active, clinically significant chronic infection
5. Recent infection requiring hospitalization or treatment with parenteral anti-infective within 4 weeks of randomization (Study Day 1) or completion of oral anti-infective treatment within 2 weeks prior to start of Screening Period
6. Cancer within the last 5 years except for basal cell carcinoma
7. Cancer >5 years prior to screening except for non-liver cancers with no evidence of recurrence
8. Chronic kidney disease with an impaired renal function and a glomerular filtration rate <70 mL/min/1.73 m^2
9. Patient with surgical history of disruption of the enterohepatic circulation (biliary diversion surgery) within 6 months prior to start of Screening Period
10. Patient has had a liver transplant or a liver transplant is planned within 6 months of randomization
11. Decompensated liver disease, history or presence of clinically significant ascites, variceal hemorrhage, and/or encephalopathy
12. 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)
13. Serum ALT >10 × ULN at Screening
14. Serum ALT >15 × ULN at any time point during the last 6 months unless an alternate etiology was confirmed for the elevation
15. Total bilirubin >15 × ULN at Screening
16. Patient suffers from uncontrolled, recalcitrant pruritic condition other than ALGS. Examples include, but not limited to, refractory atopic dermatitis or other primary pruritic skin diseases
17. Any patient who is pregnant or lactating or who is planning to become pregnant within 24 weeks of randomization
18. Patient with a past medical history of alcohol or substance abuse. 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 gastrointestinal 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. Any other conditions or abnormalities which, in the opinion of the investigator may compromise the safety of the patient, or interfere with the patient participating in or completing the study

7.2.3 Study Withdrawal of Patients

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

Any withdrawal of consent, 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 Safety Follow-Up visit, the investigator/investigative staff will, regardless of reason for withdrawal of consent, record any patient data they receive concerning SAEs, and all drug -related non-serious AEs, and subsequently report these according to Section 10.1.3. Withdrawn patients will not be replaced.

Patients may be withdrawn in the following circumstances:

- A patient's/caregiver's desire for withdrawal for any reason (withdrawal of consent)
- Lost to follow-up: A patient will be considered lost to follow-up if both of the following occur: The patient misses 2 consecutive study visits and is subsequently unable to be contacted by telephone (3 documented attempts by telephone following the second missed visit) AND the patient does not respond within 2 weeks to a registered letter sent after the 3 attempted telephone calls

7.2.4 Individual Patient Stopping Criteria

While this study has no restrictions on patient age at enrollment, it is likely that the majority will be in the pediatric age categories. To protect the patients, administration of study drug will be stopped for the following reasons:

- An AE that, in the opinion of the investigator, necessitates treatment discontinuation. Specific instructions for the management of hepatic AEs, potential drug-induced liver injury and diarrhea are provided in Section 10.2.2
- In the opinion of the investigator there is no longer a potential benefit for continuing treatment; for example in patients who develop hepatic failure requiring liver

transplantation or in patients who undergo biliary diversion surgery for the relief of pruritus

7.2.5 Termination of Study Participation

Participation in the study will be completed for each individual patient when one of the following occurs:

- Week 24 visit: for patients who complete the treatment period and enter an open-label study within 28 days of the Week 24 visit
- Safety Follow-up visit: for patients who complete the treatment period and do not enter an open-label study within 28 days of the Week 24 visit
- For patients who withdraw consent or assent, the date of withdrawal of consent or assent
- For patients who are lost to follow-up (Section 7.2.3), the date of study participation completion will be defined as the date of the last contact

7.2.6 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

Odevixibat and placebo will be supplied as capsules for oral administration. White opaque capsules filled with pellets containing odevixibat 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 is intended to 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 odevixibat and placebo capsules will be identical in appearance and filling weight. Bottles with capsules will be given to the patient/caregiver.. Refer to the Pharmacy Manual for further details.

8.2 Administration of Study Drug

Patients will be dosed with odevixibat at a dose of 120 µg/kg/day or placebo for 24 weeks. Dose modification is permitted per Section 8.3. Study drug will be dispensed to the patient from randomization (Study Day 1) through the Week 20 visit, together with instructions on how to store and take the drug.

Odevixibat is to be taken in the morning, together with food. On clinic visit days when laboratory assessments are conducted, the study drug is to be taken after the visit and after laboratory samples are taken. The capsule(s) is/are not to be crushed or chewed. When swallowing the capsule intact, the dose is to be administered with a glass of water.

For a patient unable to swallow a 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 is to be less than what the patient/infant would be expected to eat and is to be followed by water. For infants who are not yet weaned onto solid foods, the pellets can be administered as a suspension in infant formula or breast milk using an appropriate dosing device (e.g. an oral dosing syringe).

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 mL 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 randomization (Study Day 1) and can be adjusted depending on the patient's weight (identified in Table 2) at each study visit.

Table 2 Dosing and Capsule Strength for 120 µg/kg/day Dose

Body Weight (kg)	Capsule Size	Number of Capsules per Day	Capsule Strength (µg)	Total Dose (µg)	Capsule Size, Placebo (µg)
4.0-<6	0	3	200	600	0
6.0-<8	0	4	200	800	0
8.0-<12.5	0	2	600	1200	0
12.5 to <17.5	0	3	600	1800	0
17.5 to <19.5	0	4	600	2400	0
19.5 to <25.5	3	2	1200	2400	3
25.5 to <35.5	3	3	1200	3600	3
35.5 to <45.5	3	4	1200	4800	3
45.5 to 55.5	3	5	1200	6000	3
>55.5	3	6	1200	7200	3

8.3 Dose Modification Guidelines

If, in the clinical opinion of the investigator, a dose modification may be beneficial to manage any potential AEs, dose modification is permitted and study drug may be continued at a reduced dose level of 40 µg/kg/day (Table 3). In addition, if study drug is interrupted for clinically significant diarrhea (Section 10.2.2.4) and the patient fails re-challenge at 120 µg/kg/day, the patient may be re-challenged at 40 µg/kg/day. With the exception of clinically significant diarrhea, the timing of dose increase/re-challenge at 120 µg/kg/day is at the discretion of the investigator. Two dose reductions are permitted during the study. Any dose modification must be recorded in the clinical database.

Table 3 Dosing and Capsule Strength for 40 µg/kg/day Dose

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

8.4 Study Treatment Packaging and Labeling

8.4.1 Packaging and Labeling

The capsules will be packed in high density polyethylene containers, with child-proof polypropylene caps. Study drug capsules containing odevixibat in the strengths specified in Table 2 and Table 3 above will be manufactured.

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

8.4.2 Storage

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

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

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

8.4.3 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.

An Interactive Web Response System (IWRS) will be used to assign patients to treatment. The randomization codes will be computer generated by Albireo or a qualified randomization vendor.

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 odevixibat 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 to which a patient is randomized. Traceability of the treatment is ensured by the study drug number.

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 un-blinding and will not be displayed to end users in the IWRS.

8.5 Procedure for Breaking the Randomization Code

Should a situation arise where un-blinding 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 un-blinding through IWRS. The

responsibility to break the treatment code in emergency situations resides solely with the investigator.

The study site investigator will be authorized to access the emergency un-blinding functionality within the IWRS. Emergency un-blinding takes place in the IWRS via a dedicated emergency un-blinding module. Once the randomization code is broken for a patient, he/she must be withdrawn from the study and all assessments and an EOT visit should be performed. Once a randomization code has been broken, the investigator must inform the sponsor Medical Monitor in writing within 24 hours.

8.6 Patient Compliance

The study site staff will monitor eDiary compliance by routine review of the eDiary website. If both diary entries on a day are missing, or if more than two in a week are missing, the study site staff will call the patient/caregiver to remind them to complete all scheduled entries.

Additionally, for study drug compliance, patients will return all study drug at each visit. The study site staff will count all returned drug and perform reconciliation.

Any non-compliance in reference to eDiary or study drug will be documented and explained in the source documents.

8.7 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.

8.8 Concomitant Therapy and Prohibited Medications

The investigator will note all ongoing medication and any medication recently stopped (within 28 days prior to screening) in the eCRF. At all visits, all changes in medication (stopping or starting new medication or changes in dose and/or frequency) 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.

Medications to treat pruritis, such as ursodeoxycholic acid, rifampicin, and antihistamines, are permitted provided the patient was on stable dosage at least four weeks prior to first dose, and no dosage change is planned during the treatment period.

Prohibited medications are listed in Appendix 1.

9 ASSESSMENT OF EFFICACY

9.1 Efficacy Endpoints

9.1.1 Primary Efficacy Endpoint

- Change from baseline in scratching to Month 6 (Weeks 21 to 24) as measured by the Albireo ObsRO caregiver instrument

9.1.2 Key Secondary Efficacy Endpoint

- Change in serum bile acid levels from baseline to the average of Week 20 and Week 24

9.1.3 Secondary Efficacy Endpoints:

- Change from baseline in pruritus to Month 6 (Weeks 21 to 24) as measured by the Albireo PRO instrument
- Percentage of patients achieving a clinically meaningful decrease in pruritus (pruritis responders) as measured by the Albireo ObsRO/PRO instruments
- Change from baseline through Week 24 in patient reported and observer reported itching and scratching severity scores, respectively, for the morning assessment, for the evening assessment. These endpoints will be assessed combining age groups, and by age group, 0 to <8, 8 to <12, 12 to <18, and 18 years and over
- Change from baseline to Week 24 in sleep parameters as measured with the Albireo ObsRO/PRO instruments (e.g. tiredness and number of awakenings)
- Change from baseline to Week 24 in PedsQL subdomain scores
- Assessment of Global Symptom Relief to from baseline to Weeks 4, 12, and 24 as measured by patient, caregiver, and clinician GIS (PGIS, CaGIS, CGIS) items
- Assessment of Global Symptom Relief as measured by patient, caregiver, and clinician GIC (PGIC, CaGIC, CGIC) items at Weeks 4, 12, and 24
- Patient impression of treatment effect as recorded during exit interviews at Week 24
- Change from baseline to Week 24 in xanthomatosis as assessed by the Clinician Xanthoma Scale
- Change in serum bile acid levels from baseline through Week 24
- Change from baseline to Week 24 in serum ALT concentration
- Change from baseline to Week 24 in serum AST concentration
- Change from baseline to Week 24 in gamma-glutamyl transferase concentration
- Change from baseline to Week 24 in total bilirubin concentration
- Change from baseline in biochemical markers and measures of bile acid synthesis (autotaxin, p-C4, only in patients >10 kg)

- Change from baseline in total cholesterol concentration

9.1.4 Exploratory Cohort Endpoints

- All endpoints assessed for the primary analysis population will be assessed for the cohort of patients ≥18 years of age at randomization and are considered exploratory. Of note, the PRO instead of the ObsRO will be assessed in all cases

9.2 Efficacy Assessments

All in clinic assessments will be performed pre-dose.

9.2.1 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 Screening Visit 1. During Screening Visit 2, parents and caregivers will be retrained if necessary. When logistically feasible, the same parent or caregiver should record in the eDiary, and changes to the parent or caregiver who is performing the recording should be minimized. If a new parent or caregiver will begin to complete the Albireo ObsRO eDiary during the course of the trial, they will be provided with training during a clinic visit before they begin to use the eDiary. The caregiver recording in the eDiary will be recorded.

The eDiary includes Albireo ObsRO and PRO items. Patients <8 years of age at time of signing the ICF will not be asked to complete the Albireo PRO items; only the Albireo ObsRO eDiary will be completed by caregivers of patients in this age group. Older patients, 8 to 18 years of age at time of signing the ICF, will complete the Albireo PRO items and the caregiver will complete the Albireo ObsRO items. Patients ≥18 years of age at signing of the ICF will complete the PRO; no caregiver information will be collected. 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 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 and PM score for the Albireo ObsRO scratching item will be averaged from the 2 ratings for each day. The AM score captures nighttime symptoms and the PM score captures daytime symptoms.

9.2.2 Serum Bile Acids

Blood samples for analysis of fasting total serum bile acid will be drawn at all clinic visits. Patients will fast (water intake only is permissible) for at least 4 hours prior to the collection of samples for serum bile acid. 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 post baseline serum bile acid will be blinded. Samples will be processed and transported to a central laboratory per instructions in the laboratory manual.

9.2.3 Quality of Life Questionnaire (PedsQL)

Patients and caregivers will be asked to complete a QoL questionnaire (PedsQL) at randomization (Study Day 1), Week 12, Week 24/EOT visits. Details of the questions included on the questionnaire are provided in Appendix 4.

9.2.4 Biochemical Markers and PK Samples

Blood samples for analysis of clinical chemistry will be drawn during Screening Visits 1 and 2, as well as at randomization (Study Day 1), Week 4 to 24/EOT, and Safety Follow-up visits. As outlined in Table 4, total bilirubin, AST, ALT, and gamma glutamyl transferase assessments are included as part of the routine laboratory parameters. Blood samples for autotaxin and p-C4 will be drawn at randomization (Study Day 1), Week 12, and Week 24/EOT, only for children with body weight >10 kg. Blood for Odevixibat PK assessment will be drawn at Week 4, Week 20, and EOT (only for children with body weight >10 kg). When a patient meets criteria for close observation due to suspected DILI (Section 10.2.2.2) samples will be collected for FGF19, p-C4, and PK within 3 days if possible (only for children with body weight >10 kg). Samples will be processed and transported to a laboratory per instructions in the laboratory manual.

9.2.5 Global Impression of Change and Global Impression of Symptom Measures

Patients (≥8 years of age), caregivers, and clinicians will complete GIC (PGIC, CaGIC, and CGIC) and the GIS measures (PGIS, CaGIS, CGIS) (Appendix 3) at randomization (Study Day 1; PGIS only), Week 4, 12, and 24/EOT.

The GIC items assess change in itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) since starting the study drug. The GIS items assess itch (patient version), scratching (caregiver and clinician versions), and sleep (all versions) in the past week. Caregivers and clinicians will complete the GIC and GIS for all patients; those patients ≥8 years of age will complete the patient version.

9.2.6 Clinician Assessment of Xanthoma

Changes in xanthoma as assessed by the Clinician Xanthoma Scale will be measured at randomization (Study Day 1), Week 12, and Week 24/EOT visits. The clinician's assessment of the participant's xanthomatosis is focused on the number of lesions present and the degree to which the participant's lesions interfere or limit his or her activities. The clinician xanthoma scale uses a 5-point scale, in which 0 represents no evidence of xanthomatosis, 1 represents fewer than 20 scattered individual lesions, 2 represents more than 20 lesions that do not interfere with or limit activities, 3 represents large numbers of lesions that by their large numbers or size cause distortion of the face or extremities, and 4 represents xanthomas that interfere with function (such as hand use or ability to walk) because of excess size or number.

10 ASSESSMENT OF SAFETY

The timing and frequency of safety assessments are described in Section 7.1.3 and in Table 1 above. For each study visit all assessments will be performed pre-dose.

The primary safety analysis for this study will include the incidence of total treatment-emergent AEs (TEAEs) 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, vitamins A and E, 25-hydroxy vitamin D and INR)
- Liver ultrasound and elastography (where available)
- Discontinuations due to AEs

10.1 Adverse Events

10.1.1 Safety Reporting Period

Each patient must be carefully monitored for the development of AEs during the safety reporting period.

The safety reporting period for adverse events is from the first dose of study drug through the last planned study visit or 28 calendar days after the last dose of the study drug, whichever occurs later. The safety reporting period for serious adverse events is from signing of the informed consent form through the last planned study visit or 28 calendar days after the last dose of the study drug, whichever occurs later.

An SAE that occurs following the safety reporting period and which is assessed by the investigator as related to study medication should also be reported.

10.1.2 Definitions and Investigator Assessments

10.1.2.1 Adverse Events

An AE is defined as any untoward medical occurrence in a 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 in the study whether or not the AE is related to the study drug. An AE can be a new condition or worsening of a pre-existing condition.

10.1.2.2 Clinical Significance

The investigator will review all patient-reported events, laboratory and other test results and use clinical judgement to identify events that are clinically significant.

Clinical significance is defined as any abnormal finding that results in further clinical investigation(s), treatment(s), or the diagnosis of a 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 or are otherwise judged relevant by the investigator.

10.1.2.3 Serious Adverse Events

Each TEAE must be classified by the investigator as either serious or non-serious. This assessment is made independently of severity assessment (Section 10.1.2.4). For example, the development of a severe rash may not meet serious criteria as defined below and therefore would be considered a severe, non-serious AE.

An SAE is any untoward medical occurrence that:

- Results in **death**
- Is **life-threatening** means that the patient, in the opinion of the investigator, was at immediate risk of death at the time of the event. This does not include an event which hypothetically may have caused death if it was more severe
- Requires or prolongs **inpatient hospitalization.** Inpatient hospitalization is defined as any stay in the hospital of more than 24 hours, or any admission to a hospital ward or unit as an inpatient.
    - Hospitalizations for procedures that were planned prior to study participation are not considered SAEs.
- Results in persistent or significant **disability/incapacity**. Disability means a substantial disruption of a person's ability to conduct normal life functions
- The AE results in a **congenital anomaly/birth defect**
- **Is an important medical event**. The investigator should apply medical and scientific judgement to assess whether an AE should be classified as a serious AE due to medical significance, even if no other seriousness criteria is met

  As a general guideline, medically important events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definitions above

  Example: Allergic bronchospasm requiring intensive treatment in an emergency room

10.1.2.4 Investigator Assessment of Severity

Severity is a measure of intensity, whereas seriousness is defined by criteria outlined in Section 10.1.2.3.

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 Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 grading system as follows:

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

- Grade 1: Mild; asymptomatic or mild symptoms, clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental activities of daily living.
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

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 any seriousness criteria, and therefore would be assessed as a severe non-serious AE. CTCAE Grades 4 (life-threatening) and 5 (death) should always be reported as SAEs.

10.1.2.5 Investigator Assessment of Causality

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 information. 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 a reasonable possibility that the study drug caused the event. This includes situations where 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 (positive de-challenge). 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 (positive re-challenge)

Unrelated to study drug (unlikely or unrelated)

Based on medical judgment there is no reasonable possibility that the study drug caused the event. This includes situations where 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

Default assessments of the related category without supportive evidence for a causal relationship to study drug is generally uninformative and does not contribute meaningfully to the development of the safety profile of the drug or to patient safety.

10.1.3 Recording of Adverse Events

Patients must be carefully monitored for the development of any AEs during the safety reporting period.

All observed AEs and any AEs that are spontaneously reported by the patient/caregiver will be recorded by the investigator. For all AEs, the severity, seriousness, and causality to study drug for each AE as outlined in Section 10.1.2 will be assessed and recorded in the eCRF.

Any AE assessed as serious must be reported within 24 hours of site awareness.

Any SAEs or AEs that lead to permanent discontinuation of study drug that are unresolved at the time of the last study visit will be followed up by the investigator until resolution or stabilization. Following the last study visit, the Sponsor will provide forms for reporting additional information for any patient with ongoing SAEs or AEs that led to permanent discontinuation of study drug. Albireo retains the right to request additional information for any patient with ongoing AE(s) or SAE(s) at the end of the study.

Treatment-emergent AEs are defined as any AE that occurs after first dose of study drug. All SAEs that occur in the Screening Period, i.e. after enrollment and prior to first dose of study drug, will be collected as non-treatment emergent SAEs.

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, in a patient diagnosed with hepatitis during the safety reporting period, hepatitis would be considered the AE. 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.

10.1.4 Recording and Reporting of Serious Adverse Events

Every SAE, regardless of severity and causality, that occurs during the safety reporting period must be reported by the investigator or delegate in the SAE eCRF within 24 hours of site awareness.

The SAE report is to include at least the following information:

- Patient identification information (study number, site number, and date of birth as per local country requirements for data protection)
- The event term. Provide the diagnosis term, if known. If a diagnosis is unknown, provide the signs/symptoms. Update the event term once a diagnosis is known
- Date of onset
- The action(s) taken to treat the event (i.e. treatment medications, temporary discontinuation)
- The event outcome
- Seriousness criteria
- The relationship of the event to the study drug or to the study procedure (i.e. the investigator's assessment of causality)
- A brief narrative of the clinical course of the SAE

Follow up reports to already reported SAEs should contain any new information or query responses and must be documented on the SAE eCRF using the same process and timelines as for the initial SAE report.

If the SAE eCRF is not accessible, a hardcopy SAE form should be completed within 24 hours of the site becoming aware of the event and faxed or emailed to the address on the form and later entered in the eCRF when it becomes accessible.

10.1.5 24-Hour Emergency Contact

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 call center.

Reporting of Expedited Safety Reports

A 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 (i.e. the investigator brochure).

The Sponsor and/or its designee are responsible for reporting of SUSARs to regulatory authorities, central ethics committees and investigators within required timelines.

The investigator is responsible for submitting required safety information to their local Institutional Review Board (IRB) or IEC per local regulations. This information includes, but is not limited to, any safety alert letter received from the sponsor.

10.1.5.1 Special Situations

Certain safety events are defined by regulatory authorities as Special Situations and require reporting. These Special Situations include:

- Overdose of study medication (See Section 10.2.8)
- Suspected abuse/misuse of study medication
- Medication error involving the study medication
- Drug-drug interaction
- Pregnancy (See Section 10.2.9)

Special Situations should be reported on the Special Situations eCRF, whether or not they result in an AE/SAE. If a Special Situation results in an AE or SAE, the event also should be reported on the AE/SAE eCRF following the process described in Section 10.1.2.

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 4.

At screening, alkaline phosphatase will be fractionated (liver, bone, and intestinal isoenzymes) for patients ≥ age 18 at the time of enrollment. If alkaline phosphatase is elevated during the treatment period, isoenzymes can be re-measured to assist in evaluation of the alkaline phosphatase elevation.

Blood for AFP measurement will be drawn at randomization (Study Day 1) and Week 24/EOT visits. Blood for measurement of fat-soluble vitamin levels including vitamins A and E, and 25-hydroxy vitamin D will be drawn at Screening Visit 1, randomization (Study Day 1), Week 12, and Week 24/EOT visits. Blood for INR (surrogate for vitamin K) will be drawn at Screening Visit 1, randomization (Study Day 1), Week 4 to 24/EOT, and Safety Follow-up visits. 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 Screening Visit 1. A urine pregnancy test will be collected at all other visits for females who have reached menarche. If a urine pregnancy test is positive, a serum pregnancy test is to 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 is to be discontinued (see Section 10.2.9).

All samples will be processed and transported to a laboratory per instructions in the laboratory manual. For the purposes of study conduct, only laboratory tests done in the central laboratory may be used. Local laboratories may be used at the discretion of the local investigator for management of urgent medical issues. If a local laboratory test value is found to be abnormal and clinically significant, it will be verified by the central laboratory as soon as possible after the investigator becomes aware of the abnormal result. If it is not possible to send a timely specimen to the central laboratory (e.g. the patient was hospitalized elsewhere), the investigator may base the assessment of an AE on the local laboratory value.

The observed laboratory values will be recorded and assessed according to the investigator's clinical judgment.

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 4 Routine Laboratory Parameters

Clinical Chemistry	Hematology	Urinalysis
• Albumin • ALT • Alkaline phosphatase (and isoenzymes as applicable) • AST • Bilirubin – total and direct • Calcium • Chloride • Cholesterol • 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

ALT = alanine aminotransferase; AST = aspartate aminotransferase.

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.

The liver monitoring criteria for study drug interruption are provided in Table 5. If any of the ALT, AST, bilirubin, and alkaline phosphatase criteria are met, repeat testing must be performed within 48 to 72 hours. If the criteria are confirmed upon repeat testing, study drug must be interrupted immediately.

Study drug must be interrupted immediately without waiting for confirmatory testing if any of the INR or symptom-based criteria are met in Table 5.

Table 5 Liver Monitoring - Study Drug Interruption Criteria

Laboratory value(s) or Symptoms	Interruption Criterion
AST or ALT in combination with total bilirubin (confirmed with repeat testing within 48 to 72 hours)	CCI [redacted]
Total bilirubin (confirmed with repeat testing within 48 to 72 hours)	
INR	
Symptoms	

Symptoms	CCI
For patients ≥ 18 years of age only	
Alkaline phosphatase in combination with direct (conjugated) bilirubin[a] (confirmed with repeat testing within 48 to 72 hours)	

ALT = alanine aminotransferase; AST = aspartate aminotransferase; INR = international normalized ratio; ULN = upper limit of normal.
[a] If the alkaline phosphatase is confirmed to be NOT of liver origin (e.g. of bone origin) with the use of alkaline phosphatase isoenzymes, this criterion is not applicable.

If study drug is interrupted due to one of the criteria in Table 5:

a. Initiate drug-induced liver injury work-up for alternative etiologies

b. Repeat liver profile (AST, ALT, total bilirubin, direct bilirubin) and prothrombin time or INR within 48 to 72 hours

c. Monitor the patient using close observation found in Section 10.2.2.2

d. If a patient resides in a remote area, they may be tested locally and the results communicated to the investigator site promptly. Results of any local liver-related laboratory tests and normal ranges must be shared with the medical monitor in a timely fashion

10.2.2.2 Close Observation

- Repeat liver enzyme and serum bilirubin tests 2 or 3 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 patient is asymptomatic
- Obtain FGF19, p-C4, and pharmacokinetic levels within 3 days (p-C4 and PK only for patients >10 kg)
- 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 and liver biopsy

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 (see Table 5 for definition)
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. The sponsor Medical Monitor must be notified prior to re-challenge
3. If the ALT/total bilirubin elevations are observed after re-challenge, then repeat re-challenge is discouraged

If patient is permanently discontinued, monitoring is to be continued as outlined in Section 10.1.3.

10.2.2.4 Diarrhea

Study drug is to be interrupted 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 are to 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 dose of study drug can be reduced to 40 µg/kg/day (Section 8.3). If the diarrhea persists on the lower dose of study drug, the patient will be permanently discontinued and monitored as outlined in Section 10.1.3.

Any dose modification must be recorded in the clinical database.

10.2.2.5 Fat-soluble Vitamin Deficiency (FSV)

Patients with cholestatic liver disease are at risk for the development of FSV

deficiency. The investigator should assess patients for the following signs and symptoms of FSV deficiency at screening, at each study visit, and at the end of treatment visit per the Schedule of Assessments (Table 1):

- Vitamin A deficiency: night blindness, blindness, dry eyes, hair loss
- Vitamin D deficiency: osteopenia, fractures, muscle weakness, impaired wound healing
- Vitamin E deficiency: muscle weakness, difficulty walking, tremors, vision problems, poor immune function
- Vitamin K deficiency: bleeding difficulties

If, despite vitamin supplementation, levels of one or more of the fat-soluble vitamins remain low and in the Investigator's opinion presents a significant risk to the patient, treatment with study drug should be interrupted. If the fat-soluble vitamin levels improve and are no longer considered to pose a serious risk, study drug may be restarted at the Investigator's discretion. Fat-soluble vitamin levels should be monitored.

10.2.3 Demographics/Medical and Surgical History

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

Medical and surgical history will be obtained at Screening Visit 1. This includes date of diagnosis of ALGS, prior investigational medications for ALGS, historical (2 years 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.

Medical History will be recorded at screening visits and randomization before the first dose of study drug.

10.2.4 Clinical Genetic Testing

At Screening Visit 1, clinical genetic analysis to confirm mutations in either the JAG1 or NOTCH2 genes will be performed if the results are not available in the medical record. No other diagnostic genetic testing will be offered.

10.2.5 Physical Examination including Skin Examination

A physician or suitably trained qualified assistant will perform a complete physical examination at Screening Visit 1 and Study Day 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 (if clinically indicated), extremities, skin, musculoskeletal, neurologic, and other.

Skin examinations of skin lesions due to scratching will be conducted at Screening Visit 2, Week 4 to 24/EOT, and Safety Follow-up visits (see Table 1). Skin will be examined thoroughly, and excoriations/scratch marks recorded. In addition, a symptom-directed physical exam will occur, with any findings recorded as medical history or AEs, as appropriate.

10.2.6 Liver Ultrasound and Elastography

Ultrasound of the liver and spleen will be performed at randomization (Study Day 1) and at Week 24/EOT. Liver size, echogenicity, and presence of masses/nodules will be recorded. Elastography will be performed as per institution standard practice where available.

10.2.7 Vital Signs

Evaluation of vital signs will be performed at all clinic 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.

10.2.8 Overdose

Odevixibat 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 odevixibat 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).

Any overdose is to be immediately (not later than within 24 hours of knowledge of the overdose) reported by the investigator or delegate on the Special Situations report form. In the event of an odevixibat overdose, the patient is to 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, study drug will be permanently discontinued and an EOT visit will be scheduled. 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 is to 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 Pregnancy Notification Form within 24 hours of site awareness. Date of exposure and details of the period of gestation at the time of exposure is to be provided.

The pregnancy is to be followed to determine maternal and infant outcomes, 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. Any pregnancy that occurs within 90 days of the last dose of study drug must also be reported to the Sponsor.

10.3 Individual Study Drug Interruption and/or Discontinuation

Study drug administration will be discontinued for the following:

- CTCAE Grade 3 or higher deemed possibly or probably related to study drug by the site Investigator.
- CTCAE Grade 4 or higher regardless of attribution to study drug.

10.4 Trial Discontinuation

Trial discontinuation will be considered when any of the following occur:

- Three patients develop the same Grade 3 CTCAE attributed to study drug

  OR

- Two patients develop any Grade 4 CTCAE attributed to study drug

  OR

- One patient develops a fatal adverse event (Grade 5 CTCAE)

Events that could potentially trigger trial discontinuation will undergo an expedited review by Albireo's internal Safety Review Committee, which will reach an agreement prior to dosing new patients.

11 STATISTICAL EVALUATION

All personnel involved with the analysis of the study will remain blinded to treatment assignment until database lock is completed and until major protocol violations have been identified.

All statistical analyses will be performed using SAS version 9.4 or higher.

11.1 Sample Size and Power

Forty-five (45) patients <18 years of age will be randomized at an experimental to control allocation of 2 to 1 in order to obtain approximately 36 completers, assuming an approximate drop-out rate of 20%. Subjects <18 years of age will be randomized according one age stratification factor, i.e. <10, and 10 to <18 years of age. This stratification factor is based on Kamath et al. (2020), showing an increase in the prevalence and severity of pruritus in children <10 years of age. At a 1-sided significance level of $alpha_{1\text{-sided}} = 0.025$, assuming a pooled standard deviation (SD) of 1.0, and a difference between the treatment groups of 1.2 in change of pruritus, favoring response, the power of the study is 0.909, using the exact method (Proc Power, SAS v.9.4, Cary, NC). The key secondary endpoint is also powered for a standardized treatment effect (treatment effect/SD) of 1.2.

11.1.1 Sample Size Re-Estimation

After a minimum of 18 patients have completed the Week 16 visit, the pooled SD of change from baseline to Week 16 (Weeks 13 to 16 assessments) will be calculated by an independent statistician. Samples size re-estimation is based on non-comparative blinded data for which no alpha adjustment is required. If the pooled SD was underestimated in this original sample size calculation, an adjustment will be made to maintain study power, assuming a 1.2 treatment effect. It is assumed that the Week 16 and Week 24 SDs will be equivalent; however, a multiplication factor (e.g., 1.2) on the calculated SD may be made based on current understanding of the instrument at the time of sample size re-estimation. If SD was overestimated, in this original sample size calculation, the sample size will not be decreased.

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 study drug treatment. Patients will be analyzed in the treatment group they were randomized to even if they received incorrect study drug. The FAS, excluding patients 18 years of age or older at randomization for whom the ObsRO is not utilized, will be the

primary analysis set for primary and key efficacy analyses. The FAS with all age groups will be utilized for all other secondary 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 (i.e. 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 un-blinding 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 120 µg/kg/day odevixibat in relieving pruritus in patients with ALGS.

11.2.2.2 Missing Data

There will be no imputation in the primary analyses of endpoints in this study as methods robust to missing data such as the mixed-effect model for repeated measures (MMRM) will be used. Each week, at least 4 of 7 assessments need to be completed for each of the AM and PM assessments. If these minimum assessments are not available, the week is considered missing. Monthly values will only be calculated if at least 3 weeks can be calculated. Sensitivity analyses including multiple imputation for missing data will be employed to assess the robustness of efficacy results and will be specified in the Statistical Analysis Plan.

11.2.2.3 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 Visit. 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.

11.2.2.4 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.

11.2.2.5 Patient 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 treatment
- Patients completing the study
- Patients discontinuing treatment early (including reason for discontinuation of treatment)
- Patients withdrawing early from the study (including withdrawal reason)

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

11.2.2.6 Evaluation of Primary Efficacy Variables

The FAS will be used as the primary analysis population. The primary endpoint is based on the worst scratching item 1 in the ObsRO AM and ObsRO PM (Appendix 2). The monthly (28-day) average for Months 1 through 6 in pruritis will be calculated by taking the average AM and the average PM weekly scores, then averaging the AM and PM weekly scores, and finally calculating the monthly average by averaging the 4 weeks within the month. Baseline is calculated similarly for the 14 days preceding start of treatment by taking the average AM and the average PM weekly scores, then averaging the AM and PM weekly scores, and finally calculating the average by averaging the 2 weeks. Change from baseline is calculated as the monthly score minus the baseline score.

The primary analysis for the primary endpoint will be determined from a MMRM modelling change from baseline for each four-week average pruritis score, with baseline age, baseline pruritus, baseline conjugated bilirubin, treatment group, time (in months), and treatment-by-time interaction in the model. A MMRM will be used to compare treatment effects at Month 6.

Sensitivity analyses based on different missing data mechanisms, and for different population subgroups will be conducted as described in the Statistical Analysis Plan. The PP analysis set will also be used as a sensitivity analysis.

The primary objective of the study is to assess the efficacy of odevixibat in relieving pruritus in patients with ALGS. To avoid confounding of the treatment effect, the data collected following an intercurrent event will not be included in the primary analysis. Intercurrent events will be defined in the Statistical Analysis Plan.

Additional analyses including all data collected through the end of the study regardless of intercurrent events will also be performed.

11.2.2.7 Evaluation of Additional Efficacy Variables

There will be no alpha adjustment for multiple hypothesis testing as these additional efficacy variables are supportive, only. For efficacy variables in which change from baseline is assessed, MMRM will be used as the primary analysis methodology in the FAS population. Sensitivity analyses will be performed as defined in the Statistical Analysis Plan.

11.2.2.8 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 class and World Health Organization 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.

11.2.2.9 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 Data Safety Monitoring Board

A Data and Safety Monitoring Board (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 un-blinded 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 who are responsible for responding to the recommendations of the DSMB and taking appropriate action. The investigators will be informed by the Albireo Medical Monitor 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 un-blinded 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 un-blinded DSMB statistician will have access to these data.

11.2.4 Hepatic Safety Adjudication Committee

The Hepatic Safety Adjudication Committee (HSAC) will consist of a blinded committee of at least three hepatologists with experience assessing events of potential hepatotoxicity who will review in an ongoing fashion any suspected drug related hepatic injury event. They will adjudicate any suspected event and provide a written report to the DSMB for review. The HSAC will act according to its own written standard operating procedure described in a separate charter.

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 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 the 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 including eDiary compliance, 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.

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, investigator brochure, 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 odevixibat 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 is to 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 must read and consider the statements before signing and dating them and is to 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 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.

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

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, digital versions 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 is to take measures to prevent accidental or premature destruction of these documents.

16 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 patient to the sponsor's approval requirements.

17 REFERENCE LIST

1. Diaz-Frias J, Kondamudi NP. Alagille Syndrome. [Updated 2019 Dec 30]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK507827/.
2. Emerick KM, Whitington PF. Partial external biliary diversion for intractable pruritus and xanthomas in Alagille syndrome. Hepatology. 2002:35:1501-1506.
3. Harpavat S, Heubi JE, Karpen SJ, et al. Prognostic value of serum bile acids after Kasai portoenterostomy in biliary atresia. Paper presented at: American Association for the Study of Liver Diseases (AASLD) Conference; December 2018; San Francisco, CA.
4. Hofmann AF. The enterohepatic circulation of bile acids in mammals: form and functions. Front Biosci. 2009;14:2584-2598.
5. Kamath BM, Bason L, Piccoli DA, Krantz ID, Spinner NB. Consequences of *JAG1* mutations. J Med Genet. 2003;40:891–895.
6. Kamath BM, Baker A, Houwen R, et al. Systematic review: the epidemiology, natural history, and burden of Alagille syndrome. J Pediatr Gastroenterol Hepatol. 2018;67:148-156.
7. Kamath BM, Ye W, Goodrich NP, et al. Outcomes of childhood cholestasis in Alagille Syndrome: Results of a multicenter observational study. Hepatol. Commun. 2020;4:387-398.
8. Krantz ID, Piccoli DA, Spinner NB. Alagille syndrome. J Med Genet. 1997;34:152-157.
9. Mattei P, von Allmen D, Picolli D, et al. Relief of intractable pruritus in Alagille syndrome by partial external biliary diversion. J Pediatr Surg. 2006:41;104-107.
10. 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):512–523.
11. Oda T, Elkahloun AG, Pike BL, et al. Mutations in the human *Jagged1* gene are responsible for Alagille syndrome. Nat Genet. 1997;16:235-243.
12. Pawlowska J, Socha P, Jankowska I. Factors affecting catch-up growth after liver transplantation in children with cholestatic liver diseases. Ann Transplant. 2009:15;72-76.
13. Sheflin-Findling S, Arnon R, Lee S, et al. Partial internal biliary diversion for Alagille syndrome: case report and review of the literature. J Pediatr Surg. 2012:47;1453-1456.
14. Singh SP, Pati GK. Alagille syndrome and the liver: current insights. Euroasian J Hepato-Gastroenterol. 2018:8:140-147.
15. Turnpenny PD, Ellard S. Alagille syndrome: pathogenesis, diagnosis and management. Eur J Hum Gen. 2012;20:251-257.

16. Van Wessel et al. Factors associated with the natural course of disease in patients with FC1-deficiency: the NAPPED-consortium. ABSTRACT. 2019.

17. Van Wessel DBE, Thompson RJ, Gonzales E, et al. Genotype correlates with the natural history of severe bile salt export pump deficiency. J Hepatol. 2020;73(1):84-93.

18. 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):702-707.

19. 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.