

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

215498Orig1s000

INTEGRATED REVIEW

Integrated Review

Table 1. Administrative Application Information

Category	Application Information
Application type	NDA
Application number(s)	215498
Priority or standard	Priority
Submit date(s)	11/20/2020
Received date(s)	11/20/2020
PDUFA goal date	7/20/2021
Division/office	Division of Hepatology and Nutrition (DHN)
Review completion date	7/19/2021
Established/proper name	Odevixibat
(Proposed) proprietary name	BYLVAY
Pharmacologic class	Ileal bile acid transporter (IBAT) inhibitor
Code name	Cholestatic liver diseases (i.e., PBC and PSC) (7060106)
Applicant	Albireo AB
Dosage form(s)/formulation(s)	Capsules for oral use, oral pellets
Dosing regimen	Click or tap here to enter text.
Applicant proposed indication(s)/ population(s)	Treatment of pruritus in progressive familial intrahepatic cholestasis (PFIC)
Proposed SNOMED indication	74162007 Progressive intrahepatic cholestasis (disorder)
Regulatory action	Approval
Approved dosage (if applicable)	40 mcg/kg to a maximum 6 mg/day
Approved indication(s)/ population(s) (if applicable)	Treatment of pruritus in progressive familial intrahepatic cholestasis (PFIC)
Approved SNOMED term for indication (if applicable)	Click or tap here to enter text.

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Glossary

ASBT	apical sodium-dependent bile acid transporter
ADaM	Analysis Data Model
ADME	absorption, distribution, metabolism, excretion
AE	adverse event
AESI	adverse event of special interest
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
API	active pharmaceutical ingredient
AST	aspartate aminotransferase
AUC	area under the concentration-time curve
BA	bile acid
BID	twice daily
BRIC	benign recurrent intrahepatic cholestasis
BSEP	bile salt export pump
CAC	Carcinogenicity Assessment Committee
CaGIS	Caregiver Global Impression of Symptoms
CDER	Center for Drug Evaluation and Research
CDS	clinical data scientist
CDTL	cross-disciplinary team leader
CFR	Code of Federal Regulations
CLIA	clinical laboratory improvement amendments
C _{max}	maximum plasma concentration
CMC	chemistry, manufacturing, and controls
COA	clinical outcome assessment
CRC	colonic release formulation of cholestyramine
CSR	clinical study report
CV	coefficient of variation
DB	direct bilirubin
DMPH	Division of Pediatric and Maternal Health
EOT	end-of-treatment
EPC	established pharmacologic class
FAS	full analysis set
FDA	Food and Drug Administration
FSV	fat-soluble vitamin
IBAT	ileal bile acid transporter
IC ₅₀	half maximal inhibitory concentration
IM	intramuscular
IND	investigational new drug
INR	international normalized ratio
IR	Information Request
ITT	intent-to-treat
IU	international units
IV	intravenous

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LLOQ	lower limit of quantification
LS	least squares
MAD	multiple ascending dose
MAR	missing at random
MedDRA	Medical Dictionary for Regulatory Activities
MI	multiple imputation
MMRM	mixed-effect model for repeated measures
MNAR	missing not at random
MOA	mechanism of action
MTD	maximum tolerated dose
NCI-ODWG	National Cancer Institute Organ Dysfunction Working Group
NDA	new drug application
NOAEL	no observed adverse effect level
ObsRO	observer-reported outcome
OPQ	Office of Pharmaceutical Quality
OSI	Office of Scientific Investigations
PBC	primary biliary cholangitis
PD	pharmacodynamic
PFIC	progressive familial intrahepatic cholestasis
PI	Prescribing Information
PK	pharmacokinetic
PMC	postmarketing commitment
PND	postnatal day
PO-SCORAD	patient-oriented scoring atopic dermatitis
PRO	patient-reported outcome
PT	preferred term
QD	once daily
SAD	single ascending dose
SAE	serious adverse event
SAP	statistical analysis plan
sBA	serum bile acid
SD	standard deviation
SDD	Sentinel Distributed Database
SE	standard error
SOC	system organ class
STF	safety tables and figures
TB	total bilirubin
TBM	to-be-marketed
TEAE	treatment-emergent adverse event
T _{max}	time to maximum concentration
UDCA	ursodeoxycholic acid
ULN	upper limit of normal
VAS	visual analog scale

I. Executive Summary

1. Summary of Regulatory Action

Odevixibat (BYLVAY) is being approved for the treatment of pruritus in patients with all forms of progressive familial intrahepatic cholestasis (PFIC). Pruritus that can be relentless and severely debilitating is the hallmark clinical symptom of this disease. Intractable pruritus is a reason for liver transplantation for some patients regardless of liver disease severity. Surgical biliary diversion procedures are also done for patients with intractable pruritus. There are no FDA-approved therapies for treatment of pruritus in patients with PFIC. This is an area of unmet medical need with significant morbidity.

One multicenter adequate and placebo-controlled trial of odevixibat, Trial A4250-005, showed a direct measurement of benefit for treatment of pruritus in patients with PFIC. A parent or caregiver observational assessment of pruritus was used as a primary efficacy measure of how a patient feels or functions. This measure was evaluated by the Applicant for appropriate findings of construct and content validity. Statistically persuasive findings were shown regardless of the type of efficacy endpoint or analysis of observer-reported outcome (ObsRO) (e.g., the mean of the patients' proportion of "positive pruritus assessments" during the 24-week treatment period) with definition of a "positive pruritus assessment" being a score of 1 or 0 (little or no scratching).

The statutory standard of substantial evidence of effectiveness was met with this single phase 3 trial for the following reasons: (1) statistically persuasive findings on the prespecified primary endpoint and on an alternate efficacy endpoint (mean percentage of ObsRO assessments that are ≤ 1 over the 24-week treatment period); (2) consistent benefit across centers; (3) post hoc anchor-based analyses evaluating clinically meaningful within-patient change on the endpoint based on the Worst Weekly Scratching Score suggested that both odevixibat doses yielded meaningful within-patient improvement in scratching severity relative to placebo from baseline to end-of-treatment; and (4) profound benefit in a patient who avoided a surgical procedure. Supportive evidence for the effectiveness of odevixibat is provided by the following: (1) secondary endpoint finding of reduction in serum bile acid levels (elevated serum bile acid levels are hypothesized to be related to pruritus); (2) association between improvement in pruritus scores and reduction in serum bile acid levels for many treated patients; (3) evidence of reduction in sBA levels in a proof-of-concept phase 2 trial; and (4) nonclinical proof-of-concept studies to support the mechanistic rationale for treatment of pruritus with odevixibat in patients with PFIC.

The safety information supports a favorable benefit/risk assessment. Odevixibat is minimally absorbed with low systemic exposure in patients at the recommended doses. Elevation in liver enzymes were observed in a greater proportion of odevixibat-treated patients, but there were no differences in serious adverse events compared to placebo. Diarrhea and fat-soluble vitamin deficiency were also observed but can be addressed by treatment interruption or treatment discontinuation. These observations are included in the Warnings and Precautions to alert clinicians to carefully follow patients who experience these adverse reactions. There were two notable safety findings in two individual patients: one with pancreatitis and one with a bone fracture. In a small clinical trial database of fewer than 100 persons, a single serious adverse

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reaction can have potential meaning. However, careful evaluation of these case reports did not clearly identify odevixibat as being a cause for these adverse events.

To address concerns regarding the limited safety data available following long-term treatment, the open-label Trial A4250-008, an ongoing trial in patients with PFIC, will enroll 120 patients and follow them for at least 72-weeks. This trial will assess patients for growth, and the requirement for surgical intervention (biliary diversion and liver transplantation).

The adverse cardiovascular malformations reported in one reproductive toxicology study are unexplained, given that this drug is generally not systemically absorbed. A pregnancy registry will be required as part of postmarketing evaluations, in addition to the collection of safety data in an open-label long-term trial that will include assessment of growth in children. The Applicant agreed to the postmarketing evaluation of a dosing strategy in milk or water, as only soft foods were evaluated in this NDA submission.

Two doses were evaluated in the phase 3 clinical trial, both showing persuasive findings on the prespecified primary efficacy endpoint and analysis. In sensitivity analyses of efficacy, the 40 mcg/kg/day dosing group showed findings more persuasive than the 120 mcg/kg/day dosing group. The 120 mcg/kg/day dose did not show additional benefit compared to the 40 mcg/kg/day dose in terms of efficacy and had an acceptable safety profile. Notably, the A4250-008 long-term extension trial is currently ongoing and is studying only a 120 mcg/kg dose. Considering the small number of patients over a wide range of age in the clinical trials, the availability of 120 mcg/kg/day should allow further individualized treatment. A maximum tolerated dose should be 6 mg per day.

Approval for use in children 3 to 6 months age and in patients >18 years of age will be recommended. Children 6 months and older were enrolled in the phase 3 trial and no adult patients were enrolled. However, the pathophysiology and the mechanism of action of the drug are the same for all children (including infants between 3 to 6 months of age) and adults. Patients can present with pruritus as early as 3 months of age and would benefit from treatment. Adult patients with PFIC who either present with new onset pruritus secondary to PFIC or responded to treatment as children and have turned 18 years or older would also benefit from access to treatment. Due to the extreme rarity of the disease in the children 3 to 6 months of age and adults with PFIC (as most patients with PFIC suffering from pruritus receive liver transplantation before adulthood), it will be impracticable if not impossible to conduct trials in these subgroups. Therefore, the drug will be approved for children 3 months and older.

The Applicant met all criteria for receiving a rare pediatric disease priority review voucher. Notably, labeling will include all patients with PFIC affected by pruritus.

In summary, our review of the Applicant's NDA demonstrated that odevixibat is safe and effective for the treatment of pruritus in patients with PFIC.

2. Benefit-Risk Assessment

2.1. Benefit-Risk Framework

Table 2. Benefit-Risk Framework

Dimension	Evidence and Uncertainties	Conclusions and Reasons
<p>Analysis of Condition</p>	<p>Progressive familial intrahepatic cholestasis (PFIC) is a rare, autosomal recessive condition that results in impaired bile flow due to impaired bile acid (BA) secretion leading to cholestatic liver injury. It is estimated that there might be 400-500 patients with PFIC in the United States. The disease presents during early childhood with pruritus, elevated liver tests, jaundice, and growth failure. The disease can progress to cirrhosis leading to liver failure, and one-third of patients with PFIC2 can develop hepatocellular carcinoma.</p> <p>Pruritus is a severe and disabling symptom, especially in patients with PFIC1 and PFIC2 subtypes. The physical manifestations range from scratch marks, excoriations, and scarring due to persistent and unrelenting pruritus.</p> <p>Within the PFIC2 subtype, patients can be categorized based on genotypic severity (BSEP1, BSEP2, BSEP3). The predicted residual BSEP transport function decreases with each category, with BSEP3 being the most severe variant.</p>	<p>PFIC is a rare, serious condition presenting in childhood associated with severe pruritus. The itching and scratching are disabling symptoms and can be disruptive for patients and their families, and limit patient’s ability to participate in daily activities.</p>
<p>Current Treatment Options</p>	<p>Off-label medical treatment for pruritus includes use of ursodeoxycholic acid (UDCA), cholestyramine, antihistamines, naltrexone, rifampin, and ondansetron. Most of these medical therapies do not ameliorate pruritus and some of these therapies have undesirable adverse events (AEs).</p> <p>When the pruritus is severe and quality of life is significantly impacted, the patients undergo surgical intervention.</p> <p>Surgical interventions include internal or external biliary diversion. Severe intractable pruritus, regardless of the actual disease process in the liver, is considered an indication for liver transplantation. Complications of a liver transplantation procedure are serious. Moreover, after liver transplantation, patients must</p>	<p>There is no U.S. Food and Drug Administration (FDA)-approved medical therapy for treatment of pruritus in patients with PFIC.</p> <p>Majority of patients do not respond to the available off-label medical treatments and patients continue to experience intractable pruritus.</p> <p>Surgical options (biliary diversion and liver transplantation) are invasive and associated with numerous serious adverse events.</p> <p>Treatment of pruritus in patients with PFIC remains a high unmet medical need.</p>

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Dimension	Evidence and Uncertainties	Conclusions and Reasons
	<p>be maintained on lifelong immunosuppression, which has its own complications and associated serious adverse events.</p>	
<p>Benefit</p>	<p>Trial A4250-005 enrolled 62 patients with PFIC (PFIC1 and PFIC2) in a randomized double-blind, placebo-controlled trial as follows: Placebo arm N=20; odevixibat 40 mg/kg arm N=23; odevixibat 120 mcg/kg arm N=19.</p> <p>Measures used for Trial A4250-005 were observer-reported outcome (ObsRO) and patient-reported outcome (PRO) assessments.</p> <p>Patients' pruritus was assessed by their caregiver twice daily (BID) on a single-item ObsRO with scores ranging from 0 (no scratching) to 4 (worst possible scratching).</p> <p>Patients' pruritus was assessed BID on a single-item PRO with scores ranging from 0 (no itching) to 4 (worst possible itching). PRO was used in children ages ≥8 years.</p> <p>FDA found the content validity of both ObsRO and PRO measures of pruritus to be acceptable.</p> <p>Given the young ages of enrolled patients, patient self-reports of itching severity was limited to patients aged 8 years and older. ObsRO and PRO pruritus scores aligned well in the few patients (n=9) who had both PRO and ObsRO scores during the 4 weeks immediately prior to randomization.</p> <p><i>Primary efficacy endpoint</i></p> <p>While the content validity of the ObsRO was acceptable, FDA questioned the clinical meaningfulness of the trial' prespecified endpoint i.e., "mean percentage of assessments over the 24-week treatment period that are ≤1 or at least a 1-point drop from baseline." Specifically, FDA questioned the clinical meaningfulness of a 1-point drop in pruritus, on a 0 to 4 scale. Therefore, FDA used an alternative endpoint for the primary assessment of clinically meaningful efficacy, defined as the mean percentage of assessments over the 24-week treatment period that are ≤1 (no scratching or a little scratching). Least squares (LS) mean (standard error [SE]) for placebo-treated patients was 13.2 (8.7); for odevixibat 40 mcg/kg/day treated patients was 35.4</p>	<p>The clinical trial design and FDA analyses were appropriate for evaluation of pruritus in the PFIC1 and PFIC2 populations, which is a rare disease, and pruritus is one the disabling symptoms for which there are currently no FDA approved treatment options for people with PFIC.</p> <p>On average, patients treated with odevixibat had no or a little scratching for a higher percentage of the treatment period compared to patients treated with placebo. Reduction in pruritus (as assessed by the FDA's alternative endpoint) is clinically meaningful.</p> <p>In addition, analyses were also performed using "worst scratching severity" within a day and aggregate Daily Scratching Scores within at least a week. The results of these analyses were consistent with the FDA's alternative primary efficacy assessment.</p> <p>Trial did not enroll infants <6 months of age. The Agency decided to approve odevixibat for infants 3 months and older. This decision was based on unmet medical need, to allow access to young infants and adolescents, and adult patients with PFIC; biology of disease is similar and mechanism of action (MOA) of drug is also the same across all age groups.</p> <p>Phenotypical patients with PFIC experience similar disease manifestations, i.e., pruritus. This would allow access to odevixibat.</p> <p>The Agency decided to approve odevixibat for all PFIC subtypes. The mechanism of ileal bile acid transporter (IBAT) inhibitors would allow reduction in pruritus across all patients with PFIC.</p> <p>The pathophysiology of pruritus is not completely understood and is considered multifactorial. While the complete mechanism for pruritus is not fully understood, elevated sBA levels may be associated with pruritus in patients with cholestatic liver diseases.</p> <p>In the clinical trial, patients with PFIC with elevated serum bile acid levels were enrolled and the improvement of pruritus was</p>

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	<p>(8.1); and for odevixibat 120 mcg/kg/day treated patients was 30.1 (9.0). The LS mean difference (95% CI) compared to the placebo arm was 22.2 (4.7, 39.6) for odevixibat 40 mcg/kg/day treated patients and 16.9 (-2.0, 35.7) for odevixibat 120 mcg/kg/day treated patients.</p> <p>Patients enrolled in A4250-005, after completing this trial were offered to enroll in the open-label Trial A4250-008. Majority of patients from Trial A4250-005 enrolled in this open-label trial.</p> <p><i>Pharmacodynamic (PD) effects</i></p> <p>Trial A4250-005 enrolled patients with elevated serum bile acid levels (>100 micromole/L); upon odevixibat treatment, serum bile acid levels were lowered within 4 to 6 weeks and maintained at lower levels for the rest of the trial period although sBA levels fluctuated over time.</p> <p>The reduction in sBA levels tended to trend with reduction in pruritus, however, decrease in sBA levels has not been established as a biomarker that predicts improvement in pruritus.</p>	<p>observed along with sBA level reduction by odevixibat treatment. As such, the reduction in sBA levels as a result of reduced reabsorption of bile salts may provide indirect supportive evidence of mechanism of action.</p> <p>sBA level is distal from disease course and clinical outcomes, i.e., sBA levels do not provide an accurate estimation of hepatocellular bile acid levels which are the key mediators of liver damage.</p>
<p>Risk and Risk Management</p>	<p>The safety database consisted of 62 patients, which is expected as PFIC is a rare disease, the most common adverse events included the following:</p> <p><i>Liver test abnormalities</i></p> <p>Predominantly, elevations in alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), and direct bilirubin were observed during the trial, and these elevations occurred in greater percentages of odevixibat-treated patients relative to placebo-treated patients.</p> <p>Treatment interruptions due to liver test abnormalities occurred in a higher percentage of odevixibat-treated (17%) patients relative to placebo-treated (10%) patients.</p> <p><i>Gastrointestinal TEAEs</i></p> <p><i>Abdominal pain and vomiting</i></p> <p>Odevixibat-treated patients experienced abdominal pain (14%) and vomiting (17%) relative to none in placebo-treated patients.</p>	<p><i>Liver test abnormalities</i></p> <p>A small safety database limits assessment of liver test abnormalities, which is a concerning signal.</p> <p>These AEs of liver test abnormality can be monitored i.e., liver tests should be obtained at baseline and monitored frequently while odevixibat is administered. Treatment interruption, dose reduction, or treatment discontinuation is recommended based on the seriousness of the liver test abnormalities.</p> <p><i>Gastrointestinal TEAEs</i></p> <p>The gastrointestinal TEAEs are monitorable and can be managed based on symptoms.</p> <p><i>Abdominal pain and vomiting</i></p> <p>Treatment interruption, dose reduction, or treatment discontinuation is recommended, based on the seriousness of the gastrointestinal symptoms. If the vomiting leads to dehydration or vomiting is persistent, treatment should be interrupted.</p>

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	<p><i>Diarrhea</i></p> <p>Total of 31% of the odevixibat-treated patients experienced diarrhea, relative to 5% placebo-treated patients.</p> <p>Overall, 22% odevixibat-treated patients experienced diarrhea ≥3 days duration and none in placebo arm.</p> <p>Two (11%) odevixibat 120 mcg/kg treated patients experienced diarrhea ≥7 days relative to none in odevixibat 40 mcg/kg arm or placebo arm.</p> <p>One (5%) patient dosed with odevixibat 120 mcg/kg/day, discontinued from trial due to AE of diarrhea.</p> <p><i>Fat-soluble vitamin (FSV) deficiency</i></p> <p>A clear signal for this AE could not be established due to multiple confounders present that precluded ascertainment of this AE. However, there is a potential that patients may have new onset or worsening of FSV deficiency.</p> <p><i>Risk observed in nonclinical studies, malformations in heart and great vessels in rabbit fetuses</i></p> <p>There are no available data on odevixibat use in pregnant women. The cardiovascular malformations in rabbit fetuses justify a concern for a clinically significant risk to embryo-fetal development.</p>	<p><i>Diarrhea</i></p> <p>Bile acids are irritants and presence of bile acids in the colon has a potential to cause microscopic blood loss. Moreover, there is a potential to develop bile acid diarrhea, and persistent diarrhea, in a few patients can also lead to microscopic blood loss. Therefore, patients should be monitored for iron deficiency anemia by regularly monitoring hemoglobin and hematocrit.</p> <p>Caregivers and patients should interrupt treatment if diarrhea is persistent or requires intervention. Odevixibat can be restarted at 40 mcg/kg dose and increased as tolerated.</p> <p><i>Fat-soluble vitamin deficiency</i></p> <p>FSV levels should be obtained at baseline and monitored frequently while patients are administered odevixibat. If patients develop FSV deficiency, FSV should be supplemented. If patients develop clinical symptoms of FSV deficiency and the FSV deficiency is unresponsive to supplementation, based on severity of symptoms, consider treatment interruption, dose reduction or treatment discontinuation.</p> <p><i>Fetal cardiovascular malformation</i></p> <p>While the fetal cardiovascular malformations are concerning, they were only observed in one of the two animal species tested. For a drug that is largely not absorbed systemically, and the limitation of the findings only in one animal species, the risk level in humans that can be inferred from the malformations is uncertain. Including this information in labeling should inform providers of this concern.</p>

2.2. Conclusions Regarding Benefit-Risk

PFIC is a rare, pediatric cholestatic liver disease, in which pruritus is a debilitating symptom. Patients with PFIC with intractable pruritus who do not respond to the current (off-label) standard of care treatments have no therapeutic options for symptomatic relief of pruritus. Patients with pruritus who are refractory to medical therapy undergo surgical biliary diversion or liver transplantation. Both procedures are invasive, specifically liver transplantation, for which life-long immunosuppression is required and is associated with complications related to immunosuppression. This population needs new and effective treatment that could reduce pruritus. Odevixibat is a first-in-class intestinal bile acid transport (IBAT) inhibitor that was granted fast track designation for drug development as well as a Rare Pediatric Disease Designation for the treatment of PFIC.

Patients with PFIC who had moderate to severe pruritus at baseline demonstrated clear improvement in pruritus scores after receiving odevixibat. The pivotal trial, Trial A4250-005, demonstrated the superiority of odevixibat compared to placebo with improvements in pruritus scores over a 24-week treatment period. The duration of treatment was 24 weeks. In addition, a higher percentage of patients treated with odevixibat achieved normalization of sBA levels at any timepoint during the trial and maintained lower levels at end of treatment compared to placebo.

The Agency has decided to approve both doses (odevixibat 40 mcg/kg/day and 120 mcg/kg/day). The recommended starting dose is 40 mcg/kg/day. If there is no response after 3 months, the dose could be increased by 40 mcg/kg (i.e., 80 mcg/kg/day) and further increased to 120 mcg/kg/day based on safety, tolerability, and improvement in pruritus. The maximum daily dose of odevixibat that can be administered to patients is 6 mg.

The safety of odevixibat use for greater than 24 weeks is not well characterized. In the context of a rare disease the safety data for odevixibat is acceptable for the proposed dosing regimen in the intended population for the treatment of pruritus. Overall, odevixibat has a favorable safety profile and the safety concerns can be adequately addressed in the labeling. The nature and frequency of the safety events (liver test abnormalities, diarrhea, and potential fat-soluble vitamin deficiency) can be mitigated in the target population by monitoring for adverse events. There are no current treatment options for this population.

Based upon review of all the available efficacy and safety data, the benefit of odevixibat clearly outweighs the risk of treatment in patients with PFIC, who have medically refractory pruritus. The availability of odevixibat will provide a new and effective treatment option for this patient population.

Table 3. Benefit-Risk Effects

Effect Measure and Definition	Placebo N=20	Odevixibat 40 mcg/kg/day N=23	Odevixibat 120 mcg/kg/day N=19	Uncertainties
Benefits (Favorable Effects)				
Improvement in pruritus (FDA alternative endpoint)				
LS mean (SE)	13.2 (8.7)	35.4 (8.1)	30.1 (9.0)	
LS mean difference (95% CI)		22.2 (4.7, 39.6)	16.9 (-2.0, 35.7)	
Patients' pruritus was assessed by their caregiver twice daily on a single-item ObsRO assessment with scores ranging from 0 (no scratching) to 4 (worst possible scratching). For each patient, the percentage of assessments over the 24-week treatment period that were ≤1 (no scratching or a little scratching) was calculated. The average of this outcome across patients in each treatment group was analyzed using an ANCOVA model.				For this endpoint, odevixibat 40 mcg/kg dose achieved statistical significance compared to placebo, but odevixibat 120 mcg/kg/day dose did not demonstrate superiority to placebo on FDA's endpoint analysis. There remains uncertainty of improvement in pruritus with 120 mcg/kg/day dose. Therefore, FDA recommended a starting dose of 40 mcg/kg/day. If no response is observed after 3 months of dosing, then the dose can be increased.
Risks (Unfavorable Effects)				
Abnormality of liver tests, n (%)				
ALT increase over baseline ≥150 U/L	0	2 (8.7)	2 (10.5)	The clinical impact of liver test elevations over time with chronic dose administration are uncertain
AST increase over baseline by ≥150 U/L	0	1 (4.3)	3 (15.8)	
TB increase over baseline by ≥2 mg/dL	1 (5)	4 (17.4)	1 (5.3)	
DB increase over baseline by ≥1 mg/dL	2 (10)	5 (21.7)	2 (10.5)	
Fat-soluble vitamin deficiency, n (%)				
Vitamin deficiency (total)	2 (10)	1 (4.30)	5 (26.30)	The clinical impact of vitamin deficiency is uncertain but could include effects on cognition and growth as well as contribute to anemia.
Vitamin D	1 (5)	0	3 (15.80)	
Vitamin A	0	0	1 (5.30)	
Vitamin E	0	0	1 (5.30)	
INR increased	5	1 (4.30)	0	

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Effect	Placebo	Odevixibat	Odevixibat	Uncertainties
Measure and Definition	N=20	40 mcg/kg/day	120 mcg/kg/day	
		N=23	N=19	
Diarrhea, n (%)				Could potentially contribute to anemia
Any diarrhea AE	2 (10)	9 (39.10)	4 (21.10)	Long-term effects on cognition and growth
Drug interruption due to TEAE of diarrhea	0	0	2 (10.50)	
Discontinuation due to TEAE of diarrhea	0	0	1 (5.30)	
Diarrhea ≥3 days duration	0	6 (26)	3 (15.80)	
Diarrhea ≥7 days duration	0	0	2 (10.50)	
Growth				All of the above-mentioned risks could contribute to issues with growth (height/weight)

Source: Generated by the clinical review team and decision support analysis team

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; ANCOVA, analysis of covariance; AST, aspartate aminotransferase; CI, confidence interval; DB, direct bilirubin; INR, international normalized ratio; LS, least squares; ObsRO, observer-reported outcome; SE, standard error; TB, total bilirubin; TEAE, treatment-emergent adverse event

The Applicant demonstrated a qualitatively greater reduction in sBA levels in patients treated with odevixibat relative to placebo. An association between reduction in sBA levels and improvement in pruritus was observed in Trial A4250-005. This secondary endpoint provides some evidence of a hypothesized mechanism of action for odevixibat. However, FDA questioned the clinical meaningfulness of the of 70% reduction in sBA levels. In addition, the bioanalytical assay method to measure sBA levels was not adequately validated to support the accuracy of the 70% reduction from baseline.

There are uncertainties about sBA level reduction and long-term benefits from decreasing serum bile acid levels, (i.e., sBA levels are not an established biomarker). Clinical benefits of reduction in sBA levels that pertain to liver-related outcomes using a therapeutic agent are currently unknown. sBA levels are indirect measure of bile acids in liver and do not provide accurate estimate of liver bile acid levels. One of the key mediators of liver damage include bile acids in liver which predicts liver-related clinical outcomes. Whether a therapeutic agent that reduces sBA levels could provide similar effects that surgical biliary diversion offers such as, decreased requirement of liver transplantation is uncertain at this time.

II. Interdisciplinary Assessment

3. Introduction

Albireo AB seeks approval of BYLVAY (odevixibat) for the “treatment of pruritus in patients with progressive familial intrahepatic cholestasis (PFIC).” The proposed recommended dose for odevixibat is 40 mcg/kg/day. If no response is observed in 3 months, the dose may be increased up to 120 mcg/kg/day, not to exceed 6.0 mg/day.

Odevixibat is a new molecular entity. Odevixibat is an ileal bile acid transport inhibitor and blocks the reuptake of secondary bile acid (BA) from the terminal ileum.

PFIC is a rare, autosomal recessive genetic disorder, that typically presents during infancy or early childhood and is estimated to affect one in every 50,000 to 100,000 children born worldwide. However, relatively high incidences are reported in Saudi Arabia (approximately 1:7200) (van Wessel et al. 2020). There are at least three subtypes of PFIC that are well described in literature (PFIC1, PFIC2, and PFIC3). PFIC1 and PFIC2 constitute about two-thirds of the PFIC population.

Unlike other cholestatic diseases, in PFIC1 and 2, there is no injury or obstruction to bile ducts themselves. Instead cholestasis results secondary to reduced transporters in hepatic cells, due to reduced expression of protein.

Bile is an alkaline fluid, which contains lipids, organic anions, bile acids, phospholipids, metabolites of drugs, xenobiotics, bilirubin (degradation product of hemoglobin, myoglobin etc.), among other components.

There are other subtypes of PFIC (Bull and Thompson 2018). PFIC3, is due to reduced expression of multidrug resistance MDR3, which is encoded by ABCB4. MDR3 transports phosphatidylcholine (PC) from inner to outer leaflet of canalicular membrane, for incorporation in bile. Deficiency in PC, leads to free bile acids, resulting in damage to the cholangiocyte membrane by the detergent action of bile acids. PFIC4 occurs due loss of function of tight junction protein (TJP2) etc., leading to cholestasis.

For this NDA, the population enrolled includes patients with PFIC1 and 2 (excluding BSEP3), therefore, reviewers will limit the discussions and findings in these two subtypes.

PFIC1 occurs due to mutations on the *ATP8B1* gene (Davit-Spraul et al. 2010; Pawlikowska et al. 2010) which encodes for P-type APase, FIC1, expressed in many tissues. It is likely an aminophospholipid flippase protein that facilitates movement of phospholipids (Davit-Spraul et al. 2010). Impaired FIC1 function may lead to loss of lipid asymmetry in the canalicular membranes of hepatocytes resulting in dysfunction of the bile salt export pump (BSEP), a liver-specific canalicular protein, responsible for biliary bile acid (BA) secretion, and encoded by *ABCB11*. *ATP8B1* is also expressed in the small intestine, kidney, and pancreas, thereby explaining the extrahepatic manifestations. Patients with PFIC1 can also present with sensorineural deafness.

PFIC2 occurs due to mutation on the *ABCB11* gene (insertion, deletion, nonsense, and splicing) resulting in failure of protein expression on hepatocytes i.e., deficiency of bile salt export pump (BSEP). BSEP is the main exporter of BA from hepatocytes to canaliculi; defects in BSEP leads to reduced bile acid (also called as bile salts) secretion from liver to the intestine, and accumulation of bile acids in hepatocytes and bile acid induced hepatocellular damage. PFIC2 is further subdivided in three categories: BSEP1, BSEP2, BSEP3. BSEP3 has the worst prognosis as there is either extremely limited or a complete lack of functional BSEP protein and bile acids are not secreted from the liver. The majority of patients with BSEP3 receive liver transplants before 10 years of age, and none survive to adulthood without liver transplantation. BSEP1 and BSEP2 are milder forms and about 20% (BSEP1) and 40% (BSEP2) of children survive past age 18.

Patients with PFIC1 and PFIC2 present with elevated serum bile acid (sBA) levels and cholestasis of the liver along with pruritus, abnormal liver tests including coagulopathy, and diarrhea leading to evaluation. The liver disease can progress to fibrosis, cirrhosis, and portal hypertension and related complications. About one-third of patients with PFIC2 develop hepatocellular cancer.

Pruritus is quite severe in patients with either PFIC1 or PFIC2 but is more severe in those with PFIC2. There is no FDA-approved treatment for pruritus in patients with PFIC; however, there is widespread use of off-label cholestyramine (bile acid binding agent), antihistamine agents, ursodeoxycholic acid (UDCA), rifampin, ondansetron, and naltrexone. Most of these therapies do not provide much relief and patients continue to have intense itching or scratching, leading to scratch marks, excoriations, and skin mutilation. If the symptomatic treatment does not help the patient with itching or scratching, then current alternative options include surgical biliary diversion (van Wessel et al. 2020) and liver transplantation (Agarwal et al. 2016; Perito et al. 2017). Indeed, intractable pruritus is a reason for evaluation for liver transplantation and placement on a transplant list, regardless of the extent of direct liver involvement from PFIC. Both procedures are invasive, and patients can experience surgical complications.

Albireo AB opened new investigational new drug application (IND) 130591 on June 10, 2016 and submitted the phase 3 protocol to study the effect of odevixibat on pruritus in patients with PFIC. Odevixibat was granted Rare Pediatric Disease Designation (RPDD) for the treatment of PFIC by the Office of Orphan Products Development on June 04, 2018 (designation RPD-2018-170). Fast Track designation was granted to A4250 (odevixibat) on July 18, 2018 for treatment of pruritus associated with progressive familial intrahepatic cholestasis (PFIC).

The phase 3, multicenter, randomized, double-blind, placebo-controlled trial enrolled patients with PFIC1 and PFIC2 with a history of significant pruritus; and a caregiver-reported observed

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average scratching score of ≥ 2 (on a 0 to 4 scale) in the 2 weeks prior to randomization. The primary efficacy endpoint was the proportion of positive pruritus assessments at the patient level over the 24-week treatment period, where a positive pruritus assessment was defined as a scratching score of ≤ 1 or at least a 1-point drop from baseline on the Albireo ObsRO instrument.

3.1. Review Issue List

3.1.1. Key Review Issues Relevant to Evaluation of Benefit

3.1.1.1. Acceptability of Single Trial for Approval of Indication

3.1.1.2. Uncertainty of Primary Efficacy Endpoint

3.1.1.3. Doses for Approval

3.1.1.4. Interpretation of Serum Bile Acid Level Data

3.1.1.5. Approval for Infants 3 to 6 Months of Age

3.1.1.6. Indication for Approval

3.1.1.7. Approval for Adult PFIC Population

3.1.2. Key Review Issues Relevant to Evaluation of Risk

3.1.2.1. Liver Test Abnormalities

3.1.2.2. Diarrhea

3.1.2.3. Fat-Soluble Vitamin Deficiency

3.1.2.4. Pediatric Formulation and Dosing Instructions

3.1.2.5. Malformations in Heart and Great Vessels in Rabbit Fetus (Nonclinical Study)

3.1.2.6. Maximum Dose Selected as 6 mg

3.2. Approach to the Review

[Table 4](#) provides an overview of the clinical trials conducted to support the benefit-risk assessment of odevixibat. Results of the placebo-controlled, 24-week phase 3 trial, A4250-005, provides the primary efficacy and safety data of odevixibat for treatment of pruritus in pediatric patients with PFIC.

In addition, an ongoing 72-week, phase 3, open-label extension trial, A4250-008, provides supportive evidence for durability of improvement in pruritus.

Table 4. Clinical Trials Submitted in Support of Efficacy and/or Safety Determinations for Odevixibat

Trial Identifier (NCT #)	Trial Population	Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	Number of Subjects Planned; Actual Randomized	Number of Centers and Countries
A4250-005 (NCT03566238)	Pediatric patients with PFIC1 or PFIC2	Phase 3, multicenter, randomized, double-blind, placebo-controlled	40 mcg/kg: N=23 120 mcg/kg: N=19 Placebo: N=20 Duration: 24 weeks	Primary: Proportion of positive pruritus assessments at subject level at week-24 Secondary: Proportion of ≥70% bile acid reduction	Planned: N=60 Actual: N=62	33 centers 12 countries (Europe, Middle East, Australia, Canada, and US)
A4250-008 (NCT03659916)	Pediatric patients with PFIC1, PFIC2, or PFIC3	Phase 3, open-label, long-term extension	120 mcg/kg Cohort 1: N=53 Cohort 2: N=31 Duration: 72 weeks	Primary: Proportion of positive pruritus assessment at patient level at week-72 Secondary: Change from baseline in serum bile acid level at 72 weeks	Planned: N=120 Cohort 1: N=60 Cohort 2: N=60 Actual: N=84 Cohort 1: N=53 Cohort 2: N=31 Recruitment is ongoing	33 centers 12 countries (Europe, Middle East, Australia, Canada, and US)
A4250-003 (EUDRACT2015-001157-32)	Pediatric patients with PFIC, Alagille syndrome (ALGS), biliary atresia, sclerosing cholangitis PFIC1 N=1 PFIC2, N=7 PFIC3, N=2 Alagille's, N=6 Biliary atresia, N=3 PFIC with microvillous inclusion atrophy, N=1	Phase 2, open-label, single ascending dose, and multiple ascending dose Single dose followed by 14-day washout; then daily dosing for 4 weeks	10 mcg/kg/day: N=4 30 mcg/kg/day: N=6 60 mcg/kg/day: N=4 100 mcg/kg/day: N=6 200 mcg/kg/day: N=4 Multiple dose duration: 4 weeks	Primary: Safety, tolerability, serum bile acid levels Secondary: PK, liver biochemistry, pruritus	Planned: N=24 Actual: N=20	6 centers 4 countries (Europe)

Source: Reviewer

Abbreviations: NCT, national clinical trial; PFIC, progressive familial intrahepatic cholestasis; PK, pharmacokinetic

4. Patient Experience Data

The Applicant submitted patient experience data ([Table 5](#)) to assess the efficacy of odevixibat. The ObsRO clinical outcome assessment measured patients' scratching as observed by their caregiver and scores were recorded in an eDiary in the morning and the evening. ObsRO is a 5-point ordinal response scale, with scores ranging from 0 (no scratching) to 4 (worst possible scratching). [Table 5](#) summarizes the ObsRO data as discussed in this Integrated Review as well as the Applicant's submission of other patient experience data.

Table 5. Patient Experience Data Submitted or Considered

Data Submitted in the Application		
Check if Submitted	Type of Data	Section Where Discussed, if Applicable
Clinical outcome assessment data submitted in the application		
<input checked="" type="checkbox"/>	Patient-reported outcome	Integrated Review: Section 6 : Evidence of Benefit Section 16 : Additional Information and Assessment
<input checked="" type="checkbox"/>	Observer-reported outcome	Integrated Review: Section 6 : Evidence of Benefit Section 16 : Additional Information and Assessment Submission: Module 2.5: Clinical Overview Module 2.7.3: Summary of Clinical Efficacy Module 5.3.5.1: Study Reports of A4250-005 Module 5.3.5.3: Integrated Summary of Efficacy
<input checked="" type="checkbox"/>	Clinician-reported outcome	Section 16 : Additional Information and Assessment
<input type="checkbox"/>	Performance outcome	
Other patient experience data submitted in the application		
<input type="checkbox"/>	Patient-focused drug development meeting summary	
<input checked="" type="checkbox"/>	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel)	Module 5.3.5.3: YW30004eDiary Master, including Qualitative Interview
<input type="checkbox"/>	Observational survey studies	
<input type="checkbox"/>	Natural history studies	
<input type="checkbox"/>	Patient preference studies	
<input type="checkbox"/>	Other: (please specify)	

Data Submitted in the Application		
Check if Submitted	Type of Data	Section Where Discussed, if Applicable
<input type="checkbox"/>	If no patient experience data were submitted by Applicant, indicate here.	
Data Considered in the Assessment (But Not Submitted by Applicant)		
Check if Considered	Type of Data	Section Where Discussed, if Applicable
<input type="checkbox"/>	Perspectives shared at patient stakeholder meeting	
<input type="checkbox"/>	Patient-focused drug development meeting summary report	
<input type="checkbox"/>	Other stakeholder meeting summary report	
<input type="checkbox"/>	Observational survey studies	
<input checked="" type="checkbox"/>	Other: (please specify)	Patients with PFIC listening sessions (2018 and 2021). A brief summary from the listening sessions is presented below.

Source: Reviewer

Abbreviations: PFIC, progressive familial intrahepatic cholestasis

Two, one-hour listening sessions organized by Division of Professional Affairs Stakeholder Engagement (PASE) were held between the FDA and families in 2018 and 2021(PASE 2019). Families and caregivers of patients with PFIC confirmed that pruritus is a disabling symptom and that it can be severe and unrelenting, adversely affecting the quality of life of both children with PFIC and the caregivers.

Pruritus in patients with PFIC remains an unmet medical need.

5. Pharmacologic Activity, Pharmacokinetics, and Clinical Pharmacology

The key clinical pharmacology information about odevixibat is summarized in [Table 6](#). The pharmacokinetic (PK) parameters below were primarily based on the clinical studies using the final, to-be-marketed (TBM) formulation (i.e., oral pellets and oral capsule) or a comparable formulation.

Table 6. Summary of General Clinical Pharmacology and Pharmacokinetics

Characteristics	Drug Information
	Pharmacologic Activity
Established pharmacologic class (EPC)	Ileal bile acid transporter (IBAT) inhibitor
Mechanism of action	Pruritus is a common symptom in patients with PFIC and the pathophysiology of pruritus in patients with PFIC is not completely understood. Although the complete mechanism through which odevixibat improves pruritus in patients with PFIC is unknown, it may involve inhibition of IBAT, which results in decreased reuptake of bile salts from the terminal ileum as observed by reduction in serum bile acid levels.
Active moieties	Odevixibat

Characteristics	Drug Information
QT prolongation	Odevixibat is not expected to prolong the QT interval. Based on low systemic exposure to odevixibat, $C_{max} < 1$ nmol/L at 120 mcg/kg daily dosing, a thorough QT study was not deemed necessary per the IRT-QT (see the IRT-QT review memo dated 12/4/2019 for IND 130591/SDN053).

General Information	
Bioanalysis	Plasma concentrations of odevixibat were measured using adequately validated bioanalytical methods with LC-MS/MS.
Drug exposure at steady state following the therapeutic dosing regimen (or single dosage, if more relevant for the drug)	Following oral administration of odevixibat 40 mcg/kg or 120 mcg/kg once daily in the morning with food to patients with PFIC aging 6 months to 17 years old, the measurable plasma concentrations ranged from 0.0616 to 0.723 ng/mL at 40 mcg/kg and 0.176 to 0.511 ng/mL at 120 mcg/kg. However, plasma concentrations were erratic and were not measurable (LLOQ of 0.05 ng/mL) in the majority of plasma PK samples. Therefore, PK parameters could not be estimated in pediatric patients.

In healthy adults, following single and multiple once daily doses of odevixibat up to 3 mg (≈ 42 mcg/kg for a 70-kg men), PK parameters could not be estimated because the majority of plasma concentrations were below LLOQ (i.e., < 0.05 ng/mL). At doses higher than 3 mg, odevixibat was measurable in plasma and the systemic exposure under fasted condition are shown below:

Table 7. Odevixibat Doses Greater Than 3 mg

Dose	N	C_{max}	AUC_{0-24h}	T_{max}
		Mean (SD) (ng/mL)	Mean (SD) (ng*hr/L)	Median (Range) (h)
7.2 mg single dose	21	0.47 (0.16)	2.19 (0.79)	2.5 (1.0 to 5.0)
9.6 mg single dose	17	0.61 (0.31)	2.94 (1.48)	3.0 (1.2 to 5.0)
7.2 mg QD for 4 days with midazolam	20	0.53 (0.25)	2.66 (1.14)	2.76 (1.5 to 5.0)

Source: A4250-004 CSR and A4250-013 CSR

Abbreviations: AUC, area under the concentration-time curve; C_{max} , maximum plasma concentration; QD, once a day; T_{max} , time of maximum concentration observed

Range of effective dose or exposure	At 40 mcg/kg and 120 mcg/kg, a reduction in serum bile acid levels and pruritus symptom score from baseline by odevixibat was greater than by placebo treatment in the phase 3 trial. The effect on serum bile acid levels and pruritus symptom score was similar between 40 mcg/kg and 120 mcg/kg. In the open-label, uncontrolled, phase 2 study in pediatric patients with cholestatic liver disease, serum bile acid level reduction from baseline was observed over the dose range of 10 to 200 mcg/kg QD. However, the dose-response relationships were not apparent (see Section 14.2.5).
Maximally tolerated dose or exposure	At this time FDA has accepted 6 mg/day as the maximum recommended dose. No serious safety findings were identified in the dose range in the phase 2 study (10 to 200 mcg/kg QD) and the phase 3 study (40 to 120 mcg/kg QD) under A4250-005 (see Section 7.6) In phase 3 trials (A4250-005 and A4250-008), 15 patients, aged 6 to 19 years, were administered a total daily odevixibat dose from 3.6 mg up to 7.2 mg. One patient (ID (b) (6)), who was 19 years of age, was treated with odevixibat 7.2 mg/day in Trial A4250-008, withdrew due to the serious adverse event of acute pancreatitis. This was the only patient who received odevixibat 7.2 mg/day. Another patient, a 15-year-old (ID (b) (6)), enrolled in Trial A4250-008 was treated with a daily dose of 6 mg and did not experience significant adverse events, i.e., requiring treatment interruption or withdrawal. Therefore, the maximum daily dose was determined as 6 mg.

Characteristics	Drug Information
Dosage proportionality	In healthy adults, the mean systemic exposure generally increased as dose increased; plasma drug concentrations were above LLOQ at a single dose of 7.2 mg and 9.6 mg while no systemic exposure was observed at 3 mg dose in A4250-001. Likewise, in pediatric patients with cholestatic liver disease, the number of drug concentrations above LLOQ increased as dose increased from 10 to 200 mcg/kg. However, the dose proportionality could not be reliably assessed because of the erratic systemic exposure due to low oral absorption and a high variability in A4250-003.
Accumulation	No accumulation was observed in healthy adult volunteers following multiple administration once daily.
Bridge between to-be-marketed and clinical trial formulations	The to-be-marketed formulations (i.e., 200 mcg and 600 mcg oral pellets; 400 mcg and 1200 mcg oral capsules) were used in the pivotal phase 3 study, A4250-005. There is no need to bridge the to-be-marketed formulation to the clinical trial formulation.

Absorption

Bioavailability	The absolute bioavailability of odevixibat has not been characterized. The mass balance study results indicate that oral absorption is low (<1%).
T _{max}	1 to 5 hours.
Sprinkle vs. capsule Geometric mean ratio and 90% CI	In healthy adults, when a single dose odevixibat 9.6 mg (8 X 1200 mcg oral capsules) was sprinkled on applesauce (approximately 30 mL), compared to when the capsules were taken as whole under fasted condition, the AUC and C _{max} was about 35-40% lower.

Table 8. Mean Ratio (90% CI) for AUC and C_{max} When Single Dose Odevixibat 9.6 mg Was Sprinkled on Applesauce Compared to Swallowed Whole

AUC _{0-24h}	C _{max}	T _{max}
0.65 (0.52, 0.79)	0.61 (0.51, 0.73)	Median T _{max} delayed from 3 hr (capsule) to 4.5 hr (sprinkle)

Source: A4250-013 CSR

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; T_{max}, time of maximum concentration observed

In the phase 3 trial, patients weighing <19.5 kg took odevixibat after sprinkled on soft food only. The oral capsule for patients weighing ≥19.5 kg can be either taken as a whole capsule or sprinkled on soft food.

Food effect (fed/fasted) Geometric mean ratio and 90% CI	A significant food effect was observed. When a single dose of odevixibat 9.6 mg was administered in healthy adults with a high-fat meal, mean AUC and C _{max} was 62% and 72% lower, respectively compared to the fasted state. In the phase 3 trial, odevixibat was administered with food in the morning.
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As odevixibat is locally acting in the GI tract (ileum), the effect of food on the changes of systemic exposures to odevixibat is not clinically relevant for efficacy.

Table 9. Mean Ratio (90% CI) for AUC and C_{max} When Single Dose of Odevixibat 9.6 mg Was Administered in Healthy Adults With High-Fat Meal Compared to Administered Under Fasted State

AUC _{0-24h}	C _{max}	T _{max}
0.38 (0.30, 0.47)	0.28 (0.23, 0.34)	Median T _{max} delayed from 3 hr (fasted) to 4.5 hr (fed)

Source: A4250-013 CSR

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; CI, confidence interval; T_{max}, time of maximum concentration observed
Fed state=30 minutes after start of high-fat, high-calorie breakfast

Characteristics		Drug Information	
		Distribution	
Plasma protein binding	>99% protein binding in human plasma in vitro		
Drug as substrate of transporters	Odevixibat is a substrate of P-gp, but not a substrate of BCRP.		
		Elimination	
Mass balance results	Following a single oral dose of radiolabeled odevixibat 3 mg in healthy adults, the average percentage recovery of the administered dose was 82.9% in feces (mostly within 72 hours postdose) and less than 0.002% was recovered in the urine. More than 97% of fecal radioactivity (~80% of administered radioactivity) was determined to be unchanged odevixibat. There were neither quantifiable concentrations of odevixibat nor radioactivity in plasma.		
Half-life	Mean half-life: 2.36 hours		
Metabolic pathway(s)	In human hepatocytes, odevixibat was metabolized via mono-hydroxylation.		
Primary excretion pathways (% dosage)	Fecal excretion (see 'Mass balance results' above)		
Drug Interaction Liability (Drug as Perpetrator)			
Inhibition/induction of metabolism	In vitro odevixibat is not an inhibitor of CYP isoforms 1A2, 2B6, 2C8, 2C9, 2C19, or 2D6 nor an inducer of CYP isoforms 1A2, 2B6, or 3A4. When odevixibat was co-administered with midazolam (a CYP3A4 substrate) at 7.2 mg once daily for 4 days in healthy adults, the AUC of midazolam and its metabolite, 1-OH midazolam was decreased by 33% and 13%, respectively.		
Inhibition/induction of transporter systems	In vitro odevixibat does not inhibit P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, and MATE2-K.		

Source: Section 2.7.2 Summary of Clinical Pharmacology Studies (SDN 1)

Abbreviations: AUC, area under the concentration-time curve; BCRP, breast cancer resistance protein; C_{max}, maximum plasma concentration; GI, gastrointestinal; IBAT, ileal bile Acid transporter; IRT, interdisciplinary review team; LC-MS/MS, high-performance liquid chromatography with two mass spectrometry detectors; LLOQ, lower limit of quantification; PFIC, progressive familial intrahepatic cholestasis; P-gp, permeability glycoprotein; PK, pharmacokinetic; QD, once a day; QT, measure between Q wave and T wave in the heart's electrical cycle

5.1. Nonclinical Assessment of Potential Effectiveness

The potential effectiveness of odevixibat was tested in vitro (cell culture systems) and in vivo (mouse models). In summary:

- The half maximal inhibitory concentration (IC₅₀) values for inhibition of human, mouse, and dog IBAT by AZD8294 (odevixibat) were 0.13, 0.12, and 1.4 nmol/L, respectively.
- Potency of AZD8294 for inhibition of the human IBAT was high compared to inhibition of the homologous human liver (basolateral) bile acid transporter. Inhibition of amino acid uptake via the neutral amino acid transporters in HEK293 cells by AZD8294 was much lower than that of bile acid transport via the human IBAT.
- AZD8294 inhibited intestinal bile acid absorption in ApoE knockout mice in vivo. The ED₅₀ of AZD8294 was calculated to be 0.073 mcmol/kg (54.1 mcg/kg). Up to 91% of the total recovered radiolabeled bile acid marker was measured in feces at 24.5 hours after oral odevixibat administration (compared to 37% after vehicle administration), while 9% was measured in the gall bladder/intestine (considered % body retention).

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BYLVAY (odevixibat)

- Odevixibat reduced liver/body weight ratios and serum markers of liver damage and cholestasis in Mdr2^{-/-} mice, a mouse model of cholestasis.
- “Ileal bile acid transporter (IBAT) inhibitor” was selected as the Established Pharmacologic Class (EPC) of odevixibat based on the criteria in the Manual of Policies and Procedures (Determining the Established Pharmacologic Class for Use in the Highlights of Prescribing Information). Various terminology for the pharmacological target of odevixibat are used in the scientific literature. IBAT and apical sodium-dependent bile acid transporter (ASBT) are the two most frequently used terms and thus, both IBAT inhibitor and ASBT inhibitor are scientifically valid. The term IBAT inhibitor has the added benefit of the inclusion of the tissue localization of the transporter, and therefore may be more clinically meaningful to physicians. The key studies that support the scientific validity for the selection of “ileal bile acid transporter (IBAT) inhibitor” as the EPC are summarized below.

In Vitro Potency and Selectivity of Odevixibat

AZD8294 In Vitro Potency and Selectivity Studies (25881)

The potency of AZD8294 (odevixibat) was tested using a cell line with stable expression of the human IBAT or in HEK293 cells after stable or transient transfection with expression vectors for mouse or dog IBAT. The specificity of AZD8294 was tested using cells expressing the homologous human liver (basolateral) sodium/bile acid cotransporter and by measuring the uptake of α -aminoisobutyric acid by the endogenous neutral amino acid transporter present in the HEK293 cells. The IC₅₀ values for inhibition of human, mouse, and dog IBAT by AZD8294 (odevixibat) were 0.13, 0.12, and 1.4 nmol/L, respectively. The potency of AZD8294 for inhibition of the human ileal (apical) transporter was over 700-fold higher than the potency for inhibition of the homologous human liver (basolateral) sodium/bile acid cotransporter. Inhibition of amino acid uptake via the neutral amino acid transporter(s) in HEK293 cells was at least 1000 times lower than that of bile acid transport via the human ileal bile acid transporter.

In Vivo Effects of Odevixibat

AZD8294: The ED₅₀ of AZD8294 on ⁷⁵SeHCAT (Taura-23-selena-25-homocholeic acid) intestinal absorption in ApoE knockout mice In Vivo (24546)

ApoE knockout mice were orally administered AZD8294 (odevixibat), followed by dosing with a radiolabeled bile acid analogue (⁷⁵SeHCAT) 30 minutes later. After 24 hours the gall bladder, whole intestine, and feces (from the intestine) were collected from each mouse. The gamma radioactivity of ⁷⁵SeHCAT in the feces and the combined gamma radioactivity in gall bladder and intestine were measured. Up to 91% of the total recovered radiolabeled bile acid marker was measured in feces at 24.5 hours after oral odevixibat administration (compared to 37% after vehicle administration), while 9% was measured in the gall bladder/intestine (considered % body retention). The ED₅₀ of AZD8294 was calculated to be 0.073 mcmol/kg (54.1 mcg/kg).

IBAT/ASBT Inhibition With A4250 in the Mdr2 KO Model (ARR4250000117)

Mdr2 knockout mice (model of cholestasis) were administered A4250 (odevixibat) in their diet for 4 weeks. Compared to control-fed mice, relative liver weight was reduced; serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)

activities were decreased; and serum total bile acids were reduced by treatment with A4250. There was no significant effect of A4250 on serum bilirubin.

6. Assessment of Effectiveness

6.1. Dose and Dose Responsiveness

In the placebo-controlled phase 3 trial (A4250-005), two doses of odevixibat, i.e., 40 and 120 mcg/kg once daily, were studied. Odevixibat was administered with food in the morning. The total daily dose was determined based on patient body weight targeting 40 or 120 mcg/kg/day (Table 10).

Patients weighing 5 to <19.5 kg received odevixibat sprinkled on soft food using 200 mcg or 600 mcg capsules for total doses of either 40 mcg/kg or 120 mcg/kg, respectively. Patients ≥19.5 kg received odevixibat 400 mcg or 1200 mcg capsules for total doses of either 40 mcg/kg or 120 mcg/kg, respectively. Patients who could not swallow whole capsules were permitted to open the capsule and sprinkle the contents onto soft food. There was no appreciable systemic exposure from either dosage regimen in Trial A4250-005.

Of note, the 200 mcg and 400 mcg strength capsules contain (b) (4) used for the 40 mcg/kg dosing schedule and the 600 mcg and 1200 mcg capsule strengths contain (b) (4) used for the 120 mcg/kg dosing schedule (see Section 9).

Table 10. Dosing Based on Body Weight, Trial A4250-005

Body Weight (kg)	Formulation	Number of Capsules	Total Daily Dose (mcg) (Capsule Strength X Number of Capsules)		
			Placebo	Odevixibat 40 mcg/kg	Odevixibat 120 mcg/kg
5.0 to <7.5	Sprinkle capsule (oral pellets)	1		200 (200 X1)	600 (600 X1)
7.5 to <12.5		2		400 (200 X2)	1200 (600 X2)
12.5 to <17.5		3		600 (200 X3)	1800 (600 X3)
17.5 to <19.5		4		800 (200 X4)	2400 (600 X4)
19.5 to <25.5	Oral capsule	2		800 (400 X2)	2400 (1200 X2)
25.5 to <35.5		3		1200 (400 X3)	3600 (1200 X3)
35.5 to <45.5		4		1600 (400 X4)	4800 (1200 X4)
45.5 to <55.5		5		2000 (400 X5)	6000 (1200 X5)
>55.5		6		2400 (400 X6)	7200 (1200 X6)

Source: A4250-005 CSR, Table 4

The patients in Trial A4250-005 were 6 months to 15.9 years of age with body weight ranging from 5.7 to 55.2 kg. The total daily dose given to those patients ranged from 200 to 2400 mcg and from 600 to 4800 mcg in the odevixibat 40 and 120 mcg/kg arms, respectively. The actual body weight-normalized daily dose (total daily dose supplied/individual body weight) ranged from 25.48 to 51.28 mcg/kg/day and 81.08 to 143.37 mcg/kg/day, in 40 and 120 mcg/kg arms, respectively.

Dose Rationale for the Pivotal Phase 3 Trial

The doses for the pivotal phase 3 trial were selected based on an open-label, uncontrolled phase 2 trial (A4250-003), in which 24 pediatric patients ranging from 1 to 17 years of age with cholestatic liver disease (including 13 patients with PFIC) received odevixibat 10, 30, 60, 100, or

200 mcg/kg/day with food in the morning. Following a 4-week course of treatment with odevixibat, serum bile acid levels decreased from baseline and pruritus appeared to be improved based on patient diary data (i.e., visual analog scale (VAS)-itch score, patient-oriented scoring atopic dermatitis (PO-SCORAD) itching score, Whittington Scale, and PO-SCORAD sleep disturbance score) in all dosage groups. Reduction in serum bile acid levels in patients receiving 30 and 60 mcg/kg/day and improvement in VAS-itch score at 100 and 200 mcg/kg/day were numerically greater than other doses. For either of the two endpoints (serum bile acid levels and pruritus score), no dose-response relationships were apparent over the dosage range of 10 to 200 mcg/kg/day (see Section 14.2.5).

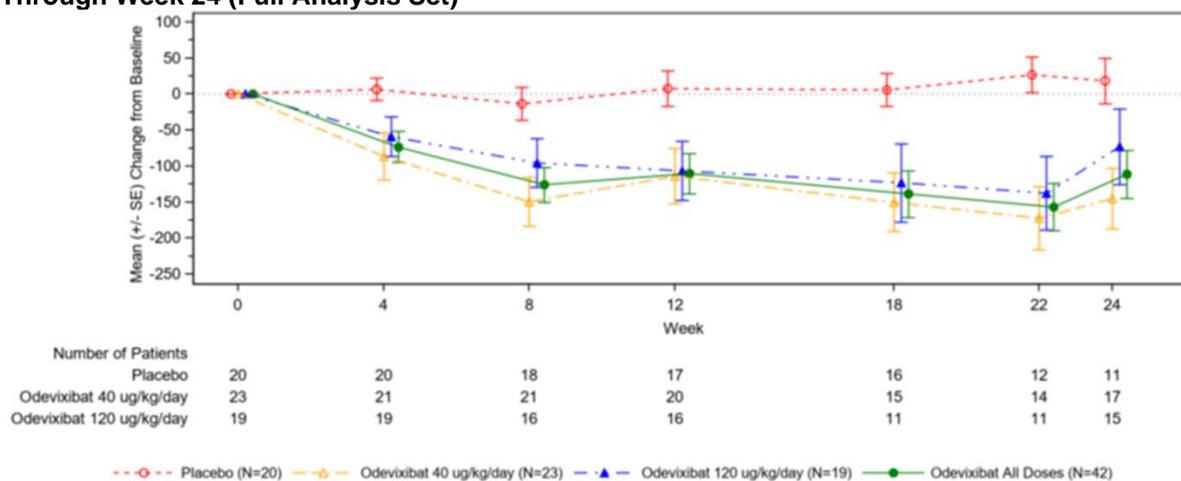
In Trial A4250-001 which had healthy volunteers receiving odevixibat 1 and 3 mg/day for 7 days, a dose-response relationship for a decrease in serum bile acid levels was not apparent (see Section 14.2.1).

Dose-Response Between 40 and 120 mcg/kg/day

A total of 62 pediatric patients with PFIC received odevixibat 40 mcg/kg, 120 mcg/kg or matching placebo once daily for 24 weeks in a double-blind trial (see Section 15). While both the 40 and 120 mcg/kg/day dose met the Applicant’s prespecified primary efficacy endpoint over placebo (i.e., the mean proportion of positive pruritus assessments that are ≤ 1 or at least a 1-point drop from baseline), there was no dose-dependent increase in the response rate between two doses. The response with pruritus improvement at 40 mcg/kg/day was numerically better than that of the patients who received 120 mcg/kg/day, both with the protocol prespecified primary endpoint and the FDA’s efficacy alternative endpoint (see Section 6.2.1.4).

In addition, the reduction of serum bile acid levels from baseline was numerically greater with patients receiving 40 mcg/kg/day compared to 120 mcg/kg/day (Figure 1). By Weeks 22/24, mean serum bile acids levels had decreased 54.5% (-141.5 micromole/L) in the 40 mcg/kg/day group and 19.4% (-83.7 micromole/L) in the 120 mcg/kg/day group while it was 8.8% (13.1 micromole/L) greater than baseline in the placebo group (see Section 14.2.6.1).

Figure 1. Mean (SE) Change From Baseline in Serum Bile Acid Levels (micromole/L) by Visit Through Week 24 (Full Analysis Set)



Source: A4250-005 CSR, Figure 3.
 Abbreviations: SE, standard error

On the other hand, a greater percentage of patients who received odevixibat 120 mcg/kg/day experienced adverse events (AEs) including diarrhea and liver test abnormalities compared to

those who received 40 mcg/kg/day, although safety profiles for both doses were generally acceptable. A greater percentage of patients in the odevixibat 120 mcg/kg/day group had dose reductions or dose interruptions due to AEs compared to those in the 40 mcg/kg/day group (31.6%, 6/19 versus 13%, 3/20) (see Section [7.6](#)).

Additionally, there was no clear evidence supporting better efficacy responses at 120 mcg/kg/day in patients who did not respond to 40 mcg/kg/day after dose escalation in the open-label trial. Even though a dose change was not allowed during Trial A4250-005, the patients who completed or discontinued Trial A4250-005 were rolled over to the open-label, long-term extension trial (A4250-008, currently ongoing) and received odevixibat 120 mcg/kg/day. A total of 19 patients each received placebo or 40 mcg/kg/day in Trial A4250-005 and escalated to 120 mcg/kg/day, whereas 15 patients who had received 120 mcg/kg/day continued to receive 120 mcg/kg/day.

Per the Applicant, after transition to Trial A4250-008, four of nine (44%) nonresponders in the 40 mcg/kg/day group (i.e., patients who did not have a >1-point reduction in monthly pruritus score, as defined by the Applicant) showed a favorable response after the dose was increased to 120 mcg/kg/day. Three of eight (38%) nonresponders in the 120 mcg/kg/day group showed a favorable response when therapy was continued at 120 mcg/kg/day during the open-label trial. These results suggest that the apparent improvement from initial nonresponders may not be attributed to the dose increase from 40 mcg/kg/day to 120 mcg/kg/day as a similar proportion of patients improved after rolling over to the open-label trial regardless of the dose increase. More importantly, given the open-label, uncontrolled design of Trial A4250-008, the pruritus assessment data from this trial are deemed inadequate to be used in efficacy evaluation due to potential bias.

Serum bile acid levels—a more objective measure did not further decrease after the dose increased from 40 mcg/kg/day to 120 mcg/kg/day. Out of six nonresponders in terms of serum bile acid level reduction in the 40 mcg/kg/day group (i.e., less than 70% reduction or did not reach ≤ 70 micromole/L per the Applicant's definition), none became responders after the dose was increased to 120 mcg/kg/day.

Recommended Dosing Regimen

(b) (4)
However, based on the similar efficacy between

40 mcg/kg and 120 mcg/kg in Trial A4250-005, we recommend 40 mcg/kg as the starting dose.

The 40 mcg/kg is to be given once daily with food in the morning.

In addition, the Applicant's proposal to keep the 120 mcg/kg dose available as an option was found acceptable. Although the 120 mcg/kg/day dose did not show additional benefit compared to the 40 mcg/kg/day dose in terms of the efficacy, the 120 mcg/kg dose showed similar efficacy to 40 mcg/kg compared to placebo and had an acceptable safety profile. Of note, the A4250-008 long-term extension trial is currently ongoing and only studying a 120 mcg/kg dose. Considering the small number of patients over a wide range of age in the clinical trials, the availability of 120 mcg/kg should allow further individualized treatment.

Treatment should start at 40 mcg/kg and the dose may be increased up to 120 mcg/kg for patients who did not respond to 40 mcg/kg after the initial 3 months of treatment as the improvement of pruritus was generally shown within 3-4 months of treatment in Trial A4250-005 ([Figure 2](#)).

Treatment should start at 40 mcg/kg and the dose may be increased up to 120 mcg/kg for patients

who did not respond to 40 mcg/kg after the initial 3 months of treatment as the improvement of pruritus was generally shown within 3 to 4 months of treatment in Trial A4250-005 (Figure 2). We recommend the dose be increased in increments of 40 mcg/kg based on tolerability to allow for assessment of tolerability and responsiveness at 80 mcg/kg dose before dose escalation to 120 mcg/kg.

Notably, the recommended body weight-based dosing table includes patient weights from ≤ 7.4 kg to >55 kg whereas the dosing table used in Trial A4250-005 was designed for patients ≥ 5 kg. To allow the use of odevixibat in patients as young as 3 months of age who typically weigh 3.5 to 4 kg, it is reasonable to expand the body weight range down to '7.4 kg and below' for the 40 mcg/kg/day dose. Of note, in patients weighing 3.5 to 90 kg, the expanded dosing table for 40 mcg/kg/day will result in the actual body weight-normalized dose (mcg/kg) ranging from 26.7 to 57.153 mcg/kg, which is within the dose range that was actually given in Trial A4250-005 (25.48 to 51.28 mcg/kg for 40 mcg/kg/day; 81.08 to 143.37 mcg/kg for 120 mcg/kg/day).

6.2. Clinical Trials Intended to Demonstrate Efficacy

6.2.1. Trial A4250-005

Title

A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC1)

6.2.1.1. Design, Trial A4250-005

Trial A4250-005 (NCT03566238) was a double-blind, randomized, placebo-controlled phase 3 trial to evaluate the efficacy and safety of odevixibat compared to placebo in children with progressive familial intrahepatic cholestasis types 1 and 2.

The trial consisted of a 35- to 56-day screening period, a 24-week treatment period, and a 4-week follow-up period. The protocol planned to enroll approximately 60 eligible patients who were randomized in a 1:1:1 ratio to receive 40 mcg/kg/day of odevixibat, 120 mcg/kg/day of odevixibat, or a matching placebo. The protocol specified that randomization was stratified according to PFIC class (types 1 and 2) and age group (6 months to 5 years, 6 to 12 years, and 13 to ≤ 18 years).

Patients were treated for up to 24 weeks in Trial A4250-005. Patients who completed the 24-week treatment period and the Day 168 visit were invited to participate in Trial A4250-008, a 72-week open-label extension trial in which all patients received 120 mcg/kg/day of odevixibat. Trial A4250-005's protocol originally allowed patients to roll over to Trial A4250-008 early at Week 12 if the patient had no improvement or intolerable symptoms. This option of early roll over was removed in the protocol amendment dated June 24, 2019.

Trial visits during the double-blind treatment period of Trial A4250-005 were scheduled to occur at baseline, Days 28, 56, 84, 126, 154, and 168 with a follow-up visit 28 days after the last dose of study drug, unless patients entered Trial A4250-008.

Throughout the trial period (i.e., between Visits 1 [screening] and 10 [follow-up visit 28 days after last dose], inclusive), a single-item ObsRO was used to measure the severity of patients' scratching (pruritus) as observed by their caregiver twice daily (once in the morning [AM] and once in the evening [PM]). The AM ObsRO measure was administered as item 1 of the ObsRO daily *morning* electronic diary (see screen 2 in [Figure 42](#)), and the PM ObsRO measure was administered as item 4 of the ObsRO daily *evening* electronic diary (see screen 5 in [Figure 43](#)). Both the AM and PM ObsRO measures of scratching utilize the same 5-point ordinal response scale:

- 0 = "No scratching"
- 1 = "A little scratching"
- 2 = "Medium scratching"
- 3 = "A lot of scratching"
- 4 = "Worst possible scratching"

A higher score on the AM ObsRO measure indicates a higher level of *nighttime* scratching severity (experienced the previous night), and a higher score on the PM ObsRO measure indicates a higher level of *daytime* scratching severity (experienced that day).

Throughout the trial period (i.e., between Visits 1 and 10, inclusive), patients aged 8 to 18 years completed analogous AM and PM patient-reported outcome (PRO) measures of pruritus. According to page 60/248 of the clinical study report (CSR),

"...the PRO items were only required to be completed for patients 8 to 18 years of age. For patients 8 to 12 years of age, the caregivers were to read the PRO items along with the child and record the child's response. A guide was to be provided to the caregivers that had standardized explanations of the PRO items in case the patient was confused or required clarification. The ObsRO items were completed by the caregivers of all patients."

The AM PRO measure was administered as item 1 of the PRO daily morning electronic diary (see screen 2 in [Figure 40](#)), and the PM PRO measure was administered as item 1 of the PRO daily evening electronic diary (see screen 2 in [Figure 41](#)). Both the AM and PM PRO measures of pruritus utilize the same 5-point ordinal response scale:

- 0 = "No itching"
- 1 = "A little itching"
- 2 = "Medium itching"
- 3 = "A lot of itching"
- 4 = "The worst itching"

A higher score on the AM PRO measure indicates a higher level of *nighttime* itching severity (experienced the previous night), and a higher score on the PM PRO measure indicates a higher level of *daytime* itching severity (experienced that day).

All scores computed from the PRO and ObsRO measures of pruritus—including scores used to construct the focal efficacy endpoints considered by the Agency (discussed below)—are enumerated in [Table 129](#).

The protocol prespecified the primary efficacy endpoint as the proportion of “positive pruritus assessments” at the patient level over the 24-week treatment period, where a “positive pruritus assessment” is defined as a scratching score of ≤ 1 or at least a 1-point drop from baseline on the ObsRO instrument. The primary outcome for each patient is a proportion, and the mean of the patient-level proportions is compared across treatment groups. Hereinafter, we refer to this endpoint as the mean percentage of assessments over the 24-week treatment period that are ≤ 1 or at least a 1-point drop from baseline. To determine whether each pruritus assessment met the definition of a “positive pruritus assessment,” the AM score was compared to the AM baseline, and the PM score was compared to the PM baseline. The AM scores from the 14 days prior to the first dose of study medication were averaged to calculate AM baseline; PM baseline was calculated similarly.

The protocol specified a different primary endpoint for the European Union (EU) and rest of the world (RoW), which was the “proportion of patients experiencing at least a 70% reduction in fasting sBA level from baseline to the end of treatment or reaching a level ≤ 70 micromole/L compared to placebo after 24 weeks of treatment.” Serum bile acid levels can be a clinically important biomarker to provide supportive evidence of efficacy because it indirectly reflects the mechanism of action (MOA) of odevixibat. However, in Trial A4250-005, the bioanalytical methods for serum bile acid measurements were not adequately validated according to the FDA guidance for industry *Bioanalytical Method Validation* (2018) (see Section [14.4.2](#) for further details) even though the bioanalysis performed using a commercial kit from clinical laboratory improvement amendments (CLIA)-certified laboratories might have fit the purpose of diagnosis in a routine clinical setting (i.e., However, in Trial A4250-005, the bioanalytical methods for serum bile acid measurements were not adequately validated according to the FDA guidance for industry *Bioanalytical Method Validation* (2018) (see Section [14.4.2](#) for further details) even though the bioanalysis performed using a commercial kit from clinical laboratory improvement amendments (CLIA)-certified laboratories might have fit the purpose of diagnosis in a routine clinical setting (i.e., abnormality of serum bile acid level, >10 micromole/L). Therefore, the reliability of the reported serum bile acid levels was not sufficiently demonstrated to be able to support primary or secondary efficacy findings. With this limitation, the results of serum bile acid levels in this trial were considered exploratory and they were only interpreted in a qualitative manner based on the overall trend. For results relating to serum bile acid levels, refer to Section [14.2.6.1](#).

The protocol also specified many secondary and exploratory endpoints that were not adjusted for multiplicity.

Due to concerns with the prespecified primary endpoint (refer to Section [6.3.2](#)), the Agency analyzed various endpoints to further evaluate the treatment effect of odevixibat on pruritus. This review will cover results for two additional endpoints determined to be clinically meaningful by the Agency and four exploratory endpoints prespecified by the Applicant.

Additional Endpoints Determined To Be Meaningful by the Agency

1. Mean percentage of assessments over the 24-week treatment period that are ≤ 1
A " ≤ 1 " is defined as a scratching score of 0 (no scratching) or 1 (a little scratching), regardless of baseline average on the ObsRO instrument. This differs from the Applicant's prespecified endpoint in that it does not consider a 1-point drop from baseline alone to be a "positive pruritus assessment" (see Section [6.3.2](#) for further discussion).
2. Change in Worst Weekly Scratching Score from baseline (Study Days [-28, -1]) to Weeks 21–24 (Study Days [141, 168])

The Worst Weekly Scratching Score for an assessment period of 4 consecutive weeks (28 days) is computed per [Table 129](#) as the maximum (worst) Weekly Average Worst Scratching Score within the 4-week interval, where the Weekly Average Worst Scratching Score for a given assessment week is computed as the average of the Worst Daily Scratching Scores across the 7 days comprising the week. A Worst Daily Scratching score is considered to be missing if both the Daytime and Nighttime Scratching Scores for that day are missing; a Weekly Average Scratching Score is considered to be missing when \geq four Worst Daily Scratching Scores are missing for that week; and the Worst Weekly Scratching Score is considered to be missing if all four Weekly Average Scratching Scores are missing. This endpoint was considered by the review team to provide additional support to endpoint 1 described above and to facilitate evaluation of clinically meaningful within-patient change in pruritus (see Section [16.1.7](#) for in-depth discussion) by the following:

Being constructed from a daily summary of pruritus (the Worst Daily Scratching Score; defined in [Table 129](#)) that accommodates differences between daytime and nighttime scratching severity within a given day (see Section [16.1.4](#)) while also capturing the patient's worst pruritus experience that day; and

Aligning the implied recall periods (1 week) of the Worst Weekly Scratching Score and the Caregiver Global Impression of Symptoms (CaGIS; [Figure 44](#))—the anchor measure administered in Trial A4250-005 with the least potential for recall bias that aligns with the caregiver perspective captured by the ObsRO pruritus measures used to construct the focal efficacy endpoints.

Exploratory Efficacy Endpoints Prespecified by the Applicant

3. Change from baseline in monthly average of the scratching scores

Monthly average of the scratching scores is computed per [Table 129](#) as the average of the Daytime (PM ObsRO) and Nighttime (AM ObsRO) Scratching Scores across the 28 days (56 assessments) comprising a period of 4 consecutive weeks. The monthly average of the scratching scores is considered to be missing if ≥ 29 of the 56 assessments are missing.

4. Change from baseline in monthly average of Nighttime Scratching Scores

Monthly average of Nighttime Scratching Scores is computed per [Table 129](#) as the average of the Nighttime (AM ObsRO) Scratching Scores across the 28 days (28 assessments) comprising a period of 4 consecutive weeks. The monthly average of the Nighttime Scratching Scores is considered to be missing if ≥ 15 of the 28 assessments are missing.

5. Change from baseline in monthly average of Daytime Scratching Scores

Monthly average of Daytime Scratching Scores is computed per [Table 129](#) as the average of Daytime (PM ObsRO) Scratching Scores across the 28 days (28 assessments) comprising a period of 4 consecutive weeks. The monthly average of Daytime Scratching Scores is considered to be missing if ≥ 15 of the 28 assessments are missing.

6. Change from baseline in monthly average of Worst Daily Scratching Scores

Monthly average of the Worst Daily Scratching Scores is computed per [Table 129](#) as the average of the Worst Daily Scratching Scores—i.e., the worst/maximum of the Daytime (PM ObsRO) and Nighttime (AM ObsRO) Scratching Scores on a given day—across the 28 days comprising a period of 4 consecutive weeks. The monthly average of the Worst Daily Scratching scores is considered to be missing if ≥ 15 of the 28 Worst Daily Scratching Scores are missing.

6.2.1.2. Eligibility Criteria, Trial A4250-005

Key Inclusion Criteria

- Pediatric patients, between ages ≥ 6 months and < 18 years; with a clinical diagnosis of PFIC types 1 or 2 with genetic confirmation
- Elevated serum bile acid levels ≥ 100 micromole/L as the average of two samples prior to randomization collected at least 7 days apart
- Significant pruritus and a caregiver-reported observed scratching in electronic diary average of ≥ 2 (in a scale of 0 to 4) in the 2 weeks prior to randomization

Key Exclusion Criteria

- Pathologic variation of the *ABCCB11* gene that predict complete absence of the BSEP protein
- Other types of cholestatic liver diseases such as biliary atresia or Alagille's Syndrome

- Medically refractory pruritus caused by conditions other than PFIC, for example, refractory atopic dermatitis or primary pruritic skin disease
- Decompensated liver disease (i.e., presence of ascites, variceal bleeding, coagulopathy, encephalopathy)
- International normalized ratio (INR) >1.4, ALT >10 x upper limit of normal (ULN), total bilirubin (TB) >10 x ULN at screening
- Pregnancy or lactation
- Medical history of chronic diarrhea (i.e., >3 months), conditions that may interfere with action of drug or bile acid metabolism (for example, surgical resection of terminal ileum, inflammatory bowel disease); HIV; confirmed cancers; and chronic kidney disease (glomerular filtration rate <70 mL/min/1.73m²), previous liver transplant or liver transplant that was planned within 6 months of randomization
- Use of bile acid binding agents, such as cholestyramine, colesevelam, colestipol; drugs known to affect gastrointestinal motility (sucralfate, loperamide, codeine); other investigation products to treat PFIC (e.g., 4-phenylbutyrate).

6.2.1.3. Statistical Analysis Plan, Trial A4250-005

The protocol-specified primary population for efficacy analyses was based on all randomized patients who received at least one dose of assigned treatment. As all randomized patients received at least one dose of assigned treatment, this is hereinafter referred as the intent-to-treat (ITT) population. The safety population was defined as all patients who received at least one dose of randomized treatment in Trial A4250-005.

The protocol specified that for the primary endpoint (proportion of positive pruritus assessments at the patient level over the 24-week treatment period), the comparisons between the treatment groups is evaluated using an analysis of covariance (ANCOVA) model. The model includes treatment arm, AM baseline pruritus score, PM baseline pruritus score, and the randomization stratification factors, i.e., PFIC class and age class. Least squares (LS) means (standard errors [SEs]) by treatment arm, LS mean difference (SE), 95% confidence intervals, and p-values (where applicable) between treatments (120 mcg/kg/day and 40 mcg/kg/day) versus placebo are estimated from the ANCOVA model. All missing scratching assessments were considered to be negative pruritus assessments (i.e., not meeting the criteria of a “positive pruritus assessment”). Similarly, all planned assessments after intercurrent events (i.e., premature treatment discontinuation, death, or initiation of rescue treatments) are also considered to be negative pruritus assessments. The same model and method to handle missing data was used to evaluate the Agency’s recommended endpoint using an alternative definition of a “positive pruritus assessment” (see endpoint 1 in Section [6.2.1.1](#)).

The protocol/SAP (statistical analysis plan) specified a multiple testing procedure to control the type I error rate for evaluating the primary efficacy endpoint for each of the two dose groups compared to the placebo group. In the closed testing procedure, the average effect of the 40 mcg/kg/day and 120 mcg/kg/day dose groups was first compared with the placebo group. If the one-sided p-value was ≤ 0.025 , the one-sided p-values are calculated for the comparisons of the 40 mcg/kg/day dose versus placebo and the 120 mcg/kg/day dose versus placebo. If both individual p-values are ≤ 0.025 , a significant treatment effect is declared for both dose groups. If

only one p-value is ≤ 0.025 , a significant treatment effect is declared for the corresponding dose group.

No adjustments were specified for multiple comparisons when testing secondary and exploratory efficacy variables.

For the primary endpoint calculation, the SAP dated October 23, 2019, stated that all 336 assessments planned over the 24-week treatment period (two assessments per day x 7 days per week x 24 weeks) would be included in the denominator. However, an amended SAP dated August 25, 2020, stated that if the last pruritus assessment for a patient who completes treatment (i.e., completes Week 24 visit) is reported prior to Day 168, then the denominator used for analysis would be 2 times the last report day of the pruritus assessment; for example, if a patient completed the treatment period and their caregiver last reported a scratching score on Day 160, then the Applicant used a denominator of 2 x 160 when calculating the primary endpoint. For patients who did not complete the treatment period, the denominator was 336 as planned in the previous version of the SAP.

In the pre-NDA (new drug application) meeting minutes dated October 26, 2019, the Agency expressed concern that using two times the last reported day for the denominator was an attempt to address two distinct issues: (1) missing pruritus assessments at the end of the treatment period and (2) patients who completed the Week 24 visit prior to Day 168. The Agency did not agree that this was the most appropriate strategy to handle missing pruritus assessment data at the end of the treatment period, and this did not align with the specified methodology for handling other missing pruritus assessment data. As a sensitivity analysis for the Agency's recommended endpoint using an alternative definition of a "positive pruritus assessment," the Agency calculated the denominator as the smaller number of (1) 336 to represent all 336 assessments planned over the 24-week treatment period or (2) 2 times the total number of days up to the patient's Week 24 visit day. This sensitivity analysis used the same ANCOVA model described above for the prespecified primary endpoint.

For the prespecified exploratory endpoints (endpoints 3–6 in Section [6.2.1.1](#)), the protocol specified analyses based on a mixed-effect model for repeated measures (MMRM) with an unstructured covariance matrix to compare treatment effects at the last 28 days (i.e., Month 6). The MMRM model includes baseline pruritus score, treatment group, month, treatment-by-baseline pruritus score interaction, treatment-by-month interaction, and the stratification factors (i.e., PFIC type and age group). The baseline pruritus score included in the MMRM model for endpoints 3–5 in Section [6.2.1.1](#) are, respectively, both AM and PM baseline scores, the AM baseline score, and the PM baseline score. The baseline pruritus score included in the MMRM model for the endpoint 6 in Section [6.2.1.1](#) is calculated by averaging the worst daily scores for 14 days prior to or on the first dose day of study medication.

A multiple imputation (MI) procedure imputed missing data based on outcomes from the placebo arm completers adjusted for PFIC type and age category as covariates. The Applicant retained the first 200 imputed datasets that consisted of imputed values only in the range of scratching scores (i.e., 0 to 4). To evaluate the potential impact of the Applicant's approach, the Agency performed an additional analysis where the first 200 imputed datasets were used, regardless of whether the imputed scratching scores were within the range of 0 to 4.

For the endpoint "change from baseline in Worst Weekly Scratching Score" suggested by the Agency (see second endpoint in Section [6.2.1.1](#)), the MMRM model was applied with the same

model structure used for the prespecified exploratory continuous endpoints 3–6 in Section [6.2.1.1](#).

For handling missing data, the Agency evaluated four different approaches:

1. No imputation, using only the observed data in the MMRM model (assumes data are Missing at Random [MAR])
2. MI approach under an MAR assumption where missing values are imputed for each treatment group by an MI model including the baseline pruritus score and the stratification factors (i.e., PFIC type and age category)
3. MI approach under a Missing Not at Random (MNAR) assumption as specified by the Applicant (described above) where only imputed values within the range of 0 to 4 are utilized
4. Alternative MI approach under an MNAR assumption as conducted by the Agency where the first 200 imputed datasets were utilized regardless of whether the imputed scratching scores were within the range of 0 to 4

6.2.1.4. Results of Analyses, Trial A4250-005

A total of 107 patients were screened and 62 patients were randomized, 23 to odevixibat 40 mcg/kg/day, 19 to odevixibat 120 mcg/kg/day, and 20 to placebo. This trial was an international trial, and patients were enrolled at 28 study centers: 5 centers in the United States, 15 centers in Europe, and 8 centers in the rest of world (RoW). The majority of patients were enrolled from the United Kingdom (13 patients), Turkey (11 patients), Germany (nine patients), and the United States (eight patients).

[Table 11](#) presents the demographics and baseline clinical characteristics for the patients in Trial A4250-005. Both the demographics and clinical characteristics were generally balanced across the treatment arms. Approximately 76% of the patients were younger than 6 years old, and both males and females were equally represented. Approximately 84% of the patients were white and approximately 73% of the patients had PFIC2.

The average AM and PM baseline pruritus scores for each patient was obtained by averaging the AM and PM pruritus scores for 14 days prior to or on the first dose day of study medication (28 assessments). Patients had an average baseline pruritus score of approximately 2.9.

Table 11. Baseline Demographic and Clinical Characteristics, ITT Population, Trial A4250-005

Characteristic	Placebo N=20	Odevixibat 40 mcg/kg N=23	Odevixibat 120 mcg/kg N=19
Sex, n (%)			
Male	12 (60)	11 (48)	8 (42)
Female	8(40)	12 (52)	11 (58)
Age, years			
Mean (SD)	3.7 (3.9)	3.9 (3.7)	5.2 (4.2)
Median (min, max)	2.8 (0.5, 15.0)	3.2 (0.6, 15.9)	4.9 (1.0, 13.2)
Age groups (years), n (%)			
6 months to 5 years	16 (80)	17 (74)	14 (74)
6 to 12 years	3 (15)	5 (22)	4 (21)
13 to 18 years	1 (5)	1 (4)	1 (5)

Characteristic	Placebo N=20	Odevixibat 40 mcg/kg N=23	Odevixibat 120 mcg/kg N=19
Race, n (%)			
White ¹	17 (85)	18 (78)	17 (89)
Asian	1 (5)	0 (0)	1 (5)
Black/African American	0 (0)	2 (9)	0 (0)
Other	2 (10)	3 (13)	1 (5)
Ethnicity, n (%)			
Hispanic	1 (5)	0	0
Non-Hispanic	19 (95)	23 (100)	19 (100)
Region of participation, n (%)			
United States	3 (15)	2 (9)	3 (16)
Europe	12 (60)	13 (57)	10 (53)
Rest of world	5 (25)	8 (35)	6 (32)
PFIC type, n (%)			
Type 1	5 (25)	7 (30)	5 (26)
Type 2	15 (75)	16 (70)	14 (74)
Serum bile acid level, n (%)			
<250 micromole/L	10 (50)	14 (61)	13 (68)
≥250 micromole/L	10 (50)	9 (39)	6 (32)
AM baseline pruritus score			
Mean (SD)	3.0 (0.2)	3.1 (0.1)	2.9 (0.1)
Median (min, max)	3.0 (1.5, 4.0)	3.1 (2.1, 4.0)	2.9 (2.0, 3.5)
PM baseline pruritus score			
Mean (SD)	2.9 (0.1)	2.9 (0.1)	2.6 (0.1)
Median (min, max)	3.0 (1.7, 4.0)	2.9 (1.9, 4.0)	2.8 (1.1, 3.5)
Average of AM and PM baseline pruritus			
Mean (SD)	3.0 (0.1)	3.0 (0.1)	2.7 (0.1)
Median (min, max)	3.0 (1.9, 4.0)	3.0 (2.0, 4.0)	2.9 (1.6, 3.4)

Source: Statistical Reviewer's Analysis (same as the Applicant's results)

Abbreviations: ITT, intent-to-treat; PFIC, progressive familial intrahepatic cholestasis; SD, standard deviation
N, number of subjects; n, number of subjects with at least one event

¹ The trial population from Middle Eastern countries was included under race category "white" by the Applicant.

The numbers of patients screened and randomized are presented in [Table 12](#) and patient disposition is presented in [Table 13](#). There were a total of 11 patients (five in the placebo arm, four in the odevixibat 40 mcg/kg/day group, and two in the odevixibat 120 mcg/kg/day group) who did not complete dosing in Trial A4250-005 in order to roll over early into Trial A4250-008 where all patients received open-label treatment with 120 mcg/kg/day of odevixibat. There was one patient in the odevixibat 120 mcg/kg/day arm who discontinued the trial due to an adverse event (AE).

Table 12. Patient Screening and Randomization, Trial A4250-005

Disposition	No. Patients
Screened	107
Not randomized	45
Screening failures, n (%)	45 (100)
Randomized	62

Source: Statistical Reviewer's Analysis (same as the Applicant's results)

Table 13. Patient Disposition, Trial A4250-005

Disposition Category	Placebo N=20 n (%)	Odevixibat 40 mcg/kg N=23 n (%)	Odevixibat 120 mcg/kg N=19 n (%)
Patients randomized	20 (100)	23 (100)	19 (100)
ITT population	20 (100)	23 (100)	19 (100)
Safety population	20 (100)	23 (100)	19 (100)
Discontinued study drug	5 (25)	5 (22)	3 (16)
Adverse event	0 (0)	0 (0)	1 (5)
Lack of efficacy	5 (25)	4 (17)	2 (11)
Other ¹	0 (0)	1 (4)	0 (0)
Early discontinuation of the treatment period of A4250-005	5 (25)	5 (22)	2 (11)
Rolled over early to Trial A4250-008	5 (25)	4 (17)	2 (11)
Other ^{1, 2}	0 (0)	1 (4)	0 (0)
Completed the treatment period of A4250-005 and rolled over to Trial A4250-008	14 (70)	17 (74)	14 (74)

Source: Statistical Reviewer's Analysis (same as the Applicant's results)

Abbreviation: ITT, intent-to-treat; N, number of subjects; n, number of subjects with at least one event

¹ Noncompliance/inability to travel to the site

² Noncompliance with visits, eDiary, and dosing

Efficacy Results for the Mean Percentage of “Positive Pruritus Assessments” Endpoints

[Table 14](#) shows the efficacy results for both versions of the mean percentage of “positive pruritus assessments” over 24 weeks of the treatment period endpoint, using the Applicant’s prespecified definition of a “positive pruritus assessment” as a scratching score of ≤ 1 or at least a 1-point drop from baseline and the Agency’s alternative definition of a “positive pruritus assessment” as a scratching score of ≤ 1 (not including the 1-point drop from baseline criterion). Two analyses are presented for the alternative definition using different calculations of the denominator (see [Section 6.2.1.3](#)).

The Agency’s definition of a positive pruritus assessment is more conservative, so the LS mean estimates for each treatment arm for this endpoint are smaller than when using the Applicant’s definition.

Based on the primary endpoint specified by the Applicant, both the 40 mcg/kg/day and 120 mcg/kg/day doses of odevixibat demonstrated superiority to the placebo arm (adjusted one-sided p-values < 0.025 for both arms) according to the closed testing procedure described in [Section 6.2.1.3](#). For the endpoint using the alternative definition recommended by the Agency, the 40 mcg/kg/day dose had a nominal one-sided p-value less than 0.025, but the 120 mcg/kg/day dose had a nominal one-sided p-value greater than 0.025. The LS mean differences compared to the placebo group for the Agency’s endpoint were smaller than the Applicant’s endpoint. The results for the Agency’s alternative endpoint using different calculations of the denominator are consistent with each other.

Table 14. Efficacy Results Over 24-Week Treatment Period in Trial A4250-005¹

Results	Placebo N=20	Odevixibat 40 mcg/kg/day N=23	Odevixibat 120 mcg/kg/day N=19
Prespecified primary endpoint:			
Mean percentage of assessments that are ≤1 or at least a 1-point drop from baseline ²			
LS mean (SE)	30.1 (9.1)	58.3 (8.6)	51.8 (9.5)
LS mean difference (SE) ²		28.2 (9.2)	21.7 (9.9)
95% CI for LS mean difference		(9.8, 46.6)	(1.9, 41.5)
One-sided unadjusted p-value		0.0016	0.0163
One-sided adjusted p-value ³		0.0019	0.0163
FDA alternative endpoint definition:			
Mean percentage of assessments that are ≤1			
LS mean (SE)	13.2 (8.7)	35.4 (8.1)	30.1 (9.0)
LS mean difference (SE) ²		22.2 (8.7)	16.9 (9.4)
95% CI for LS mean difference		(4.7, 39.6)	(-2.0, 35.7)
One-sided unadjusted p-value		0.0069	0.0390
FDA alternative endpoint definition:			
Mean percentage of assessments that are ≤1 (based on the last visit day)			
LS mean (SE)	13.2 (8.6)	35.3 (8.1)	29.9 (8.9)
LS mean difference (SE) ²		22.1 (8.7)	16.8 (9.3)
95% CI for LS mean difference		(4.7, 39.4)	(-2.0, 35.5)

Source: Statistical Reviewer's Analysis and Table 20 of clinical study report (pg. 119) - confirmed by the statistical reviewer

Abbreviations: CI, confidence interval; LS, least squares; SE, standard error; FDA, Food and Drug Administration

¹ Results presented for the randomized set as intent-to-treat (ITT) population.

² Difference represents odevixibat – placebo. The analysis was based on an ANCOVA model with rounded AM and PM baseline scores as covariates, and treatment group and stratification factors (PFIC type and age category) as fixed effects.

³ For an individual dose, the adjusted p-value is calculated as the maximum value of the unadjusted p-value for odevixibat combined doses and the unadjusted p-value for the individual dose.

Efficacy Results for Supportive Pruritus Endpoints

[Table 15](#) presents results at Month 6 for the endpoint evaluating the change from baseline in Worst Weekly Scratching Score (see [Table 129](#) and Section [6.2.1.1](#)). The results are based on MMRM analyses under four different approaches for handling missing data, as described in Section [6.2.1.3](#). Both the 40 mcg/kg/day and 120 mcg/kg/day odevixibat groups had greater decreases in LS mean Worst Weekly Scratching Score compared to the placebo group, and the point estimates appeared consistent across the different methodologies for handling missing data. However, the 95% confidence intervals for the comparisons of each odevixibat group with the placebo group do not exclude 0 for some of the analyses.

Table 15. Efficacy Results for Change in Worst Weekly Scratching Score From Baseline to Month 6 (Weeks 21–24)^{1, 2}

Results	Placebo N=20	Odevixibat 40 mcg/kg N=23	Odevixibat 120 mcg/kg N=19
Baseline mean (SE)	3.4 (0.1)	3.4 (0.1)	3.2 (0.1)
No imputation prior to MMRM analysis (MAR assumption)			
LS mean (SE)	-0.3 (0.3)	-1.1 (0.3)	-1.1 (0.3)
LS mean difference, (95% CI)		-0.7 (-1.4, 0.0)	-0.8 (-1.5, -0.1)
MI under MAR assumption ³			
LS mean (SE)	-0.4 (0.3)	-1.1 (0.3)	-1.1 (0.3)
LS mean difference, (95% CI)		-0.7 (-1.3, 0.0)	-0.7 (-1.4, 0.0)
MI with MNAR assumption ⁴			
LS mean (SE)	-0.4 (0.3)	-1.2 (0.3)	-1.1 (0.3)
LS mean difference, (95% CI)		-0.7 (-1.4, 0.0)	-0.7 (-1.4, 0.1)
Alternative MI method with MNAR assumption ⁵			
LS mean (SE)	-0.3 (0.3)	-1.1 (0.3)	-1.3 (0.4)
LS mean difference, (95% CI)		-0.8 (-1.6, 0.1)	-0.9 (-1.9, 0.1)

Source: Statistical Reviewer's Analysis

Abbreviations: CI, confidence interval; LS, least squares; MAR, missing at random; MI, multiple imputation; MMRM, mixed-effect model for repeated measures; MNAR, missing not at random; SE, standard error

¹ Results presented for the randomized set as the intent-to-treat (ITT) population

² Difference represents odevixibat – placebo. The analysis was based on a mixed model for repeated measures (MMRM) with baseline score as a covariate, and treatment group, time (in month), treatment-by-time interaction, treatment-by-baseline interaction, and stratification factors (PFIC type and age category) as fixed effects

³ Missing data were imputed under the MAR assumption using the baseline pruritus score and the stratification factors as covariates (refer to Section [6.2.1.3](#))

⁴ Missing data were imputed under the MNAR assumption as specified by the Applicant, where only datasets with imputed values within the range of 0 to 4 are utilized (refer to Section [6.2.1.3](#))

⁵ Missing data were imputed under the MNAR assumption, and the first 200 imputed datasets were utilized regardless of whether the imputed scratching scores were within the range of 0 to 4 (refer to Section [6.2.1.3](#))

[Table 16](#) presents the results for the prespecified exploratory endpoints evaluating change in Worst Weekly Scratching Score from baseline to Month 6 (Weeks 21–24), as described in Section [6.2.1.3](#), based on the MMRM analysis with the multiple imputation method prespecified by the Applicant (refer to Section [6.2.1.3](#)). The odevixibat 40 mcg/kg/day group had greater decreases in the LS mean score (improvement in pruritus) than the odevixibat 120 mcg/kg/day group. When comparing the odevixibat 40 mcg/kg/day group to the placebo group, the 95% confidence interval for the LS mean difference excluded 0 for all endpoints; when comparing the odevixibat 120 mcg/kg/day group to the placebo group, the 95% confidence interval for the LS mean difference included 0 for all endpoints.

Table 16. Efficacy Results for LS Mean Change From Baseline to Month 6 (Week 21 to Week 24) in Different Types of Scratching Scores (MI)^{1, 2, 3}

	Placebo N=20	Odevixibat 40 mcg/kg N=23	Odevixibat 120 mcg/kg N=19
Monthly Average			
Scratching scores			
Baseline mean (SE)	3.0 (0.1)	3.0 (0.1)	2.7 (0.1)
Change from baseline at month 6			
LS mean (SE)	-0.3 (0.3)	-1.2 (0.3)	-0.8 (0.3)
LS mean difference, (95% CI)		-0.9 (-1.5, -0.2)	-0.5 (-1.2, 0.2)
Nighttime scratching scores			
Baseline mean (SE)	3.0 (0.2)	3.1 (0.1)	2.9 (0.1)
Change from baseline at month 6			
LS mean (SE)	-0.5 (0.3)	-1.3 (0.3)	-1.1 (0.3)
LS Mean Difference, (95% CI)		-0.8 (-1.5, -0.1)	-0.6 (-1.3, 0.1)
Daytime Scratching Scores			
Baseline mean (SE)	2.9 (0.1)	2.9 (0.1)	2.6 (0.1)
Change from baseline at month 6			
LS mean (SE)	-0.2 (0.3)	-1.2 (0.3)	-0.7 (0.3)
LS mean difference, (95% CI)		-0.9 (-1.6, -0.2)	-0.4 (-1.2, 0.3)
Worst Daily Scratching Scores			
Baseline mean (SE)	3.2 (0.1)	3.2 (0.1)	3.0 (0.1)
Change from baseline at month 6			
LS mean (SE)	-0.4 (0.3)	-1.3 (0.3)	-1.1 (0.3)
LS mean difference, (95% CI)		-0.8 (-1.5, -0.1)	-0.7 (-1.4, 0.1)

Source: Statistical Reviewer's Analysis (same as the Applicant's results)

Abbreviations: CI, confidence interval; LS, least squares; MI, multiple imputation; SE, standard error

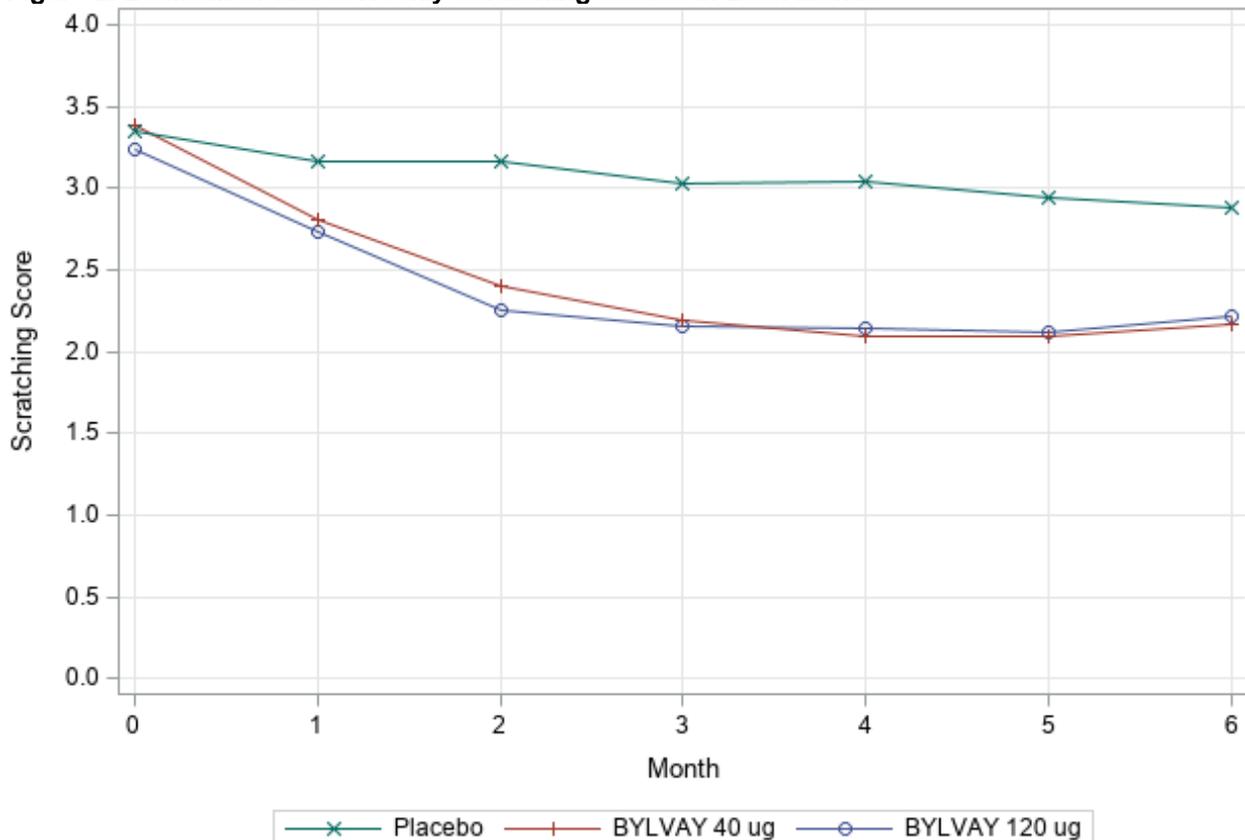
¹ Results presented for the randomized set as the intent-to-treat (ITT) population

² The analysis was based on a mixed model for repeated measures (MMRM) with baseline score as a covariate, and treatment group, time (in month), treatment-by-time interaction, treatment-by-baseline interaction and stratification factors (Progressive Familial Intrahepatic Cholestasis (PFIC) type and age category) as fixed effects

³ A multiple imputation (MI) was applied for handling missing data based on the placebo observed data

[Figure 2](#) shows the LS mean of the Worst Weekly Scratching Scores for each month, where missing data were handled using the Applicant's prespecified MI approach under the MNAR assumption. The odevixibat and placebo arms show separation over time, and the pruritus severity appears similar between the 40 mcg/kg/day odevixibat and 120 mcg/kg/day odevixibat groups across the 6-month treatment period. The trajectories over time when utilizing the other three approaches for handling missing data have a similar pattern. The trajectory plot depicting the LS mean of the change from baseline in Worst Weekly Scratching Score is depicted in [Figure 45](#).

Figure 2. LS Mean of Worst Weekly Scratching Scores in Each Month^{1, 2, 3}



Source: Statistical Reviewer's Analysis
Abbreviations: LS, least squares

¹ Results presented for the randomized set as the intent-to-treat (ITT) population.

² The analysis was based on a mixed model for repeated measures (MMRM) with baseline score as a covariate, and treatment group, time (in months), treatment-by-time interaction, treatment-by-baseline interaction, and stratification factors (PFIC type and age category) as fixed effects.

³ A placebo-based multiple imputation approach under a MNAR assumption was conducted as specified by the Applicant (refer to Section [6.2.1.3](#)).

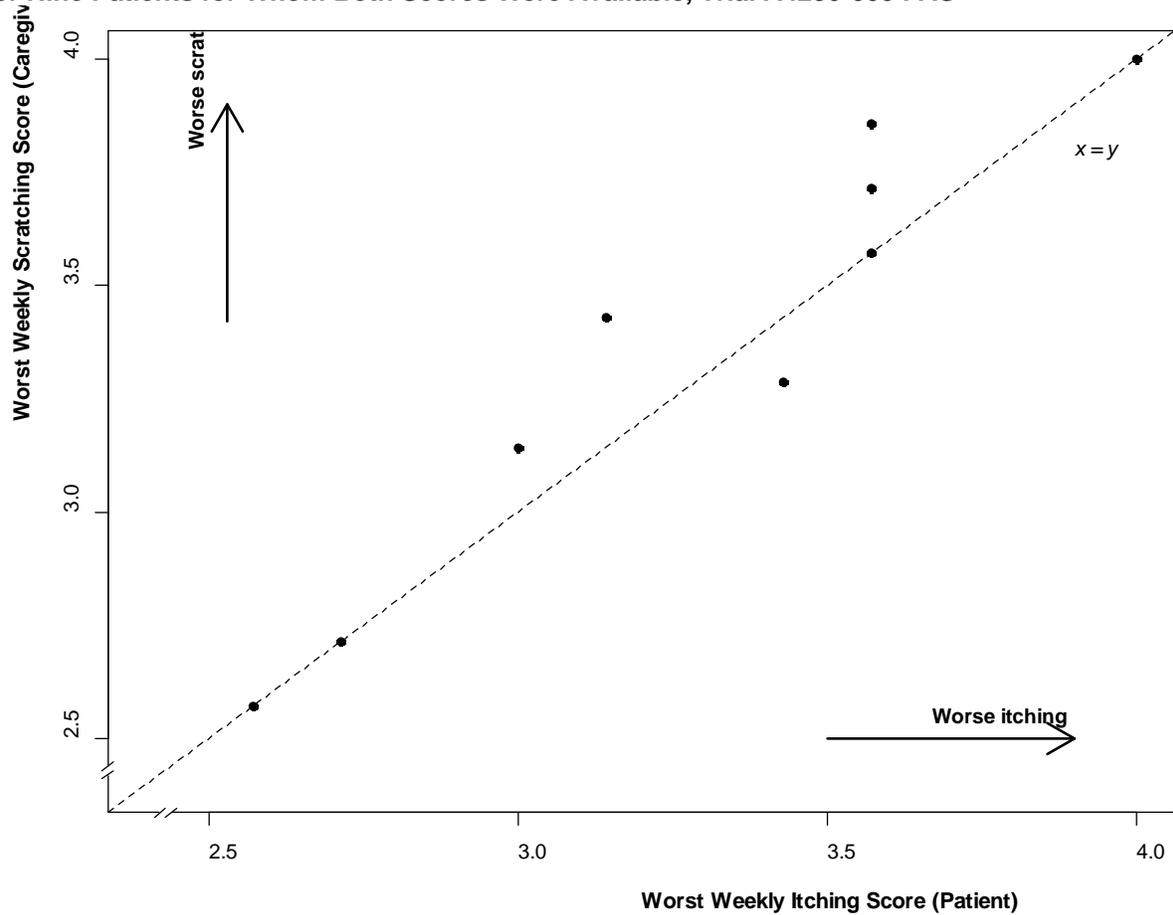
Patient-Reported Outcome Results

Few patients were able to self-report scratching on the PRO instrument (i.e., a total of nine patients with two in the placebo arm, two in the odevixibat 40 mcg/kg/day arm, and five in the odevixibat 120 mcg/kg/day arm). As a result, formal statistical analyses were not conducted.

Comparability of Patient and Caregiver Pruritus Assessments

The Agency conducted post hoc descriptive analyses to evaluate the relationship between patient and caregiver assessments of pruritus severity among patients (n=9) in the full analysis set of Trial A4250-005 with both PRO and ObsRO score data during the 4 weeks immediately prior to randomization (i.e., during Study Days [-28, -1]). In the Agency's analyses, patients were pooled across trial arms and missing data were not imputed. Visual inspection of [Figure 3](#) suggests general alignment between Worst Weekly Itching Scores (directly measuring pruritus from the patients' perspective) and Worst Weekly Scratching Scores (measuring patients' scratching as observed by the caregiver), although some uncertainty surrounds these findings due to the very small number of patients (n=9) with both PRO and ObsRO score data available during Study Days [-28, -1]. Refer to Section [16.1.5](#) for further discussion.

Figure 3. Scatterplot of Worst Weekly Scratching Score (Representing Caregiver Perspective) by Worst Weekly Itching Score (Representing Patient Perspective) at Baseline (Study Days [-28, -1]) for Nine Patients for Whom Both Scores Were Available, Trial A4250-005 FAS



Source: PFSS Reviewer's figure
Abbreviations: FAS, full analysis set
The Worst Weekly Itching Score and Worst Weekly Scratching Score are defined in [Table 129](#).

Subgroup Analyses

[Table 17](#) presents efficacy results by subgroups for the endpoint capturing the mean percentage of assessments over the 24-week treatment period that are ≤ 1 . Model-based estimates and 95% confidence intervals are not presented due to insufficient sample sizes. There was one Hispanic patient randomized to the placebo group and no other Hispanic patients enrolled in the trial; therefore, results by ethnicity are not presented. It is difficult to make conclusions regarding the potential of differential efficacy across subgroups due to small sample sizes; however, overall trends were marginally in favor of the odevixibat arms compared to the placebo arm based on sample mean estimates.

Table 17. Subgroup Efficacy Results: Mean Percentage of Assessments Over 24-Week Treatment Period That Are ≤1¹

Subgroup type	Placebo		Odevixibat 40 mcg/kg		Odevixibat 120 mcg/kg	
	N	Mean (SE)	N	Mean (SE)	N	Mean (SE)
Overall	20	8.9 (3.1)	23	32.0 (7.2)	19	28.3 (7.4)
Sex						
Male	12	10.9 (4.5)	11	22.7 (10.2)	8	35.7 (12.3)
Female	8	6.0 (3.8)	12	40.6 (9.8)	11	22.9 (9.4)
Age groups (years)						
6 months to 5 years	16	7.4 (3.4)	17	29.4 (8.6)	14	28.1 (8.6)
6 to 12 years	3	9.8 (7.0)	5	41.1 (16.7)	4	35.8 (19.6)
13 to 18 years	1	31.3 (-)	1	31.5 (-)	1	0.9 (-)
Race						
White ²	17	9.0 (3.5)	18	31.0 (8.5)	17	27.1 (7.6)
Non-white	3	8.1 (7.5)	5	35.7 (14.3)	2	38.2 (37.3)
Region						
United States	3	0.3 (0.2)	2	55.4 (23.8)	3	29.1 (1.7)
Europe	12	7.3 (3.7)	13	27.4 (9.2)	10	34.2 (12.1)
Rest of World	5	17.9 (7.5)	8	33.6 (13.6)	6	18.0 (12.6)
PFIC type						
Type 1	5	9.5 (9.2)	7	31.1 (14.5)	5	49.1 (12.6)
Type 2	15	8.7 (3.1)	16	32.4 (8.5)	14	20.8 (8.4)

Source: Statistical Reviewer's Analysis

Abbreviations: PFIC, progressive familial intrahepatic cholestasis; SE, standard error

¹ Results presented for the randomized set as intent-to-treat (ITT) population.

² The trial population from Middle Eastern countries was included under race category White by the Applicant.

6.3. Key Review Issues Relevant to Evaluation of Benefit

6.3.1. Acceptability of Single Trial for Approval of Indication

Issue

Trial A4250-005 is one adequate and well-controlled investigation. Is one trial sufficient as confirmatory evidence for the purposes of approval?

Background

PFIC is a rare disease and there are few patients in the US, disease prevalence is between 1:50,000 to 1:100,000.

Trial A4250-005 was a global, multicenter phase 3 trial. Patients were enrolled at 33 trial centers, including 17 in Europe (Belgium, France, Germany, Italy, Netherlands, Poland, Sweden, the United Kingdom), eight in the United States, and eight in the rest of world (RoW) (Australia, Canada, Israel, Saudi Arabia, and Turkey).

The Applicant conducted a single, double-blind, placebo-controlled trial demonstrating a highly persuasive treatment effect on relief of pruritus in patients with PFIC. Patients could roll over to open-label Trial A4250-008, if they either completed Trial A4250-005 and volunteered to enroll

in this open-label trial, or if they experienced intolerable symptoms (especially pruritus) during Trial A4250-005 and were unable to complete Trial A4250-005.

Assessment

Primary evidence of effectiveness from Trial A4250-005 supporting approval:

- Trial A4250-005 results and review evidence. Evidence from a clinical meaningful endpoint that is a direct measure of how a patient feels and functions. Severe pruritus is a detrimental symptom for pediatric patients with PFIC as young as 3 months of age and is a reason for evaluation and placement of a patient on a liver transplantation list. FDA’s alternative primary endpoint analysis is meaningful as it captures minimal or no remaining pruritus (score ≤ 1), avoiding a “one-point drop” which is not a clear indication of clinical meaningfulness (see Section [6.3.2](#)). For the Applicant’s prespecified primary endpoint, statistically persuasive findings were observed.
- Each of the 33 centers contributed an average of one to two patients and the efficacy results were not driven by one center or one region (see [Table 17](#)). A placebo-controlled trial, accounting for patients who discontinued Trial A4250-005 early by imputing assessments after discontinuation as “failures” demonstrated favorable results for odevixibat-treated patients on pruritus. Sensitivity analyses supported these results.
- Additional evidence of treatment benefit was provided by evaluating differences between trial arms in meaningful improvement in Worst Weekly Scratching Score (see [Table 15](#), [Table 16](#), and [Figure 2](#)). There was concordance between the observational pruritus outcome assessments in younger children and a direct patient-reported outcome instrument for older children (See [Figure 3](#)).
- Evidence of efficacy in one patient to be so profound as to avert a surgical procedure. The Applicant provided information (Information Request (IR) response submitted SD #10) on one patient (Patient (b) (6)) who was initially enrolled to the placebo treatment arm and then prematurely discontinued from Trial A4250-005 (at week 12) to enroll in Trial A4250-008 due to intractable pruritus. The patient was scheduled for biliary diversion surgery. However, when the patient received odevixibat 120 mcg/kg/day, patient and family were able to avoid biliary diversion surgery.

Supportive evidence of effectiveness is provided by:

- Evidence of reduction of sBA levels. Reduction of sBA levels was observed in greater percentage of odevixibat-treated patients relative to placebo. In some patients sBA levels decreased to near-normal range (ULN is 10 micromole/L) during Trial A4250-005. Spontaneous normalization of sBA levels generally is not observed in patients with PFIC, especially if the baseline sBA levels are >100 micromole/L (see Section [14.2.6.1](#)).
- There was an association between reduction in sBA level and improvement in pruritus in some patients but not all (see Section [14.2.6.1](#)).
- Early phase development. The phase 2 Trial A4250-003 was a 4-week, proof-of-concept trial in which patients with PFIC were enrolled and change in sBA levels was the endpoint. Reduction in sBA levels was observed in this trial (see Section [14.2.5](#)).
- Nonclinical evidence of proof-of-concept. IBAT/ASBT Inhibition with A4250 in the Mdr2 KO Model (ARR4250000117): Mdr2 knockout mice (model of cholestasis) were

administered A4250 (odevixibat) in the diet for 4 weeks. Compared to control-fed mice relative liver weight was reduced, serum ALT, AST, and ALP activities were decreased, and total serum bile acid levels were reduced by treatment with A4250. There was no significant effect by A4250 on serum bilirubin levels (see Section [5.1](#)).

Conclusions

The main source of evidence to support the approval came from one adequate and well controlled trial, i.e., Trial A4250-005.

The primary endpoint is related to how a patient feels and functions. Whether the efficacy was evaluated by the Applicant's prespecified primary endpoint or by FDA's alternative endpoint, treatment with odevixibat demonstrated statistically persuasive results in improvement of pruritus. The results were consistent across multiple global sites and showed a clinical benefit, i.e., improvement in pruritus was observed in patients recruited across different sites in a population that is refractory to existing medical treatment. Findings of reduction in pruritus are persuasive and lend credibility.

In addition to the clinical endpoint of improvement in pruritus, which was sustained in multiple sensitivity analyses, the reduction in sBA levels observed in phase 3 trials further supported the mechanism of action and provided supportive evidence for efficacy. Although the pathophysiology for pruritus is multifactorial and not fully understood, elevated serum bile acid levels, likely reflecting increase in overall bile acid pool, is often observed in patients with pruritus and considered one of putative factors. In the phase 3 trial, patients with elevated sBA levels >100 micromole/L were enrolled and 50% of patients had sBA levels >250 micromole/L at baseline. Upon odevixibat treatment but not with placebo treatment, sBA levels substantially decreased and remained low over time. In some patients, sBA levels decreased to near normal range (<10 micromole/L) with odevixibat treatment. The decrease in sBA levels was associated with the improvement in pruritus in an exploratory analysis.

The nonclinical studies are supportive of the mechanistic rationale.

Therefore, the Agency accepts a single adequate and well-controlled trial with confirmatory evidence as sufficient to support a recommendation for approving odevixibat for "treatment of pruritus."

6.3.2. Uncertainty of Primary Efficacy Endpoint

Issue

The prespecified primary efficacy endpoint was the proportion of "positive pruritus assessments" at the patient level over the 24-week treatment period, where a *positive pruritus assessment* for each patient on each assessment day was a dichotomous outcome defined as occurring when one or both of the following two criteria were met: "a scratching score of ≤ 1 or at least a 1-point drop from baseline on the Albireo ObsRO instrument" (Trial A4250-005 CSR Section 9.7.1.13.1, page 80/248). The Agency had concerns about the Applicant's definition of a *positive pruritus assessment*. Specifically, the Agency questioned the interpretability of comparisons made between scores aggregated over different time intervals that carry different interpretations as a result (e.g., a patient's daytime scratching severity on a given day is a different clinical outcome than the average daytime scratching severity experienced over the course of 2 weeks). The Agency also pointed out that the Applicant's prespecified endpoint involved comparing an

integer that can take values of 0, 1, 2, 3, or 4 to an average that can take any real number value that is between 0 and 4, inclusive (e.g., 3.6). The Agency conveyed concerns about including the criterion of “at least 1-point drop from baseline” in the Applicant’s definition of a *positive pruritus assessment* to the Applicant during review of the Trial A4250-005 protocol in the IND phase. In the NDA submission, the Applicant stated that this criterion was supported by a blinded psychometric analysis; however, these analyses addressed neither (a) the interpretability of comparisons made between scores aggregated over different time intervals nor (b) the meaningfulness of a 1-point drop from baseline as it is used in the prespecified primary endpoint.

Background

The second criterion in the prespecified primary efficacy endpoint used to determine whether a patient had achieved a *positive pruritus assessment* on a given day during the 24-week treatment period involved comparing the patient’s Daytime (PM ObsRO) and Nighttime (AM ObsRO) Scratching Scores each *day* during treatment to a baseline *biweekly average* of the Daytime and Nighttime Scratching Scores, respectively, taken over the 14 days immediately prior to first dose of study medication. The Agency questioned the interpretability of comparisons made between scores aggregated over different time intervals that carry different interpretations as a result, as noted above under “Issue.” The Applicant specified rounding the baseline score in order to compare integers with integers; however, under this approach a reduction in scratching score as low as 0.5 may be considered a “positive” pruritus assessment. For example, a baseline average score of 3.6 would be rounded to a score of 4, and then a score of 3 during the trial would be classified as a “positive” pruritus assessment, even though the reduction in score was 0.6 instead of a prespecified 1-point reduction. If using the unrounded baseline average score of 3.6, then a score of 3 would not be classified as a “positive” pruritus assessment. The Applicant conducted a sensitivity analysis using the unrounded baseline score, and there was still evidence of a beneficial treatment effect. However, using the rounded baseline scores results in more scores being considered “positive pruritus assessments” than using the unrounded baseline scores. While this should not affect the treatment arms in a differential manner, it is not clear that such a summary measure that is impacted by rounding is a meaningful summary of outcomes.

Furthermore, the Agency had concerns with whether a “1-point drop from baseline” connotes a meaningful improvement in scratching severity during review of the Trial A4250-005 protocol in the IND phase. The Applicant did not provide a justification for this criterion in the IND phase but stated in the NDA submission that this criterion was supported by a blinded psychometric analysis. The Applicant’s psychometric analysis plan states that the primary purpose of the blinded analysis was to estimate a threshold of clinically meaningful change (i.e., a responder definition) based on PRO itching and ObsRO scratching scores. However, these analyses were intended to support the evaluation of meaningful within-patient change for endpoints such as “the proportion of patients who achieve a clinically meaningful reduction in ObsRO score at Weeks 12 and 24,” which compares an average score at baseline to an average score at Week 12 or 24. The Agency did not concur that the blinded psychometric analyses supported the interpretability or meaningfulness of a 1-point drop from a biweekly average (AM or PM ObsRO) score at baseline to a daily (AM or PM ObsRO) score as utilized in the prespecified primary endpoint definition.

Evaluation of clinically meaningful within-patient change (i.e., improvement and deterioration from the patients’/caregivers’ perspective) in the Applicant’s prespecified primary efficacy endpoint was further impeded by defining the endpoint in terms of the patient-level *proportion of*

days with a positive pruritus assessment, which makes no reference to nor otherwise permits comparison to baseline (i.e., there is no baseline proportion of positive pruritus assessments). Lastly, misalignment between (a) the assessment periods of the ObsRO scores used to construct all prespecified secondary and exploratory efficacy endpoints constructed from ObsRO pruritus scores (enumerated in Trial A4250-005 CSR Section 9.7.1.1.1) and (b) the recall periods implied by anchor measures implemented in Trial A4250-005 (e.g., the CaGIS; see [Figure 44](#)) limited the interpretability of anchor-based analyses conducted to quantitatively evaluate clinically meaningful within-patient change in prespecified secondary and exploratory efficacy endpoints constructed from ObsRO pruritus scores (enumerated in Trial A4250-005 CSR Section 9.7.1.1.1) using anchor-based methods (FDA 2019).

Assessment

The Agency proposed and analyzed the following two additional efficacy endpoints to address the issues described above, neither of which were included in the testing hierarchy:

- An alternative primary efficacy endpoint—the “mean percentage of assessments over the 24-week treatment period that are ≤ 1 ” (endpoint 1 in Section [6.2.1.1](#)) utilizing a definition of a “positive pruritus assessment” based solely on the first criterion specified by the Applicant of “a scratching score of ≤ 1 ” (Trial A4250-005 CSR Section 9.7.1.13.1, page 80/248), where achieving Daytime (PM ObsRO) or Nighttime (AM ObsRO) Scratching Score of 0 (no scratching) or 1 (a little scratching)—was considered by the Agency to be a more interpretable and inherently meaningful criterion for clinical benefit.
- The endpoint “change in Worst Weekly Scratching Score from baseline (Study Days [-28, -1]) to Weeks 21–24 (Study Days [141, 168])” (endpoint 2 in Section [6.2.1.1](#)) was considered by the Agency to provide additional support to the Agency’s alternative primary efficacy endpoint and to facilitate evaluation of clinically meaningful within-patient change in pruritus (see Section [16.1.7](#) for in-depth discussion). Note that a meaningful change analyses should not be construed to represent evaluation of the statistical significance of group-level differences in treatment effects.

Conclusion

- The analysis results for the alternative endpoint considered by FDA confirmed that treatment with odevixibat is more effective than placebo which can be seen from a larger proportion of pruritus assessments (over a 24-week double-blind treatment period) with minimal or no scratching (refer to [Table 14](#)). Analysis results for the endpoint based on the Worst Weekly Scratching Score also supported the FDA’s alternative endpoint results (refer to [Table 15](#)).
- The Agency’s post hoc anchor-based analyses evaluating clinically meaningful within-patient change in the endpoint based on the Worst Weekly Scratching Score (see Sections [16.1.7.1](#) and [16.1.7.2](#)) suggested that overall:
 - Both the low dose (40 mcg/kg/day) and high dose (120 mcg/kg/day) of odevixibat yielded meaningful within-patient improvement in scratching severity for a notably higher proportion of patients than the placebo; and
 - The low and high doses of odevixibat yielded comparable levels of meaningful within-patient improvement in scratching severity from baseline to Weeks 21–24.

- Potentially substantive differences between odevixibat arms in meaningful within-patient improvement in scratching severity were noted for different levels of scratching severity at baseline (as measured by baseline CaGIS score). Specifically:
 - The low dose of odevixibat yielded the highest level of meaningful within-patient improvement in scratching severity for patients with “moderate” scratching severity at baseline (as measured by baseline CaGIS score); and
 - The high dose of odevixibat yielded the highest level of meaningful within-patient improvement in scratching severity for patients with “severe” or “very severe” scratching severity at baseline (as measured by baseline CaGIS score), with gains in meaningful improvement appearing to increase as baseline scratching severity and/or threshold magnitude increases.

Note: Meaningful change analyses should not be construed to represent evaluation of the statistical significance of group-level differences in treatment effects.

6.3.3. Doses for Approval

Issue

Consideration for the recommended doses: 40 mcg/kg or 120 mcg/kg or both

Background

The Applicant proposed 120 mcg/kg as the recommended dose for labeling. The review team (clinical, clinical pharmacology, and statistics) performed analyses of Trial A4250-005 for interpreting whether one or both doses were efficacious and safe. Overall, results suggest that odevixibat 120 mcg/kg dose did not provide better efficacy relative to odevixibat 40 mcg/kg dose, although exploratory analyses suggest better efficacy for the 40 mcg/kg dose in patients with moderate disease and for the 120 mcg/kg dose in severe disease. Therefore, the challenges for the dose recommendation include (1) whether to approve only the 40 mcg/kg dose or the 120 mcg/kg dose, (2) whether to approve both the 40 and 120 mcg/kg doses; and, (3) how to provide dosing instruction in the labeling if two doses were approved.

Assessment

Based on the Applicant’s prespecified primary endpoint, mean percentage of assessments over the 24-week treatment period that are ≤ 1 or at least a 1-point drop from baseline, both the odevixibat 40 mcg/kg and odevixibat 120 mcg/kg arms demonstrated superiority to the placebo arm (see [Table 14](#)).

However, for the alternative definition of the primary endpoint evaluated by FDA, i.e., the mean percentage of assessments over the 24-week treatment period that are ≤ 1 , only the odevixibat 40 mcg/kg arm demonstrated superiority to the placebo arm (see [Table 14](#)). Although odevixibat 120 mcg/kg/day did not demonstrate a statistically significant effect for this endpoint, it showed numerical improvement on pruritus scores compared to the placebo arm. For other endpoints (e.g., change in Worst Weekly Scratching Score from baseline) odevixibat 40 mcg/kg and odevixibat 120 mcg/kg arms performed similarly (see [Figure 2](#)).

A greater percentage of patients treated with odevixibat 120 mcg/kg experienced AEs (liver test abnormalities and diarrhea) requiring treatment interruptions compared to odevixibat 40 mcg/kg

(see Sections [7.4](#), [7.5](#), [7.6](#), and [7.7](#)). One patient treated with odevixibat 120 mcg/kg/day discontinued from the trial due to the AE of diarrhea (three events of positive dechallenge and two events of positive rechallenge).

While the Agency was keen on approving the odevixibat 40 mcg/kg dose, the Applicant stated that there might be patients who may benefit from a dose increase if treatment response is not observed at the 40 mcg/kg dose. Trial A4250-005 design did not allow assessment whether dose increase would be a reasonable strategy. As odevixibat 40 mcg/kg dose had a better safety and tolerability profile, the Agency recommended to approve 40mcg/kg/day as a safe and effective starting dose.

Additionally, FDA conducted analyses in patients who either prematurely discontinued or completed Trial A4250-005 and enrolled into A4250-008. Two groups of patients were identified: (1) those initially treated with the odevixibat 40 mcg/kg/day (N=19) dose and then switched to odevixibat 120 mcg/kg/day; and (2) those who were on odevixibat 120 mcg/kg/day (N=15) and then maintained on odevixibat 120 mcg/kg/day. For the purpose of this assessment, the Applicant defined a “responder” as a patient who achieved a 1-point reduction in monthly pruritus score. Of the nine patients treated with 40 mcg/kg/day who were nonresponders in Trial A4250-005, four patients subsequently became responders after switching to 120 mcg/kg/day in Trial A4250-008. Of the eight patients treated with 120 mcg/kg/day who were nonresponders in Trial A4250-005, three patients subsequently became responders while maintaining 120 mcg/kg/day in Trial A4250-008.

The number of patients analyzed were few and did not provide interpretable results given the open-label design of Trial A4250-008 and nonrandomized comparison to evaluate the effect of increasing the dose after initial nonresponse. While these two groups of patients cannot be directly compared, these results show that improvement in initial nonresponders cannot necessarily be attributed to the dose increase from 40 mcg/kg/day to 120 mcg/kg/day. Some patients may have become responders due to the natural variation in their symptoms over time. Although the 120 mcg/kg/day dose does not seem superior to the 40 mcg/kg/day dose in terms of overall efficacy and safety, a decision was made to keep the 120 mcg/kg dose available as an option for patients because 120 mcg/kg still showed efficacy compared to placebo, had an acceptable safety profile, and may be a more effective dose for patients with severe disease.

The review team also performed analyses to identify the time to improvement in pruritus scores. It seemed that a majority of patients achieved maximum reduction in pruritus scores by 3 to 4 months. Although a dose in between 40 mcg/kg/day and 120 mcg/kg/day was not evaluated in the Applicant’s clinical development program, it stands to reason that a patient might achieve a desirable outcome with the use of 80 mcg/kg/day. For the purpose of labeling instructions, if the patient does not respond to 40 mcg/kg dose, the dose could be increased to 80 mcg/kg/day. If there was no response with this dose, the dose could be increased to 120 mcg/kg/day. The safety and tolerability concerns could be mitigated via labeling, i.e., via treatment interruption, dose reduction, and permanent drug discontinuation. The safety and efficacy of an 80 mcg/kg/day dose is bracketed by the results from Trial A4250-008.

Conclusion

Odevixibat 40 mcg/kg/day and 120 mcg/kg/day demonstrated treatment efficacy relative to placebo. Both odevixibat 40 mcg/kg/day and 120 mcg/kg doses had acceptable safety profiles for approval. Therefore, the review team recommends approving a starting dose of 40 mcg/kg with

the option to increase the dose to 80 mcg/kg/day for nonresponders as the next step and if there is still no improvement then increase the dose to 120 mcg/kg/day.

6.3.4. Interpretation of Serum Bile Acid Level Data

Issue

Does the reduction in serum bile acids levels provide supportive evidence for efficacy?

Background

Odevixibat inhibits IBAT resulting in decreased reabsorption of bile acids from the terminal ileum. Serum bile acid levels were expected to decline because of inhibition of reabsorption of bile acids, so it was considered a pharmacodynamic biomarker in the odevixibat clinical program. Serum bile acid levels were expected to decline because odevixibat inhibits reabsorption of bile acids, so it was considered a pharmacodynamic biomarker in the odevixibat clinical program.

Reduction of total serum bile acid levels was a secondary endpoint in the pivotal phase 3 trial (A4250-005) and it is also being monitored in the long-term extension trial (A4250-008). In the phase 2 dose-ranging trial (A4250-003), total serum bile acid levels were level was the primary endpoint. Spontaneous normalization in sBA levels are generally not observed in this population.

Of note, sBA levels and a change in sBA levels is not a proven surrogate endpoint for reducing hepatic decompensation events, survival with native liver etc. There are uncertainties about long-term benefits from decreasing serum bile acid levels. sBA levels do not provide accurate estimation of hepatocellular bile acid levels which are the key mediators of liver damage. sBA levels are distal from PFIC disease course and there are uncertainties whether therapeutic reduction in sBA levels would provide clinical outcomes specifically, liver related outcomes.

Pathogenesis of pruritus has not been fully understood. Cholestatic pruritus is considered multifactorial and sBA has been suggested as a pruritogen. Surgical biliary diversion, an alternative option to liver transplant for patients with PFIC reduces sBA levels. Biliary diversion allows removal the bile, i.e., biliary fluid secreted by liver into intestines, and bile acids are one of the many substances secreted in bile and are removed from the body. Whether a therapeutic agent that reduces sBA levels could provide similar effects that surgical biliary diversion confers is uncertain.

Assessment

In clinical trials where serum bile acid levels were measured in patients (i.e., A4250-003, A4250-005, and A4250-008) Generally, sBA levels are collected after 12 hours fasting, as postprandial sBA levels are higher than fasting sBA levels.) sBA levels are generally collected after fasting for 12 hours as postprandial sBA levels are higher than fasting sBA levels. In Trial A4250-005, patients were required to fast for at least 4 hours prior to the collection of samples for serum bile acid testing given it is not feasible to keep young children in a prolonged fasted state. In infants <12 months of age serum bile acid levels were collected at any time point without regard to the feeding status.

The bioanalytical methods for serum bile acid levels were not adequately validated according to the FDA guidance for industry *Bioanalytical Method Validation* (2018). In particular, the assays

were not validated for the accurate measurement of high bile acid levels (e.g., >180 micromole/L) for which the samples should be diluted to bring down the concentrations to within the calibrated concentration range. However, the dilution integrity of samples was not validated (see Section [14.4.2](#) for further details). As such, the assessment of percent change from baseline is not considered reliable because 50% of patients had sBA levels greater than 250 micromole/L at baseline. The bioanalysis using a commercial kit in CLIA-certified laboratories may be fit for the purpose of diagnosis in routine clinical setting (i.e., abnormality of serum bile acid levels, >10 micromole/L). However, the reliability of the reported serum bile acid levels was not sufficiently demonstrated to be able to support this evaluation as a secondary efficacy endpoint (proportion of patients at least a 70% reduction in sBA levels from baseline or reaching a level < 70 micromole/L by the Applicant). However, the reliability of the reported serum bile acid levels was not sufficiently demonstrated to be able to support this evaluation as a secondary efficacy endpoint (proportion of patients with at least a 70% reduction in sBA levels from baseline or reaching a level < 70 micromole/L as defined by the Applicant).

Nevertheless, the observed change of sBA levels over time during treatment is considered interpretable as the samples were analyzed using the same assay in a consistent manner during the trial. In general, in Trial A4250-005, serum bile acid levels were reduced from baseline within 4 to 8 weeks of odevixibat treatment compared to placebo treatment. In general, serum bile acid levels were reduced from baseline within 4 to 8 weeks of odevixibat treatment compared to placebo treatment during Trial A4250-005. The decreased levels of serum bile acids fluctuated over time, but generally were maintained during the treatment over 24 weeks. The decreased levels of serum bile acids fluctuated over time, but generally were maintained during the 24 week treatment period. Among the 62 patients, 15 patients achieved sBA levels below 10 micromole/L (the upper limit of the normal range for serum bile acid levels) one or more times during the trial while spontaneous normalization of sBA levels are generally difficult to achieve in severely cholestatic patient population, like those with PFIC.

Keeping these limitations in mind, the reviewers explored the relationship between improvement in pruritus scores and reduction in sBA levels. The reduction in serum bile acid levels and the improvement in pruritus scores appears to be generally associated in patients treated with odevixibat although did not always move in the same direction in every patient, i.e., The reduction in serum bile acid levels and the improvement in pruritus scores appears to be generally associated in patients treated with odevixibat although trends did not always move in the same direction in every patient, i.e., some patients experienced improvement in pruritus scores, but had an increase in sBA levels and vice versa (see Section [14.2.6.1](#)).

Conclusions

- Overall decrease in sBA levels over time from baseline and an association between reduction in sBA levels and improvement in pruritus provides supportive evidence for the mechanism of action of odevixibat. In addition, reduction of sBA levels below 10 micromole/L in some patients whose sBA levels were greater than 100 micromole/L at baseline associated with improvement in pruritus may provide supportive evidence of efficacy.
- However, due to inadequate validation of sBA analytical methods, the reduction of sBA levels was interpreted in a qualitative manner based on the overall trend. Quantitative comparison (e.g., 70% reduction) is not appropriate for the labeling.

6.3.5. Approval for Infants 3 to 6 Months of Age

Issue

Trial A4250-005 did not enroll infants 3 to 6 months of age. Infants are generally diagnosed with PFIC by the age of approximately 3 months. The most common presenting symptoms include pruritus and jaundice. There remains an unmet medical need to treat pruritus in infants 3 to 6 months of age.

Background

PFIC is a rare disease with an incidence ranging between 1:50,000 to 1:100,000, therefore, newly diagnosed cases for enrollment in a trial would be limited, and not all patients experience pruritus, so it would be impracticable to conduct clinical trials in patients between 3 and 6 months of age. According to the literature, infants as young as 2 months of age have signs of itching, although they lack the motor skills to scratch properly, but infants present with coarse scratching movement and irritability.

As odevixibat has been demonstrated to be efficacious in reducing pruritus in older infants (6 to 12 months), infants 3 to 6 months of age may also benefit with treatment that has a potential to improve pruritus. As odevixibat has been demonstrated to be efficacious in reducing pruritus in older infants (6 to 12 months), infants 3 to 6 months of age may also benefit from treatment that has a potential to improve pruritus. Approving odevixibat in infants 3 to 6 months of age will allow access to odevixibat for this pediatric subgroup with intractable pruritus.

Assessment

The mechanism of action of odevixibat remains the same, and in infants IBAT inhibition is expected to reduce intestinal bile acid re-absorption. Physiologically, a 3-month old infant with PFIC, who has very elevated sBA levels, is expected to respond in a similar manner as a 6-month-old infant with PFIC would respond. However, it is unknown whether IBAT expression is up or down regulated in infants 3 to 6 months of age. Based on published data, it appears, that IBAT does not fully function i.e., is immature in infants (who do not have any underlying liver pathology) less than 9 months of age (de Belle et al. 1979). In Trial A4250-005, there were seven children 9 months of age or younger at the start of the trial. The expectation is that infants between 3 and 6 months of age would have similar biology and physiology as 6- to 9-month-old infants. Even though the response may be partial, infants may benefit, similar to the response observed in the clinical trial.

A total of 10 children between 6 and 11 months of age, were enrolled in Trial A4250-005, (five infants were treated with placebo, five infants were treated with odevixibat 40 mcg/kg/day)). [Table 18](#) presents summary statistics for various age patients for the endpoint evaluating the mean percentage of ObsRO assessments over the 24-week treatment period that are ≤ 1 .

Table 18. Mean Percentage of ObsRO Assessments That Are ≤ 1 for Subgroups of Patients Between Ages 6 Months and 12 Months

Subgroup type	Placebo		Odevixibat 40 mcg/kg		Odevixibat 120 mcg/kg	
	N	Mean (SE)	N	Mean (SE)	N	Mean (SE)
Overall	20	8.9 (3.1)	23	32.0 (7.2)	19	28.3 (7.4)
Age groups (years)						
6 months to 5 years	16	7.4 (3.4)	17	29.4 (8.6)	14	28.1 (8.6)
6 months to less than 1 year	5	12.3 (8.8)	5	37.8 (17.2)	0	- (-)
1 to 5 years	11	5.1 (3.2)	12	25.9 (10.1)	14	28.1 (8.6)
6 to 12 years	3	9.8 (7.0)	5	41.1 (16.7)	4	35.8 (19.6)
13 to 18 years	1	31.3 (-)	1	31.5 (-)	1	0.9 (-)

Source: Statistical Reviewer's Analysis
 Abbreviations: ObsRO, observer-reported outcome; SE, standard error

In the subgroup of patients 6 to 11 months of age, patients who were treated with odevixibat 40 mcg/kg had on average 37.8% assessments with either no or minimal scratching (i.e., ≤ 1 scratching score) compared to the placebo arm with mean of 12.3%. Overall, the trend of improvement in pruritus was in favor of odevixibat-treated patients.

However, interpretation of these results is limited by the small sample sizes in both arms.

Additionally, the drug is minimally absorbed. The site of action is in the gastrointestinal lumen, thereby safety of odevixibat use in younger children would be monitorable.

If the infant experiences any adverse reactions, those would be monitored in a similar manner as infants ≥ 6 months of age and are managed as a part of standard clinical care. Most of these infants are followed by physicians who monitor their liver tests (biochemical, imaging, endoscopic etc.), complete blood count, fat-soluble vitamin (FSV) levels, growth, nutrition, and other clinical signs and symptoms. Most adverse reactions can be addressed by providing guidance to provider via the labeling.

Conclusion

Based on an understanding of the biology of disease and physiologic action of IBAT inhibition (even though functioning is partial), there is a reasonable expectation that infants 3 to 6 months of age with PFIC, will have a similar response as infants ≥ 6 -months, and can be included in the target population for which the drug will be indicated. Conducting a trial in this age-subgroup of a rare disease population will be impracticable if not impossible.

Therefore, the decision to approve was supported by pathophysiology of disease, pharmacological action of drug, ontogeny of IBAT, and dose and efficacy data in infants ≥ 6 months of age. The AEs are monitorable and treatment can be interrupted if an infant experiences any adverse reaction. Therefore, the Agency has decided to approve odevixibat for infants ages 3 to 6 months.

6.3.6. Indication for Approval

Issue

- Should the drug be approved for PFIC1 and PFIC2 subtypes only (population tested), or all PFIC subtypes?
- Should odevixibat be approved for use in patients who have experienced hepatic decompensation event or in patients with compensated cirrhosis with evidence of portal hypertension?
- Should the drug be approved for populations with benign recurrent intrahepatic cholestasis (BRIC)1 and BRIC2?

Background

All patients with PFIC phenotypically present with elevated serum bile acid levels and with progression of liver failure over time. A limitation of use has been added for BSEP3, which is a subpopulation in PFIC2, as these patients have no expression of BSEP. However, itching is quite severe in populations with PFIC1 and PFIC2. In patients with PFIC3 itching/scratching is generally mild to moderate in intensity.

Data for safe use of odevixibat in children with hepatic decompensation is not available, i.e., whether the drug would be safe. Data on safety of odevixibat use in patients with portal hypertension is very scant. There were at least two patients enrolled in the trial who had positive findings of splenomegaly, low platelet count, and elevated Fibroscan reading (>16 KPa). FDA encourages the Applicant to collect data for safety in this population through a registry. In addition, the Applicant is also conducting a trial in the biliary atresia population to seek approval for treatment of biliary atresia. About 70% of children with biliary atresia have varying degrees of fibrosis or cirrhosis at baseline, data from this trial may also characterize safe use in cirrhotic patients.

Both BRIC1 and BRIC2 disorders occur on the same gene as patients with PFIC1 and PFIC2, respectively. Both BRIC1 and BRIC2 disorders occur on the same gene in patients with PFIC1 and PFIC2, respectively. The BRIC1 gene defect is on *ATP8B1* and the BRIC2 gene defect is on *ABCB11* gene. BRIC1 and BRIC2 are milder phenotypes of PFIC1 and PFIC2, respectively.

BRIC is characterized by recurrent episodes of cholestasis that lasts for a couple of weeks, without progression to liver failure; therefore, the nomenclature is “benign recurrent.” Within one patient, the timing of a cholestatic event might occur may not be known, i.e., the episodes are unpredictable. Patients present with severe pruritus, elevated liver enzymes, and jaundice. The cholestatic attacks vary in severity and duration, and patients are asymptomatic between the episodes, both clinically and biochemically. Exact prevalence is unknown, and the disorder may not be diagnosed in many patients as the symptoms are intermittent and episodic.

Assessment

PFIC3 or other newly detected subtypes of PFIC (e.g., PFIC4, etc.) are extremely rare disorders, the incidence is significantly lower than PFIC1 and PFIC2, and conducting studies in these populations would be impracticable. Phenotypically, patients with all PFIC subtypes present with similar manifestations, i.e., pruritus (of varying degrees), and the expectation would be the itching caused by PFIC disorder (with any genotype) would also respond in a similar manner.

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Providing a labeling indication would allow for ease of access to odevixibat in these patients who also experience pruritus. However, in patients with BSEP3 who lack BSEP protein expression, bile acid secretion in intestine is impaired and it is expected there will be no efficacy.

Currently, the labeling states that odevixibat dosing should be discontinued in patients who experience hepatic decompensation. However, if there are safety data (for an appropriate dose) available in patients with hepatic decompensation, the Agency remains open to adding information about dosing and safety to the labeling in the future.

Both BRIC1 and BRIC2 are rare disorders and are milder versions of PFIC1 and 2 respectively. The cholestatic events are recurrent and are unpredictable. Conducting studies in these patients would be very impracticable.

There are no data providing estimated prevalence of the BRIC1 and BRIC2 and the safety of doses that should be administered is unknown. There's also uncertainty whether the duration of disease would offset treatment benefit.

However, if the Applicant could collect data under a registry, the FDA would be amenable to reviewing the data, even if data are collected in limited number of patients. If these data can demonstrate reasonable safety profile of doses used and the benefit outweighs the risks, FDA could potentially approve odevixibat for populations with BRIC1 and BRIC2.

Conclusion

The Agency is recommending approval of odevixibat for all PFIC subtypes, however, odevixibat is not recommended for BSEP3 (subpopulation within PFIC2), and limitations of use has been added for BSEP3 in labeling.

FDA recommends caution when dosing odevixibat in patients with cirrhosis with portal hypertension and in patients with prior hepatic decompensation event(s). The labeling states that the drug was not studied in cirrhotic patients with portal hypertension and in patients who have experienced prior hepatic decompensation event. However, if the Applicant submits adequate safety data to support safety of dosing in this population or similar pediatric population, such as biliary atresia (with evidence of clinically significant portal hypertension or prior hepatic decompensation event), the Agency remains open to discussing with Applicant to revise sections in labeling.

The Applicant could collect data in a registry, treating patients with BRIC1 and BRIC2, and submit data to the FDA for review, for potential addition of this indication with dosing instructions accounting for the intermittent pruritus.

6.3.7. Approval for Adult PFIC Population

Issue

Whether to approve odevixibat for adult patients with PFIC.

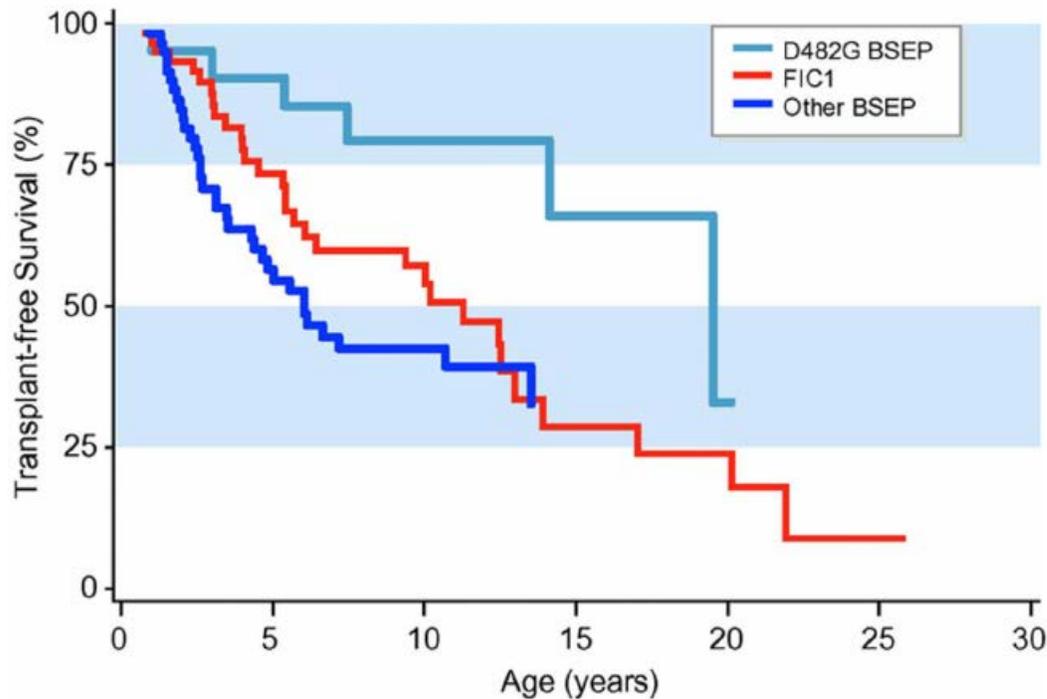
Background

The Applicant proposed to approve odevixibat for pediatric as well as adult patients with PFIC although there were no efficacy and safety data in Trial A4250-005 in adult participants. The Applicant intended to extrapolate pediatric efficacy and safety data to allow use in adults post approval, i.e., for the purposes of labeling.

The majority of patients with PFIC receive liver transplant before they reach adulthood.

Pawlikowska et al. reported 20 to 30% of patients with PFIC1 reach adulthood with their native livers (Pawlikowska et al. 2010) (see [Figure 4](#)). The NATURAL course and Prognosis of PFIC and Effect of biliary Diversion (NAPPED) study also shows that there may be a need for adult dosing (see [Figure 5](#)). In addition, if we assume that patients who were censored are alive, there might be adult patients with PFIC who may require treatment for pruritus. However, there remains some uncertainty as pruritus in these patients is not characterized, i.e., there is paucity of published literature on pruritus in adult patients with PFIC.

Figure 4. Kaplan-Meier Analysis for Survival With Native Liver

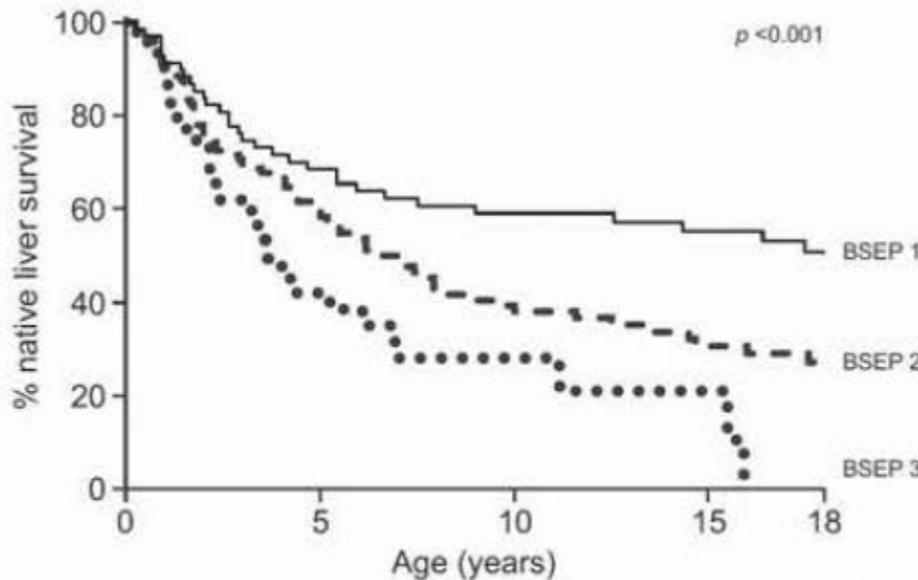


Source: Copied and electronically reproduced from (Pawlikowska et al. 2010)

Abbreviations: BSEP, bile salt export pump

In each case FIC1, D482G-BSEP and other BSEP are plotted. Fig.4A shows survival without liver transplant (LT). 26/61 (43%) FIC1 patients, and 38/84 (45%) patients with BSEP underwent LT. Among patients with BSEP, 5/21 (24%) with D482G and 30/60 (50%) without D482G underwent LT.

Figure 5. Observed Native Liver Survival and Diversion-Free Survival Per Genotypic Severity Category in Patients With PFIC2



N° at risk					
BSEP 1	72	43	36	29	18
BSEP 2	136	53	31	20	12
BSEP 3	56	13	8	2	0

Source: Copied and electronically reproduced from (van Wessel et al. 2020)
 Abbreviations: BSEP, bile salt export pump; PFIC, progressive familial intrahepatic cholestasis
 Proportion of patients alive with native liver over time with a BSEP1 (solid line), BSEP2 (dashed line), or BSEP3 (dotted line) genotype. Log-rank test.

IBAT inhibition biologically would lead to a similar response and the pathophysiology of the disease is expected to be similar. There is no expectation that IBAT receptors might be up regulated in adult patients compared to adolescents. Therefore, doses required to achieve reduction in pruritus are expected to be similar to approved doses.

Assessment

There is a paucity of data on the estimated number of adult patients with PFIC and native livers who might require treatment with odevixibat. Additionally, there are a few other concerns regarding dosing adult patients with PFIC with odevixibat, as noted below:

- While data on efficacy can be extrapolated, there are no data for safety for the adult PFIC population in Trial A4250-005.
- In Trial A4250-005, the safety and tolerability of doses >2.4 mg is not well characterized in patients with PFIC, with the exception of five patients, ages 9-12 years, who received doses ranging from 2.4 mg to 4.8 mg daily. All these patients who received odevixibat doses >2.4 mg experienced adverse reaction(s) (mostly elevation of liver tests).
- Data from a phase 2 trial in adults with primary biliary cholangitis (PBC) submitted by the Applicant (Trial A4250PBC pruritus-Investigator’s Brochure, IND 130591) with this NDA package and from the Al-Dury 2018 publication showed a lack of tolerability with higher doses of odevixibat. Nine adult patients with PBC were treated with 0.75 mg (N=4) and

1.5 mg (N=5) for 4 weeks. Five patients discontinued from the trial prematurely due to an AE of abdominal pain and diarrhea, the authors concluded that the starting dose of odevixibat may have been too high for adult patients (Al-Dury et al. 2018).

The Agency acknowledges that there might be patients who would start odevixibat administration before their 18th birthday and show favorable response, and once they reach adulthood, they might need to be continue therapy with odevixibat. If the labeling is restricted to children, this would be problematic for odevixibat-treated 17-year-olds who demonstrate improvement in pruritus and all of a sudden on their 18th birthday the treatment is stopped because of issues related to health insurance coverage based on FDA-approved indications in labeling. The Agency recommends allowing continued access to odevixibat treatment into adulthood.

Conclusion

Despite absence of data in the adult population, the Agency recommends approval of odevixibat for adult patients with PFIC to allow access and continuity of medical care. Moreover, the disease pathophysiology remains the same, and pharmacological response to IBAT inhibition is expected to be similar. The dose could be adjusted by the physician in clinical settings based on tolerability and safety.

7. Risk and Risk Management

The overall assessment of safety of odevixibat is informed by variety of sources, including nonclinical toxicology, safety pharmacology studies, and early phase clinical studies. The safety assessment for the intended population of patients with PFIC is based primarily on the A4250-005 trial. Prior to NDA submission, the clinical review team identified several potential risks based on mechanism of action of drug and AE reporting to IND. These AEs of special interest (AESIs) included the following clinical safety issues:

- Liver test abnormalities
- Diarrhea
- Fat-soluble vitamin deficiency

7.1. Potential Risks or Safety Concerns Based on Nonclinical Data

The nonclinical safety profile of odevixibat has been extensively explored in (1) single and repeated dose toxicity studies in rats (up to 6 months) and dogs (up to 9 months), (2) in vitro and in vivo genotoxicity studies, (3) reproductive and developmental toxicity studies including fertility studies in male and female rats, embryo-fetal developmental studies in rats and rabbits, and a pre- and postnatal developmental study in rats, (4) juvenile animal studies in rats, (5) carcinogenicity studies in mice and rats (2-year bioassays) (see Section [13](#) for complete details). In addition, pharmacokinetics (PK) were studied as part of the toxicity studies or in separate animal PK studies for animal to human comparison of systemic exposure. There were no nonclinical safety issues of significant concern as assessed by the general toxicology studies conducted during the development program. However, there were developmental toxicity effects

that may be relevant to human risk which are highlighted below. Additional information regarding the developmental toxicity effects can be found in Sections 8.4 and 13. From a nonclinical perspective, the human risks appear to be manageable. We defer to the Pediatric and Maternal Health teams for evaluation of benefit versus risk in the target patient population (PFIC), related to the cardiovascular malformations in fetal rabbits.

There were no nonclinical safety issues of significant concern as assessed by the secondary pharmacology screens or the cardiovascular, respiratory, central nervous system, gastrointestinal, or renal safety pharmacology studies conducted during the development program.

Pharmacokinetic and absorption, distribution, metabolism, excretion (ADME) studies with odevixibat demonstrated low bioavailability in marmosets and rats after oral administration. After oral administration in rats, radiolabeled odevixibat and/or its metabolites were poorly absorbed and the concentration in blood was below the detection limit. Most of the radioactivity was found in the content of the gastrointestinal tract, whereas none was detected in the central nervous system (CNS). After oral administration of radiolabeled odevixibat in rats, radioactivity was mainly detected in the feces (mean recovery of 88.6%) while urine and carcass accounted for a very small proportion of total radioactivity (0.07% and 0.10%, respectively).

In pregnant rats dosed intravenously with radiolabeled odevixibat on Day 18 of gestation, the radioactivity was rapidly distributed throughout the body of the dam (including the placenta and amnion membrane). Radioactivity passed through the placenta and was detectable in low concentrations at 4 hours postinjection in the fetal liver only. Radiolabeled odevixibat was slowly and minimally metabolized in cultured hepatocytes from rats, mice, and humans, primarily by hydroxylation. Up to six radiolabeled metabolites (M1–M6), in addition to parent drug (the most abundant component of the samples) were detected. Radiolabeled odevixibat was metabolized to a greater extent by dog hepatocytes.

There were no unique human metabolites. Plasma protein binding of odevixibat was greater than 99.7% (>99.7% at 4 micromoles/L and >99.97% at 40 micromoles/L) in mouse, rat, dog, rabbit, marmoset, and human plasma. Odevixibat was generally excluded from the blood cell fraction.

General toxicology studies were performed in mice, rats, dogs, and marmosets. No drug-related deaths occurred in these studies, with the exception of deaths in male mice treated orally with 300 mg/kg/day in a 13-week study. Deterioration of clinical condition (decreased activity, cold body surface, piloerection and/or slow/labored breathing) prior to euthanasia and distended gastrointestinal tract at necropsy were observed in the decedents, but no specific microscopic lesion could be identified as a cause of death. The deaths in this study were considered related to drug administration and occurred at a systemic exposure of 1440 ng•hr/mL (490 times the AUC in humans at the maximum recommended dose, 120 mcg/kg). Clinical signs observed in most species included vomiting (marmosets and dogs), fluid/soft feces, and/or increased salivation after dosing. Slight, nonadverse reductions in body weight/body weight gain were mostly observed at high doses in marmosets, rats, and dogs. Changes in hematology parameters and clinical chemistry parameters were generally not observed, were of low magnitude, were not considered adverse, or were expected to occur as pharmacological effects (e.g., decreased HDL, LDL, and/or total cholesterol in dogs, due to diversion of cholesterol from lipoprotein synthesis to bile acid synthesis in liver).

Drug-related microscopic changes were mainly observed in rats. In a one-month oral toxicity study with a recovery period in rats (27, 270 and 2700 mcg/kg/day, equivalent to 20, 200 and 2000 mg/kg/day), drug-related microscopic changes included mild mucosal hypertrophy of

cecum and colon at ≥ 270 $\mu\text{mol/kg/day}$, and mild diffuse basophilia of the parotid salivary gland acinar cells in females at 2700 $\mu\text{mol/kg/day}$. However, these findings were not observed in the 26-week oral toxicity study in rats at doses up to 300 mg/kg/day.

Drug-related macroscopic findings including dark cecum, red discoloration of duodenum, and/or thickening of thymus were observed in dogs at the maximum tolerated dose (MTD) (1000 mg/kg/day) in a 14-day oral study. However, no drug-related macroscopic findings were noted in the 13-week and 39-week oral toxicity studies in dogs, which used maximum doses of 300 mg/kg/day and 150 mg/kg/day, respectively. No drug-related microscopic findings were observed in the 39-week oral toxicity study in dogs. There were no drug-related effects on urinalysis, ophthalmoscopy, electrocardiography, and/or organ weights in the pivotal oral toxicity studies in rats and dogs. The NOAEL (no observed adverse effect level) in the 26-week oral toxicity study in rats and the 39-week oral toxicity study in dogs was the high dose (300 mg/kg/day and 150 mg/kg/day, respectively).

Reproductive and developmental toxicology studies included a fertility study in male and female rats, embryo-fetal development studies in rats and rabbits, and a pre- and postnatal development study in rats. A juvenile animal toxicity study was performed in rats. No adverse findings were observed in the fertility study in male and female Wistar rats, the embryo-fetal development study in rats, or the juvenile toxicity study in rats. In an embryo-fetal development study in pregnant rabbits, fetuses from all maternal groups treated orally with odevixibat showed an increase in cardiovascular malformations, which occurred at low maternal systemic exposures (6.28 ng•hr/mL) to odevixibat. Detailed information regarding the juvenile animal study and reproductive and developmental toxicology studies can be found in Sections [8.3](#) and [8.4](#), respectively, with supporting details in Section [13](#). Detailed information regarding the juvenile animal study and reproductive and developmental toxicology studies can be found in Sections [8.3](#) and [8.4](#), respectively, with supporting details in Section [13](#).

In the 2-year carcinogenicity studies, odevixibat was not tumorigenic in rats or mice at oral doses up to 100 mg/kg/day. Systemic exposure to odevixibat area under the concentration-time curve (AUC) at the maximum dose studied was approximately 680 ng•hr/mL in rats and 1440 ng•hr/mL in mice. Odevixibat tested negative in the bacterial reverse mutation (Ames) assay, the in vitro mouse lymphoma cell forward mutation assay, and the in vivo rat micronucleus test. There was no evidence of ocular or cutaneous phototoxicity in the repeat dose phototoxicity study in rats.

7.2. Potential Risks or Safety Concerns Based on Drug Class or Other Drug-Specific Factors

Not applicable. Odevixibat is a first-in-class drug.

7.3. Potential Safety Concerns Identified Through Postmarket Experience

Not applicable. Odevixibat is not yet commercially available in any country.

7.4. FDA Approach to the Safety Review

7.4.1. Sources of Data for Clinical Safety Assessment

Basis for Safety Evaluation

- Primary safety database: Data from single phase 3 trial, A4250-005, a randomized, double-blind, placebo-controlled trial of 24 weeks duration conducted in patients with PFIC1 or PFIC2 was the primary database for safety evaluations. A summary of the A4250-005 trial design can be found in [Table 4](#), Section [6.2.1.1](#), Section [6.2.1.2](#), and Section [15](#).
- Supportive safety database: Data from phase 3, A4250-008, an open-label, long-term safety extension, 72-week trial conducted in patients with PFIC1 or PFIC2 were also reviewed. Trial A4250-008 is currently ongoing. The data from Trial A4250-008 served as supportive safety database. For safety analyses the reviewer utilized the pooled safety dataset (A4250-008 and A4250-005). A brief synopsis of the design of A4250-008 can be found in Section [15](#).

Both Trials A4250-005 and A4250-008 were conducted at multiple sites including sites in Australia, Canada, Europe, Middle East, and North America.

In Trial A4250-005, a total of 62 patients were enrolled and were dosed with either placebo, odevixibat 40 mcg/kg/day, or odevixibat 120 mcg/kg/day for 24 weeks. Patients with PFIC1 or PFIC2 (early stage disease, i.e., no prior hepatic decompensations) were enrolled in this trial. Only 13% (8 of 62 patients) were enrolled from the United States. Of the 62 patients enrolled in Trial A4250-005, 56 (90%) rolled over to Trial A4250-008.

Data from foreign countries is acceptable as the diagnoses of PFIC was confirmed by molecular testing. The disease severity was defined by laboratory and clinical criteria prespecified in the inclusion and exclusion criteria (Section [6.2.1.2](#)), therefore, significant differences between US and non-U.S. population are not expected. The clinical practice in the US and at the non-U.S. sites vary. The Applicant states that prior to starting the trial, meetings were held with investigators to align the standard of care and trial expectations. Therefore, clinical care was expected to be similar for the majority of patients for the duration of trial. However, some differences still existed, for example, use of medication for supportive treatment of disease, such as use of intramuscular or intravenous fat-soluble vitamins. Another example is use of medications to treat diarrhea. At some of the non-U.S. sites probiotics and antidiarrheal agents are used aggressively, whereas in US these drugs are not used as commonly. However, not all variables for standard of care could be controlled, therefore, the alignment strategy for most standard of care procedures was deemed acceptable. Moreover, all these differences in standard of care would not impact the assessment of primary efficacy endpoint, therefore were acceptable.

In A4250-008, ongoing, open-label trial (supportive for safety), a total of 84 patients with PFIC1, PFIC2, or PFIC3 have been enrolled and treated with odevixibat 120 mcg/kg/day. Only 13% (11 of 84 patients) are enrolled from US, i.e., most data are from foreign sites and foreign data are acceptable (see explanation noted in paragraph above).

The primary data cutoff for this trial was July 15, 2020, and the data cutoff for the 120-safety update was December 4, 2020. After this data cutoff of July 15, 2020, a total of seven additional patients were enrolled in Trial A4250-008. As of December 4, 2020, a total of 66 (79%) patients are continuing the trial and 14 (17%) (two patients discontinued from Trial A4250-005 and 12

from A4250-008) have discontinued from treatment. For details of patients who have discontinued from the trial, please see Section [7.6.1.6](#).

7.4.2. Safety Analysis Plan and Definitions

The prespecified safety analysis plan and definitions were reviewed during protocol development and were acceptable to the clinical review team. Use of descriptive statistics was predefined in the protocol for summarizing the safety outcomes. The review team agreed with the proposed approach. All adverse events (AEs) were classified by Primary System Organ Class (SOC) and preferred term (PT) according to Medical Dictionary for Regulatory Activities (MedDRA) version 23.0 throughout the study.

- An AE is defined as any untoward medical occurrence in an enrolled patient regardless of causal relationship with study drug
- AEs were classified as treatment-emergent adverse events (TEAEs), defined as follows: An AE (PT) that occurs during the treatment period will be considered a TEAE if it has a start date on or after the first dose date of study drug, or it has a start date before the date of the first dose date of study drug, but worsened in severity on or after the date of the first dose date of study drug.
- Adverse events of special interest (AESIs) were classified based on preclinical and clinical experience. The following AESIs were prespecified in the protocol:
 - Diarrhea
 - Liver test abnormalities
 - Fat-soluble vitamin deficiency
- Serious adverse events (SAEs) were protocol-defined as any untoward medical occurrence that, at any dose:
 - Results in an outcome of death
 - Is life threatening (defined as, in the opinion of the investigator, at immediate risk of death from the reaction as it occurred)
 - Results in persistent or significant disability/incapacity (defined as disruption of person's ability to conduct normal life function)
 - Requires or prolongs hospitalization
 - Results in congenital anomaly or birth defect
 - Is an important medical event (defined as representing a significant hazard, requires medical and surgical intervention to prevent any of the outcomes listed above).
- Severity assessments were classified as follows:
 - Mild (awareness of sign and symptom, but easily tolerated)
 - Moderate (discomfort sufficient to cause interference with normal activities)
 - Severe (incapacitating, with inability to perform normal activities)

An AE of severe intensity need not necessarily be considered serious.

7.4.3. Reviewer's Approach to the Safety Evaluation

Clinical trial data were analyzed using JMP, JMP clinical, and Python software. Clinical data scientists (CDS) from the Division of Biomedical Informatics generated safety tables and figures. These include safety tables and figures for patient disposition, exposure data, adverse events, and serious adverse events. CDS also generated tables for drug-induced liver injury assessments. The Division of Biomedical Informatics, Research & Biomarker Development, also provided technical support for data analysis using JMP and JMP Clinical software.

Data from Trials A4250-005 and A4250-008 were not pooled due to differences in trial design. A4250-005 is randomized, double-blind, placebo-controlled trial for 24 weeks where as A4250-008 is open-label extension trial for 72 weeks. Therefore, the safety data were analyzed separately for both these trials.

No major data quality or integrity issues were identified that would preclude the clinical review team from performing a safety review for this NDA. Data quality and integrity were verified by the FDA's CDS team as well as the clinical reviewer. There were no major identified issues with respect to recording, coding, and categorizing AEs. However, a few PTs were recoded to analyze AESI as shown in [Table 19](#). These recoded terms were used by the CDS team for analyzing the safety data, in addition to the analyses performed by the Applicant coding and categorization of AE.

Table 19. Reviewer's Recoding From AE Terms

Medical Officer Recoding	AE Terms
Abdominal pain	Gastric distress; abdominal cramps; transient abdominal pain; stomachache
Aminotransferase increased	ALT increased; elevated alanine aminotransferase; elevated ALT; raised ALT; elevation of ALT
Blood bilirubin increased	Bilirubin increased; elevated bilirubin; total bilirubin increased
Diarrhea	Diarrhea; loose stools
Pruritus	Increased pruritus; itching; worsening of itching; scratches on face; worsening scratch marks
Vitamin D deficiency	Low vitamin D; worsening fat-soluble vitamin D deficiency

Source: Adapted from CDS Excel file: Trial A4250-005 Overall Combination of Verbatim LLTs and PTs CDS
Abbreviations: AE, adverse event; ALT, alanine aminotransferase

7.5. Adequacy of Clinical Safety Database

The exposure data are presented in [Table 20](#) for Trial A4250-005. About 50% patients across all treatment arms received investigational agent for at least 18 weeks i.e., 11 (55%) patients on placebo, 12 (52%) patients on odevixibat 40 mcg/kg arm, and 10 (53%) patients on odevixibat 120 mcg/kg treatment arm. The median duration of exposure was 24 weeks and ranged from 4 to 27.6 weeks.

A total of 11 patients prematurely discontinued from Trial A4250-005 and rolled over into Trial A4250-008, prior to completion of 24 weeks of treatment. The 11 patients included 5 (25%), 4 (17%), and 2 (11%) patients randomized to placebo, odevixibat 40 mcg/kg/day, and odevixibat 120 mcg/kg/day, respectively.

For assessment of reduction in pruritus, a 24-week trial is acceptable, and the Agency had agreed upon this duration. However, the safety profile of odevixibat use for >6 months is not well characterized. While it is not ideal to have a limited safety characterization for a drug that would

be administered chronically (>6 months), the data for safety, although not adequate, are acceptable. It is taken into consideration that PFIC is a rare pediatric disease and patient recruitment and retention can be difficult. Moreover, we understand the MOA of odevixibat as well as the potential adverse effects of long-term dosing based on the MOA. The short-term use (<6 months) has been well characterized in this rare disease population.

The COVID-19 pandemic resulted in a delay of “nonessential” clinic visits under Trial A4250-005, and the Agency found it acceptable to extend the periods of in-person visits for safety and efficacy evaluations. The two doses that were tested were 40 mcg/kg/day and 120 mcg/kg/day, however, doses in-between these two were not explored for safety or efficacy.

A4250-008, is an ongoing 72-week, an open-label extension that provides additional exposure and longer safety follow-up and supports the safety findings of A4250-005. Interpreting safety signals from an open-label trial are challenging, especially as the AE causality assessment is generally confounded due to underlying disease and its progression.

Table 20. Duration of Exposure, Safety Population, Trial A4250-005

Variable	Placebo N=20	Odevixibat 40 mcg/kg/day N=23	Odevixibat 120 mcg/kg/day N=19	Total Odevixibat N=42
Duration of treatment, (weeks)				
Mean (SD)	21.6 (4.6)	21.7 (5.0)	21.8 (5.8)	21.7 (5.3)
Median (min, max)	23.8 (11.7, 29.1)	23.9 (10.7, 25.9)	23.9 (4.0, 27.6)	23.9 (4.0, 27.6)
Patients treated, by duration, n (%)				
<6 weeks	0	0	1 (5.3)	1 (2.4)
≥6 to <12 weeks	1 (5.0)	3 (13.0)	1 (5.3)	4 (9.5)
≥12 to <18 weeks	4 (20.0)	1 (4.3)	1 (5.3)	2 (4.8)
≥18 to <24 weeks	11 (55.0)	13 (56.5)	10 (52.6)	23 (54.8)
≥24 weeks	4 (20.0)	6 (26.1)	6 (31.6)	12 (28.6)

Source: generated by CDS; adex.xpt; Software: Python; verified from CSR Table 32.
Abbreviations: SD, standard deviation

In pooled safety data for Trial A4250-008, a total 84 patients have received odevixibat 120 mcg/kg/day, as stated by Applicant in 120-day safety update (data cut-off December 4, 2020). A total of 70 (83%) patients have received odevixibat for ≥6 months (24 weeks), 44 (52%) patients have received odevixibat for ≥12 months (52 weeks), and 26 (31%) patients have received odevixibat for ≥18 months (76 weeks).

Of the 62 patients with PFIC who were enrolled on the basis of molecular diagnoses i.e., confirmed for biallelic variants, there were 17 patients with PFIC1, i.e., *ATP8B1* variant, and 47 patients were *ABCB11* variants, i.e., had PFIC2. Of the patients who had PFIC2 (BSEP deficiency), none of the patients with BSEP3 subtype were enrolled, 12 patients were BSEP1 subtype, and 33 patients were BSEP2 subtype. The patients with BSEP3 subtype were excluded from enrollment as the bile salt export pump expression is almost nonfunctional and these patients have poor outcomes compared to BSEP1 or BSEP2 subtypes (Pawlikowska et al. 2010). The percentage of patients with PFIC1 and PFIC2 enrolled in the trial reflect the overall prevalence in PFIC subtypes, therefore, it is acceptable for greater number of patients with PFIC2 to be enrolled relative to those with PFIC1.

7.6. Safety Findings and Concerns Based on Review of Clinical Safety Database

7.6.1. Safety Findings and Concerns, Trials A4250-005 and A4250-008

7.6.1.1. Overall Treatment-Emergent Adverse Events, Trials A4250-005 and A4250-008

Trial A4250-005

Overall TEAE's were balanced across treatment arms, a total of 17 (85%) patients treated with placebo; 19 (83%) patients treated with odevixibat 40 mcg/kg/day and 16 (84%) patients treated with odevixibat 120 mcg/kg/day experienced at least one TEAE (see [Table 21](#)). Most TEAEs observed during the clinical trial were mild to moderate in intensity.

Table 21. Overview of Treatment-Emergent Adverse Events, Safety Population, Trial A4250-005

Event Category	Placebo	Odevixibat 40 mcg/kg/day	Odevixibat 120 mcg/kg/day	Total Odevixibat
	N=20 n (%)	N=23 n (%)	N=19 n (%)	N=42 n (%)
Any AE	17 (85.0)	19 (82.6)	16 (84.2)	35 (83.3)
Grades 3-4	2 (10.0)	1 (4.3)	2 (10.5)	3 (7.1)
Any SAE	5 (25.0)	0	3 (15.8)	3 (7.1)
SAE with fatal outcome	0	0	0	0
AE leading to discontinuation of study drug	0	0	1 (5.3) ¹	1 (2.4)
AE leading to dose modification of study drug	1 (5.0)	3 (13.0)	6 (31.6)	9 (21.4)
AE leading to interruption of study drug	1 (5.0)	3 (13.0)	6 (31.6)	9 (21.4)
AE leading to reduction of study drug ²	0	0	0	0
AE leading to delay of study drug ²	0	0	0	0

Source: Generated by CDS; adae.xpt; Software: Python; CSR Table 33, verified.

Abbreviations: AE, adverse event; SAE, serious adverse event

¹ AE=diarrhea

² Dose reduction or delay was not allowed in Trial A4250-005

Severe AEs

Two (10%) patients treated with placebo, one (4.3%) patient treated with odevixibat 40 mcg/kg/day, and two (10.5%) patients treated with odevixibat 120 mcg/kg/day experienced a protocol-defined severe AE not reported as an SAE, and narratives are summarized below:

- Placebo
 - Patient (b) (6) experienced hand, foot, and mouth disease (viral infection), the clinical review team assessed this event to be unrelated to investigational agent (IA) use.
 - Patient (b) (6), an 8-month old white male with PFIC2, experienced insomnia, pruritus, and poor weight gain (all severe in intensity) on Days 29–36 that resolved on Day 36; patient also experienced viral infection (severe in intensity) on Day 40 which resolved by Day 43 spontaneously, both events were deemed unrelated to IA administration, and IA was not discontinued.
- Odevixibat 40 mcg/kg/day

- Patient (b) (6), an 8-month-old male with PFIC1, experienced two events of total bilirubin elevation (baseline TB =65 micromole/L). First event occurred on Day 85 (TB =164.1 micromole/L) and TB stabilized by Day 98 (TB =96.5 micromole/L), and IA was interrupted. The second event of TB elevation occurred on Day 130 (202.8 micromole/L) and TB stabilized on Day 137 (118 micromole/L), the IA was interrupted and not restarted. Both events were graded as AE of severe intensity. On Day 135 TB levels returned to 32 micromole/L, indicating positive dechallenge. Investigator assessed both events as possibly related to IA use. Review team concurs and assessed AE as probably associated with IA use. The patient was rolled over to Trial 008; however, bilirubin and liver enzyme data were not provided after Day 175, i.e., after patient was enrolled in Trial 008.
- Odevixibat 120 mcg/kg/day
 - Patient ID (b) (6), a 9-year-and-3-month-old with PFIC2, who had history of supraventricular tachycardia, underwent cardiac ablation. This event though graded as severe, is deemed as unrelated to IA use.
 - Patient ID (b) (6) was hospitalized on Day 67 for acute dehydration (severe) and increased liver transaminases (severe). Prior to hospitalization, the patient had experienced a 7-day history of watery diarrhea (three episodes per day), no blood observed, a 3-day history of intermittent, nonbilious vomiting (four episodes per day), no blood observed in vomitus, with poor oral intake, and decreased urine output. The investigator and Applicant assessed the events of dehydration, increases in liver tests, diarrhea, and vomiting as unrelated to study drug. The patient resumed treatment with odevixibat on Day 79 and completed Trial A4250-005. The patient is continuing odevixibat in the open-label extension Trial A4250-008. Data and Safety Monitoring Board reviewed the case and assessed that the elevations in liver tests were related to underlying disease. However, the reviewer assessed that the diarrhea is probably related, and liver test abnormalities as possibly related to odevixibat use, but with subsequent odevixibat introduction after the AE, the patient might have experienced adaptation, and did not experience further events of diarrhea or liver enzyme and bilirubin elevations.

Treatment-Related TEAEs (Primarily Secondary to Liver Enzyme Abnormalities and Diarrhea)

A greater percentage of patients treated with odevixibat experienced treatment related TEAE, i.e., 14 (33%) of the 42 patients who received odevixibat (30% patients treated with odevixibat 40 mcg/kg dose; 37% patients treated with 120 mcg/kg dose) compared to 3 (15%) patients who received placebo (see Section 7.6 for details).

Treatment-Emergent SAEs (see Section 7.6.1.5 for details)

- Five (25%) patients treated with placebo
- Zero patients treated with odevixibat 40 mcg/kg arm
- Three (16%) patients treated with odevixibat 120 mcg/kg dose

Trial A4250-005 Discontinuations (see Section 7.6.1.6 for details)

The majority of the discontinuations were related to lack of perceived efficacy (increase in pruritus) and not due to safety concerns. Notably, treatment discontinuation due to perceived lack of efficacy was observed across all three arms.

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- Five (25%) patients on placebo arm discontinued from Trial A4250 due to lack of efficacy.
- Five (22%) patients on odevixibat 40 mcg/kg arm discontinued from Trial A4250-005. Of the five patients who discontinued, four (17%) discontinued due to lack of efficacy. Of the five who discontinued, one (4%) discontinued due to lack of compliance i.e., inability to travel to site (PT ID [REDACTED]^{(b) (6)}).
- Three (16%) patients on odevixibat 120 mcg/kg arm discontinued treatment. Of the three patients, two (11%) discontinued due to lack of perceived efficacy. Of the three patients who discontinued treatment, one (5%) patient discontinued due to TEAE of diarrhea.

TEAEs Leading to Treatment Interruption (see Section 7.6.1.7 for details)

- One (5%) patient treated with placebo arm experienced one event of TEAEs leading to treatment interruption.
- Three (13%) patients treated with odevixibat 40 mcg/kg experienced seven TEAEs leading to treatment interruption.
- Six (32%) patients treated with odevixibat 120 mcg/kg experienced 10 TEAEs leading to treatment interruption.

TEAEs Leading to Dose Reduction (Odevixibat 120 mcg/kg to 40 mcg/kg) (see Section 7.6.1.8 for details)

Dose reduction for TEAEs was not performed during Trial A4250-005. However, four patients required dose reduction from 120 mcg/kg to 40 mcg/kg dose due to occurrence of adverse events (see Section 7.6.1.8 on Dose Reduction for details). All these dose reductions occurred in Trial A4250-008.

TEAEs Observed During Trial A4250-008

Applicant reports no patient experienced drug-related serious adverse events. Patients with any liver-related AE were experienced by 27 (47%) out of 57 patients dosed with odevixibat.

Similar SOC and preferred term TEAEs and SAEs were reported in Trial A4250-008. Gastrointestinal disorders (diarrhea, vomiting, abdominal pain, etc.), jaundice, hepatomegaly, cholelithiasis, and fat-soluble vitamin deficiency were reported, i.e., safety profile was similar as observed in Trial A4250-005. TEAE of microcytic anemia was reported in three patients, and they were treated for iron-deficiency anemia.

New AEs which were not reported in Trial A4250-005 but reported in Trial A4250-008 include neurological AEs, i.e., irritability and sleep disorders.

7.6.1.2. Deaths or Liver Transplant, Trials A4250-005 and A4250-008

Death

No deaths occurred during phase 1 and 2 trials (A4250-001 and A4250-003), phase 3 trials (Trials A4250-005 and A4250-008) (see details for trial in Section [15](#)).

Liver Transplant

One patient (ID (b) (6)) received a liver transplant from the U.S. site. The brief narrative is summarized below.

Patient was a 2-year-and-4-month-old, white male with PFIC2 and a past medical history of pruritus, milk allergy, and rectal prolapse, and no history of surgery. Baseline medication included UDCA, rifampicin, and alimemazine for pruritus. Patient's baseline liver tests included ALT 53 U/L (ULN 30 U/L); AST 51 U/L (ULN 55 U/L); TB 7.7 micromole/L (ULN 20.5 micromole/L); average of last two sBA levels was 325 micromole/L. Patient was initially enrolled in Trial A4250-005 and received placebo treatment. After 168 days of treatment with placebo, rolled over to Trial A4250-008 to receive odevixibat 120 mcg/kg/day. On Study Day 129 of Trial A4250-008, the patient discontinued odevixibat to undergo elective liver transplantation due to lack of improvement in pruritus. No SAE or treatment interruption were reported.

Based on the biochemical profile and clinical history provided in narrative there was no medically compelling reason, (i.e., liver function deterioration) for patient to receive liver transplantation. However, the family chose elective liver transplant due to lack of improvement in pruritus to odevixibat. This case illustrates need for liver transplantation in patients with refractory pruritus.

7.6.1.3. Treatment-Emergent Adverse Events, Trials A4250-005 and A4250-008

[Table 22](#) is adapted from Applicants CSR and safety datasets analyzed by CDS, summarizes treatment-emergent adverse events that were observed during the A4250-005 clinical trial. The AEs were observed in an active arm at a higher incidence compared to the placebo arm.

Overall, TEAEs of gastrointestinal disorders was observed in higher frequency in the odevixibat-treated arm:

- TEAE of abdominal pain was observed in six (14.3%) odevixibat-treated patients relative to 0% placebo-treated patients.
- TEAE of vomiting was observed in seven (16.7%) odevixibat-treated patients relative to 0% placebo-treated patients.
- TEAE of diarrhea occurred in 14 (33%) patients treated with odevixibat-treated patients relative to two (10%) placebo-treated patients.

A greater percentage of odevixibat-treated patients experienced an increase in aminotransferases as compared to those treated with placebo (45% versus 5%). An increase in total bilirubin was greater in odevixibat-treated patients relative to placebo-treated patients (10% versus 5%). The majority of these liver TEAEs were deemed as drug-related (see details in Section [7.7](#)).

Fat-soluble vitamin deficiency (vitamins A, D, and E) occurred in the 17% odevixibat-treated patients relative to 5% placebo-treated patients. Vitamin K deficiency as assessed by increase in increase in INR occurred equally across both the odevixibat and placebo treatment arms (4.3% versus 5%) (see details in Section 7.7). The TEAE of pruritus occurred in 7% odevixibat-treated versus 5% placebo-treated patients and were balanced across all treatment arms.

Table 22. Common Adverse Reactions, Trial A4250-005

Preferred Term	Placebo	Odevixibat	Odevixibat	Total
	N=20 n (%)	40 mcg/kg N=23 n (%)	120 mcg/kg N=19 n (%)	Odevixibat N=42 n (%)
Any AE	17 (85.0)	19 (82.6)	16 (84.2)	35 (83.3)
Diarrhea	2 (10.0)	9 (39.1)	4 (21.1)	14 (33.3)
Liver test abnormality (increased AST, ALT)	1 (5.0)	3 (43.5%)	9 (47.4)	19 (45.2)
Abdominal pain	0	3 (13.0)	3 (15.8)	6 (14.3)
Vomiting	0	4 (17.4)	3 (15.8)	7 (16.7)
Vitamin deficiency (A, D, and E)	1 (5.0)	0	3 (15.8)	7 (16.7)
Splenomegaly	0	0	2 (10.5)	2 (4.8)
Weight decreased	0	1 (4.3)	0	1 (2.4)
GERD	0	1 (4.3)	0	1 (2.4)
Cholelithiasis	0	0	1 (5.3)	1 (2.4)
Fracture	0	1 (4.3)	0	1 (2.4)
Dehydration	0	0	1 (5.3)	1 (2.4)
Dizziness	0	0	1 (5.3)	1 (2.4)

Source: Derived from Table 8 CDS analyses (adae.xpt; Software: Python); and Summary of Clinical Safety (Page 22 of 218)
 Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GERD, gastro esophageal reflux disease

Based on the 120-day safety update for Trial A4250-008, common adverse reactions presented in [Table 22](#) have been observed in Trial A4250-008. The most common reason for odevixibat treatment interruption was elevation of liver tests (bilirubin, ALT, AST increased). In the pooled data, four patients were diagnosed with iron deficiency anemia and received iron supplements.

During Trial A4250-008, the following patients either averted biliary diversion surgery, or underwent biliary diversion surgery, or underwent liver transplant.

Avoided Biliary Diversion Surgery

Patient ID (b) (6), a 4.6-year-old female with PFIC2, enrolled in Trial A4250-005, and was listed for biliary diversion surgery due to intractable pruritus, received placebo for approximately 12 weeks before the patient rolled over early to Trial A4250-008, due to intolerable symptoms. The biliary diversion surgery was cancelled 32 days after the patient had started treatment with odevixibat 120 mcg/kg/day.

Required Biliary Diversion Surgery

Patient ID (b) (6), a 1.4-year-old male with PFIC2/BSEP1, prematurely discontinued from Trial A4250-005 (was assigned to placebo) due to pruritus and rolled over to Trial A4250-008 and received odevixibat 120 mcg/kg/day. Patient was started on odevixibat on (b) (6), after ~3 months from enrollment in Trial A4250-008 (and day 196 after enrollment in Trial A4250-005) of dosing the patient experienced cholestasis (graded as mild) and the event resolved in 5 days and odevixibat was restarted. The patient experienced a second event of cholestasis at ~8 months (Day 343) after starting odevixibat, and odevixibat was discontinued. The patient then underwent external biliary diversion due to lack of improvement in pruritus.

This patient did not benefit with odevixibat treatment, and experienced worsening of cholestasis, after starting odevixibat (graphical patient profile is presented in [Figure 46](#), Section 17). There might be worsening of cholestasis, possibly related to underlying disease progression but cannot rule out a possibility odevixibat related injury. Superimposed injury, i.e., worsening of underlying disease occurring concurrently with odevixibat related injury also, cannot be ruled out either.

Liver Transplantation

Patient (b) (6), a 2.8-year-old male with PFIC2/BSEP1, underwent liver transplantation (see narrative in Sections [7.6.1.2](#), [7.6.1.3](#), and [7.6.1.2](#)).

Adverse Event of Microcytic Anemia

In Trial A4250-005, one patient was reported to have developed anemia and was treated with placebo (ID (b) (6)). In Trial A4250-008, three (ID (b) (6), ID (b) (6), and (b) (6)) patients developed iron deficiency anemia. [Table 23](#) summarizes the demographics and AE. Two of three patients with iron deficiency anemia also experienced adverse events of diarrhea. All patients were assessed as AE resolved after treatment of iron supplementation; however, follow-up hemoglobin levels were not provided for review. Applicant attributed the iron deficiency anemia related to poor dietary intake of iron.

Iron deficiency anemia in patients with PFIC can occur due to one or combination of the following reasons: poor dietary intake, malabsorption of micronutrients, microscopic bleeding secondary to odevixibat-induced diarrhea, underlying disease related factors, or gastrointestinal bleeding due to portal hypertension.

Therefore, when patients are treated with odevixibat chronically, hemoglobin and hematocrit should be monitored. Etiology for anemia should be evaluated and identified, in addition to iron supplementation.

Table 23. Adverse Events of Iron Deficiency Anemia, Trials A4250-005 and A4250-008

ID	Age Gender PFIC	Odevixibat (dose) or Placebo	AE Hemoglobin (Hb) Hematocrit (Hct)	Treatment of Anemia & Outcome	Other AEs
(b) (6)	1Y4M Male PFIC2	40 mcg/kg in 005 120 mcg/kg in 008	AE: Hb =10.2/Hct =31.8 Onset: Day 1 of 008	Oral ferrous glycine sulfate 10 drops twice daily Resolved	Diarrhea that was reported during 005 and 008.
	1Y4M Female PFIC1	120 mcg/kg in 005 120 mcg/kg in 008	Baseline: Hb =10.6/Hct =31.8 AE: Hb =9.8/Hct =31/8	Ferrous sulfate (220 mg/5mL) a dose of 0.25 tsp twice daily Resolved	Diarrhea, dehydration, liver test increased; required dose interruption during 005 and 008 due to liver test abnormality.
	4Y Male PFIC1	Placebo in 005 120 mcg/kg in 008	Baseline: Hb =10.4/Hct =33.2 AE: Hb =8.3/Hct =26.3	Oral ferrous sulfate 4.6 mL daily Resolving	Hypertension, fever, vitamin D deficiency, irritability, diarrhea hemorrhagic; H1N1 influenza, SAE of neurodermatitis.

ID	Age Gender PFIC	Odevixibat (dose) or Placebo	AE Hemoglobin (Hb) Hematocrit (Hct)	Treatment of Anemia & Outcome	Other AEs
(b) (6)	3Y3M Male PFIC1	40 mcg/kg in 005 120 mcg/kg in 008	Baseline: Hb =10.1/Hct =34.8 AE: Hb =7.3/Hct =27.6	Resolved	Bilirubin increased in both 005 and 008; dose interruption in 008; gastroenteritis with dose interruption.

Source: Applicant Response to Information Request (March 24, 2021)

Abbreviations: 005, Trial A4250-005; 008, Trial A4250-008; AE, adverse event; Hb, hemoglobin, Hct, hematocrit; M, months; SAE, serious adverse event; PFIC, progressive familial intrahepatic cholestasis; Y, years

Reports of pancreatitis and bone fracture in individual patients receiving odevixibat.

Patient (b) (6) experienced pancreatitis in Trial A4250-008, a 19 year, 7-month-old while female with PFIC2. The baseline ALT was 33 U/L, AST was 35 U/L and TB was 15 micromole/L. Past medical history was relevant for tonsillectomy, cholelithiasis, cholecystectomy in (b) (6), attention deficit hyperactivity disorder, abdominal pain, generalized anxiety disorder and major depression, hepatitis, abdominal distension, diarrhea, and flatulence, pancreatitis, trichotillomania, pruritus. She was on rifampin and UDCA at baseline. Odevixibat was started at 120 mcg/kg/day and on day 139 patient developed acute pancreatitis. Laboratory tests (transaminases, bilirubin, INR, lipase, amylase), imaging findings and clinical picture were not provided in the narrative, and it is not known whether patient was admitted to intensive care unit requiring intervention or did the event spontaneously resolved.

Patient ID (b) (6), a three-year-old female, with PFIC1, who was enrolled in Trial A4250-005 and received odevixibat 40 mcg/kg/day, was then rolled over to Trial A4250-008 and received odevixibat 120 mcg/kg/day. On day 141 the patient was reported to have tibia fracture which was noted by Applicant as resolved on Day 167. Experienced a bone fracture in Trial A4250-008.

In this very small safety database, a single serious adverse event has potential to be a meaningful safety signal. In these cases, the safety report and case summaries do not clearly point toward odevixibat as having potential causality for these events. We did not consider these events to be meaningful safety signals. Close monitoring of routine and spontaneous safety reports is needed in the postmarketing period. These events do not warrant inclusion as Warnings and Precautions.

7.6.1.4. Adverse Events of Special Interest, Trials A4250-005 and A4250-008

The adverse events of special interest (AESI) were also identified as the key risk issues. These AESI are discussed in detail in Section [7.7](#).

7.6.1.5. Serious Adverse Events, Trials A4250-005 and A4250-008

[Table 24](#) summarizes SAEs experienced by placebo and treatment group in Trial A4250-005. All the SAEs occurred either in the placebo arm and 120 mcg/kg treatment arm.

Table 24. Serious Adverse Events, Safety Population, Trial A4250-005

Preferred Term	Placebo	Odevixibat		Total
	N=20 n (%)	40 mcg/kg/day N=23 n (%)	120 mcg/kg/day N=19 n (%)	Odevixibat N=42 n (%)
Any SAE	5 (25.0)	0	3 (15.8)	3 (7.1)
Dehydration	0	0	1 (5.3)	1 (2.4)
Elevated transaminase or bilirubin	0	0	1 (5.3)	1 (2.4)
Supraventricular tachycardia	0	0	1 (5.3)	1 (2.4)
Urinary tract infection	1 (5.0)	0	1 (5.3)	1 (2.4)
Auricular hematoma	1 (5.0)	0	0	0
Fever	1 (5.0)	0	0	0
Gastroenteritis	1 (5.0)	0	0	0
Insomnia	1 (5.0)	0	0	0
Weight gain poor	1 (5.0)	0	0	0
Pruritus	2 (10.0)	0	0	0
Viral infection	4 (20.0)	0	0	0

Source: Generated by CDS; adae.xpt; Software: Python

Abbreviations: SAE, serious adverse event

Treatment-emergent adverse events defined as an AE that started on or after the first dose date of study drug, or started before the first dose date, but worsened in severity on or after the first dose.

Preferred term recoding was based on verbatim terms, and some verbatim terms associated with the same preferred term were recoded differently or not recoded. Recoding was performed for 10 verbatim term/preferred term combinations as follows. Fever (recoded) includes pyrexia; gastroenteritis (recoded) includes gastroenteritis, gastroenteritis adenovirus, gastroenteritis norovirus, gastroenteritis viral; pruritus (recoded) includes neurodermatitis, pruritus, scratch; viral infection (recoded) includes adenovirus infection, H1N1 influenza, hand-foot-and-mouth disease, influenza, influenza like illness, viral infection, viral upper respiratory tract infection.

[Table 25](#) below summarizes individual SAE cases, four SAEs were observed in 120 mcg/kg dose group and eight SAEs were observed in the placebo from placebo. Most SAEs were related to the underlying diseases or concurrent acute illnesses, such as the case of supraventricular tachycardia (SVT), flu, and viral infection. In all patients, the SAEs resolved. Odevixibat may be the cause of prolong diarrhea in two patients (ID (b) (6) and (b) (6)), both treated with 120 mcg/kg/day.

Table 25. Serious Adverse Events Narratives (N=8), Trial A4250-005

ID	Age Gender Race PFIC	Drug or Placebo	SAE (Onset)	Dose Change	Outcome	Reviewer Comment
(b) (6)	1Y4M Female White PFIC1	120	Diarrhea (D62-68) Dehydration (D67-70) Elevated LFT (D67-77)	No	Recovered	Diarrhea and dehydration possibly, related to the drug
	6M Male White PFIC1	120	Diarrhea (D11-50) Gastroenteritis (adenovirus, D72-81) URI (D11-34)	No	Recovered	Prolonged diarrhea and "gastroenteritis" possibly related to the drug
	9Y3M Male White PFIC2	120	SVT (D24)	No	Recovered	Not related
	4Y11M Female Middle Eastern PFIC2	120	UTI, E Coli (D116-118) Influenza A H1N1	No	Recovered	Not related
	2Y6M Female Middle Eastern PFIC2	Placebo	H1N1 influenza A (D94-102) UTI (E Coli) (D94-102)	No	Recovered	Not related
	6Y8M Female Greek PFIC1	Placebo	D63: viral infection Auricular hematoma, pyrexia	No	Recovered	Not related
	4Y Male White PFIC1	Placebo	Neurodermatitis (D64-153)	No	Recovered	Rashes/neurodermatitis may be due to severe pruritus
	8M Male White PFIC2	Placebo	Insomnia (D29-36) Pruritus (D29-36) Weight gain poor (D29-36) Influenza A (40-43)	No	Recovered	Insomnia, severe itch, dehydration, possibly due to underlying disease

Source: Reviewer generated. Adapted from "Patient Journey" submitted to Module 5.3.5
 Abbreviations: LFT, liver function test; M, months; PFIC, progressive familial intrahepatic cholestasis; SAE, serious adverse event; SVT, supraventricular tachycardia; URI, upper respiratory tract infection; UTI, urinary tract infection; Y, years

In Trial A4250-008 three additional patients experienced SAEs.

- Patient (b) (6), 19 year 7-month-old female, with PFIC2, developed acute pancreatitis. This event is possibly related to odevixibat use.
- Patient (b) (6), one-year old, white male with PFIC2, developed cholestasis and jaundice on Day 253. The SAEs seems to be probably related to odevixibat use. The patient also experienced recent episodes of exacerbation of pruritus that led to partial external biliary diversion (see [Figure 46](#), Section 17).
- Patient (b) (6), 7.5-year-old with PFIC2, assessed as moderate in intensity developed complications around the biliary stoma (dislocation of stoma device while changing catheter). The AE is unlikely to be due odevixibat.

7.6.1.6. Treatment Discontinuation, Trials A4250-005 and A4250-008

A total of 11 patients discontinued from Trial A4250-005 prematurely (see [Table 13](#)). However, most treatment discontinuations were related to lack of efficacy and not secondary to safety concerns.

- Five (25%) out of 20 patients, enrolled to placebo treatment arm discontinued Trial A4250-005 due to lack of efficacy. All five patients rolled over to Trial A4250-008.
- Five (22%) out of 23 patients enrolled to odevixibat 40 mcg/kg treatment arm discontinued Trial A4250-005, of which four patients discontinued treatment due to lack of efficacy. All four of these patients rolled over to Trial A4250-008. Fifth patient discontinued treatment due to “other reason” (unable to come for regular follow-up) and did not continue in Trial A4250-008.
- Three (16%) of the 19 patients enrolled to odevixibat 120 mcg/kg treatment arm discontinued from Trial A4250-005. Two patients discontinued due to lack of efficacy. Both patients rolled over to Trial A4250-008. Third patient (ID [REDACTED]^{(b) (6)}, 5.5-year-old, PFIC2) discontinued due to AE (diarrhea related to odevixibat use, severity graded as mild).

The trial discontinuation rates 25%, 22%, and 16%, in the placebo, odevixibat 40 mcg/kg, and odevixibat 120 mcg/kg treatment arm, are high. The reasons for discontinuation were predominantly, patients’ perception of lack of improvement in pruritus and early enrollment in open-label, Trial A4250-008 to receive odevixibat 120 mcg/kg. This option of early enrollment into open-label extension for treatment with odevixibat 120 mcg/kg was available to all patients in Trial A4250-005 per protocol, which was submitted in protocol amendment version 02 to FDA on January 1, 2018, which became available to all investigators after Trial A4250-008 was opened. Only one patient (ID [REDACTED]^{(b) (6)}) from odevixibat 120 mcg/kg group withdrew due to AE of prolonged diarrhea and reason for trial discontinuation in second patient (ID [REDACTED]^{(b) (6)}) dosed with odevixibat 40 mcg/kg included inability to come regularly to the site for follow-up visits.

Conclusion

Overall, the trial discontinuation rates were high, with the most common reason for treatment discontinued being lack of efficacy in all arms. There was an option to enroll in Trial A4250-008 and receive odevixibat as a rescue measure. Notably, six (14%) out of 42 patients who were dosed with odevixibat in the double-blind trial, also discontinued to enroll in Trial A4250-008, as the caretaker perception was that the child was not responding to treatment.

In the 120-day safety update, total of 14 patients (17%) discontinued the treatment, of which two patients discontinued from Trial A4250-005 and 12 patients discontinued from Trial A4250-008. In the pooled safety database, patient treatment discontinuation data noted below are summarized from line listings. Of these 14 patients:

- One (7%) patient (ID [REDACTED]^{(b) (6)}) discontinued the treatment due to AE of diarrhea and subsequently requires biliary diversion surgery
- Patient [REDACTED]^{(b) (6)} discontinued due to SAE of acute pancreatitis. The patient also experienced worsening of underlying disease
- Six (42.9%) discontinued due to withdrawal of consent

- One (7%) discontinued due to physician decision
- Five (35.7%) experienced a TEAE for other reasons, such as patient not responding to treatment. Applicant did not clarify whether the two patients, once who underwent liver transplantation and second who underwent biliary diversion surgery are included in this category.

7.6.1.7. Treatment Interruptions, Trials A4250-005 and A4250-008

A total of 12 patients experienced treatment interruptions during Trial A4250-005.

A greater percentage of patients who received odevixibat (10 of 42, 23.8%) underwent treatment interruptions compared to patients who received placebo (2 of 20, 10.0%).

Of the 12 patients who experienced treatment interruptions, seven of 12 (58.3%) patients were dosed with odevixibat 120 mcg/kg/day and three of 12 (25.0%) patients on odevixibat 40 mcg/kg/day and two of 12 (16.6%) patients with placebo.

In nine patients, treatment interruption was due to liver test abnormalities. In the remaining patients, the treatment interruption was due to diarrhea.

Table 26. Treatment Interruptions, Trial A4250-005

Patient ID	Age	Treatment Arm	Reason for Treatment Interruption	Days Treatment Was Interrupted	AE Resolution	Treatment Resumed
(b) (6)	0.5Y PFIC1	Placebo	Bilirubin increased	46	Recovered/ resolved	Yes
(b) (6)	6.6Y PFIC1	Placebo	Bilirubin increased	16	Recovered/ resolved	Yes
(b) (6)	3.2Y PFIC1	40 mcg/kg	Viral gastroenteritis	3	Recovered/ resolved	Yes
(b) (6)	0.6Y PFIC1	40 mcg/kg	Bilirubin increased	34	Recovered/ resolved	Yes
(b) (6)	0.6Y PFIC1	40 mcg/kg	Bilirubin increased	2	Recovered/ resolved	Yes
(b) (6)	2.0Y PFIC2	40 mcg/kg	Alanine aminotransferase increased and aspartate aminotransferase increased	124	Not resolved, not recovered	No
(b) (6)	1.5Y PFIC2	120 mcg/kg	Diarrhea	5	Recovered	Yes
(b) (6)	5.5Y PFIC2	120 mcg/kg	Diarrhea	3	Recovered	Yes
(b) (6)	5.5Y PFIC2	120 mcg/kg	Diarrhea	7	Recovered	Yes

Patient ID	Age	Treatment Arm	Reason for Treatment Interruption	Days Treatment Was Interrupted	AE Resolution	Treatment Resumed
(b) (6)	5.5Y PFIC2	120 mcg/kg	Diarrhea	1	Resolved	No
(b) (6)	5Y PFIC2	120 mcg/kg	Alanine aminotransferase increased, aspartate aminotransferase increased, and blood bilirubin increased	16	Recovered/resolved	Yes
(b) (6)	10.2Y PFIC1	120 mcg/kg	TB increase	15	Recovered/resolved	Yes
(b) (6)	10.2Y PFIC2	120 mcg/kg	TB increase	22	Recovered/resolved	Yes
(b) (6)	1.3Y PFIC1	120 mcg/kg	Liver test elevations	11	Recovered/resolved	Yes
(b) (6)	1Y PFIC1	120 mcg/kg	Alanine aminotransferase increase	6	Recovered/resolved	Yes
(b) (6)	5.5Y PFIC1	120 mcg/kg	Hepatic test elevation	2	Recovered/resolved	Yes

Source: Adapted from Applicant's Information Request response SD 0019, submitted on March 1, 2021
Abbreviations: AE, adverse event; PFIC, progressive familial intrahepatic cholestasis; TB, total bilirubin; Y, years

7.6.1.8. Dose Reduction, Trials A4250-005 and A4250-008

Dose reduction secondary to TEAE were not prespecified in the protocol A4250-005. However, four (5%) out of 84 patients enrolled in Trial A4250-008 experienced AE leading to dose reduction from 120 mcg/kg to 40 mcg/kg. The dose reduction observed in Trial A4250-008 is relevant as (b) (4)

Patient ID (b) (6)

A 3-year-and-3-month-old white male with PFIC1 enrolled in Trial A4250-005 and was randomized to 40 mcg/kg treatment arm. The patient prematurely discontinued from A4250-005 due to lack of efficacy. The patient was started on odevixibat 120 mcg/day when elevation in bilirubin levels were experienced and there was a suspected event of liver injury on Day 161. Treatment was interrupted from Day 168 to Day 261 (93 days) after which the drug was resumed. The treatment was again interrupted from Day 339 to 342 due to adverse event of diarrhea and the drug was resumed on Day 342 (see [Figure 47](#) in Section 17).

The study drug was restarted at odevixibat 40 mcg/day. However, whether the dose was reduced after first AE or second is not very clear from the narrative.

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Patient ID (b) (6)

A 4-year-and-10-month-old Middle Eastern male with PFIC2, was randomized to placebo arm in Trial A4250-005. The baseline ALT 236, AST 219, TB 34 micromole/L, and sBA level 305 micromole/L.

The patient prematurely discontinued from Trial A4250-005 and enrolled in A4250-008. On Day 87 patient experienced increase in aminotransaminases (AST and ALT) and on Day 91 total bilirubin also increased. Study drug was stopped for 11 days and resumed at a lower dose (odevixibat 40 mcg/kg) on Day 102. On Day 349 the patient again experienced elevations in liver enzymes, and met the drug discontinuation criteria, and treatment was interrupted on Day 349 for 17 days. Odevixibat was resumed on Day 354. The study drug was again interrupted on Day 400, the reason was not provided. It seems the TB started to increase again around Day 400. It is also not clear why the study drug was not stopped when AST (334 U/L) and ALT (417 U/L) and TB (86 micromole/L) all increased around Day 147 (see graphical profile in [Figure 48](#)).

The patient had recurrent treatment interruptions and dose reduction secondary to elevations in aminotransferases and bilirubin. The reviewer assesses the liver injury to be related to odevixibat use due to positive dechallenge and positive rechallenge, i.e., worsening of liver tests over the existing baseline elevations.

Patient ID (b) (6)

A 1-year-and-4-month-old white female patient with PFIC2, enrolled to Trial A4250-005 was randomized to odevixibat 120 mcg/kg. The patient discontinued treatment prematurely from Trial A4250-005 due to lack of improvement/intolerable symptoms. The patient rolled over to Trial A4250-008 and received odevixibat 120 mcg/day. On Day 59 patient experienced adverse event of constipation leading to study drug interruption for 21 days (Day 59 to Day 80). After the patient was restarted on lower dose of odevixibat 40 mcg/kg the patient continued to do well, and the dose was increased to 120 mcg/kg on Day 179.

This patient did not experience adverse event again after restarting odevixibat; an increase in dose was tolerated well.

Patient ID (b) (6)

A 2-year-and-9-month-old Arabic male with PFIC2, who was enrolled in Trial A4250-005 to odevixibat 120 mcg/kg was enrolled over to Trial A4250-008 and had dose reduction from 120 to 101 mcg/kg (reduced dose from four capsules to three capsules) due to AE of diarrhea.

Conclusions

At least two patients (IDs (b) (6) and (b) (6)) treated with odevixibat 120 mcg/kg experienced resolution of adverse events and upon rechallenge tolerated odevixibat at a lower dose. However, two patients (IDs (b) (6) and (b) (6)) experienced the recurrent adverse event of liver enzyme elevation and required more than one treatment interruption and dose reduction. Therefore, physicians would have to ascertain the severity of the AE, its recurrence with restarting the drug, and decide whether continuing the treatment would not pose a risk to the patient. Moreover, consider restarting odevixibat at a lower dose after an AE and increase as tolerated. This strategy has been specified in the labeling.

7.6.1.9. Adverse Events Leading to Study Drug Interruption, Trials A4250-005 and A4250-008

See Section [7.6.1.7](#) for details.

7.6.1.10. Laboratory and Imaging Findings, Trials A4250-005 and A4250-008

The laboratory tests for safety monitoring in Trial A4250-005 include clinical chemistry, hematology, urinalysis, vitamin A, E, D, INR, alpha fetoprotein, and abdominal ultrasound. The major laboratory information related to the safety are discussed in other sections—liver test abnormalities (Section [7.7.1](#)) (Appendix, Section [17.1](#), [17.2](#), and [17.3](#)), fat-soluble vitamin deficiency (Section [7.7.3](#)), and iron deficiency anemia (Section [7.6.1.3](#)). Safety implication of these data were discussed in labeling (Section [5](#), and Section [21](#)). Otherwise, no significant abnormal trends were detected from analyses of the rest of the laboratory tests between odevixibat treatment arms and placebo arm in Trial A4250-005.

Abdominal Ultrasound Findings

In Trial A4250-005, hepatomegaly, splenomegaly and hepatic fibrosis was observed at baseline in few patients, as noted in [Table 27](#). Most patients had no changes from baseline to Week 24 in abdominal ultrasound findings including liver size, spleen size, Fibroscan readings. These were considered exploratory analyses for the reasons stated below.

Table 27. Medical and Surgical History Relevant to Potential Portal Hypertension and Cirrhosis

Preferred Term	Odevixibat, Once Daily Dosing				
	Placebo N=20 n (%)	40 mcg/kg N=23 n (%)	120 mcg/kg N=19 n (%)	All Doses N=42 n (%)	Overall N=62 n (%)
Patients with medical/surgical history	6 (30)	1 (4.3)	2 (10.5)	3 (7.1)	9 (14.5)
Ascites	0	0	0	0	0
Portal hypertension	0	0	0	0	0
Hepatomegaly	3 (15.0)	1 (4.3)	1 (5.3)	2 (4.8)	5 (8.1)
Splenomegaly	3 (15.0)	0	1 (5.3)	1 (2.4)	4 (6.5)
Hepatic fibrosis	0	1 (4.3)	1 (5.3)	2 (4.8)	2 (3.2)

Source: Applicant's Information Request response SD 19

[Table 27](#) includes data collected from medical charts. At baseline, prior to enrollment, hepatomegaly or splenomegaly was observed in patients as noted in [Table 27](#).

Repeat (pre- and post-trial) Fibroscan was only performed in a subset of patients (N=11). There were only three patients with Fibroscan read of >14 kPa (IDs ^{(b) (6)}). However, it is not clear whether these infants were fed prior to collecting the Fibroscan reading, which may impact the readings obtained.

At enrollment and end-of-treatment, abdominal ultrasound was performed to assess liver, portal vein flow, and spleen. However, the procedures for these measurements were neither prespecified nor standardized across multiple sites. Moreover, the exact size of spleen was not provided in the datasets. A binary outcome (yes/no) of enlarged spleen was coded, which is not helpful. Viral infection as the cause of spleen enlargement was not specified. In younger children, slight increase in liver and spleen size can be observed on ultrasound, which may not be pathological. Liver size was provided, but it not useful, as the size of liver varies across children

of different ages, and liver enlargement (especially right lobe) has to be interpreted be caution as well contextualized relative the anatomic position the enlargement was measured. Moreover, patients with PFIC generally have hepatomegaly.

The Applicant also stated, based on the liver ultrasound at baseline, about 70% of patients across each treatment arm had hepatomegaly. Most patients had no changes from baseline to Week 24 in abdominal ultrasound findings including liver size, Riedel's lobe, or portal vein size. A change in liver size was not expected in the 24-week trial. Not all patients were evaluated for all components of ultrasound to assess baseline disease. For example, echogenicity was reported only in 38 patients, which was normal in the majority of patients.

On the enrollment ultrasound, splenomegaly was noted at baseline in 35% of patients in the overall odevixibat group (13 of 37 with data available) and 25% of patients in the placebo group (5 of 20 patients). Spleen size was not provided in the CSR or IR response, whether it was 1 cm or 10 cm below the costal margin, which has consequences whether these finding should be interpreted as pathological enlargement.

Protocol lacked prespecified, standardized information for (1) measuring spleen size; (2) whether ultrasound-based spleen assessments (specifically for reported enlargement) are operator dependent; and (3) probe's position while measuring spleen. These factors limit the interpretability of the results on spleen size, even if size was captured. The results of spleen enlargement findings at baseline are inconsistent from [Table 27](#), therefore, to estimate if patients truly had portal hypertension at baseline, the reviewer further assessed for low platelet count and enlarged spleen (see below).

According to the Applicant, worsening in spleen size was noted in eight patients, including six of 14 patients (43%) on odevixibat and two of five (40%) on placebo. Applicant noted, despite worsening of spleen size, which may be indicative of increase in portal hypertension, hematological (leukocyte count and platelet count) parameters were normal and remained stable in all patients. No viral infection or liver decompensation events were noted in these patients. The narratives of eight patients were reviewed and no significant liver test changes or hematological indices were noted. Brief narratives for these nine patients are described in [Section 17.1](#).

Three patients, one patient in each treatment group had low platelet count that were lower than $<150 \times 10^9/L$ at more than one timepoint during Trial A4250-005, as described below.

Patient (b) (6)

An 11.3-year-old male with PFIC2, randomized on (b) (6), completed 24 weeks of treatment with placebo. Relevant past medical history included cerebral hemorrhage (b) (6) (resolved), epistaxis (b) (6) (ongoing), splenomegaly (b) (6) (ongoing) and liver with multiple liver lesions, likely regenerative, nonmalignant (b) (6) (abdominal ultrasound showed hepatomegaly). Out of the six values collected, patient's week 4 platelet counts level was $113 \times 10^9/L$, follow-up values were Week 8=192; Week 12=180; Week 18=147 $\times 10^9/L$; and Week 24. These findings are consistent with the presence of cirrhosis and/or portal hypertension.

Patient (b) (6)

A 3-year-and-3-month-old white female with PFIC2, randomized (b) (6) and completed 24 weeks with odevixibat 40 mcg/kg/day. No relevant past medical history was recorded. Of the six values for platelet count obtained, patient's Week 12 value was $70 \times 10^9/L$,

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rest all values were normal i.e., Week 18 value was 258, and Week 24 value was $158 \times 10^9/L$. Spleen size was normal per the Analysis Data Model (AdaM) dataset. These findings suggest patient did not have portal hypertension.

Patient (b) (6)

A 10.2-year-old male with PFIC1, randomized on (b) (6) completed 24 weeks of treatment with odevixibat 120 mcg/kg/day in Trial A4250-005. Relevant past medical history included the following (all ongoing); jaundice (b) (6), vitamin deficiency (b) (6), biliary diversion surgery (b) (6), hepatomegaly (b) (6), splenomegaly (b) (6), anemia (b) (6), leukopenia (b) (6), thrombocytopenia (b) (6). Of the six values obtained for assessing platelet counts, all values were low, including baseline value $114 \times 10^9/L$, and all other values obtained during the trial: 107, 77, 81, 77, 71, $80 \times 10^9/L$. The patient had splenomegaly and a low platelet count at baseline indicating that this patient had portal hypertension at baseline. Increase in bilirubin was also observed during the treatment. Whether the portal hypertension worsened after initiating treatment is difficult to assess, however, the patient's platelet count did decrease while patient received odevixibat treatment. It is not clear whether, odevixibat has potential to worsen the underlying portal hypertension or liver disease, and this cannot be ascertained based on a single patient's narrative, as underlying disease progression might also contribute to this worsening. This patient did experience events of liver injury requiring treatment interruption during Trial A4250-005, see graphical patient profile in Section 17, Figure 49. The patient enrolled in Trial A4250-008, however, as of the 120-day safety data cutoff date, had not initiated the treatment.

Conclusion

The findings noted above indicate two (3.22%) of the 62 patients enrolled in Trial A4250-005 had portal hypertension; one was enrolled to placebo and the second patient to odevixibat 120 mcg/kg/day. Complications of cirrhosis were not observed in the patients enrolled in the trial, i.e., neither at baseline nor during the trial. Most patients enrolled in the trial had early stage disease.

7.7. Key Review Issues Relevant to Evaluation of Risk

7.7.1. Liver Test Abnormalities

Issue

Liver test abnormalities were observed across all treatment arms during the clinical trial. Most of these abnormalities included elevation of AST, ALT, total and direct bilirubin, and ALP. However, the abnormalities occurred more frequently in the odevixibat-treated arm.

Background

Elevated aminotransferases and bilirubin are observed in patients with PFIC1 and 2. Liver tests are generally higher in patients with PFIC2 (BSEP) compared to patients with PFIC1.

Noted are laboratory data in patients with PFIC (Pawlikowska et al. 2010) who received standard of care only. In PFIC1 (N=~30) reported the AST, ALT, ALP, GGT, TB, and direct bilirubin (DB) as 1.4x, 1.25x, 8x, 0.55x, 6.48x, 3.35x ULN, respectively. In the PFIC2 population (N=~30) reported the AST, ALT, ALP, GGT, TB, and DB as 5.33x, 5.63X, 4x ULN, 0.42x, 6.2x, 4.6x ULN, respectively.

In Trial A4250-005, transaminases and total bilirubin baseline values were as follows:

Table 28. Trial A4250-005, Baseline Transaminases and Total Bilirubin

Parameter	Placebo N=20	Odevixibat 40 mcg/kg/day N=23	Odevixibat 120 mcg/kg/day N=19	Odevixibat All Doses N=42
ALT (U/L)				
Mean (SD)	76.9 (56.21)	127.7 (165.81)	89.1 (86.94)	110.2 (135.83)
Median	55.5	83.0	59.0	70.0
Min, max	19, 236	21, 798	16, 314	16, 798
AST (U/L)				
Mean (SD)	90.2 (51.85)	114.2 (82.67)	96.0 (70.30)	106.0 (76.95)
Median	75.5	90.0	83.0	85.0
Min, max	32, 219	37, 405	38, 320	37, 405
TB (mg/dL)				
Mean (SD)	3.12 (3.392)	3.05 (2.840)	3.34 (4.603)	3.18 (3.695)
Median	1.80	2.77	1.54	2.21
Min, max	0.3, 11.4	0.3, 12.7	0.2, 18.6	0.2, 18.6

Source: Copied and electronically reproduced from the Applicant's submission of clinical study report (Trial A4250-005), page 110 of 248

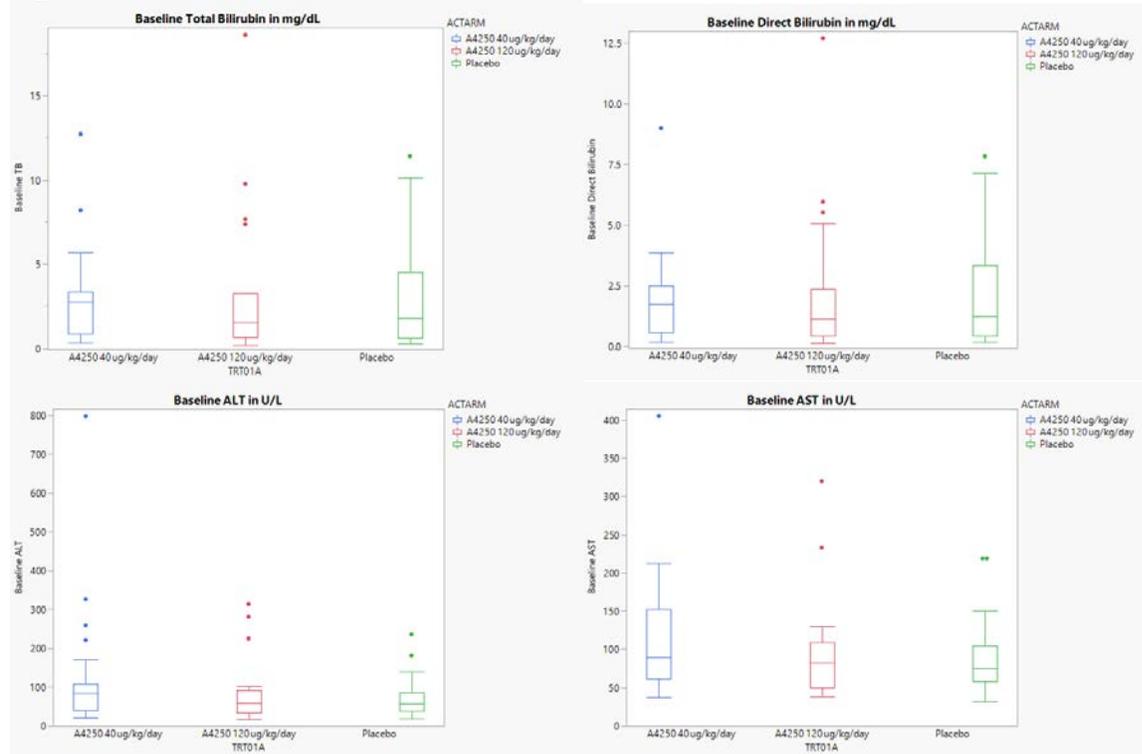
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; SD, standard deviation; TB, total bilirubin

The baseline liver enzymes and bilirubin (TB and DB) were elevated in most patients. There were few patients with normal values for these tests at baseline. There were few outliers in each treatment arm, leading to higher mean and median values.

In the odevixibat 40 mcg/kg treatment arm, the ALT and TB were higher in greater percent patients relative to patients enrolled to placebo or odevixibat 120 mcg/kg/day. However, for AST and DB, patients baseline values were similar across three treatment arms, with exception, that there were outliers, that skewed the results.

Moreover, in the patients enrolled to placebo arm, liver tests values were generally higher in greater percent patients relative to patients enrolled in odevixibat 120 mcg/kg/day.

Figure 6. Baseline ALT, AST, TB, and DB in Patients Enrolled to Trial A4250-005



Source: Generated by the clinical reviewer from adam datasets
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase

For enrollment based on ALT, AST, and TB, there are protocol violations, as in all three treatment arms patients had ALT, AST, and TB >10x ULN. However, the patients were clinically stable at baseline, therefore, the enrollment of these patients is acceptable.

Liver tests were collected on Day 1, Weeks 4, 8, 12, 18, 24, and 28 (follow-up).

The protocol prespecified thresholds for treatment interruption for liver injury was as follows:

- AST or ALT >3 x baseline or 800 U/L and total bilirubin elevation >2 x ULN,
- AST or ALT elevation >10 x ULN,
- TB doubling if baseline value <3 mg/dL, and increase by >3 mg/dL if the baseline TB \geq 3 mg/dL,
- INR increase refractory vitamin K administration
 - a. INR \geq 1.5 if INR was normal at baseline,
 - b. increase >0.4 if INR was elevated at baseline
- Any magnitude increases in AST, ALT or total bilirubin accompanied with symptoms of clinical hepatitis. Rechallenge could be considered if liver test returned to baseline and with consultation of hepatologist except in cases of drug-induced liver injury or liver decompensation event.

The thresholds for treatment interruption selected were liberal. These thresholds were based on longitudinal data, collected from a single center, in patients with PFIC submitted by Albireo AB. The overtime liver test data were collated for FDA's review by the Applicant. The limitation of these submitted data included, (1) lack of the accompanying information (i.e., intercurrent events

or disease progression) when the liver tests became abnormal (2) whether all patients were included i.e., early stage, moderately advanced stage, and advanced stage disease. However, Trial A4250-005 was conducted only in early stage disease population. The FDA reviewer identified overtime fluctuations of liver tests in all patients with PFIC. Due to lack of published longitudinal data on liver tests in the population with PFIC (other than what was submitted by the Applicant), there were no data to assess. The natural variability of liver tests in PFIC population. These test results, without information on intercurrent illness or disease progression, were used to identify thresholds and triggers for treatment interruption, and these values were considered as baseline fluctuations i.e., intercurrent events or stage of disease were not factored in and all values submitted became a part of the assessment for selecting the triggers for treatment interruption. The Agency agreed with liberal thresholds for treatment interruption because of following reasons:

- In a rare disease, prespecifying restrictive thresholds for treatment discontinuation, would have led to high rates of treatment interruption and discontinuations, and the safety of the drug would not be well characterized. It is known that there is variability of liver tests overtime, i.e., that liver tests become abnormal with intercurrent events, restrictive thresholds would not allow adequate exposure, and treatment discontinuations would occur when the event was unrelated to use of investigational agent.
- Most site investigators were hepatologists, who were experienced in providing care to patients with PFIC, and that investigators would factor in intercurrent events when liver tests become abnormal, which is part of routine clinical practice, and was specified in protocol.

Assessment

At baseline, ALT and TB were elevated (above ULN) in greater percent in patients enrolled to odevixibat 40 mcg/kg relative to placebo or odevixibat 120 mcg/kg. However, the natural fluctuations are not reflective in the baseline data, which are important consideration for fluctuations. in the phase 3 trial, elevations in transaminases and total bilirubin were observed in both placebo and treatment groups, however, there was an imbalance across the treatment arms and a greater percentage of patients who received odevixibat (especially in odevixibat 120 mcg/kg/day dose) experienced liver test abnormalities during the trial (see [Table 29](#)).

With the datasets submitted using prespecified thresholds for identifying patients who experienced liver injury, it was difficult to characterize extent of liver injury. Because the baseline values of ALT, AST, TB, and DB were variable in patients enrolled, assessing liver test increases based on fold elevation in ULN or in baseline value could not identify and characterize the liver injury. Liver tests were assessed and analyzed using a modified Hy's graph (used in patients with elevations in baseline liver tests) and other thresholds, however, none were adequate to characterize the AE of liver injury; these analyses can be found in Section [17.1](#). The liver test abnormalities were assessed using these thresholds to characterize the imbalances across treatment arms, see [Table 29](#). A higher percentage of patients dosed with odevixibat experienced isolated transaminases or isolated bilirubin increases.

Table 29. Liver Enzyme Elevations Over Baseline, Safety Population, Trial A4250-005

Liver Enzyme Elevations	Placebo	Odevixibat	Odevixibat
	N=20 n (%)	40 mcg/kg/day N=23 n (%)	120 mcg/kg/day N=19 n (%)
ALT increase over baseline ≥150 U/L	0	2 (10.5)	2 (10.5)
AST increase over baseline by ≥150 U/L	0	1 (4.3)	3 (15.8)
TB increase over baseline by ≥2 mg/dL	1 (5)	4 (17.4)	1 (5.3)
DB increase over baseline by ≥1 mg/dL	2 (10)	5 (21.7)	2 (10.5)
ALT increase over baseline by ≥150 u/L + TB increase over baseline by ≥2 mg/dL	0	0	0
ALT increase over baseline by ≥150 u/L + DB increase over baseline by ≥1 mg/dL	0	0	0
ALT increase over baseline by ≥150 u/L + TB increase over baseline by ≥2 mg/dL + INR >1.5	0	0	0

Source: adlbco.xpt; Software: R

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; INR, prothrombin international normalized ratio; TB, total bilirubin

A total of 14 patients had treatment interruption during Trial A4250-005 as stated in [Table 30](#). Treatment interruption criteria that were prespecified in the protocol are noted above (in the background section) of this risk issue. Using even the liberal thresholds for treatment interruptions, a higher percentage of odevixibat-treated i.e., seven (16%) patients experienced the protocol's prespecified definition of liver injury relative to placebo-treated i.e., two (10%) patients, requiring treatment interruption.

A total of seven (41.1%) out of 17 patients with PFIC1 experienced liver injury relative to two (4.4%) out of 45 patients with PFIC2. It seems patients with PFIC1 experienced liver injury more frequently compared PFIC2; however, the sample size is too small to draw firm conclusions.

Patient ^{(b) (6)}: AST (66→79 U/L on Day 51), ALT (50→57 U/L on Day 85), and TB (145→339 micromole/L on Day 125) that were elevated on three different occasions in placebo patient, however, the events were related in presence of intercurrent illness (viral upper respiratory tract infections).

Time to event was variable, i.e., occurrence of elevation in liver enzymes and/or total bilirubin occurred on average at Day 93 (range 3 to 147 days) after starting odevixibat. Number of days the drug was interrupted on average was 23.5 days (range 3 days to 123 days). Therefore, liver tests should be monitored on an ongoing basis while the patient is being actively dosed with odevixibat.

In patients treated with odevixibat 120 mcg/kg, a total of four patients (IDs ^{(b) (6)}) experienced isolated transaminase elevations (ranging from 2x to >14x ULN).

Patient ^{(b) (6)}, with PFIC1, a 0.6-year-old male, was treated with odevixibat 40 mcg/kg/day, had TB elevation >2 x baseline, i.e., baseline TB was 4.4 mg/dL and at time of event was 11.9 mg/dL.

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Patient (b) (6), a 10.2-year-old, PFIC1, who was treated with odevixibat 120 mcg/kg/day experienced elevation in ALT (59→114 U/L) and AST (87→ 124 U/L) on Day 28. And on Day 158 the patient experienced elevation in TB (7.57 mg/dL→11.58 mg/dL).

Patients with concurrent TB and ALT or AST elevations who did not meet the thresholds in [Table 29](#):

- Patient (b) (6), PFIC2, a 2-year-old male, treated with odevixibat 40 mcg/kg/day, on Day 71 experienced concurrent elevation in TB >2x baseline (8.19 mg/dL→16.72 mg/dL) and also experienced ALT (326 U/L→414 U/L) and AST (405→544 U/L), the treatment was interrupted for 124 days and eventually discontinued. Despite concurrent ALT and TB elevation, the thresholds specified in the either of the above noted criteria were not met. The patient does not meet the Hy's Law criteria (ALT >3x ULN and TB >2x ULN), as it is applicable only in patients with normal baseline liver tests, or modified Hy's Law criteria (ALT >3x baseline AND TB >2x baseline), as the ALT or AST elevation were <2x baseline, although TB reached >2x baseline value. Patient experienced cholestatic injury.
- Patient (b) (6), PFIC2, a 5-year-old male, treated with odevixibat 120 mcg/kg/day, on day 128 experienced elevations of ALT (92→226 U/L), AST (114→281), and TB (3.27 mg/dL→4.61mg/dL). This patient also did not meet the criteria for Hy's Law or modified Hy's Law or above stated thresholds.

The treatment interruption after liver injury ranged from 3 to 124 days.

In two patients treated with placebo, isolated TB elevations were observed. One patient (ID (b) (6)) had isolated elevation in TB (>10 ULN), however, the patient experienced upper respiratory tract infection at the same time. The second patient treated with placebo (ID (b) (6)) experienced isolated TB elevation (>14X ULN) and required drug discontinuation for 17 days.

Table 30. Odevixibat Interruption Due to Liver Test Abnormalities, Trial A4250-005

Patient ID	Age (years)/ Gender	PFIC Type	Arms	Study Day of Interruption	Interruption Duration (Days)	Positive Rechallenge ¹
(b) (6)	0.5/M	Type 1	Placebo	67	12	Yes (elevation in TB), event was related to URT infection
	0.5/M	Type 1	Placebo	82	15	Yes (elevation in TB), event was related to URT infection
	0.5/M	Type 1	Placebo	105	22	No
	6.6/F	Type 1	Placebo	28	17	No
	2/M	Type 2	40 mcg/kg	N/A	123	N/A
	0.6/M	Type 1	40 mcg/kg	127	35	Yes (elevation in TB)
	0.6/M	Type 1	40 mcg/kg	137	3	No
	5/M	Type 2	120 mcg/kg	146	17	No
	10.2/M	Type 1	120 mcg/kg	43	16	No
	10.2/M	Type 1	120 mcg/kg	174	23	No
	1.3/F	Type 1	120 mcg/kg	79	12	No
	1/M	Type 1	120 mcg/kg	91	7	No
	5.5/M	Type 1	120 mcg/kg	35	3	No

Source: Adopted from excel file for question 5, Applicant Information Request response on March 24, 2021.

Abbreviations: F, female; M, male; PFIC, progressive familial intrahepatic cholestasis; TB, total bilirubin; URT, upper respiratory tract infection

¹ TB was eventually stabilized for both patients with positive rechallenge and treatment was ultimately resumed.

² Patient never resumed treatment before termination from the trial due to the family's difficulty to bring the patient to study visits.

Biological Plausibility

Reduced re-absorption of bile acids have a negative feedback effect on intestinal and liver FXR. FGF19 was not measured, which would have provided more insights to autocrine pathway activation. However, FGF19 was expected to have decreased. The plasma C4 was measured, only at baseline and EOT, and increase in C4 were observed. Elevations in C4 indicate increased liver bile acid synthesis. During Trial A4250-005, C4 elevations were observed in patients who also had normalization in sBA levels (see Section 18.1 and Figure 21). Increase synthesis of bile acids in liver, and inability of the liver to secrete bile acids in intestines would lead to increase in cholestasis leading to liver injury. However, it is not clear why most patients did not experience liver injury.

Conclusion

Treatment with odevixibat may cause elevation in liver enzymes and bilirubin. A greater percentage of odevixibat-treated patients experienced cholestasis or hepatocellular injury relative to placebo. The number of patients requiring treatment interruption based on protocol prespecified thresholds was seven patients (in odevixibat treatment arms) relative to two patients on placebo arm. There were two odevixibat-treated patients who experienced concurrent elevation of aminotransferases and TB but did not meet modified Hy's Law criteria relative to none in placebo arm.

Treatment interruptions occurred in a greater percentage of patients treated with odevixibat 120 mcg/kg dose. A greater percentage of patients with PFIC1 experienced protocol-defined liver injury relative to patients with PFIC2, despite the fact that 73% of those enrolled were patients with PFIC2.

Time to liver injury after starting odevixibat was variable and ranged between 3 to 147 days. The number of days the drug was interrupted was also variable i.e., ranging between 3 to 124 days. Therefore, monitoring of liver tests should be indicated on an ongoing basis while the patient is dosed with odevixibat.

The risk of liver injury has been described in Section 5 of labeling (Warnings and Precautions); monitoring of liver tests, is recommended prior to starting odevixibat, and during treatment with odevixibat. Treatment interruption and discontinuations are recommended in the event abnormal liver tests are detected.

7.7.2. Diarrhea

Issue

Diarrhea and other gastrointestinal symptoms such as vomiting and abdominal pain occurred in a higher percentage of odevixibat-treated patients relative to placebo-treated patients. Odevixibat is an IBAT inhibitor and promotes bile acid excretion. Based on mechanism of action odevixibat can cause BA-induced diarrhea. Due to IBAT inhibition, the BA re-uptake by the small intestine is decreased, leading to BA secretion in the colon and eventually excretion via feces. An increase in bile acid excretion in the colon creates a clinical picture akin to BA diarrhea. BA causes an increase in mucosal permeability (Camilleri 2015; Walters 2020), induces water and electrolyte secretion, and accelerates colonic transit partly by stimulating propulsive high-amplitude colonic contractions.

Background

Diarrhea, abdominal pain, and vomiting were the most commonly occurring AEs in the SOC of gastrointestinal disorder. There was an imbalance across the treatment arm for all three AEs (see Sections [7.6.1.1](#)). In this section, the reviewer will focus specifically on the TEAE of diarrhea.

Protocol prespecified that the study drug should be discontinued if a patient develops diarrhea with at least one of the following concomitant signs or symptoms: grossly bloody stools, vomiting, dehydration requiring treatment with oral or intravenous (IV) rehydration and/or electrolyte imbalances, fever ($\geq 38^{\circ}\text{C}$), and/or the diarrhea persists for 7 or more days. If there is gross blood in stool, INR and platelet counts should be measured.

Study drug should be reintroduced (rechallenge) when the symptoms have resolved. If the diarrhea re-occurs within 1 week after the rechallenge with no alternate etiology, the drug should be permanently discontinued and monitored if patient develops any severe AE related to study drug.

Assessment

Patients with past medical history or ongoing chronic diarrhea were excluded from enrollment, or who required intravenous fluids or nutritional intervention for treatment of diarrhea and its sequelae were excluded from enrollment. In Trial A4250-005, overall, one (5%) placebo-treated patient versus 13 (31%) odevixibat-treated patients experienced the treatment-emergent AE of diarrhea, i.e., there was an imbalance across the treatment groups. However, Applicant states that neither in the odevixibat-treated nor placebo-treated patients experienced severe or serious AE of diarrhea.

Diarrhea of ≥ 3 Days

Nine (21.4%) of 42 patients had diarrhea of ≥ 3 days duration, including six (26%) out of 23 patients who received odevixibat 40 mcg/kg/day and three (15.8%) out of 19 who received 120 mcg/kg/day. None of the patients who received placebo had diarrhea of ≥ 3 days duration in a month.

Diarrhea > 7 Days

Three (7%) patients treated with odevixibat experienced diarrhea > 7 days relative to none in placebo.

One (4.34%) patient treated with odevixibat 40 mcg/kg, experienced diarrhea > 7 days (ID (b) (6), 3.2-year-old). The patient developed viral gastroenteritis requiring treatment interruption for 3 days (not noted in [Table 31](#)), after diarrhea was resolved, treatment was resumed after which the patient tolerated odevixibat.

Two (10.5%) patients treated with odevixibat 120 mcg/kg, experienced diarrhea lasting > 7 days, which required treatment interruption. Of these two patients, one patient ((b) (6)) required one treatment interruption for 5 days, and when the AE of diarrhea was resolved, odevixibat 120 mcg/kg/day was restarted, and patient tolerated the dose. Patient (b) (6) required three treatment interruptions during the trial, two interruptions lasting 3 days, 7 days, and last treatment interruption lasted 1 day, which further resulted in treatment discontinuation following the third treatment rechallenge.

Patient Experiencing Diarrhea Needing Intravenous Hydration

One patient ((b) (6)) who received odevixibat 120 mcg/kg/day experienced diarrhea that led to dehydration requiring intravenous hydration. It is not clear why this patient did not meet the SAE criteria, as hospitalization or requiring intravenous hydration due to diarrhea per protocol should be classified as SAE.

Patients Experiencing Diarrhea and Concurrent Fever (>100.4F)

Three patients experienced diarrhea concurrent with fever, including two patients ((b) (6)) and ((b) (6)) who received odevixibat 40 mcg/kg treatment arm and one patient ((b) (6)) who received odevixibat 120 mcg/kg.

In Trial A4250-008, patients are dosed with odevixibat 120 mcg/kg. Diarrhea has been observed in patients in this trial. Treatment interruption and dose reduction resulted when patients experienced diarrhea that met the trial prespecified trigger; however, this is open-label trial and interpreting safety is rather difficult. However, the dose-reduction strategy utilized in Trial A4250-008 seems to help mitigate AE of diarrhea.

Table 31. Summary of Adverse Reaction: Diarrhea, Trial A4250-005

Preferred Term	Placebo	Odevixibat	Odevixibat	Odevixibat
	N=20 n (%)	40 mcg/kg/day N=23 n (%)	120 mcg/kg/day N=19 n (%)	Total N=42 n (%)
Any diarrhea AE	1 (5.0)	9 (39.1)	4 (21.1)	13 (31)
Severe diarrhea AE	0	0	0	0
Serious diarrhea AE	0	0	0	0
Serious AE	0	0	0	0
Drug Interruption due to diarrhea AE	0	0	2 (10.5)	2 (4.8)
Discontinuation due to diarrhea AE	0	0	1 (5.3)	1 (2.4)

Source: Adopted from Table 11 of D-120 Safety Update
 Abbreviations: AE, adverse event
 Criterion for drug interruption: diarrhea lasting >7days

In Trial A4250-005 or A4250-008, four patients (IDs (b) (6) (PFIC1); (b) (6) (PFIC1); (b) (6) (PFIC1); and (b) (6) (PFIC2)) presented with microcytic anemia (hemoglobin ranging from 9.2 to 10.4 g/dL), however, it is difficult to ascertain whether odevixibat induced diarrhea led to microscopic blood loss resulting in anemia. Three of the four patients who developed anemia had PFIC1; in patients with PFIC1 small intestinal involvement due to underlying disease can cause malabsorption of iron, therefore, signal ascertainment is difficult.

Increased levels of bile salts in the colon might be associated with risk of developing colon cancer (Ocvirk and O'Keefe 2017; Nguyen et al. 2018). Although, the risk is theoretical, but physicians should keep this in mind, while treating patients. In patients who are on long-term dosing, should pay particular attention to development of colon cancer; and in patients who do not respond to treatment, discontinuation should be considered.

Conclusion

One-third (n=13, 31%) of the patient population treated with odevixibat experienced diarrhea relative to 5% (n=1) in placebo arm. The AE of greater than three bowel movement per day was observed only in odevixibat-treated patients, i.e., none in the placebo arm. Majority of the odevixibat-treated patients who reported to have experienced diarrhea, lasted for ≤7 days, however, in two (10.5%) of 19 odevixibat-treated (120 mcg/kg) patients experienced diarrhea

that lasted for >7 days. Most patients tolerated restarting the drug after treatment interruption, however, a few patients required recurrent treatment interruptions (see Section 7.6.1.7), and one patient required treatment discontinuation.

The AE of diarrhea has been described in the Warnings and Precautions section (Section 5) of labeling. Patients and caregivers should be educated to recognize new-onset or worsening of existing diarrhea and interrupt the treatment. Treatment should be restarted at odevixibat 40 mcg/kg/day dose and dose could be increased as tolerated.

In the event, patient presents with microcytic anemia that is observed after odevixibat use, an evaluation of blood loss should be performed as per standard of care.

7.7.3. Fat-Soluble Vitamin Deficiency

Issue

FSV deficiency can lead to growth abnormalities. Table 32 presented below shows, a numerically greater percentage of odevixibat-treated patients relative to placebo-treated patients experienced FSV deficiency during Trial A4250-005. Five (26.3%) odevixibat 120 mcg-treated patients, one (4.3%) odevixibat 40 mcg-treated patients, and two (10%) placebo- treated patients experienced vitamin A, D, E, or K (assessed by INR) deficiency.

If vitamin K deficiency is not included in FSV deficiency, then one (5%) patient in placebo arm, one (4.3%) patient in odevixibat 40 mcg/kg treated arm and five (26.3%) patients in the odevixibat 120 mcg/kg treated arm experienced FSV deficiency. The FDA reviewer chose to not include vitamin K assessment for two reasons: (1) it is an indirect measure, i.e., INR is assessed and assumption is made that elevated INR is the cause of vitamin K deficiency and (2) laboratory errors for INR occur frequently and it would not be clear whether an improvement in INR is a reflection of vitamin K deficiency resolution or correct assessment on repeat measure. Moreover, in the event the INR was observed to be abnormal, the Applicant did not confirm the abnormality. The imbalance in AE of FSV deficiency triggered analyses of this AE.

Background

Patients with PFIC at baseline have fat-soluble vitamin deficiency, due to reduced bile acid secretion in the intestines, fat and fat-soluble vitamins are poorly absorbed.

Table 32. Fat-Soluble Vitamin Deficiency, Trial A4250-005

Preferred Term	Placebo	Odevixibat	Odevixibat	Odevixibat
	N=20 n (%)	40 mcg/kg/day N=23 n (%)	120 mcg/kg/day N=19 n (%)	Total N=42 n (%)
Vitamin D deficiency	1 (5)	0	3 (15.8)	3 (7.1)
Vitamin A deficiency	0	0	1 (5.3)	1 (2.4)
Vitamin E deficiency	0	0	1 (5.3)	1 (2.4)
INR increased	1 (5)	1 (4.3)	0	1 (2.4)
Total	2 (10)	1 (4.3)	5 (26.3)	6 (14.3)

Source: Generated by CDS using Python software from dataset provided by the Applicant
 Abbreviations: INR, international normalized ratio

Table 32 represents what was submitted by the Applicant in the A4250-005 CSR. These results demonstrate that a greater percentage of odevixibat-treated patients developed FSV deficiency. However, when the reviewer analyzed the datasets multiple factors were not accounted for,

before concluding this imbalance in AE. Although it appears that odevixibat-treated patients are developing more FSV deficiency, once all factors were reviewed, causality ascertainment was challenging.

The following findings were submitted by the Applicant to the IR “Clinical Information Amendment, M1.11.3, dated March 1, 2021.” During Trial A4250-005, the following patients received intravenous FSV:

- Twelve patients received supplementation with one or more fat-soluble vitamins (FSV) prior to enrollment and continued these supplements during Trial A4250-005; all doses were administered intramuscularly. The Applicant did not provide information regarding which group these 12 patients were randomized to, limiting FDA’s ability to interpret these data in the context of patients who eventually developed FSV deficiencies. Four additional patients received a one-time dose of FSV supplementation just prior to enrollment, and were administered FSV via intramuscular (IM), or intravenous (IV) or subcutaneous route. Again, information regarding which group these patients were randomized, was not provided.
- Nine patients received intravenous or intramuscular supplementation with one or more FSV after starting the investigational agent, including five (25%) patients in the placebo arm and four (9.52%) patients who received odevixibat (two in each dose group). All IV or IM FSV supplementation were administered as a one-time dose; no patients started an ongoing IM or IV regimen after starting study treatment. Vitamin supplementation was administered in response to decrease in vitamin levels (i.e., less than LLN). Following FSV supplementation, in most patients, the vitamin levels, came back to normal levels or baseline levels. Two patients (IDs (b) (6) and (b) (6)) in the placebo group had a worsening of their vitamin E levels despite supplementation. However, the reviewer notes, that these two patients are not counted in the TEAE table, and both patients completed Day 167 of trial (i.e., end-of-treatment) and did not discontinue investigational agent prematurely. So, it is not clear why these patients were not captured as being deficient in vitamin E, unless these patients did not meet the threshold of reaching less than LLN. The Applicant did not provide clarity on this issue (IR response submitted on March 19, 2021).

The following patients received additional oral FSV supplementation, in addition to multivitamin preparation, that was started either before or after initiating the investigational agent:

- 49 patients received oral vitamin E including 16 (80%), 18 (78.3) and 15 (78.9%) patients in the placebo, 40 mcg/kg/day and 120 mcg/kg/day dose groups, respectively.
- 50 patients received oral vitamin K supplementation either before or after starting study treatment, including 17 (85%), 17 (73.9%) and 16 (84.2%) patients in the placebo, 40 mcg/kg/day and 120 mcg/kg/day dose groups, respectively.
- 43 patients received oral vitamin A supplementation either before or after starting study treatment, including 16 (80%), 16 (69.6%) and 11 (57.9%) patients in the placebo, 40 mcg/kg/day and 120 mcg/kg/day dose groups, respectively.
- 54 patients received oral vitamin D supplementation either before or after starting study treatment, including 18 (90%), 19 (83%) and 17 (89.5%) patients in the placebo, 40 mcg/kg/day and 120 mcg/kg/day dose groups, respectively

In the IR response that the Applicant submitted (SD 0019), in which data for FSV intake and deficiency observed were submitted in sortable excel sheet. The FSV supplementation data are summarized below:

- Out of 62 patients with PFIC enrolled in Trial A4250, 55 patients were supplemented with oral FSV and/or multivitamin supplement at baseline, i.e., prior to start of investigational agent.
- Patients were receiving vitamin preparations, i.e., either as multivitamins or FSV vitamins alone. At baseline 45 patients had one or more FSV deficiency.
- 15 patients experienced new onset FSV deficiency, i.e., had normal FSV values at baseline and received additional FSV supplementation during Trial A4250-005.
- 11 patients experienced worsening of FSV. Patients who experienced FSV deficiency at Week 12, 22, or anytime postbaseline were balanced across the three treatment arms, as noted in [Table 33](#).

Notably, in [Table 33](#), all the fat-soluble vitamin deficiencies have been lumped together including vitamin K (assessed by INR), which the reviewer initially had intended to not include in FSV deficiency assessments. However, even if vitamin K deficiency was not included in [Table 33](#), ascertainment of FSV deficiency would still be quite challenging due to the reasons described subsequently.

Assessment

Table 33. Patients With Fat-Soluble Vitamin Deficiency at Weeks 12, 24, and Any Time Postbaseline (for Patients Who Discontinued Trial Before Week 24)

Time Point in Trial FSV Deficiency Was Experienced	Placebo N=20 n (%)	Odevixibat 40 mcg/kg N=23 n (%)	Odevixibat 120 mcg/kg N=19 n (%)
Week 12	14 (70)	12 (52.1)	8 (42)
Week 22	10 (50)	10 (43.5)	8 (42)
Any time post baseline	18 (90)	17 (74)	16 (84)

Source: Generated by the Clinical Reviewer from the Information Request Response Submitted by Applicant (SD #19)
 Abbreviations: FSV, fat-soluble vitamin

It is difficult to ascertain causality for the AE of FSV deficiency. There are many factors when considered that would make the casualty ascertainment extremely complex making it impossible to determine the impact of odevixibat for causing FSV deficiency. Some of the factors are listed below:

- Missing information as noted above in this section.
- Intravenous, intramuscular, and subcutaneous route of FSV administration: Patients received oral, intravenous, intramuscular, and subcutaneous FSV supplementations, i.e., different routes and at different doses.
 - FSV administered via nonoral route that are likely to be 100% bioavailable and FSV deficiency should not persist unless there are issues with fat binding of FSV and measurement errors.
 - Even when FSV or multivitamins were administered via nonoral route the doses administered varied hugely, for example, one patient (b) (6) received intramuscular vitamin 300,000 international units (IUs) of vitamin D every 4 months, in addition to 2 to

3 drops of oral vitamin D (each drop providing 8000 IU vitamin D) daily in addition to multivitamins supplementation (which contains vitamin D). Compared to Patient (b) (6) who received 100,000 IU every 3 months and 2 drops of FSV containing all FSVs.

- Oral route
 - All patients at baseline were on multiple oral multivitamin preparations. When oral supplements were reviewed, patient's received multiple types of preparations, i.e., as multivitamin (alone or combination of one or two vitamins such as vitamin A alone or vitamin ADEK together etc.), water soluble ADEK preparations, and other combination (all vitamins in one pill or liquids) vitamin preparations. Most of these vitamin preparations were providing suprathreshold doses of vitamins.
 - Additionally, comparability of absorption of different formulation is not possible for two reasons, water-soluble ADEK are purported to be absorbed even in absence of bile acids in gut lumen, compared to ADEK preparation that are not water soluble, secondly, different patients were getting different strengths of ADEK's.
 - Different doses of vitamins were administered, making it almost impossible to compare interpatient FSV deficiency. For example, Patient (b) (6) received 300 IU vitamin A orally per day (in addition to vitamin A included in combination multivitamin preparations) compared to Patient (b) (6) who received vitamin A 10,000 IU per day along with combination multivitamins preparation.
- Patient and other factors
 - Different patients with PFIC have variable amount of intestinal bile acid pool which is dependent on degree of BSEP or FIC1 deficiency, i.e., varying degrees of reduction in BSEP or FIC1 protein expression, lowers the bile acid secretion in intestines.
 - Intercurrent illness impacts the protein expression of BSEP and FIC1 that can further exacerbate FSV deficiency.
 - Concomitant drugs that patients with PFIC are administered may also impact the BSEP protein expression, leading to decrease secretion of bile acid in intestines, leading to reduction in intestinal bile pool.
 - Another potential mechanism includes reduction in secondary UDCA salts pool. The UDCA secondary bile salts also use IBAT for re-absorption at terminal ileum and given that IBAT inhibition no longer allows absorption of UDCA, that leads to reduction of the hydrophilic bile salts in liver. UDCA is a choleric agent and promotes bile flow, with lack of UDCA secondary salts in liver the bile flow is expected to be reduced.

Biological Plausibility

Two plausible mechanisms that can lead to FSV deficiency are discussed below. There is some evidence in the literature to support the below noted hypothesis.

The secondary bile acids are secreted in small intestines, the fat and fat-soluble vitamins are absorbed in proximal jejunum and ileum, i.e., upstream effect. Once the secondary bile salts reach the terminal ileum, instead of being reabsorbed, they are excreted.

IBATs are intended to reduce the overall bile acid pool (i.e., serum, liver, and intestinal pool). Reduction in sBA level is an indirect assessment of decreased re-uptake of bile salts. Due to bile acid malabsorption, effect on autocrine function i.e., via intestinal FXR feedback (Farrugia and Arasaradnam 2020) is exerted via FGF19, which then promotes bile acid synthesis by the liver, as evidenced by an increase in C4 (see Section [18.1](#)). C4, was measured pre and post treatment, i.e., measures were not as rigorous and were not collected at each visit. The Applicant did not collect FGF19 at any timepoint. The elevation in C4 with odevixibat-treatment indicates increased synthesis of bile acids in liver. Due to the limited ability of the liver to secrete bile acids into intestine, increased bile acid synthesis in liver would cause increased hepatocyte bile acids load, leading to more cholestasis. Thereby treatment with odevixibat, could lead to paradoxical increase in liver bile acids.

- With increase in liver bile acid concentrations, it is likely that cholestasis is exacerbated, and bile acid secretion is further impaired.
- Additionally, due to IBAT inhibition, UDCA secondary salts are not absorbed, which might impair UDCA's efficacy to varying degree, and the choleric action is reduced.

Both these factors are expected to exacerbate underlying cholestasis leading to FSV deficiency.

Assessment of Growth

The impact on growth and development was evaluated using change of height from baseline to end-of-treatment and height z-scores in Trial A4250-005. [Figure 7](#) and [Figure 8](#) show a change of height and height age z-score in various age groups during 24 weeks across the three treatment groups, 40 mcg/kg, 120 mcg/kg, and placebo. There was no difference in height increments with time (from baseline to week 24) for each age group, across the three treatment arms. The sample size for patients ages 12 years and above is too small to draw a reliable conclusion. Height Z-score, (comparing height with peers), shows that most patients across all age groups are above zero and similar across the three treatment arms. Assessing height in a 6-month (24-week) trial does not provide useful information for linear growth, as patients with PFIC have difficulties with linear growth and typically are quite stunted. However, assessing height changes over short-duration may be relevant in children less 2 years of age, but there were no differences in change in height or height z-scores across the three treatment arms in this stratum. These data do not confirm benefit, but they do allow assessment of harm over short duration (24 weeks).

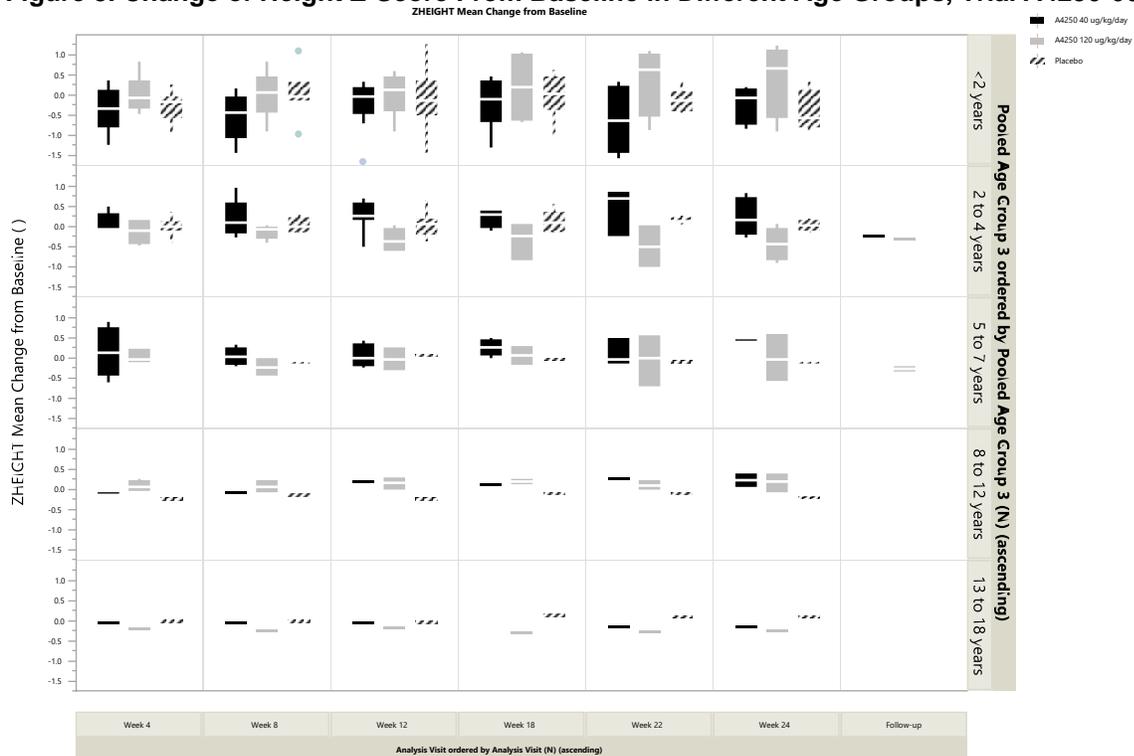
The open-label Trial A4250-008 would measure height for 72 weeks. Patients who completed the A4250-005 24-week trial and were enrolled in Trial A4250-008 will have data for 96 weeks, however, however, caution is needed when interpreting open-label trials.

Figure 7. Change of Height From Baseline in Different Age Groups, Trial A4250-005



Source: Reviewer's analyses using JMP-Clinical. Dataset: advs.xpt

Figure 8. Change of Height Z-Score From Baseline in Different Age Groups, Trial A4250-005



Source: Reviewer's analyses using JMP-Clinical. Dataset: advs.xpt

Conclusions

At baseline, there were minimal imbalances in FSV deficiency across the three-treatment arm, TEAE of FSV deficiency were observed in a greater percentage of odevixibat-treated patients relative to placebo. There is lack of clarity on the causality ascertainment for FSV deficiency due to presence of multiple confounders. Factors included nonoral routes of FSV administration which bypasses the need of bile acids in intestine, patient's phenotype (milder versus severe), use of concomitant drugs such as UDCA that might impact hepatic bile acid secretion, and intercurrent events.

There is biological plausibility that IBAT inhibitors can cause FSV deficiency, therefore, levels of FSV should be monitored periodically and patients should be supplemented with additional FSV doses as needed. This warning has been added in the Warnings and Precautions of labeling section to allow regular monitoring as well as FSV supplementation.

Effect on growth impairment was not detected in Trial A4250-005; however, the trial duration was only 24 weeks. Height usually cannot be assessed in a short-duration (6-month) trial, particularly in patients with PFIC who have stunted growth; however, in the younger infants we did not observe negative effect on growth. A longer-duration trial with a concomitant comparator arm, assessing growth in standardized manner will be required for meaningful interpretation of growth assessments.

7.7.4. Pediatric Formulation and Dosing Instructions

Issue

Different formulations (strengths) have been used in Trial A4250-005 for a specific dose regimen and body weight group. Because dose escalation from 40 mcg/kg to 120 mcg/kg will be allowed, different strengths may be used by a patient while four different strengths will be available.

Whether there are any safety or efficacy issues with changing from one strength to other strength should be addressed.

Background

Odevixibat capsule is an immediate-release capsule with four strengths: 200 mcg, 400 mcg, 600 mcg, and 1200 mcg. The four strengths are developed for two different uses: 200 mcg and 600 mcg strengths are intended for oral administration after opening the capsule shell and sprinkling the content onto a food vehicle (pellets). The 400 mcg and 1200 mcg strengths are intended for direct oral administration (b) (4)

In Trial A4250-005, patients weighing 5 to <19.5 kg received odevixibat sprinkled on soft food using the 200 mcg or 600 mcg capsules for 40 mcg/kg or 120 mcg/kg dose, respectively. Patients ≥19.5 kg received odevixibat using the 400 mcg or 1200 mcg capsules for 40 mcg/kg or 120 mcg/kg dose, respectively. (b) (4)

Refer to the Office of Pharmaceutical Quality (OPQ) review for more details.

Table 34. Odevixibat Dosage Forms and Uses, Trial A4250-005

Dosage Form	Capsule size	Strength (mcg)	(b) (4)	Use in Trial A4250-005	
				Dose Group	Body Weight
Oral pellets	0	200	(b) (4)	40 mcg/kg	<19.5 kg
		600		120 mcg/kg	<19.5 kg
Oral capsules	3	400	(b) (4)	40 mcg/kg	≥19.5 kg
		1200		120 mcg/kg	>19.5 kg

Source: Tables 1 and 2 in Description and Composition of the Drug Product and Table 4 in CSR A 4250-005

Assessment

Two types of (b) (4) used for different strengths were compared by in vitro dissolution study. The provided comparative dissolution data showed similar dissolution profiles between the (b) (4). Refer to the OPQ review for more details. In addition, in Trial A4250-005, there were no appreciable differences in the systemic exposures at the 40 mcg/kg dose provided by the dosage strengths containing (b) (4), and at the 120 mcg/kg dose provided by the dosage strengths containing (b) (4).

(b) (4) capsules can be also opened and sprinkled on soft foods for administration. Compatibility with soft foods (i.e., apple sauce, baby food, yoghurt) was supported by in vitro studies. However, compatibility with liquids such as baby formula, breast milk, or water for pediatric patients who have not started solid foods was not studied. Refer to the OPQ review for more details. Especially as the indication will go down to 3 months old infants, not all infants are expected to start solid foods at that age.

Conclusion

Based on the available in vitro dissolution data and the clinical data, there are no safety or efficacy concerns for a patient to use different strengths for dose escalation. Therefore, labeling to specify the strength for a specific dose is unnecessary.

Labeling will clarify that odevixibat should not be mixed with liquids and a postmarketing commitment (PMC) will be issued to evaluate the compatibility with liquids to further support the labeling.

7.7.5. Malformations in Heart and Great Vessels in Rabbit Fetus (Nonclinical Study)

Issue

Malformations in heart and great vessels in rabbit fetuses

Background

There are no available data on odevixibat use in pregnant women. However, a reproduction study in rabbits using oral administration showed drug-related cardiovascular malformations at clinically relevant exposures during organogenesis (see [Table 35](#)). The malformations occurred in the heart and great vessels in rabbit fetuses and were observed at maternal plasma exposures (AUC) that were approximately 2.1 times the human exposure at the maximum recommended dose of 120 mcg/kg. A NOAEL for the malformations was not identified. Also, it is noteworthy

that odevixibat was shown to cross the placenta in pregnant rats following intravenous administration.

Table 35. Summary of Cardiovascular System Malformations

Dose (mg/kg/day)		10		30			100		Fetal Incidence (%)		
		Female No.	24	39	46	62	71	76	All Test Article A4250 Groups Combined N=531	Historical Control Data	
Tissue	Fetus No.	5	1	4	4	6	5	6		2008-2012 N=2745	2013-2015 N=2300
Heart	Five chambered heart	x					x		0.4	-	0.09
	Ventricular septum defect					x			0.2	-	-
	Small ventricle				x	x		x	0.6	-	0.04
	Large atrium			x					0.2	-	0.04
	Misshapen aortic valve						x		0.2	-	-
Great blood vessels	Aortic arch dilated		x	x	x	x		x	0.9	0.04	0.04
	Aortic arch right sided and retroesophageal			x					0.2	0.04	-
	Aortic arch and pulmonary trunk fused	x							0.2	-	-
	Ductus arteriosus atretic				x			x	0.4	-	0.09
	Subclavian artery absent	x							0.2	-	-

Source: Applicant's report AB21159
 Abbreviations: X, present

Assessment

The cardiovascular malformations in rabbit fetuses justify a concern for a clinically significant risk to embryo-fetal development. These findings were observed in one of the two animal species tested. The exposure multiple at which the fetal cardiovascular malformations occurred is low (approximately 2.1 times the human AUC at 120 mcg/kg). The animal data are described in the pregnancy subsection (8.1) of the labeling. A review of the literature by the Division of Pediatric and Maternal Health (DPMH) found that pregnancy has rarely been reported in patients with PFIC (review dated 04-13-2021).

Conclusion

The risk of fetal cardiovascular malformations can be described in labeling in subsection 8.1. A statement provided by DPMH in the Risk Summary of subsection 8.1 of the labeling was included to inform patients that odevixibat may cause cardiac malformations when a fetus is exposed during pregnancy, based on findings in animal reproduction studies. The exposure margin associated with fetal cardiovascular malformations in rabbits was also included in the Risk Summary. A postmarketing pregnancy registry will be requested of the Applicant.

7.7.6. Maximum Dose Selected as 6 mg

Issue

Determination of the maximum dose

Background

In Trial A4250-005, the safety and tolerability of the maximum dose, i.e., 2.4 mg or higher, is not well characterized in patients with PFIC and pruritus, with the exception of five patients who received doses ranging from 2.4 mg to 4.8 mg daily. All these patients who received odevixibat doses >2.4 mg experienced adverse reaction(s) (mostly elevation in liver tests).

Data from a phase 2 trial in adults with PBC submitted by the Applicant (Trial A4250PBC pruritus) with the NDA package and from the Al-Dury 2018 publication showed a lack of tolerability for the 0.75 and 1.5 mg dose administered daily (Investigator’s Brochure, IND 130591). Ten adult patients with PBC were treated with 0.75 mg (N=4) and 1.5 mg (N=5) for 4 weeks. Five patients discontinued from the trial prematurely due to an AE of abdominal pain and diarrhea and the authors concluded that the starting dose may have been too high for adult patients (Al-Dury et al. 2018).

Therefore, it was not clear whether doses greater than 2.4 mg should be approved.

Assessment

Safety data in healthy volunteers at >2.4 mg is limited as all healthy volunteers were administered only single dose or multiple doses for ≤7 days. About 44.8% to 76% of healthy volunteers experienced gastrointestinal AEs, mostly abdominal pain and diarrhea, mild or moderate in severity (see [Table 36](#)).

Table 36. Clinical Trials in Healthy Adults Administered Odevixibat >2.4 mg

Study	Dose	Subjects Enrolled	Notable AE
A4250-001 Phase 1 SAD/MAD	Single dose, placebo, 0.1, 0.3, 1, 3, and 10 mg	8	Most common AE was GI related AE: diarrhea, abdominal pain
	Multiple doses for 7 days 1 mg QD, 1.5 mg BID, 3 mg QD	8	N=1 at 3 mg, ALT elevation (2.5 xULN)
A4250-007 Mass balance study	Single dose 3 mg	6	
A4250-004 Food effect/sprinkle study	Single dose 9.6 mg	17	
A4250-013 DDI study	Single dose 7.2 mg w/wo itraconazole	20	
	Multiple doses, 7.2 mg QD for 4 days w/wo midazolam	21	N=1 discontinued after taking odevixibat alone due to severe abdominal pain, muscle spasms, tremor, and vomiting

Source: Table by Clinical Pharmacology Reviewer

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; BID, twice daily; DDI, drug-drug interaction; GI, gastrointestinal; MAD, multiple ascending dose; QD, once a day; SAD, single ascending dose; ULN, upper limit of normal; w/wo, with and without

Across Trials A4250-005 and A4250-008, the majority of the patients were administered odevixibat less than 2.4 mg.

A total of five (11.9%) patients in Trial A4250-005 received doses greater than 2.4 mg. Two patients received odevixibat 4.8 mg, and four patients received 3.6 mg dose. All patients dosed greater than 2.4 mg, experienced adverse events, with the most common adverse event being increase in bilirubin (consistent with increase in cholestasis), see [Table 37](#).

In Trial A4250-008, a total of 10 (11.9%) patients received doses greater than 2.4 mg, of whom the majority received the 3.6 mg or 4.8 mg dose. One patient received the 6 mg dose, and additional one patient received 7.2 mg dose. The patient who received 7.2 mg dose experienced a serious adverse event of acute pancreatitis and discontinued the drug and withdrew from the trial (see [Table 37](#)).

Therefore, 6 mg dose was selected as a maximum recommended dose. Even though doses higher than 4.8 mg were not tested in the A4250-005, the rationale for selecting 6 mg dose as the maximum dose includes:

- Odevixibat is a nonabsorbable drug that acts in the gastrointestinal lumen.
- Majority of the SAEs and AEs including liver test abnormalities, fat soluble vitamin deficiency, and diarrhea can be addressed by close monitoring, treatment interruption, and treatment discontinuation. This has been described in Section 5, Warnings and Precautions section of labeling.
- Across both the trials (A4250-005 and A4250-008) very few adult patients were enrolled. Adult patients may potentially require higher doses relative to pediatric patients. We could not find published literature to support if IBAT receptors increase in adults, potentially, requiring higher odevixibat doses. But, if there is a need to use higher doses to achieve IBAT inhibition then, approving a maximum daily dose of up to 6 mg would allow physicians to titrate the dose, as needed.

Table 37. Patients Who Received Odevixibat >2.4 mg, Trials A4250-005 and A4250-008

Patient ID	Age/ Gender/ PFIC Subtype	Daily Dose (mg)	SAE	AE	Treatment Interruption or Withdrawal
Trial A4250-005					
(b) (6)	12Y7M, Female, PFIC2	4.8		Abdominal pain (Day 1-21)	No
	9Y3M, Male, PFIC2	3.6, SVT 4.8		Liver enzyme increased	No
	12Y, Female, PFIC2	3.6		Bilirubin and INR increased	No
	10Y3M, Male, PFIC1	3.6		Bilirubin increased	Treatment interruption
	13Y3M, Female, PFIC2	3.6		Bilirubin increased; cough	No

Patient ID	Age/ Gender/ PFIC Subtype	Daily Dose (mg)	SAE	AE	Treatment Interruption or Withdrawal
Trial A4250-008					
(b) (6)	11Y4M, Male, PFIC2	4.8		Splenomegaly; frequent bowel movements; INR increased	No
	12Y10M, Female, PFIC other type	4.8		ALT increased	No
	6Y2M, Female, PFIC2	3.6		Transaminase increased; diarrhea; gastroenteritis	Treatment interruption
	3Y3M, Male, PFIC2	3.6		UTI	No
	11Y7M, Male, PFIC1	3.6		No	No
	11Y3M, Female, PFIC3	3.6, 4.8		Bilirubin increased; INR increased	Treatment interruption
	13Y4M, Female, PFIC3	4.8		Bilirubin, ALT/AST increased	Treatment interruption x3
	15Y, Female, PFIC2	6.0		Bilirubin increased, toothache	No
	19Y7M, Female, PFIC2	7.2	Acute pancreatitis	No	Treatment withdrawn
	8Y2M, Female, PFIC2	3.6		No	No

Source: Generated by the clinical reviewer from Patient Journey and adam dataset

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; INR, international normalized ratio; M, months; PFIC, progressive familial intrahepatic cholestasis; SAE, serious adverse event; SVT, supraventricular tachycardia; UTI, urinary tract infection; Y, years

Conclusion

Data for safety and efficacy of doses greater than 2.4 mg are very limited (only five patients received doses greater than 2.4 mg). Data from Trial A4250PBC pruritus demonstrated tolerability issues with doses >1.5 mg used in adult patients with PBC. The review team recommend capping the maximal dose at 6 mg/day, given the case of acute pancreatitis during administration of a dose higher than 6 mg/day.

8. Therapeutic Individualization

8.1. Intrinsic Factors

8.1.1. Pathogenicity Determination for Patients With PFIC1/2 and Bile Salt Export Pump Subtypes

Odevixibat is not recommended in patients with BSEP3 variants (subpopulation within PFIC2 population).

There were no patients with BSEP3 in Trial A4250-005, with variants leading to a predicted, nonfunctional protein. This is consistent with the key exclusion criteria to exclude patients with pathologic variations of the *ABCB11* gene that predict complete absence or lack of function of the BSEP protein in Trial A4250-005.

Patients with BSEP3 variants lack functional BSEP in canalicular membrane to export bile salts to bile for enterohepatic circulation via biliary excretion; therefore, the pharmacological effects of odevixibat to inhibit the reabsorption of bile salts in the gastrointestinal tract cannot be expected.

Clinical genetic classification was performed for all patients enrolled in Trial A4250-005.

Classification was based on historical lab results in 81% (50/62) of patients and genetic testing was performed in a central laboratory (b) (4) in 19% (12/62) of patients, who did not have a historical clinical genetic report ([Table 38](#)). According to the Applicant, a single central expert reviewed the genetic reports and classified the variants based on the American College of Medical Genetics and Genomics criteria (Clinical Pharmacology IR, January 07, 2021). FDA conducted pathogenicity evaluation and confirmed variant pathogenicity for all patients enrolled in Trial A4250-005, based on evidence from human variant databases, literature and in silico prediction tools recommended by the American College of Medical Genetics and Genomics criteria. Refer to Section [14.4.3](#) for the detailed description of variants identified in *ABCB11* and *ATP8B1* genes and pathogenicity determination in Trial A4250-005.

For patients with PFIC1 (N=17), 18 unique variants in the *ATP8B1* gene were identified. Nonsense, large deletion variants and frameshift represented most variants in patients with PFIC1 (58.8%). The p.Ala1208ProFsTer (17.7%), a deletion variant, and p.Gly308Val (17.7%), a missense variant were the most common variants for patients with PFIC1. For patients with PFIC2 (N=45), 47 unique variants in the *ABCB11* gene were identified. The most frequent variants in patients with PFIC2 were c.890A > G p. E297G (12.2%) and c.1445A > G p.D482G (7.8%).

Patients with PFIC2 were categorized in BSEP subtypes based on specific variants. Twelve patients were categorized into BSEP1 with at least one p.D482G (c.1445A > G) or p.E297G (c.890A > G) variant. Thirty-three patients were categorized into BSEP2 with at least one missense variant, which was not p.D482G or p.E297G. The distribution of patients with BSEP1 and BSEP2 were similar in the United States, although numbers are small ([Table 39](#)). The percentage of patients with BSEP2 compared to BSEP1 were higher in “Rest of World” (i.e., non-U.S., non-EU). The percentage of patients with BSEP1 were higher in the European Union,

compared with patients with BSEP2. No formal statistical test was performed as the sample size were small for each subtype.

Table 38. Regional Distribution for Genetic Testing Methods, Trial A4250-005

Testing Method Region	Number of Patients N=62
Historical Lab Data	
European Union	33
Rest of World	12
U.S.	5
Testing performed at (b) (4) ¹	
European Union	2
Rest of World	7
U.S.	3

Source: Reviewer's table.

¹ (b) (4) was the central laboratory, which performed the genetic testing for patients where a historical clinical genetic report was not available.

Table 39. BSEP Subtypes Distribution in Regions for Patients With PFIC2, Trial A4250-005

BSEP Subtypes	EU N=25 n (%)	RoW N=16 n (%)	U.S. N=4 n (%)	Total N=45 n (%)
BSEP1	8 (66.7)	3 (25.0)	1 (8.3)	12 (100)
BSEP2	17 (51.5)	13 (39.4)	3 (9.1)	33 (100)

Source: Reviewer's table.

Abbreviations: BSEP, bile salt export pump; EU, European Union; PFIC, progressive familial intrahepatic cholestasis; RoW, rest of the world

8.1.2. Body Weight and Age

The total daily dose of odevixibat should be determined based on a patient's body weight per the recommended dosing table targeting 40 mcg/kg/day for patients ≥ 3 months of age. The dose may be increased up to 120 mcg/kg/day for nonresponders; however, should not exceed 6000 mcg. Total daily dose of odevixibat should increase as pediatric patients gain weight.

Patients weighing < 19.5 kg will receive oral pellets to be sprinkled on soft food whereas patients ≥ 19.5 kg will receive (b) (4) that can be either taken as whole or sprinkled on soft food.

Based on the minimal systemic absorption and the body weight-based dosing, significant effect of age or body weight on the systemic exposure to odevixibat is not expected although the effect of age or body weight on PK of odevixibat could not be fully evaluated due to the limited PK data.

Pediatric Patients < 6 Months of Age

Efficacy and safety in patients with PFIC < 6 months of age have not been evaluated. The literature has reported that symptoms and signs of PFIC including pruritus can occur within 0-3 months from birth (Baker et al. 2019). The literature (de Belle et al. 1979) suggests that bile acid transport in the ileum partially functional in neonates compared to pediatrics older than 8 months. While the ontogeny of IBAT activity in infants is not fully characterized, maturation of intestinal transport mechanisms resulting in normal cycling of bile salts by 3 to 7 months of age was suggested (Heubi et al. 1982).

Considering the unmet medical needs in infants with PFIC and the safety data available in the patients 6 months to 1 year of age receiving odevixibat up to 120 mcg/kg (Trials A4250-005 and A4250-008), use of odevixibat in pediatrics 3 to <6 months with 40 mcg/kg as the same dose for pediatric patients \geq 6 months is acceptable (see also Section [6.1](#), [6.3.5](#)).

8.1.3. Hepatic Impairment

Patients with PFIC may have hepatic impairment because the accumulation of the components of bile within the liver, including bilirubin and bile acids, can lead to portal hypertension, liver failure, cirrhosis, and hepatocellular carcinoma.

The patients with PFIC in Trial A4250-005, showed elevated hepatic biochemistry at baseline. The median values of hepatic biochemistry included ALT (65 U/L, approximately $2 \times$ ULN), AST (83.5 U/L, less than $2 \times$ ULN), and total bilirubin (36.8 micromole/L; 2.2 mg/dL, $1.8 \times$ ULN) (See Section [7.7.1](#)). The efficacy and safety of odevixibat in such patients with elevations in ALT and total bilirubin are supported by Trial A4250-005.

The presence of portal hypertension and cirrhosis was assessed based on clinical, imaging, and biochemical evidence with data submitted to Trial A4250-005. However, since there were only two patients with portal hypertension (see Section [7.6.1.10](#)), the safety and efficacy of odevixibat in patients with portal hypertension and decompensated cirrhosis could not be evaluated and remains unknown.

No dedicated PK trial in patients with cirrhosis (hepatic impairment) was conducted. Of note, systemic exposure to odevixibat was mostly below the lower limit of quantification (LLOQ) or very low in a few plasma samples in patients with PFIC in Trial A4250-005. Due to limited PK data, the effect of abnormal hepatic biochemistry (hepatic impairment by NCI criteria) on drug exposure could not be fully evaluated (see Section [14.2.6](#) for further PK results in A4250-005). However, given the limited systemic absorption of odevixibat and the pharmacological action in the ileum, the effect of hepatic impairment on systemic exposure to odevixibat is not expected to be clinically significant. Dose adjustment by hepatic impairment may not be necessary.

8.1.4. Renal Impairment

There are neither dedicated clinical studies in patients with renal impairment nor clinical data from patients with PFIC who have renal impairment. In Trial A4250-005, pediatric patients with PFIC showed normal range of renal function at baseline with median (range) estimated glomerular filtration rate of 166 (78.4 – 229.9) mL/min/1.73 m² estimated by the modified Schwartz equation.

Given the limited systemic absorption and negligible renal excretion of odevixibat (<0.002% of the administered dose) per the mass balance trial (A4250-007), it is unlikely that renal impairment would affect the systemic exposure to odevixibat significantly. Dose adjustment by renal impairment is unnecessary.

8.2. Drug Interactions

8.2.1. Bile Acid Binding Resins

Patients taking bile acid binding resins should take odevixibat at least 4 hours before or 4 hours after taking a bile acid binding resin. Bile acid binding resins (e.g., cholestyramine, colesevelam, colestipol) may bind odevixibat in the gut if odevixibat and bile acid binding resins are given concurrently and may potentially reduce efficacy of odevixibat. In Trial A4250-005, concomitant bile acid binding resins were prohibited due to potential influences on the efficacy assessment.

In Trial A4250-001, when healthy volunteers took Questran (cholestyramine) 1 g twice daily at 4 hours and 14 to 15 hours after taking odevixibat 3 mg once daily in the morning for 7 days, the decrease in serum total bile acid level was similar compared to when odevixibat 3 mg was given alone once daily (see Section [14.2.1](#)).

8.2.2. UDCA and Rifampicin

UDCA and rifampicin are commonly prescribed in patients with PFIC, i.e., off-label use. Drug interaction potential between odevixibat and UDCA and/or rifampicin has not been evaluated.

In Trial A4250-005, the patients were allowed to continue taking UDCA and rifampicin concomitantly; out of 62 patients, 55 (89%) were receiving UDCA and/or rifampicin at study entry with 50 patients (81%) on UDCA and 41 (66%) on rifampicin. Trial A4250-005 supports the safety and efficacy of odevixibat in patients with PFIC who concomitantly take UDCA and/or rifampicin.

8.2.3. Drug Interaction Via Drug Metabolizing Enzymes and Drug Transporters

Odevixibat as a Victim Drug

Odevixibat is a P-glycoprotein (P-gp) substrate. When odevixibat was administered with itraconazole (a strong P-gp inhibitor), odevixibat maximum plasma concentration (C_{max}) and AUC was increased by 52% and 66%, respectively. In the phase 2 trial, odevixibat was administered at 200 mcg/kg for 4 weeks to pediatric patients with cholestatic liver disease with measurable systemic exposure but without notable AEs. It is not deemed clinically significant and dose adjustment is not required for coadministration with P-gp inhibitors (see Section [14.2.4](#)).

Other than that, there is no evidence suggesting drug interaction of odevixibat as a victim drug at the clinically relevant exposure (see Section [14.1](#)).

Odevixibat as a Perpetrator Drug

Based on potential inhibition of intestinal CYP3A4 by odevixibat at 7.2 mg, an in vivo drug-drug interaction study with midazolam (a CYP3A4 substrate) was conducted. When midazolam was coadministered orally with odevixibat following multiple doses of 7.2 mg odevixibat once daily for 4 days, mean AUC of midazolam and its metabolite, 1-OH midazolam were decreased by 33% and 13%, respectively. It was not considered clinically significant (see Section [14.2.4](#)).

Other than that, there is no evidence that odevixibat influences on pharmacokinetics other drugs (see Section [14.1](#)).

8.3. Plans for Pediatric Drug Development

Not applicable; this program is being developed in all pediatric populations affected by PFIC.

Animal Data

In the juvenile rat toxicity study, animals were treated orally with 10, 30, or 100 mg/kg/day from postnatal day (PND) 14 to 64 and sacrificed on PND 65. The human age equivalent at the initiation and termination of dosing was approximately one year and 14 years, respectively. Animals in the main study were evaluated for general toxicity parameters. Long bone growth, bone mineral content, area and density of the left femur, and lumbar vertebral column were also evaluated. In subsets of animals, the following fertility parameters in females were evaluated: pregnancy status, corpora lutea, and implantations (early resorptions, dead embryos, or live embryos). The following fertility parameters in males were evaluated: sperm concentration, motility, and morphology. Behavioral performance which included learning potential, auditory function, and motor activity was evaluated towards the end of the treatment period. In general, there were limited drug-related findings after administration in juvenile rats. No drug-related deaths occurred. Loose feces occurred in the 100 mg/kg/day group during PND 15 through approximately PND 21 (weaning). No other toxicologically meaningful effects were observed; therefore, the NOAEL was 100 mg/kg/day. Additional information regarding the juvenile animal study can be found in Section [13](#).

Table 40. Juvenile Toxicity Safety Margins

Study	Nonclinical Data	NOAEL (mg/kg/day)	Nonclinical Exposure (ng•hr/mL)	Safety Margins ¹ (Multiples)
Rat (juvenile) 51 days (PND 14-64)	Loose feces observed in the 100 mg/kg/day group from PND 15 to PND 21 (weaning)	100	361	123

Source: Reviewer's table

Abbreviations: AUC, area under the concentration-time curve; NOAEL, no observed adverse effect level; PND, postnatal day; QD, once a day

¹ Exposure multiples were based on population pharmacokinetics analysis where the maximum recommended dose of 0.12 mg/kg QD resulted in $AUC_{0-24\text{ hr}}=2.94\text{ ng}\cdot\text{hr/mL}$.

8.4. Pregnancy and Lactation

Animal Data

The following nonclinical information was used in support of the labeling (Package Insert) and formed the basis for a pregnancy registry PMR. Additional details are available in Section [13](#).

Table 41. Nonclinical Data Supporting Labeling on Fertility, Pregnancy and Lactation

Labeling Section	Nonclinical Data
8.1 Pregnancy	<p>In an embryo-fetal development study, pregnant rabbits received oral doses of 10, 30, or 100 mg/kg/day odevixibat during the period of organogenesis. Fetuses from all maternal groups treated with odevixibat showed a nondose dependent increase in cardiovascular malformations, which included 5-chambered heart, small ventricle, large atrium, ventricular septum defect, misshapen aortic valve, dilated aortic arch, right sided and retroesophageal aortic arch, fusion of aortic arch and pulmonary trunk, ductus arteriosus atresia, and absence of subclavian artery. Cardiovascular defects were not observed in the control group, and the cardiovascular malformations occurred at a higher incidence than expected for normal background incidence. This indicates a potential drug-related teratogenic effect. Maternal systemic exposure to odevixibat at the lowest dose tested was 6.28 ng•hr/mL in rabbits. Therefore, the malformations occurred at doses 2.1 times the maximum recommended dose and higher, based on AUC (area under the plasma concentration-time curve).</p> <p>No adverse effects on embryo-fetal development were observed following oral administration of 100, 300, or 1000 mg/kg/day in pregnant rats during organogenesis. A small number of fetuses with external, visceral, and/or skeletal malformations were noted in the 100 and 1000 mg/kg/day groups. These findings were not attributed to drug administration due to their isolated incidence and observation in historical data. No malformations were observed in the 300 mg/kg/day group. Drug-related skeletal variations were observed at 1000 mg/kg/day, which included a higher incidence of delayed ossification of several bones (i.e., squamosal, metacarpal, sternebra, and caudal vertebral arches) and thick ribs, as compared to the concurrent control and historical control data. These findings were previously shown to be transient and do not result in permanent structural changes. Therefore, they are not considered adverse. Maternal systemic exposure to odevixibat at the maximum dose tested was 799 ng•hr/mL in rats.</p> <p>No adverse effects on postnatal development were observed in a pre- and postnatal development study, in which female rats were treated orally with up to 1000 mg/kg/day during organogenesis through lactation. The maternal AUC for odevixibat at 1000 mg/kg/day was 1274 ng•hr/mL.</p>
8.2 Lactation	<p>Although the pre- and postnatal development study in rats produced clear evidence of odevixibat excretion in milk, the relevant data was not included in the label for reasons explained here. Odevixibat was detected in rat pup plasma at all tested doses, which was likely due to transfer from rat milk rather than prenatal exposure. However, the lactating rats were given doses of odevixibat at human exposure (AUC) multiples that were 34 to 434 times the maximum recommended dose. Therefore, the clinical significance of this finding is uncertain.</p>
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility	<p>There were no effects on fertility or reproductive function in male and female rats at oral doses of up to 1000 mg/kg/day.</p>

Source: Reviewer's table

Abbreviations: AUC, area under the concentration-time curve

Table 42. Reproductive Toxicity Safety Margins

Study	NOAEL (mg/kg/day)	Nonclinical Exposure (ng•hr/mL)	Safety Margins ¹ (Multiples)
Fertility rat	1000	Males: 4149 Females: 3038	1413 1034
EFD rat	Fetal: 1000 Maternal: 1000	Maternal: 799	272
EFD rabbit	Fetal: not identified Maternal: 10	Maternal: 6.28	Not established
PPND rat	F1 offspring: 1000 Maternal: 1000	Maternal: 1274	434

Source: Reviewer's table

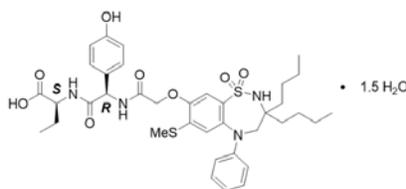
Abbreviations: EFD, embryo-fetal development; NOAEL, no observed adverse effect level; PPND, pre- and postnatal development

¹ Exposure multiples were based on population pharmacokinetics analysis where the maximum recommended dose of 0.12 mg/kg QD resulted in $AUC_{(0-24 \text{ hr})} = 2.94 \text{ ng}\cdot\text{hr/mL}$

9. Product Quality

The drug substance in BYLVAY is odevixibat sesquihydrate. It is a white to off-white solid. It is a chiral molecule with two chiral centers and produced (b) (4)

It is insoluble in low pH but its solubility increases in pH above 5.



Molecular formula: $C_{37}H_{48}N_4O_8S_2$
Molecular weight: 768.0 g/mol

Odevixibat sesquihydrate is manufactured, tested, and packaged (b) (4). The Applicant has provided satisfactory chemistry, manufacturing, and controls (CMC) information regarding the raw materials, intermediates, drug substance manufacturing process, critical steps, and in-process controls. Its chemical structure was determined by various modern spectroscopic methods as well (b) (4). The identity, strength, purity, and quality of odevixibat sesquihydrate is controlled by its specification.

Based on the long-term and accelerated stability testing per International Conference for Harmonisation Q1A(R2) of registration and supporting batches of drug substance a retest period of (b) (4) months is granted when stored at (b) (4)

Odevixibat is an IBAT inhibitor and is supplied in two dosage forms: oral pellets and capsules. 200 mcg and 600 mcg oral pellets are filled in size 0 capsules. 400 mg and 1200 mg capsules are supplied as size 3 capsules. Both dosage forms contain the following ingredients: drug substance odevixibat sesquihydrate, hypromellose and microcrystalline cellulose. Four different strengths of capsules are different in color and imprinted with unique codes for easy identification. The capsule contents can be mixed with soft food such as applesauce, baby food, yogurt, etc.

The overall quality of each strength of the drug product is controlled by its specification. The release and stability specification include the following tests: description, identity, assay, degradation products, microbial limits, uniformity of dosage and dissolution.

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Based on the satisfactory long-term and accelerated stability results a 24-month expiration dating period from the date of the (b) (4) is granted when the drug products are stored at 20 to 25°C (68 to 77°F) with excursions permitted to 15 to 30°C (59 to 86°F).

The Applicant has provided sufficient CMC information to assure the identity, strength, purity, and quality of the proposed BYLVAY (odevixibat) capsules and BYLVAY (odevixibat) oral pellets.

The Office of Pharmaceutical Manufacturing Assessment (OPMA) has made a final overall “Approval” recommendation for the facilities involved in this application.

The claim for the Categorical Exclusion for the Environmental Assessment is granted.

The label/labeling is satisfactory from the CMC perspective.

Therefore, from the OPQ perspective, this NDA is recommended for approval.

To optimize the dissolution method for quality control we will request that the Applicant to commit to address the deficiencies as a PMC and submit the information with the first annual report. Refer to the OPQ review for more details.

9.1. Device or Combination Product Considerations

Not applicable

10. Human Subjects Protections/Clinical Site and Other Good Clinical Practice Inspections/Financial Disclosure

The Applicant stated that “The study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki and with good clinical practice guidelines as denoted in the International Conference for Harmonisation E6 requirements. These practices included Institutional Review Board/Independent Ethics Committee procedures, informed consent, protocol adherence, administrative documents, drug supply accountability, data collection, patient records (source documents), adverse event recording and reporting, inspection and audit preparation, and record retention. The investigator was made aware that regulatory authorities and representatives of the Applicant could inspect the documents and patient records at any time. All patient identities were kept confidential. Each patient was assigned a unique patient number, which in turn was used in the electronic case report form (eCRF) instead of the patient’s name.”

The Office of Scientific Investigations inspected two clinical investigator sites who participated in Trial A4250-005 as well as an inspection of the Applicant Albireo Pharma, Inc. There were no concerns identified and the efficacy and safety data in this NDA appear reliable.

Financial Disclosures

See Section [23](#).

11. Advisory Committee Summary

An Advisory Committee meeting was not held for this NDA because the evaluation of the application did not raise significant safety or efficacy issues that were unexpected in the indicated population.

III. Appendices

12. Summary of Regulatory History

Table 43. Summary of Regulatory History

Date	Activity	Outcome
4/30/2012	Orphan Drug Designation request submitted to the Agency	Orphan Drug Designation granted for treatment of progressive familial intrahepatic cholestasis on 10/31/2012
	(b) (4)	
5/12/2016	IND 130591 for A4250 was submitted for the treatment of (b) (4)	Safe to Proceed
1/22/2018	Type A, meeting to discuss the drafted phase 3 protocol	The Agency provided guidance on the primary endpoint and the PRO and ObsRO tools used in the proposed phase 3 trial
7/18/2018	Fast Track Designation request	Fast Track Designation granted 9/14/2018
10/30/2018	Type C: Chemistry, Manufacturing and Controls (CMC) data to support future NDA filing for A4250	FDA communicated that the information needed to support the qualification of impurities may be submitted in the NDA. The final determination of the drug product specification will be based on the thorough review of the data provided in this NDA.
2/20/2019	Type C, Guidance to discuss the Agency's 4/5/2018 advice/Information Request letter about the protocol for Trial A4250-005 (pivotal phase 3 trial), particularly the primary endpoint and its statistical analysis	FDA communicated concerns with the proposed primary endpoint (the proportion of positive pruritus assessments (AM & PM) at the subject level over the 24-week treatment period, where a positive pruritus assessment is defined as a scratching score of ≤ 1 or at least a one-point drop from baseline on the Albireo ObsRO instrument). While it appeared to be a reasonable way to capture itching episodes across the entire treatment period the Applicant did not provided evidence that a one-point drop from baseline is clinically meaningful. The FDA recommended measuring serum bile acid levels by an adequately validated assay.
3/31/2020	Treatment protocol titled Odevixibat (A4250) for the Treatment of Progressive Familial Intrahepatic Cholestasis (Expanded Access Program)	Expanded access protocol deemed safe to proceed.
5/21/2020	Proprietary name submitted BYLVAY	11/17/2020 conditional approval was granted.
10/19/2020	Type B, pre-NDA meeting request to discuss the planned NDA for odevixibat for progressive familial intrahepatic cholestasis (PFIC).	The Agency communicated comments on the sufficiency of the safety database and that a rationale on the applicability of the trial data to the United States population would be needed for sine the pivotal studies were conducted outside the US. The Agency communicated that they did not agree that there are adequate data to support an indication "for the treatment of PFIC."

Source: Generated by reviewer based on review of interactions with the Applicant

Abbreviations: ObsRO, observer-reported outcome; PFIC, progressive familial intrahepatic cholestasis; PRO, patient-reported outcome; FDA, Food and Drug Administration

13. Pharmacology Toxicology: Additional Information and Assessment

13.1. Summary Review of Studies Submitted Under the IND

13.1.1. Pharmacology (Primary and Secondary)

Table 44. Primary and Secondary Pharmacology Studies

Study/Study No.	Findings
The ED₅₀ of AZD8294¹ on ⁷⁵SeHCAT (Taura-23-selena-25-homochoholic acid) Intestinal Absorption in ApoE Knockout Mice In Vivo (24546) Mouse/ApoE knockout 3/females/group Dose: 0.625, 0.156, 0.039 or 0.01 mcmol/kg (micromoles/kg) Route of administration and dosing frequency: oral, single	AZD8294 (odevixibat) inhibited intestinal bile acid absorption in ApoE knockout mice in vivo. The ED ₅₀ was calculated to be 0.073 mcmol/kg. Up to 91% of the total recovered radiolabeled bile acid marker was measured in feces at 24.5 hours after oral odevixibat administration (compared to 37% after vehicle administration), while 9% was measured in the gall bladder/intestine (considered % body retention).
AR-H064974 (AZD8294), AR-H073582, AR-H064965 and AR-H073559: Lipid Lowering Effect in ApoE/LDL-Receptor Knockout Mice In Vivo (24052-23) Mouse/ApoE/LDL-receptor double knockout 7/group Dose: 0.625 mcmol/kg Route of administration and dosing frequency: oral, once daily for 8 days	AZD8294 reduced plasma cholesterol levels by 40% in ApoE/LDL-receptor double knockout mice, a model of hypercholesterolemia and atherosclerosis. Reduction of cholesterol was mainly due to the VLDL (45-61% reduction) and LDL (7-24% reduction) fractions.
AZD8294, Duration of the Inhibitory Effect on the Intestinal Bile Salts in ApoE Knockout Mice In Vivo (24872) Mouse/ApoE knockout 3 females/group Dose: 0.625 mcmol/kg Route of administration and dosing frequency: oral, single	Inhibition of intestinal bile salt absorption (as measured using a radiolabeled bile acid marker) was around 83% up to 3 hours after AZD8294 administration and diminished to 28% at 10 hours after AZD8294 administration.
AZD8294 In Vitro Potency and Selectivity Studies Performed Within AstraZeneca (25881) Potency was measured using human, mouse, or dog IBAT expressed in HEK293 cells. Specificity was tested using cells expressing the homologous human liver (basolateral) bile acid transporter and by measuring the uptake of α-aminoisobutyric acid by the endogenous neutral amino acid transporter.	The IC ₅₀ values for AZD8294 were 0.13 nmol/L, 0.12 nmol/L, and 1.4 nmol/L for inhibition of human, mouse, and dog IBAT, respectively. The IC ₅₀ for inhibition of human liver (basolateral) bile acid transporter was 93 nmol/L. Potency of AZD8294 for inhibition of the human ileal (apical) transporter was over 700-fold higher than inhibition of the homologous human liver (basolateral) sodium/bile acid cotransporter. Inhibition of amino acid uptake via the neutral amino acid transporter(s) in HEK293 cells was at least 1000 times lower than that of bile acid transport via the human ileal bile acid transporter.

Study/Study No.	Findings
A4250*, Substance B, Substance C Fecal Evaluation Study in Dogs (74519) Dog/Beagle 4 animals/crossover design Dose: 30 mg/kg Route of administration and dosing frequency: oral, single	Rectal administration of cholestyramine suspension in dogs administered with oral A4250 (odevixibat) generally resulted in feces with normal consistency scores compared to very soft to watery feces observed in dogs administered oral odevixibat and rectal placebo. These observations indicate that fecal changes caused by odevixibat were directly mediated by increased bile acid levels in the large intestine.
Charcoal Propulsion Test in the Rat After Single Oral Administration (AA19205) Rat/Wistar 10 males/group Dose: 1, 10, or 100 mcmol/kg Route of administration and dosing frequency: oral, single	In a charcoal propulsion test, AZD8294 did not significantly alter intestinal transit, compared to vehicle-treated rats. Mean intestinal transit was 60.2% in the vehicle control group and 61.0%, 65.6%, and 65.7% in the AZD8294 treated groups (1, 10, and 100 mcmol/kg, respectively).
IBAT/ASBT Inhibition With A4250 in the Mdr2 KO Model (ARR4250000117) Mouse/ Mdr2-/- mice 5-6 males/group Dose: 0.03% (w/w) Route of administration and dosing frequency: dietary administration for 4 weeks	A4250 (odevixibat) reduced liver/body weight ratios and serum markers of liver damage and cholestasis in Mdr2-/- mice, a mouse model of cholestasis.
AZD8294, Selectivity Studies Performed at an External Contract Research Laboratory In Vitro (25882) The bioselectivity of AZD8294 was tested in a panel of 17 enzyme and binding assays along with two tissue models (L-type calcium channel and sodium channel site 2).	AZD8294 (1 micromole/L) showed 66% inhibition of the protein serine/threonine kinase, ERK2, in a secondary pharmacology screen. However, no significant effect (>50%) was detected at any concentration, when odevixibat was retested at 0.1 to 10 micromoles/L (74.09 to 7409 mcg/L) in the ERK2 assay.

Source: Table prepared by nonclinical reviewer

Abbreviations: ApoE, apolipoprotein E; ERK, extracellular signal-regulated kinase; hERG, the human Ether-à-go-go-Related Gene; IBAT, ileal bile acid transporter; IC₅₀, half maximal inhibitory concentration; LDL, low-density lipoprotein; ObsRO, observer-reported outcome; PFIC, progressive familial intrahepatic cholestasis; PRO, patient-reported outcome

¹ AZD8294 and A4250 are code names for odevixibat.

13.1.2. Safety Pharmacology

Table 45. Safety Pharmacology Studies

Study/Study No.	Findings
Study Report For AR-H064974¹ (odevixibat) and AR-H064965: The Effect on hERG Potassium Channel (0062SZ)	Odevixibat had no effect on hERG potassium currents at 1 micromole/L (740.9 mcg/L). hERG potassium currents in odevixibat-treated cells were at least 100% of control-treated cells.
Evaluation of the Hemodynamic Effects Following Intravenous Dosing in the Anesthetized Dog (20040107PCC) Dog/Beagle 6/sex/group Dose: 0.001, 0.01, or 0.1 mcmol/kg (0.741, 7.41, or 74.09 mcg/kg) Route of administration and dosing frequency: single intravenous infusion	At 0.001 mcmol/kg, there was a slight but statistically significant increase in QTc interval (Bazett's formula). At 0.01 mcmol/kg, a slight but statistically significant decrease in PQ interval duration was observed, lasting from 12.5 to 27.5 minutes after the start of infusion. Due to the absence of a clear dose-dependency, these changes were not attributed to drug administration.

Study/Study No.	Findings
Evaluation of Effects on Blood Pressure and Heart Rate After Single Oral Administration to Conscious Rats (20040105PCR) Rat/Wistar 6 males/group Dose: 1, 10, or 100 mcmol/kg Route of administration and dosing frequency: oral, single	There were no statistically significant changes in arterial blood pressure or heart rate during the 24-hour period of measurement after dosing. Heart rate decreased up to -13% and -11% in the 10 and 100 mcmol/kg groups, respectively, compared to the control group at 20 hours postdose. However, given the low magnitude of change and isolated occurrence, this change was not considered adverse.
Evaluation of Effect on Respiration in the Unrestrained Conscious Rat Following Single Oral Administration (20040106PCR) Rat/Wistar 8 males/group Dose: 1, 10, or 100 mcmol/kg Route of administration and dosing frequency: oral, single	There were no adverse changes in respiratory parameters (rate, peak inspiratory and expiratory flows, inspiration and expiration times, airway resistance, tidal volume, and minute volume) during the 4-hour period of measurement at doses up to 100 mcmol/kg.
Effect On Spontaneous Locomotor Activity in the Rat Following Single Oral Administration (20040102PGR) Rat/Wistar 8 males/group Dose: 1, 10, or 100 mcmol/kg Route of administration and dosing frequency: oral, single	There was a slight decrease in locomotor activity at ≥ 10 mcmol/kg which was not considered adverse due to the low magnitude of change.
Evaluation of Any Possible Effect on Motor Coordination in the Rotarod Test in the Rat Following Single Oral Administration (20040103PGR) Rat/Wistar 10 males/group Dose: 1, 10, or 100 mcmol/kg Route of administration and dosing frequency: oral, single	There was no effect on motor coordination, as evaluated in the rotarod test.
Behavioral Irwin Test and Effect on Body Temperature Following Single Oral Administration in the Rat (20040104PGR) Rat/Wistar 8 males/group Dose: 1, 10, or 100 mcmol/kg Route of administration and dosing frequency: oral, single	There were no adverse effects observed in the behavioral Irwin test or on body temperature.
Evaluation of Effect on Urine Output, Electrolyte Balance, Glomerular Filtration Rate in the Rat With Saline Overload Following Single Oral Administration (20040181PGR) Rat/Wistar 8 males/group Dose: 1, 10, or 100 mcmol/kg Route of administration and dosing frequency: oral, single	There were no drug-related effects on urine output, urinary pH, electrolyte balance, or glomerular filtration rate.

Source: Table prepared by nonclinical reviewer

Abbreviations: hERG, the human Ether-à-go-go-Related Gene; PQ, performance qualification; QTc, QT interval adjusted for the patient's heart rate

¹ AR-H064974 is a code name for odevixibat.

13.1.3. ADME/PK

Table 46. ADME/PK Studies

Study/Study No.	Findings
<p>AZD8294: Pharmacokinetic Study in the Marmoset (D8294 KPP001) Male and female marmosets were given a single intravenous dose of 10 mcmol/kg AZD8294 and a single oral dose of 25 mcmol/kg AZD8294.</p>	<p>After IV administration of 10 mcmol/kg in marmosets, the AUC was 22400 nmol•hr/L. The total plasma clearance was estimated to 7.5 mL/min/kg and $t_{1/2}$ was at 8.6 hours. The volume of distribution was 0.9 L/kg. After oral administration of 25 mcmol/kg, the AUC was 518 nmol•hr/L and the $t_{1/2}$ was estimated to be 3.3 hours. The C_{max} was 93.6 nmol/L and was observed at 4 hours after administration. The oral bioavailability was calculated to be 0.9%.</p>
<p>[¹⁴C]AZD8294: Quantitative Whole-Body Autoradiography Study on Distribution of Radioactivity in Rats After Single Intravenous or Oral Administration (0667PR) A single dose of 2.5 mcmol/kg (5 MBq/kg) [¹⁴C]AZD8294 was given intravenously to Wistar Hannover Galas (albino) and Long Evans black hooded male rats. A single oral administration of 5 mcmol/kg (10 MBq/kg) was given to albino male rats. Pregnant albino rats received a single intravenous dose of 2.5 mcmol/kg (5 MBq/kg).</p>	<p>After IV administration, [¹⁴C]AZD8294 and/or its metabolites were distributed throughout the body. Maximal levels were reached in most tissues after 5 minutes, with high concentrations in bile and liver, followed by blood. Radioactivity markedly decreased in all tissues after one hour, except in certain parts in the skin of the neck and in the pineal body of the CNS. Within 24 hours, radioactivity was detected only in the wall of small intestine, bile, liver, skin, and gut content due to excretion in bile. After 7 days, radioactivity was no longer detected. After oral administration, [¹⁴C]AZD8294 and/or its metabolites were poorly absorbed. The concentration in blood was below the detection limit throughout the study. Most of the radioactivity was found in the content of the GI tract whereas none was detected in the CNS. In pregnant rats given [¹⁴C]AZD8294 IV on Day 18 of gestation, the radioactivity was rapidly distributed throughout the body of the dam (including the placenta and amnion membrane). Radioactivity passed through the placenta and was detectable in low concentrations at 4 hours postinjection in the fetal liver only.</p>
<p>In Vitro Binding of AZD8294¹ to the Plasma Proteins of Rat, Dog, Mouse, Marmoset, Rabbit, and Man (178257) Plasma protein binding (mouse, rat, dog, rabbit, marmoset, and human) was determined by equilibrium dialysis at actual concentrations of 4 and 40 micromoles/L following incubation at 37°C for 2 hours.</p>	<p>The levels of AZD8294 were below the limit of detection (0.012 micromoles/L) in the buffer compartments for all matrices, indicating that the free fraction of AZD8294 was very low (<0.3%). Therefore, protein binding was greater than 99.7% (>99.7% at 4 micromoles/L and >99.97% at 40 micromoles/L) in mouse, rat, dog, rabbit, marmoset, and human plasma.</p>
<p>In Vitro Assessment of Plasma Protein Binding and Red Blood Cell Partitioning of A4250 (XS-1439) The in vitro plasma protein binding and red blood cell distribution of A4250 at 1.35 nmol/L was evaluated in mouse, rat, rabbit, dog, marmoset, and human plasma. Plasma protein binding was assessed by the ultracentrifugation method. Red blood cell distribution was assessed by incubating the blood of each species containing A4250 at 37°C for 15 min. The samples were analyzed by LC-MS/MS.</p>	<p>The plasma protein binding of A4250 in mouse, rat, rabbit, dog, marmoset, and human plasma was >99.6%, >99.6%, 98.0%, 99.4%, 99.2%, and 99.4%, respectively, indicating high plasma protein binding in all species tested. The blood cell/plasma partition coefficient (Kb/p) of A4250 in mouse, rat, rabbit, dog, marmoset, and human was 0.159, 0.151, 0.076, 0.091, 0.136, and 0.080, respectively. These results indicated that A4250 is generally excluded from the blood cell fraction of each species.</p>

Study/Study No.	Findings
A4250: Metabolism by Freshly Isolated Rat, Mouse, Dog, and Human Hepatocytes (ALB/05) ¹⁴ C]A4250 at a final concentration of 10 micromoles/L and nonradiolabeled A4250 at a final concentration of 1 micromole/L were incubated with freshly isolated hepatocytes (rat, mouse, dog, and human donors 1 and 2) and cryopreserved hepatocytes (human donor 3) for up to 4 hours at 37°C. Incubation samples were analyzed by HPLC with on-line radio-detection and LC-MS/MS.	A4250 metabolic turnover in all species was minimal. ¹⁴ C]A4250 (10 micromoles/L) was slowly metabolized in rat, mouse, and human hepatocytes, primarily by hydroxylation. Up to six radiolabeled metabolites (M1-M6) in addition to the parent drug (the most abundant component in the samples) were detected. ¹⁴ C]A4250 was metabolized to a greater extent by dog hepatocytes.
¹⁴C]A4250 Preclinical Enabling Studies for Clinical Metabolism Program (ALB/06) A single oral dose of ¹⁴ C]A4250 at a target level of 4 mg/kg was administered to partially pigmented male rats.	Five days after oral administration of ¹⁴ C]A4250 in rats, radioactivity was mainly detected in the feces (mean recovery of 88.6%), while urine accounted for a very small proportion of total radioactivity (0.07%). A very low proportion of the dose (0.10%) was associated with the carcass after 5 days. Twenty-four hours after oral administration of ¹⁴ C]A4250 to rats, radioactivity was mainly detected in the contents of the cecum and large intestine. At both 5 and 10 days postdose, the highest concentrations of radioactivity were measured in the small intestine mucosa. By 15 days post dose, radioactivity in all tissues was below the limit of quantification. Radioactivity was not associated with melanin-containing structures at any time after dose administration.

Source: Table prepared by nonclinical reviewer
 Abbreviations: ADME, absorption, distribution, metabolism, excretion; AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; LC-MS/MS, high-performance liquid chromatography with two mass spectrometry detectors; PK, pharmacokinetic
 AZD8294 and A4250 are code names for odevixibat.

Table 47. Toxicokinetic Data

Study/Study No.	Major Findings																																									
General Toxicology Studies																																										
26-Week Oral Toxicity Study in the Rat (TEA0001) Accumulation: No Dose proportionality: Peak plasma exposure (C _{max}) and total systemic exposure (AUC ₀₋₂₄) on Day 1, Week 13, and Week 26 all increased in a less than dose-proportional manner. NOAEL: 300 mg/kg Safety margin: 1376 (males), 732 (females)	Table 48. Week 26 TK Parameters in Rats <table border="1"> <thead> <tr> <th rowspan="2">Parameter</th> <th colspan="6">Dose (mg/kg/day)</th> </tr> <tr> <th colspan="2">10</th> <th colspan="2">100</th> <th colspan="2">300</th> </tr> <tr> <th></th> <th>M</th> <th>F</th> <th>M</th> <th>F</th> <th>M</th> <th>F</th> </tr> </thead> <tbody> <tr> <td>C_{max} (ng/mL)</td> <td>27.9</td> <td>23.8</td> <td>63.9</td> <td>160</td> <td>464</td> <td>362</td> </tr> <tr> <td>T_{max} (hr)</td> <td>1</td> <td>1</td> <td>1</td> <td>1</td> <td>1</td> <td>1</td> </tr> <tr> <td>AUC₀₋₂₄ (ng•hr/mL)</td> <td>230</td> <td>311</td> <td>680</td> <td>992</td> <td>4040</td> <td>2150</td> </tr> </tbody> </table>	Parameter	Dose (mg/kg/day)						10		100		300			M	F	M	F	M	F	C _{max} (ng/mL)	27.9	23.8	63.9	160	464	362	T _{max} (hr)	1	1	1	1	1	1	AUC ₀₋₂₄ (ng•hr/mL)	230	311	680	992	4040	2150
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Source: Report TEA0001, page 22
 Abbreviations: AUC, area under the concentration-time curve; C, concentration; F, females; M, males; T, time of concentration observed; TK, toxicokinetic

Study/Study No.	Major Findings					
39-Week Oral (Gavage) Toxicity Study in the Dog Followed by a 4-Week Recovery Period (8348308) Accumulation: Yes Dose proportionality: Systemic exposure (AUC and C _{max}) generally increased proportionally to dose increment. NOAEL: 150 mg/kg Safety margin ¹ 31 (males), 41 (females)	Table 49. Week 39 TK Parameters in Dogs					
	Dose (mg/kg/day)					
	3		30		150	
Parameter	M	F	M	F	M	F
C _{max} (nmol/L)	1.81	1.34	31.9	19.9	60.2	62.8
T _{max} (hr)	4	1	1	1	1	1
T _½ (hr)	NR	NR	NR	2.02	1.39	7.32
AUC _{0-t} (nmol•hr/L)	5.05	5.85	39.9	31.2	102	150
AUC ₀₋₂₄ (nmol•hr/L)	13.7	7.12	43.3	81.3	123	162
Source: Applicant's report 8348308, page 28						
Abbreviations: AUC, area under the concentration-time curve; C, concentration; F, females; M, males; NR, no result calculable; T, time of concentration observed; TK, toxicokinetic						

Study/Study No.	Major Findings					
13-Week Oral (Capsule) Toxicity Study in the Dog (TEA0002) Accumulation: No Dose proportionality: Both C _{max} and AUC ₀₋₂₄ were low relative to the dose levels, and they increased in a less than dose proportional manner. NOAEL: 300 mg/kg Safety margin: 17	Table 50. Week 13 TK Parameters in Dogs					
	Dose (mg/kg/day)					
	3		30		300	
Parameter	M	F	M	F	M	F
C _{max} (ng/mL)	3.42	0.640	4.05	1.77	5.80	6.96
C _{max} (nmol/L)	4.62	0.864	5.47	2.39	7.83	9.39
T _{max} (hr)	2-2	2-2	1-4	2-6	2-4	2-2
T _½ (hr)	NR	NR	1.6	NR	1.9	10.0
AUC _{0-tlast} (ng•hr/mL)	4.60	0.320	9.43	1.4	31.5	49.5
AUC ₀₋₂₄ (ng•hr/mL)	7.22	0.961	10.5	3.17	39.3	49.5
AUC ₀₋₂₄ (nmol•hr/L)	9.75	1.30	14.2	4.28	53.0	66.8
AUC _{NF} (ng•hr/mL)	NR	NR	15.5	NR	23.4	42.2
Source: Applicant's report TEA0002, page 327						
Abbreviations: AUC, area under the concentration-time curve; C, concentration; F, females; M, males; NR, no result calculable; T, time of concentration observed; TK, toxicokinetic						

Study/Study No.	Major Findings																																	
13-Week Oral Toxicity Study in the Mouse (TEA00013)	Table 51. Week 13 TK Parameters in Mice																																	
Accumulation: None, except in the 300 mg/kg/day females.	<table border="1"> <thead> <tr> <th rowspan="3">Parameter</th> <th colspan="6">Dose (mg/kg/day)</th> </tr> <tr> <th colspan="2">10</th> <th colspan="2">100</th> <th colspan="2">300</th> </tr> <tr> <th>M</th> <th>F</th> <th>M</th> <th>F</th> <th>M</th> <th>F</th> </tr> </thead> <tbody> <tr> <td>C_{max} (ng/mL)</td> <td>53.2</td> <td>40.2</td> <td>188</td> <td>323</td> <td>683</td> <td>707</td> </tr> <tr> <td>AUC₀₋₂₄ (ng•hr/mL)</td> <td>209</td> <td>256</td> <td>1440</td> <td>1350</td> <td>2760</td> <td>8860</td> </tr> </tbody> </table>	Parameter	Dose (mg/kg/day)						10		100		300		M	F	M	F	M	F	C _{max} (ng/mL)	53.2	40.2	188	323	683	707	AUC ₀₋₂₄ (ng•hr/mL)	209	256	1440	1350	2760	8860
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AUC ₀₋₂₄ (ng•hr/mL)	209	256	1440	1350	2760	8860																												
Dose proportionality: AUC increased with dose in a less than dose-proportional manner in both males and females, except during week 13 in the 300 mg/kg/day females.	Source: Applicant's report TEA0013, page 24 Abbreviations: AUC, area under the concentration-time curve; C, concentration; F, females; M, males; TK, toxicokinetic																																	
NOAEL: 100 mg/kg in males, 300 mg/kg in females																																		
Safety margin: 490 based on male NOAEL, 3017 based on female NOAEL																																		

Study/Study No.	Major Findings																																			
Reproductive Toxicology Studies	Table 52. Rat EFD TK Parameters on GD 17																																			
Embryo-Fetal Toxicity Study by the Oral Route in the Rat (AB21161)	<table border="1"> <thead> <tr> <th rowspan="3">Parameter</th> <th colspan="3">Dose (mg/kg/day)</th> </tr> <tr> <th>100</th> <th>300</th> <th>1000</th> </tr> </thead> <tbody> <tr> <td>T_{last} (hr)</td> <td>24</td> <td>24</td> <td>24</td> </tr> <tr> <td>T_{max} (hr)</td> <td>2</td> <td>0¹</td> <td>4</td> </tr> <tr> <td>C_{max} (ng/mL)</td> <td>27.1</td> <td>57.3</td> <td>76.8</td> </tr> <tr> <td>C_{max_D} (kg•ng/mL/mg)</td> <td>0.271</td> <td>0.191</td> <td>0.0768</td> </tr> <tr> <td>AUC_{last} (hr•ng/mL)</td> <td>303</td> <td>420</td> <td>799</td> </tr> <tr> <td>AUC_{last_D} (hr•kg•ng/mL/mg)</td> <td>3.03</td> <td>1.40</td> <td>0.799</td> </tr> <tr> <td>T_½ (hr)</td> <td>NR</td> <td>6.65</td> <td>NR</td> </tr> </tbody> </table>	Parameter	Dose (mg/kg/day)			100	300	1000	T _{last} (hr)	24	24	24	T _{max} (hr)	2	0 ¹	4	C _{max} (ng/mL)	27.1	57.3	76.8	C _{max_D} (kg•ng/mL/mg)	0.271	0.191	0.0768	AUC _{last} (hr•ng/mL)	303	420	799	AUC _{last_D} (hr•kg•ng/mL/mg)	3.03	1.40	0.799	T _½ (hr)	NR	6.65	NR
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T _½ (hr)	NR	6.65	NR																																	
Accumulation: No	Source: Applicant's report Ab21161, page 27 Abbreviations: AUC, area under the concentration-time curve; C, concentration; EFD, embryo-fetal development; NR, no result calculable; T, time of concentration observed; TK, toxicokinetic ¹ Predose																																			
Dose proportionality: On G6, the systemic exposure (C _{max} and AUC) increased in a dose-proportional manner from 100 to 300 mg/kg, whereas saturation of exposure was observed from 300 to 1000 mg/kg. On G17, the increase in exposure was less than dose proportional from 100 to 1000 mg/kg.																																				
NOAEL: 1000 mg/kg (fetal and maternal)																																				
Safety margin: 272																																				

Study/Study No.	Major Findings																																	
A4250- Embryo-Fetal Toxicity Study by the Oral Route (Gavage) in the Rabbit (AB21159) Accumulation: A tendency towards accumulation was noted for both C _{max} and AUC _{last} after repeated administration of 30 and 100 mg/kg. Dose proportionality: On G6 and G19, systemic exposure (C _{max} and AUC) increased in a less than dose proportional manner over the dose range of 10 to 100 mg/kg. NOAEL: not determined for fetuses; 10 mg/kg for maternal Safety margin: not determined for fetuses; 2.1 for maternal	Table 53. Rabbit EFD TK Parameters on GD 19																																	
	<table border="1"> <thead> <tr> <th rowspan="2">Parameter</th> <th colspan="3">Dose (mg/kg/day)</th> </tr> <tr> <th>10</th> <th>30</th> <th>100</th> </tr> </thead> <tbody> <tr> <td>T_{last} (hr)</td> <td>24</td> <td>24</td> <td>24</td> </tr> <tr> <td>T_{max} (hr)</td> <td>4</td> <td>4</td> <td>8</td> </tr> <tr> <td>C_{max} (ng/mL)</td> <td>0.405</td> <td>0.727</td> <td>1.45</td> </tr> <tr> <td>C_{max_D} (kg·ng/mL/mg)</td> <td>0.0405</td> <td>0.0242</td> <td>0.0145</td> </tr> <tr> <td>AUC_{last} (hr·ng/mL)</td> <td>6.28</td> <td>13.6</td> <td>26.2</td> </tr> <tr> <td>AUC_{last_D} (hr·kg·ng/mL/mg)</td> <td>0.628</td> <td>0.454</td> <td>0.262</td> </tr> </tbody> </table>				Parameter	Dose (mg/kg/day)			10	30	100	T _{last} (hr)	24	24	24	T _{max} (hr)	4	4	8	C _{max} (ng/mL)	0.405	0.727	1.45	C _{max_D} (kg·ng/mL/mg)	0.0405	0.0242	0.0145	AUC _{last} (hr·ng/mL)	6.28	13.6	26.2	AUC _{last_D} (hr·kg·ng/mL/mg)	0.628	0.454
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Pre- and Postnatal Development Study by the Oral Route in the Rat (AB22204) Accumulation: No Dose proportionality: On L4 and L20 (Lactation Days 4 and 20), the exposure (C _{max} and AUC) increased with dose in a less than dose proportional manner. NOAEL: 1000 mg/kg (F1 offspring and maternal) Safety margin: 434	Table 54. Rat PPND TK Parameters on L20																																	
	<table border="1"> <thead> <tr> <th>Day</th> <th>Dose (mg/kg/day)</th> <th>C_{max} (ng/mL)</th> <th>T_{max} (hr)</th> <th>AUC_{last} (ng·hr/mL)</th> </tr> </thead> <tbody> <tr> <td rowspan="3">L20</td> <td>10</td> <td>31.0</td> <td>2</td> <td>99.9</td> </tr> <tr> <td>100</td> <td>58.9</td> <td>1</td> <td>610</td> </tr> <tr> <td>1000</td> <td>114</td> <td>4</td> <td>1274</td> </tr> </tbody> </table>				Day	Dose (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hr)	AUC_{last} (ng·hr/mL)	L20	10	31.0	2	99.9	100	58.9	1	610	1000	114	4	1274												
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Source: Applicant's report AB21159, page 28
 Abbreviations: AUC, area under the concentration-time curve; C, concentration; EFD, embryo-fetal development; T, time of concentration observed; TK, toxicokinetic

Source: Applicant's report AB22204, page 37
 Abbreviations: AUC, area under the concentration-time curve; C, concentration; L, lactation days; PPND, pre- and postnatal development; T, time of concentration observed; TK, toxicokinetic

Study/Study No.	Major Findings						
Oral (Gavage) Toxicity Study in the Juvenile Rat (TEA0010) Accumulation: No. of exposures were markedly higher after initial dose administration on PND 14 than after repeated administration. This was likely due to the immaturity of the GI tract on PND 14. Dose proportionality: Exposure increased in a generally dose proportional manner from 10 to 30 mg/kg/day, and in a less than dose proportional manner from 30 to 100 mg/kg/day. NOAEL: 100 mg/kg/day Safety margin ² : 123	Table 55. Juvenile Rat TK Parameters on Postnatal Day 63						
		Dose (mg/kg/day)					
	Parameter	10		30		100	
	C _{max} (ng/mL)	10.8	10.8	21.1	36.9	62.2	26.0
	C _{max} (nmol/L)	14.6	14.6	28.5	49.8	84.0	35.1
	T _{max} (hr)	2	4	2	2	1	6
	T _½ (hr)	3.8	NR	6.7	6.5	5.8	7.4
	AUC _{0-tlast} (ng•hr/mL)	72.7	103	228	238	345	376
	AUC ₀₋₂₄ (ng•hr/mL)	72.7	103	228	238	345	376
	AUC ₀₋₂₄ (nmol•hr/L)	98.1	139	308	321	466	507
AUC _{NF} (ng•hr/mL)	73.9	NR	252	257	363	426	

Source: Applicant's report TEA 0010, page 826
 Abbreviations: AUC, area under the concentration-time curve; C, concentration; NR, no result calculable; T, time of concentration observed; TK, toxicokinetic

Source: Table prepared by nonclinical reviewer
 Abbreviations: AUC, area under the concentration-time curve; G, gestation day; GI, gastrointestinal; L, lactation days; NOAEL, no observed adverse effect level; PND, postnatal day
¹ Based on AUC ng•hr/mL values
² Based on average of male and female AUC ng•hr/mL

13.1.4. Toxicology

13.1.4.1. Single-Dose Toxicity

AZD8294: Single-Dose Oral Toxicity Study in the Male Mouse (0153LM)

Male mice (N=5) were administered a single oral dose of AZD8294 at 2699 mcml/kg (2000 mg/kg). No adverse effects were observed. Plasma levels of AZD8294 were 1005 ±955 nmol/mL and 320 ±154 nmol/mL at 1 and 2 hours after dosing, respectively.

AZD8294: Single-Dose Oral Toxicity Study in the Rat (0662LR)

Rats (5/sex/group) received a single oral dose of 2699 mcml/kg (2000 mg/kg) AZD8294. No adverse effects were observed. At 1 hour, plasma drug levels in males and females were 359 ±210 nmol/L and 432 ±160 nmol/L. At 2 hours, plasma drug levels in males and females were 401 ±139 nmol/L and 588 ±368 nmol/L.

13.1.4.2. Repeat-Dose Toxicity

13.1.4.2.1. Dog

A4250: 39-Week Oral (Gavage) Administration Toxicity Study in the Dog Followed by a 4-Week Recovery Period (8348308)

Key Study Findings

- There were no adverse effects.
- The NOAEL (no observed adverse effect level) was considered to be 150 mg/kg/day.

Table 56. Study Information, 39-Week Oral (Gavage) Administration Toxicity Study in the Dog Followed by a 4-Week Recovery Period (8348308)

Study Features and Methods	Details
GLP compliance	Yes
Dose and frequency of dosing	3, 30, and 150 mg/kg once daily
Route of administration	Oral gavage
Formulation/vehicle	Solution/20% v/v propylene glycol in purified water
Species/strain	Dog/Beagle
Number/sex/group	4
Age	24 to 32 weeks
Satellite groups/unique design	Recovery: 2/sex in control and 150 mg/kg/day groups; toxicokinetic
Deviation from study protocol affecting interpretation of results	None

Source: Table prepared by nonclinical reviewer
Abbreviations: GLP, good laboratory practice

Table 57. Observations and Results, 39-Week Oral (Gavage) Administration Toxicity Study in the Dog Followed by a 4-Week Recovery Period (8348308)

Parameters	Major Findings
Mortality	Two non-drug-related deaths
Clinical signs	Limited or infrequent signs of soft/liquid feces and vomiting
Body weights	Overall body weight gain at the end of the dosing phase was reduced compared to the control group (-37%, -18%, and -36% for males in the 3, 30, and 150 mg/kg/day groups, respectively).
Ophthalmoscopy	None
ECG	None
Hematology	None
Clinical chemistry	Reductions in HDL, LDL, and total cholesterol at 150 mg/kg/day which were attributed to the pharmacological activity of A4250
Urinalysis	None
Gross pathology	None
Organ weights	None
Histopathology	None
Adequate battery: Yes	

Source: Table prepared by nonclinical reviewer
Abbreviations: ECG, electrocardiogram; HDL, high-density lipoprotein; LDL, low-density lipoprotein

13-Week Oral (Capsule) Toxicity Study in the Dog (TEA0002)

Key Study Findings

- There were no adverse effects.

- The NOAEL was considered to be 300 mg/kg/day.

Table 58. Study Information, 13-Week Oral (Capsule) Toxicity Study in the Dog (TEA0002)

Study Features and Methods	Details
GLP compliance	Yes
Dose and frequency of dosing	3, 30, and 300 mg/kg once daily
Route of administration	Oral
Formulation/vehicle	Gelatin capsule
Species/strain	Dog/Beagle
Number/sex/group	3
Age	4 to 7 months
Satellite groups/unique design	Toxicokinetic
Deviation from study protocol affecting interpretation of results	None

Source: Table prepared by nonclinical reviewer
Abbreviations: GLP, good laboratory practice

Table 59. Observations and Results, 13-Week Oral (Capsule) Toxicity Study in the Dog (TEA0002)

Parameters	Major Findings
Mortality	One non-drug-related death.
Clinical signs	Increased incidence of stool softening and color changes occurred in all treatment groups, and a slight increase in incidence of vomiting was observed in the 300 mg/kg/day group.
Body weights	None
Ophthalmoscopy	None
ECG	None
Hematology	None
Clinical chemistry	A marked decrease (up to -88.1%) in plasma low density lipoprotein concentration and a moderate reduction in total cholesterol levels were observed at all doses and were attributed to the pharmacological activity of odevixibat.
Urinalysis	None
Gross pathology	None
Organ weights	None
Histopathology Adequate battery: Yes	Prominent vacuolation of the luminal epithelium of the gall bladder was observed in all drug-treated groups (0/6, 3/6, 2/6, and 3/6 in the control, 3, 30, and 300 mg/kg/day groups, respectively). The Applicant indicated that prominent epithelial vacuolation is also usually found in control dogs. Vacuolation of the epithelium of the gall bladder is a known spontaneous finding in dogs. Since there was no dose-dependence in the finding, and the pathogenesis and significance of this type of lesion are unknown, the toxicological significance of this finding is unclear.

Source: Table prepared by nonclinical reviewer
Abbreviations: ECG, electrocardiogram

A4250: 14-Day Oral (Gavage) Administration Toxicity Study in the Dog (8220869)

A4250 (30, 300, or 1000 mg/kg/day) or vehicle was administered by daily oral (gavage) to Beagle dogs (3/sex/group) for 14 days. The toxicokinetic profile of the test article was also assessed. There were no mortalities considered to be treatment related. One animal in the 30 mg/kg/day group was humanely euthanized due to gavage injury. Clinical signs during postdosing observations in all drug-treated groups included mucoïd/liquid/soft feces and vomiting. These clinical signs were noted throughout the treatment period, but the incidence of vomiting was reduced during Week 2. Mucoïd/liquid/soft feces were also noted to have a later onset in Week 2. Males and females treated with 1000 mg/kg/day had a slight reduction in

bodyweight gain. This was associated with slight reduction in mean food consumption in the 1000 mg/kg/day females on Week 1.

There were no drug-related effects on hematology, urinalysis, ophthalmoscopy, electrocardiography, organ weights, or histopathology. Changes in clinical chemistry parameters included a decrease in cholesterol in animals at ≥ 300 mg/kg/day compared to controls. Based on the overall data, the NOAEL was considered to be 1000 mg/kg/day. Exposure to A4250 generally increased with dose level from 30 to 1000 mg/kg/day. The increases in mean maximum plasma concentration (C_{\max}) and AUC_{0-24} were generally less than dose proportional. Sex differences were generally less than 2-fold for mean C_{\max} and AUC_{0-24} values. Accumulation of A4250 was observed after multiple dosing.

A4250: Maximum Tolerated Dose (MTD) Followed by a 7-Day Fixed-Dose Oral (Gavage) Administration Toxicity Study in the Dog (8220870)

Dogs (2/sex/group) were administered vehicle or increasing doses of A4250 (50, 100, 200, 400, or 1000 mg/kg/day) by oral gavage. The toxicity of repeated daily administration of the MTD for 7 days was then determined using naïve animals. The toxicokinetic profile of the test article was also assessed. Due to problems with the solubility of the test article in the vehicle (pH adjusted [8-10] purified water and 2% ethanol) at dose levels higher than 400 mg/kg, the MTD phase was stopped on Day 14 and further trials were performed using alternative vehicles. The formulation using 20% v/v propylene glycol was deemed acceptable. The MTD phase was restarted on Day 49 with a single dose at 400 mg/kg followed by 3 days at 1000 mg/kg/day. The MTD was determined to be 1000 mg/kg/day. Therefore, the fixed dose phase evaluated administration of 1000 mg/kg/day for 7 days.

During the MTD phase and the fixed dose phase, clinical signs including loose and/or soft feces were noted after dosing, generally at 4 hours, in all dose groups. In addition, vomiting was noted in a couple of animals during the fixed dose phase. Body weight loss or reduced bodyweight gain was noted in females during the MTD phase with no correlating changes in food consumption. Changes in hematology parameters included a reduction in reticulocytes at the end of the MTD phase and fixed dose phase, compared to pretreatment values. Macroscopic findings included a slight/moderate dark discoloration of cecum in $\frac{3}{4}$ animals and a red discoloration (slight) of duodenum in $\frac{2}{4}$ animals in the MTD phase. In addition, a thick thymus (slight to moderate in severity) was recorded in $\frac{3}{4}$ animals.

During the fixed dose phase, darkened cecum (minimal) was noted in $\frac{2}{4}$ animals and a slight to moderate thickened thymus occurred in $\frac{3}{4}$ animals. Systemic exposure increased with dose from 400 mg/kg (MTD phase) to 1000 mg/kg (Day 1 of fixed dose phase). Sex-related differences in exposure (C_{\max} and AUC_{0-24}) were less than 2-fold. Accumulation of A4250 was observed after repeated dosing.

13.1.4.2.2. Rat

26-Week Oral (Gavage) Toxicity Study in the Rat (TEA0001)

Key Study Findings

- There were no adverse effects.
- The NOAEL was 300 mg/kg/day.

Table 60. Study Information, 26-Week Oral (Gavage) Toxicity Study in the Rat (TEA0001)

Study Features and Methods	Details
GLP compliance	Yes
Dose and frequency of dosing	10, 100, and 300 mg/kg once daily
Route of administration	Oral gavage
Formulation/vehicle	Solution/20% v/v propylene glycol in purified water
Species/strain	Rat/Crl:WI(Han)
Number/sex/group	12
Age	7-8 weeks of age
Satellite groups/unique design	Toxicokinetic
Deviation from study protocol affecting interpretation of results	None

Source: Table prepared by nonclinical reviewer
 Abbreviations: GLP, good laboratory practice

Table 61. Observations and Results, 26-Week Oral (Gavage) Toxicity Study in the Rat (TEA0001)

Parameters	Major Findings
Mortality	None
Clinical signs	None
Body weights	None
Ophthalmoscopy	None
ECG	N/A
Hematology	None
Clinical chemistry	At weeks 13 and 26, slight but statistically significant decreases (up to -11.9%) in plasma total protein were noted in the 100 and 300 mg/kg/day males and in all A4250-treated females. In females, the changes in total protein were more pronounced and were accompanied by slight but statistically significant decreases in plasma albumin (up to -12.3%) and calcium (up to -4.2%).
Urinalysis	None
Gross pathology	None
Organ weights	None
Histopathology	Basophilic foci of cellular alteration in the liver were observed in two control females and in six 300 mg/kg/day females; severity was minimal in all affected animals. The incidence of basophilic foci in the liver generally increases with age, and they are more commonly found in females than in males. In the absence of indications of liver toxicity, the increased incidence of this finding in the 300 mg/kg/day females was considered to be due to normal biological variation.
Adequate battery: Yes	

Source: Table prepared by nonclinical reviewer
 Abbreviations: ECG, electrocardiogram

1-Month Oral Toxicity Study, With Assessment of Recovery, in the Rat (0664AR)

Key Study Findings

- Nonadverse microscopic findings included mild mucosal hypertrophy of cecum and colon at ≥ 200 mg/kg/day, and mild diffuse basophilia of the parotid salivary gland acinar cells in females at 2000 mg/kg/day.
- The NOAEL was considered to be 2000 mg/kg/day.

Table 62. Study Information, 1-Month Oral Toxicity Study, With Assessment of Recovery, in the Rat (0664AR)

Study Features and Methods	Details
GLP compliance	Yes
Dose and frequency of dosing	27, 270, and 2700 mcmol/kg (20, 200, and 2000 mg/kg); twice daily (i.e., 10, 100, and 1000 mg/kg BID)
Route of administration	Oral gavage
Formulation/vehicle	Solution/distilled water
Species/strain	Rat/ Wistar Hannover
Number/sex/group	10
Age	72 \pm 3 days
Satellite groups/unique design	Recovery: 5/sex/group; Toxicokinetic
Deviation from study protocol affecting interpretation of results	None

Source: Table prepared by nonclinical reviewer
 Abbreviations: GLP, good laboratory practice; BID, twice daily

Table 63. Observations and Results, 1-Month Oral Toxicity Study, With Assessment of Recovery, in the Rat (0664AR)

Parameters	Major Findings
Mortality	One non-drug-related early euthanization
Clinical signs	Increased salivation, reflex salivation following dosing, and persistent soft feces at 2700 mcmol/kg/day
Body weights	Body weight gain was reduced in males at 2700 mcmol/kg/day (mean body weight was reduced by 6.8% compared to control animals, on Day 28).
Ophthalmoscopy	None
ECG	N/A
Hematology	Small reductions in red cell parameters (hemoglobin, hematocrit, red cell count, and reticulocytes) were observed in males and females. Males at ≥ 27 mcmol/kg/day showed slight increases in total white cell, neutrophil, and lymphocyte counts and in APTT.
Clinical chemistry	A small reduction in plasma total protein and albumin was observed at ≥ 27 mcmol/kg/day in females and mainly at 2700 mcmol/kg/day in males, without a clear relationship to dose.
Urinalysis	On Day 23 or Day 24, there was a decrease in volume and related changes in urine composition (6 hours after dosing) in males at ≥ 270 mcmol/kg/day. This was associated with an increase in specific gravity, protein, and ketones. Urine pH was reduced at 2700 mcmol/kg/day.

Parameters	Major Findings
Gross pathology	None
Organ weights	None
Histopathology Adequate battery: Yes	Mild mucosal hypertrophy of cecum and colon at ≥ 270 mcmol/kg/day, and mild diffuse basophilia of the parotid salivary gland acinar cells in females at 2700 mcmol/kg/day.
Water consumption	Water consumption was markedly increased (approximately 1.6 to 1.8-fold) in both sexes at 2700 mcmol/kg/day (Day 15 to Day 22).

Source: Table prepared by nonclinical reviewer

Abbreviations: ECG, electrocardiogram; APTT, activated partial thromboplastin time

AR-H064974XX and AR-H064965XX: 7-Day Oral Toxicity Study in the Rat (02263)

Three groups of Han Wistar rats (4/sex/group) were given ARH064974XX (odevixibat) at doses of 14, 140, and 1400 mcmol/kg/day (10, 100, and 1000 mg/kg/day) by oral gavage for 7 days. Three other groups were given ARH064965XX at doses of 13, 110, and 420 mcmol/kg/day (10, 88, and 320 mg/kg/day) by oral gavage. The data for ARH064965XX treatment groups are not presented here. The vehicle control group received tap water.

Additional groups were used for analysis of plasma concentration of the test compounds. Animals were evaluated for clinical signs, body weight, food consumption, hematology, clinical chemistry, liver enzyme induction, gross pathology, organ weights, and microscopic pathology. There were no drug-related mortalities, effects on body weight or food consumption, or changes in hematology parameters. Clinical signs included increased salivation in a few animals at 1400 mcmol/kg ARH064974XX and ploughing in all animals at 1400 mcmol/kg.

Compared to the control group, there was a slight decrease in the mean plasma concentration of total protein and albumin noted in all female groups. No significant changes in the levels of CYP450 (1A, 2B, 3A, 4A) isoenzymes were observed. There were no drug-related effects on organ weights, macroscopic findings, or microscopic findings. C_{max} of AR-H064974XX was observed between 1 and 8 hours after dosing. Exposure to AR-H064974XX area under the concentration-time curve (AUC) increased in a less than proportional manner with increasing dose. There was no obvious change in exposure to AR-H064974XX after repeated administration for 7 days. Exposure (C_{max} and AUC) were similar in males and females.

13.1.4.2.3. Mouse

A4250: Oral (Gavage) 13-Week Dose Range-Finding Study in the Mouse With Toxicokinetic Sampling (TEA0013)

Key Study Findings

- Four main study 300 mg/kg/day males were sacrificed before scheduled termination, and one toxicokinetic 300 mg/kg/day male was found dead on Day 71. No specific findings could be identified as a cause of death in any of the five decedents. The five deaths are considered drug related.
- The NOAELs in the male and female mice were considered to be 100 and 300 mg/kg/day A4250, respectively.

Table 64. Study Information, Oral (Gavage) 13-Week Dose Range-Finding Study in the Mouse With Toxicokinetic Sampling (TEA0013)

Study Features and Methods	Details
GLP compliance	Yes
Dose and frequency of dosing	10, 100, and 300 mg/kg once daily
Route of administration	Oral gavage
Formulation/vehicle	Solution/20% v/v propylene glycol in purified water
Species/strain	mouse/Crl:CD-1 (ICR)
Number/sex/group	12
Age:	4 to 5 weeks
Satellite groups/unique design	Toxicokinetic
Deviation from study protocol affecting interpretation of results	None

Source: Table prepared by nonclinical reviewer
 Abbreviations: GLP, good laboratory practice

Table 65. Observations and Results, Oral (Gavage) 13-Week Dose Range-Finding Study in the Mouse With Toxicokinetic Sampling (TEA0013)

Parameters	Major Findings
Mortality	Four main study 300 mg/kg/day males were sacrificed before scheduled termination, and one toxicokinetic 300 mg/kg/day male was found dead on Day 71. In the four main study decedent males, decreased activity, cold body surface, piloerection and/or slow/labored breathing generally were observed before sacrifice. Distended abdomen was noted in two of the four males. At necropsy, regions of the GI tract were distended in all four animals; however, no specific microscopic lesion could be identified as a cause of death in any of the five decedents. No specific findings could be identified as a cause of death in any of the five decedents. The five deaths are considered as drug-related.
Clinical signs	None
Body weights	Bodyweight was decreased, compared to controls, in the 300 mg/kg/day males starting on week 5. Terminal bodyweight on week 13 in the 300 mg/kg/day males decreased significantly by 7.8%. Bodyweight gain in the 300 mg/kg/day males was decreased by 23.7% (not statistically significant). Transient decreases in bodyweight, compared to controls, also were observed in the 100 mg/kg/day males.
Food consumption	None
Ophthalmoscopy	None
ECG	N/A
Hematology	A statistically significant decrease in MCH was noted in the 100 and 300 mg/kg/day males (-5.4% and -3.0%, respectively). The changes are not considered to be of toxicological significance because they occurred in a single sex and the magnitude of the changes was small.
Clinical chemistry	None
Urinalysis	N/A
Gross pathology	None
Organ weights	None
Histopathology	None
Adequate battery: Yes	None

Source: Table prepared by nonclinical reviewer
 Abbreviations: ECG, electrocardiogram; MCH, mean corpuscular hemoglobin

A4250: Preliminary and 14-Day Oral (Gavage) Dose Range Finding Study in the Mouse (TEA0012)

In phase 1 (MTD phase) of this study, mice (3/sex/group) were dosed at 500, 1000, or 1500 mg/kg/day A4250 by oral gavage. Dosing was staggered to assess reactions before the next dose administration. In phase 2 (14-day fixed dose phase), mice (5/sex/group) were dosed once daily with 0 (vehicle), 100, 300, 750, or 1500 mg/kg/day at a dose volume of 10 mL/kg body weight for 14 days, until the day before necropsy. Additional animals were included for toxicokinetic blood samples on Days 1 and 14.

In phase 1, there were no deaths, clinical signs, or effects on body weight in animals given 500, 1000, or 1500 mg/kg/day. There were no drug-related macroscopic findings. Based on these observations, a high dose of 1500 mg/kg/day was chosen for phase 2. Three females given 300 mg/kg/day (one main study animal and two satellite animals) in phase 2 were killed on Day 8 of dosing due to a decline in clinical condition. The findings at necropsy indicated that these deaths were due to gavage-related dosing error.

There were no drug-related clinical signs, effects on body weight/body weight gain, or changes in food intake. There were no drug-related changes in organ weights or macroscopic findings. After single and repeat dose administration, systemic exposure increased with dose in a less than dose-proportional manner. Higher exposures were observed in females compared to males at all dose levels on Day 1 and were more variable between sexes on Day 14. No accumulation was seen after 14 days of repeated administration, except for females administered 750 mg/kg/day and males administered 1500 mg/kg/day. There was no appreciable increase in systemic exposure above 300 mg/kg/day.

13.1.4.2.4. Marmoset

AZD8294: Tolerability and 7-Day Oral Toxicity Study in the Marmoset (0011DT)

In the tolerability phase, marmosets (1/sex/group) were dosed at 67.5 or 135 mcmol/kg/day AZD8294 over Days 1 to 6. In the repeated dose phase, marmosets (1/sex/group) were administered vehicle or 350 mcmol/kg AZD8294 for 7 days. During the tolerability phase, no adverse clinical signs or notable effects on body weight or food consumption (qualitative) were observed. During the repeated dose phase, fluid or soft feces were observed (6 days in males and 2 days in females), and slight reductions in weight were observed (both males lost approximately 4 to 8% of body weight over Days 1 to 8). The body weight changes suggest that a dose of 350 mcmol/kg/day (259 mg/kg/day) would be too high for use in studies of longer duration in marmosets. However, there were no notable effects on food consumption (qualitative) or changes in hematology or clinical chemistry parameters. There were no drug-related macroscopic or microscopic histopathology changes. No clinical signs were observed during the 14-day recovery period. At termination, no drug-related findings were observed in the necropsies.

13.1.4.3. Genetic Toxicology

Table 66. Genetic Toxicology

Study No./Study Title	Key Study Findings
AZD8294: Genetic Toxicity Evaluation Using a Bacterial Reverse Mutation Test (0332BV) GLP compliance: Yes Study is valid: Yes	Mutagenic activity was tested in <i>Salmonella</i> strains TA1535, TA1537, TA98, TA100, and <i>Escherichia</i> strain uvrA/pKM101 in the absence and presence of S9 mix. The highest dose tested was 5050 mcg (6.82 micromoles) AZD8294, and the treatment duration was 3 days at 37°C. AZD8294 was not mutagenic for strains of <i>Salmonella typhimurium</i> LT2 or <i>Escherichia coli</i> WP2
AZD8294: Genetic Toxicity Evaluation Using the Mouse Lymphoma Cell Thymidine Kinase Locus Assay GLP compliance: Yes Study is valid: Yes	The ability to induce forward mutation at the thymidine kinase (tk) locus was tested in mouse lymphoma L5178Y TK [±] 3.7.2C cells. The concentrations tested in the presence of S9 for 3 hours of exposure were 0.120 to 0.219 mmol/L (88.7-162 mcg/mL). The concentrations tested in the absence of S9 for 3 hours of exposure were 0.0506 to 0.0708 mmol/L (37.5 to 52.4 mcg/mL). The drug concentration range tested for 24 hours of exposure in the absence of S9 was 0.0607 to 0.121 mmol/L (45.0 to 89.9 mcg/mL). Relative total growth was 10-13% at the highest doses analyzed. AZD8294 was not mutagenic in the mouse lymphoma cell forward mutation assay.
A4250: Oral (Gavage) Rat Micronucleus Test (TEA0008) GLP compliance: Yes Study is valid: Yes	In vivo clastogenicity or aneugenicity was tested in polychromatic erythrocytes in the bone marrow of rats. Rats [CrI:WI(Han)] were administered 2000 mg/kg/day for 2 days, and bone marrow was harvested 24 hours after the second dose. AZD8294 was not clastogenic or aneugenic in the in vivo rat micronucleus test.

Source: Table prepared by nonclinical reviewer
Abbreviations: GLP, good laboratory practice

13.1.4.4. Reproductive Toxicology

A4250: Fertility Toxicity Study by the Oral Route (Gavage) in the Rat (AB21162)

Key Study Findings

- There were no adverse effects.
- The NOAEL was considered to be 1000 mg/kg/day.

Table 67. Study Information, Fertility Toxicity Study by the Oral Route (Gavage) in the Rat (AB21162)

Parameter	Method Details
GLP compliance	Yes
Dose and frequency of dosing	100, 300, and 1000 mg/kg once daily
Route of administration	Oral gavage
Formulation/vehicle	Solution/20% v/v Propylene glycol in purified water
Species/strain	Rat/ Wistar: CrI: WI (Han)
Number/sex/group	20
Satellite groups	None

Parameter	Method Details
Study design	Males and females were treated for 2 weeks before pairing, throughout mating, and up to the day before necropsy (males) or until Day 7 of gestation (females). A minimum of 14 days for the male pre-mating treatment period was selected based on the absence of organ weight changes or microscopic findings in reproductive organs in a previous 6-month study in rats.
Deviation from study protocol affecting interpretation of results	None

Source: Table prepared by nonclinical reviewer
Abbreviations: GLP, good laboratory practice

Table 68. Observations and Results, Fertility Toxicity Study by the Oral Route (Gavage) in the Rat (AB21162)

Parameters	Major Findings
Mortality	None
Clinical signs	None
Body weights	None
Fertility and pregnancy	One pair of rats at 100 mg/kg/day did not mate within 14 days of pairing. One mated female at 300 mg/kg/day which had a positive vaginal smear for sperm did not become pregnant. These isolated cases were considered incidental as they did not occur at the high dose. Mating performance and fertility parameters are shown in the table below.

Table 69. Summary of Mating Performance and Fertility

Variable	Dose (mg/kg/day)			
	0	100	300	1000
Number of females				
Paired	20	20	20	20
Inseminated	20	19	20	20
Pregnant	20	19	19	20
Precoital interval (days)				
Mean	3.1	3.1	3.8	2.6
SD	2.6	0.8	2.9	0.9
N	20	19	18 ¹	20
Copulation index, n (%) ²	100	95	100	100
Fertility index, n (%) ³	100	100	95	100

Source: Report AB21162, page 66.

Abbreviations: SD, standard deviation

¹ Mating not detected for two pairs of rats (pregnancy did occur in the females)

² Copulation index = (# inseminated ÷ # paired) x 100

³ Fertility index = (# pregnant ÷ # inseminated) x 100

Necropsy	None
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Source: Table prepared by nonclinical reviewer

A4250: Embryo-Fetal Toxicity Study by the Oral Route (Gavage) in the Rabbit (AB21159)

Key Study Findings

- Drug-related cardiovascular defects occurred in a nondose dependent manner at all doses (10, 30, and 100 mg/kg/day). These malformations included five-chambered heart, small ventricle, large atrium, ventricular septum defect, misshapen aortic valve, dilated aortic arch, right sided and retroesophageal aortic arch, fusion of aortic arch and pulmonary trunk, ductus arteriosus atresia, and absence of subclavian artery.

- Two females were prematurely euthanized after aborting/delivering early on Day 27 or Day 29 of gestation (one at 30 mg/kg/day and one at 100 mg/kg/day). Severe body weight loss and reduced food consumption were observed in both females.
- A NOAEL for embryo-fetal development could not be determined. The NOAEL for maternal toxicity was 10 mg/kg/day.

Table 70. Methods, Oral Embryo-Fetal Developmental Study in Rabbits (AB21159)

Parameter	Method Details
GLP compliance	Yes
Dose and frequency of dosing	10, 30, and 100 mg/kg once daily
Route of administration	Oral gavage
Formulation/vehicle	Solution/20% v/v Propylene glycol in purified water
Species/strain	Rabbit/New Zealand White
Number/sex/group	22 mated females
Satellite groups	None
Study design	Animals were treated from Day 6 to 19 of gestation (G6-G19) inclusive
Deviation from study protocol affecting interpretation of results	None

Source: Table prepared by nonclinical reviewer
 Abbreviations: G, gestation day; GLP, good laboratory practice

Table 71. Observations and Results, Oral Embryo-Fetal Developmental Study in Rabbits (AB21159)

Parameters	Major Findings
Mortality	One female at 30 mg/kg/day (#53) and one female at 100 mg/kg/day (#73) were prematurely euthanized after aborting/delivering early on Day 27 or Day 29 of gestation. Clinical signs were observed in the 100 mg/kg/day female including red fluid in the cage, red colored urine, few and pale feces, and thin appearance. Severe body weight loss and reduced food consumption were observed in both females. Macroscopic findings included pale kidneys with dark areas (#53). One female at 10 mg/kg/day died with macroscopic findings consistent with gavage error.
Clinical signs	There was an increased incidence of reduced/no fecal output in all drug-treated groups. Thin appearance was observed in 3 of 22 females at 100 mg/kg/day. Pale, soft, and/or mucoid feces were noted in one female at 30 mg/kg/day and six females at 100 mg/kg/day. Red colored urine and/or red traces in the cage was noted for two females at 100 mg/kg/day.
Body weights	Dose-dependent body weight loss was observed from G6 to G9 in the 10, 30, and 100 mg/kg/day groups (-26 g, -42 g, and -58 g, respectively, compared to -8.5 g in the control group). After G9, body weight gain was comparable or greater than the control group and/or historical control data. Mean terminal body weight was comparable in all groups.
Food consumption	Dose-dependent reduction in food consumption was observed from G6 to G9 in the 10, 30, and 100 mg/kg/day groups (-6%, -10%, and -26%, respectively, compared to the control group). This effect persisted to G20 in the 100 mg/kg/day group. Mean food consumption in the control group was slightly lower than the historical control range (127 g compared to a historical range of 129 to 191 g) from G6 to G9.

Parameters	Major Findings
Necropsy findings Cesarean section data	The percentage of early resorptions was increased in females at 100 mg/kg/day compared to the control group (13% vs. 3% in the control group). This was mainly due to one female with 15 early resorptions and no viable fetuses. This also disproportionately influenced the percentage postimplantation loss in the 100 mg/kg/day group (13.4% vs. 4.8% in the control group and 7.4% in the historical control data). When the female with 15 early resorptions was omitted from the data, the percentage postimplantation loss in the 100 mg/kg/day group was 7.6%. Mean fetal weight in all groups including the control group was slightly below the historical control data range.
Necropsy findings Offspring	Drug-related cardiovascular defects (5-chambered heart, small ventricle, large atrium, ventricular septum defect, misshapen aortic valve, dilated aortic arch, right sided and retroesophageal aortic arch, fusion of aortic arch and pulmonary trunk, ductus arteriosus atresia, and absence of subclavian artery) occurred in a nondose dependent manner at all doses (see Table 35 in Section 7.7.5). Cardiovascular defects were not observed in the control group, and the cardiovascular malformations occurred at a higher incidence than expected for normal background incidence. This indicates a potential drug-related teratogenic effect.

Source: Table prepared by nonclinical reviewer
Abbreviations: G, gestation day

A4250: Embryo-Fetal Toxicity Study by the Oral Route (Gavage) in the Rat (AB21161)

Key Study Findings

- Drug-related skeletal variations were observed at 1000 mg/kg/day (higher incidence of delayed ossification of several bones and thick ribs as compared to the concurrent control and historical control data). These findings have been previously shown to be transient and do not result in permanent structural changes. Therefore, they are not considered adverse.
- The NOAEL for embryo-fetal development and maternal toxicity was 1000 mg/kg/day.

Table 72. Methods, Oral Embryo-Fetal Developmental Study in Rats (AB21161)

Parameter	Method Details
GLP compliance	Yes
Dose and frequency of dosing	100, 300, and 1000 mg/kg once daily
Route of administration	Oral gavage
Formulation/vehicle	Solution/20% v/v Propylene glycol in purified water
Species/strain	Rat/ Wistar: Crl: WI (Han)
Number/sex/group	22 mated females
Satellite groups	None
Study design	Animals were treated from Days 6 to 17 of gestation (G6-G17) inclusive
Deviation from study protocol affecting interpretation of results	None

Source: Table prepared by nonclinical reviewer
Abbreviations: G, gestation day; GLP, good laboratory practice

Table 73. Observations and Results, Oral Embryo-Fetal Developmental Study in Rats (AB21161)

Parameters	Major Findings
Mortality	There were no unscheduled deaths.
Clinical signs	There were no drug-related clinical signs.
Body weights	Lower body weight gain was observed during the first 3 days of dosing (G6 to G9) in all treatment groups, compared to the control group (-21%, -21%, and -34% in the 100, 300, and 1000 mg/kg/day groups, respectively). Terminal mean body weight was comparable in all groups (-4%, -0.8%, and -5% in the 100, 300, and 1000 mg/kg/day groups, respectively).
Food consumption	Reduced food consumption was noted during the first 3 days of dosing (G6 to G9) in all treatment groups compared to the control group (-7%, -8%, and -15% in the 100, 300, and 1000 mg/kg/day groups, respectively). Reduced food consumption in animals treated with 1000 mg/kg/day persisted to G18 (from G6 to G18, mean food consumption was 9% lower than the control group).
Necropsy findings Cesarean section data	The mean number of corpora lutea and mean number of implantation sites in the 1000 mg/kg/day group were slightly lower than in the concurrent control group but were comparable with the mean historical control values. Mean live litter size was slightly lower in the 1000 mg/kg/day group (10.8) as compared to the concurrent control (11.6) but was the same as the mean historical control value (10.8). Total litter weight in the 1000 mg/kg/day group was slightly lower than the control group (-9%) but was within the range of the historical control values. Gravid uterus weight in the 1000 mg/kg/day group was slightly lower than the control group (-9%). This may be attributed to the slight decrease in mean live litter size.
Necropsy findings Offspring	A small number of fetuses with external, visceral, and/or skeletal malformations were noted in the 100 and 1000 mg/kg/day groups. These findings were not attributed to drug administration due to their isolated incidence, and their occurrence in historical data. No malformations were observed in the 300 mg/kg/day group. Drug-related skeletal variations were observed at 1000 mg/kg/day, which included a higher incidence of delayed ossification of several bones (i.e., squamosal, metacarpal, sternebra, and caudal vertebral arches) and thick ribs as compared to the concurrent control and historical control data (see table below). These findings have been previously shown to be transient and do not result in permanent structural changes. Therefore, they are not considered adverse.

Table 74. Summary of Skeletal Variations

Finding	Historical Control Data 2012-2014	Control	Dose (mg/kg/day)		
			100	300	1000
Number of fetuses	1403	128	123	130	126
Parietal, incomplete ossification (%)	12.5	14.1	9.8	8.5	23.8
Squamosal, incomplete ossification (%)	5.5	7.0	8.1	5.4	14.3
Metacarpal, 5 th digit unossified (%)	8.7	16.4	19.5	8.5	29.4
Sternebra, 5 th unossified (%)	5.8	6.3	4.9	4.6	11.1
Caudal vertebral arch, 1 st and 2 nd unossified	27.8	18.8	20.3	20.8	40.5
Ribs, thick (%)	1.2	3.1	2.4	0	9.5

Source: Report AB21161, page 32
 Values represent % of fetuses with finding.

Source: Table prepared by nonclinical reviewer
 Abbreviations: G, gestation day

A4250: Pre- and Postnatal Development Study by the Oral Route (Gavage) in the Rat (AB22204)

Key Study Findings

- There were no adverse effects.
- The NOAEL was considered to be 1000 mg/kg/day in the offspring and F0 females.

Table 75. Study Information, Pre- and Postnatal Development Study by the Oral Route (Gavage) in the Rat (AB22204)

Parameter	Method Details
GLP compliance:	Yes
Dose and frequency of dosing:	10, 100, and 1000 mg/kg once daily
Route of administration:	Oral gavage
Formulation/vehicle:	Solution/20% v/v Propylene glycol in purified water
Species/strain:	Rat/ Wistar: Crl: WI (Han)
Number/sex/group:	F0 generation: 22 females F1 generation: 20
Satellite groups:	None
Study design:	F0 females were treated from Day 6 of gestation until Day 20 of lactation. The F1 generation was maintained without dosing for evaluation of postweaning development, behavioral testing, and mating. These animals were maintained for approximately 8 weeks after weaning (i.e., age 11 weeks) and then were mated for up to 2 weeks.
Deviation from study protocol affecting interpretation of results:	None

Source: Table prepared by nonclinical reviewer
 Abbreviations: GLP, good laboratory practice

Table 76. Observations and Results, Pre- and Postnatal Development Study by the Oral Route (Gavage) in the Rat (AB22204)

Parameters	Major Findings
Mortality	Two females at 100 mg/kg/day were unable to deliver on G23 (gestation day 23) and were humanely sacrificed. No unscheduled terminations occurred in the 1000 mg/kg/day dose group. Since there was no relationship to dose, these deaths were not considered drug-related.
Clinical signs	None
Body weights	None
Food consumption	A slight nonadverse reduction in food consumption occurred in the 1000 mg/kg/day (-11%) group during the first 3 days of dosing (G6 to G9), compared to the control group.
F0 pregnancy parameters	Prebirth loss was slightly increased in the 1000 mg/kg/day group compared to the concurrent control group (11.9% vs. 8.3% in controls) but was within the range observed in the historical control data. Pup weaning index (ratio of pups alive on Day 21 to Day 4) was slightly lower in the 1000 mg/kg/day group (98.8%) compared to the concurrent control group (100%) and historical control data (99.9-100%). However, given the low magnitude of change, this was not considered adverse.

Parameters	Major Findings
F0 necropsy	At necropsy, dark foci on the glandular mucosa of the stomach was observed in the 10 and 1000 mg/kg/day groups (one and two females, respectively). Given the low incidence and absence of a clear dose relationship, this finding was not attributed to the drug. One pup at 100 mg/kg/day and one pup at 1000 mg/kg/day had dilated kidney(s) and/or dilated ureter. Given that this finding is a known background change and occurred at a low incidence, it was not attributed to the drug.
F1 neurobehavioral tests	<p>In the water maze test, there were no drug-related changes in mean swimming times and/or errors during the straight channel trials (to test swimming ability) or the learning trials, compared to the control group. In the 1000 mg/kg/day females, mean escape times were increased by 60% and mean number of errors were increased by 70% in the memory trial, compared to the control group. The individual mean escape time and mean number of errors was highly variable in all female groups, therefore these effects were not clearly demonstrated to be drug-related. Mean escape times and mean number of errors in the memory trial were comparable among all male groups.</p> <p>Animals were evaluated for locomotor activity at 10 weeks of age. Mean ambulatory activity and/or fine movements (i.e., locomotor activity) were increased in females (up to 47% and 38% of total, respectively) and males (up to 29% and 20% of total, respectively) in all treatment groups, compared with the concurrent control group. This generally occurred in a dose-dependent manner in the first trial, but a dose relationship was not present in subsequent trials. By the sixth trial, no significant differences in mean ambulatory activity or fine movements were noted. Since all groups showed a normal habituation response during the six trials, these effects were not considered adverse.</p>
F1 reproductive parameters	The fertility index was 100%, 90%, 95%, and 95% in the 0 (vehicle control), 10, 100, and 1000 mg/kg/day groups, respectively. These values were within the range observed in the historical control data. One female in the 1000 mg/kg/day group did not have viable embryos. This female had only one resorbed implantation resulting in a postimplantation loss of 100%, which disproportionately influenced the group mean value. This isolated finding was considered to be incidental.
F1 necropsy	None

Parameters	Major Findings
F1 drug plasma concentrations	Drug exposure was demonstrated in all pups one hour after dosing, and in most pups at four hours after dosing. Generally, plasma concentrations increased with dose in a less than dose-proportional manner on PND 4 and PND 20.

Table 77. Drug Plasma Concentrations in F1 Pups

Occasion	Sex	Dose (mg/kg/day)	Time (h)	Mean Concentration (ng/mL)
PND 4	Not specified	10	1	7.00
			4	BLQ
		100	1	4.61
			4	1.32
		1000	1	33.7
			4	10.8
PND 20	Male	10	1	1.22
			4	1.28
		100	1	3.64
			4	4.40
		1000	1	55.1
			4	10.6
PND 20	Female	10	1	2.48
			4	0.620
		100	1	8.50
			4	1.45
		1000	1	24.9
			4	50.9

Source: Report AB22204, page 1039
 Abbreviations: BLQ, below limit of quantification; PND, post-natal day

Source: Table prepared by nonclinical reviewer
 Abbreviations: PND, postnatal day

A4250: Oral (Gavage) Toxicity Study in the Juvenile Rat (TEA0010)

Key Study Findings

- There were no adverse effects.
- The NOAEL was considered to be 100 mg/kg/day.

Table 78. Study Information, Oral (Gavage) Toxicity Study in the Juvenile Rat (TEA0010)

Parameter	Method Details
GLP compliance	Yes
Dose and frequency of dosing	10, 30, and 100 mg/kg once daily
Route of administration	Oral gavage
Formulation/vehicle	Solution/20% v/v Propylene glycol in purified water
Species/strain	Rat/Crl:CD(SD)
Number/sex/group	Subset 1: 12 Subset 2: 6 Subset 3: 20
Study design	Subset 1 (12/sex/group): treated from PND 14 to 64, sacrificed on PND 65 Subset 2 (6/sex/group): treated from PND 14 to 63 and sacrificed after a 28-day recovery period Subset 3 (20/sex/group): treated from PND 14 to 63 for assessment of behavior and reproduction (mating and fertility) and toxicokinetics on PND 63; after PND 63, the subset 3 animals were maintained without treatment Subset 4 (18/sex/group): treated on PND 14 and sacrificed on PND 14 for the toxicokinetic study Starting on PND 91, each female in subset 3 was paired with a male in the same dose group (20/sex/group) for a maximum of 5 days. A subgroup of the control and 100 mg/kg/day animals (15/sex/group) in subset 3 was used to assess behavioral performance.
Deviation from study protocol affecting interpretation of results	None

Source: Table prepared by nonclinical reviewer

Abbreviations: GLP, good laboratory practice; PND, postnatal day

Table 79. Observations and Results, Oral (Gavage) Toxicity Study in the Juvenile Rat (TEA0010)

Parameters	Major Findings
Mortality	One non-drug-related death.
Clinical signs	From PND 15 until around the time of weaning, most 100 mg/kg/day animals showed loose feces. No other clinical signs were observed after the pups were weaned.
Body weights	None
Food consumption	None
Ophthalmoscopy	None
Hematology	None
Clinical chemistry	None
Reproduction	None
Neurobehavioral	None
Bone evaluation	Sporadic and statistically significant reductions in long bone growth were observed. However, the small changes (up to -3.7%) were observed at isolated timepoints and did not always have a clear relationship to dose. There were no meaningful drug-related effects on bone density.

Parameters	Major Findings
Gross pathology	None
Organ weights	None
Histopathology	None

Source: Table prepared by nonclinical reviewer
 Abbreviations: PND, postnatal day

13.1.4.5. Impurities/Degradants

Drug Substance

The identified impurities were evaluated for genotoxic potential by QSAR (Derek Nexus and Leadscope). The impurities (b) (4) were predicted negative for bacterial mutagenicity in Derek Nexus. (b) (4) were predicted negative for bacterial mutagenicity in Leadscope Model Applier.

The impurities (b) (4) were predicted negative for bacterial mutagenicity in the E coli Sal 102 A-T Mut model, but they were not in the applicability domain of the Salmonella Mut model of the Leadscope Model Applier.

The impurities (b) (4) were not in the applicability domain of the E coli Sal 102 A-T Mut and Salmonella Mut models of the Leadscope Model Applier. However, the structures of the (b) (4)

Based on structural similarity and/or lack of genotoxicity alerts, the drug substance impurities are classified as nonmutagenic (Class 5).

The specifications are acceptable from a pharmacology/toxicology perspective based on the qualification of the impurities in the general toxicology studies (see [Table 80](#)).

Table 80. Drug Substance Impurities and Dose Multiples

Drug Substance Impurity	Maximum Proposed Dose (mg/kg/day)	Proposed Limit (%)	Human Impurity Dose (mg/kg/day)	Lot ¹ Impurity Level (%)	NOAEL ³ (mg/kg)	Impurity Dose at NOAEL (mg/kg)	HED ² (mg/kg)	Margin
(b) (4)								

Source: Table prepared by nonclinical reviewer
 Abbreviations: NOAEL, no observed adverse effect level; HED, human equivalent dose
¹ S0413083: Lot used in the 26-week rat, juvenile rat, and 13-week dog toxicity studies.
² HED based on rat
³ Based on the 26-week rat toxicity study

Drug Product

The impurities (b) (4) are specified degradation impurities. As discussed above, (b) (4) are classified as nonmutagenic. (b) (4) is the (b) (4)

According to the Applicant, (b) (4)

(b) (4) The proposed specification of (b) (4),
 (b) (4) s NMT (b) (4)% w/w for each impurity.

At the maximum daily dose of odevixibat (7.2 mg assuming a body weight of 60 kg), the amount of impurity at the proposed specification is at or below the qualification threshold in International Conference for Harmonisation Q3B(R2). Therefore, the proposed drug product specifications are acceptable from a pharmacology/toxicology perspective.

Table 81. Drug Product Impurities

Degradation Products	MDD (mg/day)	Impurity (mcg/day)	Qualification Threshold (b) (4)
(b) (4)			

Source: Table prepared by nonclinical reviewer

Abbreviations: MDD, maximum daily dose; (b) (4)

13.2. Individual Reviews of Studies Submitted to the NDA

13.2.1. Carcinogenicity

13.2.1.1. A4250: 104-Week Oral (Gavage) Carcinogenicity Study in the Mouse

Table 82. Study Information, 104-Week Oral (Gavage) Carcinogenicity Study in the Mouse

Parameter	Method Details
Study no.	TEA0016
Study report location:	Module 4.2.3.4
Conducting laboratory and location:	(b) (4)
Date of study initiation	10/6/2016
GLP compliance	Yes
QA statement	Yes
Drug, lot #, and % purity	A4250 / GF15000211 and GF16000059 / 98-98.6%
CAC concurrence	Yes

Source: Table prepared by nonclinical reviewer

Abbreviations: CAC, Carcinogenicity Assessment Committee; GLP, good laboratory practice; QA, quality assessment

Key Study Findings

In the 2-year carcinogenicity study in CD-1 mice, A4250 was tested at oral doses of 10, 30, and 100 mg/kg/day via oral gavage. These dose levels were recommended by the Executive Carcinogenicity Assessment Committee (CAC). The study included a water control and a vehicle control group (20% v/v propylene glycol). Treatment with A4250 did not affect animal survival.

The trend analysis of tumor incidence showed a significant increase in adenoma and combined adenoma and adenocarcinoma in pituitary gland in males. However, the pairwise comparisons did not reveal any statistically significant increase in tumor incidence in the treatment groups as compared to the vehicle control groups in either sex. Therefore, it is concluded that treatment with A4250 did not increase the incidence of neoplasms in this study.

Adequacy of Carcinogenicity Study

The carcinogenicity study was conducted appropriately.

Appropriateness of Test Models

The test model was appropriate.

Evaluation of Tumor Findings

The trend analysis of tumor incidence showed a significant increase in adenoma and combined adenoma and adenocarcinoma in pituitary gland in males. However, the pairwise comparisons did not reveal any statistically significant increase in tumor incidence in the treatment groups as compared to the vehicle control groups in either sex. Therefore, it is concluded that treatment with Trial A4250 did not increase the incidence of neoplasms in this study.

Table 83. Methods, 104-Week Oral (Gavage) Carcinogenicity Study in the Mouse

Parameter	Method Details
Doses	0 (water control), 0 (vehicle control), 10, 30, and 100 mg/kg
Frequency of dosing	daily
Dose volume	10 mL/kg
Route of administration	oral
Formulation/vehicle	20% v/v propylene glycol in water
Basis of dose selection	Males: MTD (high dose was one third of lethal dose in the dose-ranging study) Females: animal to human multiple of local drug concentration in the intestinal tract
Species/strain	CrI:CD-1 (ICR) mice Males: 27 to 40 g Females: 18 to 30 g
Number/sex/group	54
Age	4 to 5 weeks
Animal housing	groups of up to 3 for males and up to 5 for females
Paradigm for dietary restriction	None
Dual control employed	Yes
Interim sacrifice	No
Satellite groups	6/sex/group
Deviation from study protocol	Deviations did not have a significant impact on the study outcome.

Source: Table prepared by nonclinical reviewer
Abbreviations: MTD, maximum tolerated dose

Mice (54/sex/group) were treated with Trial A4250 at dose levels of 10, 30, and 100 mg/kg/day via oral gavage for 2 years. The study included a water control and a vehicle control group (20% v/v propylene glycol). The study design was reviewed by the Executive CAC under investigational new drug application (IND) 130591, and the CAC's recommended doses were used.

The toxicokinetic evaluation was conducted during Weeks 13 and 26 (6/sex/group). Clinical signs of toxicity and mortality were observed daily. Study parameters also included body weights, food consumption, hematology, and ophthalmology. All animals were necropsied and histopathological examination was performed at study termination or after premature death. The Applicant's tumor data analysis was conducted using the Peto method (chi-squared test with time adjustment).

Observations and Results

Mortality

There were no treatment-related deaths.

Clinical Signs

There were no treatment-related changes.

Body Weight

The initial and final body weights are listed in the following table.

Table 84. Initial and Final Body Weights (g)

Body Weight	Water		Vehicle		10 mg/kg		30 mg/kg		100 mg/kg	
	M	F	M	F	M	F	M	F	M	F
Initial	32.9	25.2	32.8	25.2	32.3	25.9	32.3	25.8	32.4	25.4
Final	45.9	35.9	43.4	36.2	43.9	37.8	42.0	35.2	42.1	34.1

Source: Table prepared by nonclinical reviewer
Abbreviations: M, male; F, female

The terminal body weight gains (Weeks 1 to 104) were 13.0, 11.4, 9.7, and 9.7 g in the water control, low-, mid-, and high-dose males, and 10.9, 12.0, 9.5, and 8.9 g in the water control, low-, mid-, and high-dose females, respectively.

Feed Consumption

A slight decrease in food consumption was noted in males in all treatment groups and in the high-dose females.

Ophthalmology

There were no treatment-related changes.

Hematology

No treatment-related changes were observed.

Gross Pathology

No treatment-related changes were observed.

Histopathology

Peer Review

Yes

Non-Neoplastic Changes

Treatment with A4250 produced significant increases in the incidence of cystic hyperplasia and basophilic amorphous contents in the gall bladder.

Neoplastic Changes

The trend analysis of tumor incidence by the Food and Drug Administration (FDA) showed a significant increase in adenoma and combined adenoma and adenocarcinoma in pituitary gland in males. The incidence of adenoma and combined adenoma and adenocarcinoma in pituitary gland in males is summarized in the following table.

Table 85. Incidence of Adenoma and Combined Adenoma and Adenocarcinoma in Pituitary Gland in Males

Tumor Type	Water	Vehicle	10 mg/kg	30 mg/kg	100 mg/kg
Adenoma	1/53	0/54	0/53	0/54	3/54
Adenoma + Adenocarcinoma	1/53	0/54	0/53	0/54	4/54

Source: Table prepared by nonclinical reviewer

The pairwise comparisons by FDA did not reveal any statistically significant increase in tumor incidence in the treatment groups as compared to the vehicle control groups in either sex.

Toxicokinetics

The plasma concentration of A4250 was measured at 1 and 24 hours after dosing on Weeks 13 and 26. The data are shown in [Table 86](#) (copied from the study report).

Table 86. Plasma Concentrations at 1 Hour and 24 Hours After Dosing on Weeks 13 and 26

Dose level A4250 (mg/kg/day)	Mean plasma concentrations at 1 hour after dosing (ng/mL)				Mean plasma concentration at 24 hours after dosing (ng/mL)			
	Males		Females		Males		Females	
	Week 13	Week 26	Week 13	Week 26	Week 13	Week 26	Week 13	Week 26
10	34.2	45.2	25.2	30.2	BQL	BQL	BQL	0.667
30	50.8	54.9	75.1	55.3	1.64	0.457	0.485	9.57
100	150	107.8	99.2	192	1.48	5.40	28.8	2.43

BQL = Below quantifiable limit (0.500 ng/mL)

Individual values of BQL were taken as zero for the purposes of calculations. For this reason, group mean values may appear lower than the lower limit of quantification.

Values are shown to 3 significant values.

Analytical range = 0.500 ng/mL to 200 ng/mL

Source: Table copied from Applicant's report TEA0016

Dosing Solution Analysis

The concentration analysis indicated that individual concentrations of A4250 were within 8% of the nominal concentration, and relative standard deviations (SD) were $\leq 2.6\%$.

FDA Statistical Review

Reviewer's Analysis

To verify the Applicant's analysis and to perform additional analyses suggested by the reviewing pharmacologist, this reviewer independently performed the survival and tumor data analyses. Data used in this reviewer's analyses were provided by the Applicant electronically on January 06, 2021, via SN0007.

Survival Analysis

The intercurrent mortality data is presented separately in the Statistical Review and Evaluation (authored by Malick Mbodj, PhD).

Reviewer's Findings

This reviewer's analysis showed the numbers of mice surviving to their terminal necropsy were 25 (46%), 24 (44%), 28 (52%), 32 (59%), and 25 (46%), in water control, vehicle control, low, medium, and high dose groups in male mice, respectively, and 25 (46%), 22 (41%), 21 (39%), 25 (46%), and 22 (41%), in water control, vehicle control, low, medium, medium-high, and high dose groups in female mice, respectively. This reviewer's analysis showed no statistically significant increase in mortality across the vehicle control group and the three treated groups in either sex of mice. The pairwise comparisons showed no statistically significant increase or decrease in mortality between each of the treated groups and the vehicle control group in either sex of mice.

Tumor Data Analysis

In the reviewer's analysis, the tumor data were analyzed for a dose response relationship across vehicle control group and the treated groups without including the water control group, as well as the pairwise comparisons of vehicle control group with each of the treated groups using the Poly-k method described in the paper of (Bailer and Portier 1988; Bieler and Williams 1993). In this method, an animal that lives the full study period (max w) or dies before the terminal sacrifice with development of the tumor type being tested gets a score of $h_s = 1$. An animal that dies at Week h w without development of the given tumor type before the end of the study gets a score of

$$s_h = \left(\frac{w_h}{w_{\max}} \right)^k < 1.$$

The adjusted group size is defined as $\sum h_s$. As an interpretation, an animal with score $h_s = 1$ can be considered as a whole animal, while an animal with score $h_s < 1$ can be considered as a partial animal. The adjusted group size $\sum h_s$ is equal to N (the original group size) if all animals live up to the end of the study or if each animal develops the given tumor being tested, otherwise the adjusted group size is less than N. These adjusted group sizes are then used for the dose response relationship (or the pairwise comparison) tests using the Cochran-Armitage test. One critical point for Poly-k test is the choice of the appropriate value of k. For long-term 104-week standard rat and mouse studies, a value of k=3 is suggested in the literature (Portier 1986; Moon et al. 2003; Gebregziabher and Hoel 2009).

Hence, this reviewer used k=3 for the analysis of the data. Based on the intent-to-treat (ITT) principle Wmax was considered as 104 for both male and female mice. For the calculation of p-values, if there were less than 10 tumor bearing animals across all treatment groups for a given tumor type, the exact tests based on the discrete permutation distribution were used, with dose levels (0, 10, 30, and 100 for both male and female mice) as scores. Asymptotic tests were used for tumor types with higher incidences. The tumor rates and the p-values of all tumor types tested for dose response relationship and the pairwise comparisons of vehicle control and treated groups are presented separately in the Statistical Review and Evaluation (authored by Malick Mbodj, PhD). The tumor types that achieved statistical significance in either the dose-response (trend) or pairwise tests are shown in [Table 87](#) (copied from the Statistical Review and Evaluation).

Multiple Testing Adjustment

Performed in accordance with the FDA draft guidance for carcinogenicity study design and data analysis (2001).

Reviewer's Findings

Table 87. Tumor Types With P-Values ≤0.05 for Dose-Response Relationship or Pairwise Comparisons (Treated Groups and Vehicle Control Group in Mice)

Sex	Organ Name	Tumor Name	0 mg Water (N=54) P -VC vs.W	0 mg Vehicle (N=54) P - Trend	10 mg Low (N=54) P - VC vs. L	30 mg Med(N=54) P - VC vs. M	100 mg High (N=54) P - VC vs. H
Male	Pituitary Gland	Adenoma	1/53 (41) 0.5190	0/54 (38) 0.0118*	0/53 (40) NC	0/54 (44) NC	3/54 (37) 0.1151
		Adenocarcinoma/ Adenoma	1/53 (41) 0.5190	0/54 (38) 0.0028*	0/53 (40) NC	0/54 (44) NC	4/54 (38) 0.0575
Female	Skin/Subcutis (Non-Protocol)	Fibrosarcoma	0/54 (39) NC	0/54 (37) 0.0224*	0/54 (36) NC	1/54 (38) 0.5067	3/54 (40) 0.1351
	Uterus	Polyp(S)	5/54 (41) 0.0355*	0/54 (37) 0.3621	3/54 (37) 0.1199	3/54 (39) 0.1300	2/54 (38) 0.2533

Source: Table prepared by the nonclinical statistical reviewer

Abbreviations: H, high dose; L, low dose; M, mid dose; P, p-value; VC, vehicle control; W, water

* Statistically significant at 0.025 level or 0.05 level, for rare tumors for tests of dose response relationship and pairwise comparison, respectively.

Following the multiple testing adjustment method described above, this reviewer's analyses showed statistically significant increasing dose response relationships across the vehicle control and the treated groups of male mice for the incidence of adenoma, the combined adenocarcinoma and adenoma, in pituitary gland, and in female mice for the incidence of fibrosarcoma, in skin/subcutis (p-value=0.0118, =0.0028, and =0.0224, respectively). The pairwise comparisons showed no tumor types with a statistically significant increase in tumor incidences in A4250 treated groups, when compare to the vehicle control group in either sex of mice. Also, this reviewer's analyses showed a statistical significance in the water control group for the incidence of polyp(s) in the uterus, when compared to the vehicle control group in female mice (P=0.0355).

Reviewer's Comments on the Tumor Type Nonprotocol Tissues in Skin/Subcutis

For the tumor dataset the number of animals examined for the tumor type nonprotocol tissues in the skin/subcutis in mice were 54 in each dose group. However, in the study report, the number of animals that were examined were 14, 13, 6, 5, and 6, in the water control, vehicle control group, low, medium, and high dose groups, in male mice, respectively, and 7, 4, 4, 4, and 6 in female mice, respectively. It has been advised by the pharm/tox reviewers and the Executive CAC that those discrepancies do not need to be resolved since the overall conclusions about drug-related tumorigenicity will not be impacted.

13.2.1.2. A4250: 104-Week Oral (Gavage) Carcinogenicity Study in the Rat

Table 88. Study Information, 104-Week Oral (Gavage) Carcinogenicity Study in the Rat

Parameter	Method Details
Study no.	TEA0015
Study report location	Module 4.2.3.4
Conducting laboratory and location	(b) (4)
Date of study initiation	11/3/2016
GLP compliance	Yes
QA statement	Yes
Drug, lot #, and % purity	A4250 / GF15000211 and GF16000059 / 98-98.6%
CAC concurrence	Yes

Source: Table prepared by nonclinical reviewer

Abbreviations: CAC, Carcinogenicity Assessment Committee; GLP, good laboratory practice; QA, quality assessment

Key Study Findings

In the 2-year carcinogenicity study in Wistar Han rats, A4250 was tested at oral doses of 10, 30, and 100 mg/kg/day via oral gavage. These dose levels were recommended by the Executive CAC. The study included a water control and a vehicle control group (20% v/v propylene glycol). Treatment with A4250 did not affect animal survival. Neither trend nor pairwise comparisons revealed any statistically significant increase in tumor incidence in the treatment groups as compared to the vehicle control groups in either sex. Therefore, it is concluded that treatment with A4250 did not increase the incidence of neoplasms in this study.

Adequacy of Carcinogenicity Study

The carcinogenicity study was conducted appropriately.

Appropriateness of Test Models

The test model was appropriate.

Evaluation of Tumor Findings

Neither trend nor pairwise comparisons revealed any statistically significant increase in tumor incidence in the treatment groups as compared to the vehicle control groups in either sex. Therefore, it is concluded that treatment with A4250 did not increase the incidence of neoplasms in this study.

Table 89. Methods, 104-Week Oral (Gavage) Carcinogenicity Study in the Rat

Parameter	Method Details
Doses	0 (water control), 0 (vehicle control), 10, 30, and 100 mg/kg
Frequency of dosing	Daily
Dose volume	5 mL/kg
Route of administration	Oral
Formulation/vehicle	20% v/v propylene glycol in water
Basis of dose selection	Animal to human multiple of local drug concentration in the intestinal tract
Species/strain	Crl:WI(Han) rats Males: 217 to 329 g Females: 150 to 226 g
Number/sex/group	50
Age	5-6 weeks
Animal housing	Groups of up to 3 for males and up to 4 for females
Paradigm for dietary restriction	None
Dual control employed	Yes
Interim sacrifice	No
Satellite groups	3/sex/group
Deviation from study protocol	Deviations did not have a significant impact on the study outcome.

Source: Table prepared by nonclinical reviewer

Rats (50/sex/group) were treated with A4250 at dose levels of 10, 30, and 100 mg/kg/day via oral gavage for 2 years. The study design was reviewed by the Executive CAC under IND 130591, and CAC's recommended doses were used. The toxicokinetic evaluation was conducted during Weeks 13 and 26 (3/sex/group). Clinical signs of toxicity and mortality were observed daily. Study parameters also included body weights, food consumption, hematology, and ophthalmology. All animals were necropsied and complete histopathological examination was performed at termination or after premature death. The Applicant's tumor data analysis was conducted using the Peto method (Chi-squared test with time adjustment).

Observations and Results

Mortality

Treatment with A4250 had no effect on mortality.

Clinical Signs

There were no treatment-related changes.

Body Weight

The initial and final body weights are listed in the following table.

Table 90. Initial and Final Body Weights (g), 104-Week Oral (Gavage) Carcinogenicity Study in the Rat

Initial/ Final	Water		Vehicle		10 mg/kg		30 mg/kg		100 mg/kg	
	M	F	M	F	M	F	M	F	M	F
Initial	273.8	183.2	276.1	184.9	270.1	183.1	270.2	184.4	273.3	186.2
Final	735.8	435	724.1	407.1	684.9	387.9	705.2	410.3	704.5	412.1

Source: Table prepared by nonclinical reviewer
Abbreviations: M, male; F, female

No significant effects on weight gain were observed.

Feed Consumption

There were no treatment-related changes.

Ophthalmology

There were no treatment-related changes.

Hematology

No treatment-related changes were observed.

Clinical Chemistry

The total plasma cholesterol, HDL, and LDL levels in male treatment groups were lower compared to the control group. Plasma glucose levels were lower during Week 52 but were increased at Week 103 in the treated males as compared to the controls. These changes were not observed in females.

Gross Pathology

No treatment-related changes were observed.

Peer Review

Yes

Non-Neoplastic Changes

Treatment with A4250 produced increases in the incidence of hyperplasia of the urothelium and mineralization of the pelvis in kidney, and biliary hyperplasia and basophilic foci in liver.

The summary of these changes is presented in the Applicant's tables below.

Table 91. Summary of Non-Neoplastic Changes in Kidney, 104-Week Oral (Gavage) Carcinogenicity Study in the Rat

Group	Males					Females					
	1	2	3	4	5	1	2	3	4	5	
Dose Level (mg/kg/day)	0	0	10	30	100	0	0	10	30	100	
Number of Rats Examined	50	50	50	50	50	50	50	50	50	50	
Kidney											
Hyperplasia, urothelium	Minimal	4	9	10	12	15	28	31	27	31	21
	Slight	1	2	5	0	3	3	5	9	5	16
	Moderate	0	0	0	0	0	0	0	2	0	1
	Total	5	11	15	12	18	31	36	38	36	38
Mineralisation, pelvis	Minimal	1	1	3	7	10	23	33	24	29	24
	Slight	1	6	4	0	4	6	2	8	3	10
	Moderate	0	2	1	0	0	0	1	0	0	1
	Total	2	9	8	7	14	29	36	32	32	35

Source: Table copied from Applicant's report TEA0015

Table 92. Summary of Non-Neoplastic Changes in Liver, 104-Week Oral (Gavage) Carcinogenicity Study in the Rat

Group	Males					Females					
	1	2	3	4	5	1	2	3	4	5	
Dose Level (mg/kg/day)	0	0	10	30	100	0	0	10	30	100	
Number of Rats Examined	50	50	50	50	50	50	50	50	50	50	
Liver											
Biliary hyperplasia	Minimal	1	4	6	3	3	5	5	6	6	10
	Slight	2	2	0	3	6	2	3	1	4	6
	Moderate	1	0	0	1	0	1	1	0	0	1
	Total	4	6	6	7	9	8	9	7	10	17
Foci of Alteration (basophilic)	Minimal	9	5	6	10	9	16	18	14	20	13
	Slight	0	2	0	4	1	6	6	15	14	14
	Moderate	0	1	0	1	0	2	2	6	7	8
	Marked	0	0	0	0	0	0	0	3	2	4
	Severe	0	0	0	0	0	0	0	0	0	1
	Total	9	8	6	15	10	24	26	38	43	40

Source: Table copied from Applicant's report TEA0015

Neoplastic Changes

Neither trend nor pairwise comparisons revealed any statistically significant increase in tumor incidence in the treatment groups as compared to the vehicle control groups in either sex. Therefore, it is concluded that treatment with A4250 did not increase the incidence of neoplasms in this study.

Toxicokinetics

The plasma concentrations of A4250 are summarized in [Table 93](#) (copied from the study report).

Table 93. Plasma Concentrations of A4250 at 1 Hour and 24 Hours After Dosing

Dose level A4250 (mg/kg/day)	Mean plasma concentrations at 1 hour after dosing (ng/mL)				Mean plasma concentration at 24 hours after dosing (ng/mL)			
	Males		Females		Males		Females	
	Week 13	Week 26	Week 13	Week 26	Week 13	Week 26	Week 13	Week 26
10	15.2	28.2	15.5	11.0	23.5	8.89	7.30	10.8
30	32.2	50.9	29.8	23.9	7.90	3.61	5.37	8.53
100	69.2	80.2	28.7	29.3	12.3	12.1	15.2	7.15

Source: Table copied from Applicant's report TEA0015
 Analytical range =0.500 ng/mL to 200 ng/mL

Dosing Solution Analysis

The concentration analysis indicated that individual concentrations of A4250 were within 8% of the nominal concentration, and relative standard deviations were $\leq 2.7\%$.

FDA Statistical Review

Reviewer's Analysis

To verify Applicant's analysis and to perform additional analyses suggested by the reviewing pharmacologist, this reviewer independently performed the survival and tumor data analyses. Data used in this reviewer's analyses were provided by the Applicant electronically on January 6, 2021, via SN0007.

Similar to the mouse study, this reviewer independently performed the survival and tumor data analyses of the rat study. For the analysis of the survival data and the tumor data of the rat study, this reviewer used similar methodologies that were used for the analyses of the survival and tumor data of the mouse study. Data used in this reviewer's analyses were provided by the Applicant electronically.

Survival Analysis

The intercurrent mortality data is presented separately in the Statistical Review and Evaluation (authored by Malick Mbodj, PhD).

Reviewer's Findings

This reviewer's analysis showed the numbers of rats surviving to their terminal necropsy were 33 (66%), 34 (68%), 25 (50%), 32 (64%), and 32 (64%) in the water control, vehicle control group, low, medium, and high dose groups, in male rats, respectively, and 28 (56%), 36 (72%), 32 (64%), 35 (70%), and 33 (66%) in water control, vehicle control, low, medium, and high dose groups, in female rats, respectively. This reviewer's analysis showed no statistically significant increase in mortality across the vehicle control group and the three treated groups in either sex of rats. The pairwise comparisons showed no statistically significant increase or decrease in mortality between each of the treated groups and the vehicle control group in either sex of rats.

Tumor Data Analysis

The tumor rates and the p-values of all tumor types are presented separately in the Statistical Review and Evaluation (authored by Malick Mbodj, PhD). The tumor types that achieved statistical significance in either the dose-response (trend) or pairwise tests are shown in [Table 94](#) (copied from the Statistical Review and Evaluation). (Portier 1986; Moon et al. 2003;

Gebregziabher and Hoel 2009). Following the FDA draft guidance for the carcinogenicity study design and data analysis 2001, for the two-year rat study this reviewer used significance levels of 0.005 and 0.025 for common and rare tumors, respectively in dose response relationship (trend) tests and significance levels of 0.01 and 0.05 for common and rare tumors, respectively in pairwise comparisons. A tumor is defined as a rare tumor if the published spontaneous rate or the spontaneous rate of the vehicle control of the tumor is less than 1%, and a common tumor is defined as one with tumor rate greater than or equal to 1%.

Multiple Testing Adjustment

Performed in accordance with the FDA draft guidance for carcinogenicity study design and data analysis (2001).

Reviewer’s Findings

Reviewer’s comment on the Applicant’s tumor data of rat study

For the tumor data of the rat study, this reviewer noticed that two different tumor codes were associated with the same name for three tumor types (adenoma in the adrenals, fibroadenoma in the nonprotocol mammary gland, and fibroma in the nonprotocol skin for both male and female rats; the tumor codes used were 49500 and 39700, 39700 and 49500, and 50200 and 76500, respectively). Therefore, this reviewer combined them.

Table 94. Tumor Types With P-Values ≤0.05 for Dose Response Relationship or Pairwise Comparison Treated Groups and Vehicle Control Group in Rats

Sex	Organ Name	Tumor Name	0 mg Water (N=50) P - VC vs. W	0 mg Vehicle Cont (N=50) P - Trend	10 mg Low (N=50) P - VC vs. L	30 mg Med (N=50) P - VC vs. M	100 mg High (N=50) P - VC vs. H
Male	Pituitary Gland	Adenoma	14/50 (44) 0.4078	12/48 (44) 0.9558	22/50 (46) 0.0361@	15/49 (43) 0.2963	9/48 (42) 0.8108
	Non-Protocol Skin	Papilloma, Sessile	0/50 (42) NC	0/50 (44) 0.0229*	0/50 (41) NC	2/50 (43) 0.2414	3/50 (42) 0.1122
Female	Non-Protocol Mammary Gland	Fibroadenoma	10/50 (42) 0.7629	13/50 (46) 0.0142@	9/50 (44) 0.8659	9/50 (43) 0.8525	19/50 (44) 0.1041

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;

NC = Not calculable.

*: Statistically significant at 0.005 and 0.025 level for common and rare tumor or 0.01 and 0.05 level for common and rare tumors for tests of dose response relationship and pairwise comparison, respectively.

@ = Not statistically significant in rare tumor at 0.025 level for test of dose response relationship and at 0.05 level for test of pairwise comparisons, or in common tumor at 0.005 level for test of dose response relationship and at 0.01 level for test of pairwise comparisons

Source: Table prepared by the nonclinical statistical reviewer

Abbreviations: P, p-value; VC, vehicle control; W, water; L, low dose; M, mid dose; H, high dose

Following the multiple testing adjustment method described above, this reviewer’s analysis showed statistically significant increasing dose response relationships across the vehicle control and the treated groups for the incidence of papilloma, sessile in nonprotocol skin in of male rats (p-value=0.0229). The pairwise comparisons showed no tumor types with a statistically significant increase in tumor incidences in A4250 treated groups, when compare to the vehicle control group in either sex of rats.

Reviewer's comment on the tumor types of nonprotocol tissues in skin and mammary gland

For the Applicant's electronic tumor dataset, the numbers of animals examined for the tumor types of nonprotocol tissues in the skin and in the mammary gland of rats were all 50 in each dose group. However, in the Applicant's study report, the numbers of animals that were examined in the nonprotocol tissues in the skin were 18, 25, 16, 13, and 20, in the water control, vehicle control group, low, medium, and high dose groups, in male rats, respectively, and 16, 17, 17, 12, and 17 in female rats, respectively. For the mammary gland, the Applicant's study report shows that 17, 17, 14, 17, and 16 in female rats were examined. It has been advised by the pharm/tox reviewers and the Executive CAC that those discrepancies do not need to be resolved since the overall conclusions about drug-related tumorigenicity will not be impacted.

The reviews of the two carcinogenicity study reports were presented at the Executive CAC meeting on March 23, 2021. The Executive CAC concurred that the 2-year carcinogenicity studies in mice and rats were adequate and concluded that there were no drug-related neoplasms in either males or females in these studies.

13.2.1.3. A Repeat Dosage Phototoxicity Study to Determine the Effects of Oral Administration of A4250 on Eyes and Skin in Pigmented Rats (20243334)

Key Study Findings

- There was no evidence of ocular or cutaneous phototoxicity after administration of A4250 for 3 days followed by a single exposure to UVR in female Long-Evans rats.
- The plasma concentrations demonstrated presence of A4250 in treated animals.
- The NOAEL was considered to be 1000 mg/kg/day.

Table 95. Study Information, a Repeat Dosage Phototoxicity Study to Determine the Effects of Oral Administration of A4250 on Eyes and Skin in Pigmented Rats (20243334)

Parameter	Method Details
GLP compliance	Yes
Dose and frequency of dosing	100, 300, and 1000 mg/kg once daily for 3 days
Route of administration	Oral gavage
Formulation/vehicle	Solution/20% v/v Propylene glycol in purified water
Species/strain	Rat/Crl:LE (Long-Evans)
Number/sex/group	5 females/group

Parameter	Method Details
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Study design

Table 96. Experimental Design

Group	Female Rats (N)	Treatment (mg/kg)	Dose (mg/kg)	UVA Dose (J/cm ²)	Interval Between
					Dose and UVR Exposure ¹ (hours ± minutes)
1	5	Control Article	0	10 ± 1	4 ± 10
2	5	A4250	100	10 ± 1	4 ± 10
3	5	A4250	300	10 ± 1	4 ± 10
4	5	A4250	1000	10 ± 1	4 ± 10
5	5	A4250	1000	0	N/A ²

Source: Applicant's report 20243334, page 11

Abbreviations: N/A, not applicable; UVA, ultraviolet A; UVR, ultraviolet radiation

¹ Interval based on the T_{max} of the test article. The actual exposure time of the group was based on the average dosing time of the group.

² Sham exposure; rats were sedated and restrained but not exposed to UVR.

Using a xenon lamp, a dose of 10.29 J/cm² of UVA was delivered to each rat over a period of 42 minutes. The UVB dose was 145 mJ/cm². UVR exposure occurred on Day 3, 4 hours after dosing.

Deviation from study protocol affecting interpretation of results None

Source: Table prepared by nonclinical reviewer

Abbreviations: UVA, ultraviolet A; UVB, ultraviolet B; UVR, ultraviolet radiation

Table 97. Observations and Results, a Repeat Dosage Phototoxicity Study to Determine the Effects of Oral Administration of A4250 on Eyes and Skin in Pigmented Rats (20243334)

Parameters	Major Findings
Mortality	One death, not drug-related.
Clinical signs	Sedation was observed in 2 of 5 animals/group in groups 2, 3, and 4 on Day 3. This was attributed to anesthetization used for restraint during UVR exposure.
Body weights	None
Skin Reactions	None
Ophthalmoscopy	Corneal dystrophy was observed in all treated groups. It is known to be a common background finding in this rat strain (47.7% in a dataset of 2232 Long-Evans rats age 12 weeks; (Gilger et al. 2018). Inferior focal retinopathy was noted in all groups exposed to UVR. This observation is related to the UVR exposure, as it was not noted in the sham UVR group (group 5).

Table 98. Summary of Ophthalmic Findings

Finding	Group 1	Group 2	Group 3	Group 4	Group 5
	0 (mg/kg)	100 (mg/kg)	300 (mg/kg)	1000 (mg/kg)	1000 (mg/kg)
Number animals examined (eyes)	4 (8)	5 (10)	5 (10)	5 (10)	5 (10)
Inferior focal retinopathy	3 (4)	5 (10)	5 (7)	5 (9)	0 (0)
Corneal dystrophy	0 (0)	1 (1)	2 (4)	3 (4)	1 (1)

Source: Report 20243334, page 203

Value recorded is the total number of rats with that observation with the number of eyes affected in parentheses.

Histopathology Neutrophilic infiltration in the corneal stroma was generally observed bilaterally with minimal severity at a similar incidence in all groups, including the control group. The corneal changes were suggestive of prolonged corneal exposure and desiccation, possibly as a result of manipulation of the eyes for UVR exposure. One animal at 300 mg/kg had moderate to marked infiltrates of neutrophils with minimal to mild edema in the corneal stroma, which was considered to be associated with the marked bilateral corneal dystrophy noted in the ophthalmic exam and not drug-related.

14. Clinical Pharmacology: Additional Information and Assessment

14.1. In Vitro Studies

To interpret the following in vitro studies in the context of clinically relevant concentrations of odevixibat, plasma drug concentrations were mostly below 0.05 ng/mL (=0.067 nmol/L) at around the recommended dose, 40 mcg/kg/day, in clinical studies of odevixibat. The highest C_{max} observed among pediatric patients given odevixibat 10 to 200 mcg/kg was 1.11 ng/mL (=1.48 nmol/L) following a single dose of odevixibat 100 mcg/kg in the phase 2 study.

Protein Binding (Study No. 178257)

The binding of odevixibat to human plasma proteins was determined by equilibrium dialysis at concentrations of 4 and 40 micromoles/L following incubation at 37°C for 2 hours. Odevixibat could not be detected in the buffer compartments for all matrices, with levels below the limit of detection of 0.012 micromoles/L. This suggests that the free fraction of odevixibat is very low, at least <0.3%, corresponding to a protein binding >99.7% (>99.7% at 4 micromoles/L and >99.97% at 40 micromoles/L).

Odevixibat as Substrate of CYP Enzymes (Study No. ALB/05)

In vitro metabolism was studied in freshly isolated human hepatocytes, which demonstrated both phase 1 and phase 2 metabolism suggesting that odevixibat can be metabolized via monohydroxylation. This study used both radiolabeled and non- radiolabeled odevixibat at 10 micromoles/L and 1 micromole/L, respectively.

In the human hepatocytes incubated with ¹⁴C-odevixibat at a concentration of 10 micromoles/L for 4 hours, the parent and the main metabolites M2, M3, and M6 corresponding to monohydroxy ¹⁴C-odevixibat were confirmed to be present in each sample. The proportion of metabolized odevixibat ranged from 8.2% to 22.6% of the initial concentration of odevixibat.

In the human hepatocytes incubated with nonradiolabeled odevixibat at 1 micromole/L for 4 hours, the parent drug was confirmed to be present in all samples and the presence of M2, M3, and M6 corresponding to monohydroxy odevixibat were also confirmed in two of three human hepatocyte samples.

Odevixibat as an Inhibitor/Inducer of CYP Enzymes (Study No. 25582, XT165057, and XT163049)

The direct inhibitory potency of odevixibat was evaluated on the activities of five human recombinant CYP isoforms (i.e., 1A2, 2C9, 2C19, 2D6, and 3A4) with odevixibat concentrations in the range of 0.01-30 micromoles/L. Odevixibat had no inhibitory effects on CYP1A2 and 2C19. The half maximal inhibitory concentration (IC_{50}) value obtained for CYP2C9 was 1.2 micromoles/L, CYP2D6 was 16.0 micromoles/L, and CYP3A4 was 16.2 micromoles/L. In human liver microsomes with odevixibat concentrations in the range of 0.1-100 nmol/L, odevixibat was a direct inhibitor of CYP1A2 and CYP2B6 with IC_{50} of 13 nmol/L and

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5.6 nmol/L, respectively, and of CYP3A4/5 with IC₅₀ of 4.0 micromoles/L (midazolam as probe) and 14 micromoles/L (testosterone as probe). Some inhibition was observed for CYP2C8 with IC₅₀>100 nmol/L.

In human hepatocytes incubated with odevixibat concentrations in the range of 1-30 micromoles/L, a time-dependent inhibition of CYP3A4/5 was found with an IC₅₀ decrease from 4.1 micromoles/L to 0.48 micromoles/L after pre-incubation.

Odevixibat was not an inducer of CYP enzymes at the clinically relevant concentration. Treatment of cultured human hepatocytes with up to 20 micromole/L odevixibat did not induce CYP1A2, CYP2B6, or CYP3A4 mRNA levels in all three hepatocyte preparations tested.

These studies indicated that odevixibat is neither an inhibitor nor an inducer of CYP enzymes at the expected plasma concentration of odevixibat; however, it suggested that a 7.2 mg dose of odevixibat in adults (corresponding approximately 103 mcg/kg for a 70-kg man) may inhibit intestinal CYP3A enzyme (i.e., $(1 + [I]_{gut}/K_i) = 20.5$). Subsequently, in an in vivo drug-drug (DDI) study with midazolam (a CYP3A4 substrate), no inhibition of CYP3A4 was observed (see Section [14.2.4](#)).

Odevixibat as a Substrate or an Inhibitor of Transporters (Study No. XT168064)

Odevixibat was shown to be a substrate of P-gp in Caco-2 as well as MDCKII cells at a concentration of 5 micromoles/L. It led to an in vivo DDI study with itraconazole (a strong P-gp inhibitor) (see Section [14.2.4](#)). Odevixibat was not a substrate of BCRP in MDCKII at 5 micromoles/L.

Odevixibat was not an inhibitor of transporters at the clinically relevant concentration. The inhibition of P-gp and BCRP was <50% in the presence of odevixibat up to 30 micromoles/L. The inhibition of OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1- or MATE2-K was evaluated in the presence of odevixibat at 0.1 and 1 micromole/L. Odevixibat was an inhibitor of OATP1B1, OATP1B3, and OAT3 with IC₅₀ values of 0.308, 0.697, and 0.504 micromoles/L, respectively. Approximately 50% inhibition of OAT1 and OCT2 was observed at 1 micromole/L, indicating approximate IC₅₀ values of 1 micromole/L for these transporters. The inhibition of MATE1- or MATE2-K was <50% in the presence of odevixibat up to 1 micromole/L.

14.2. In Vivo Studies

Clinical studies supporting clinical pharmacology information of odevixibat are summarized in [Table 99](#).

Table 99. Summary of Clinical Pharmacology Studies Supporting Clinical Pharmacology Information of Odevixibat

Study	Formulation	Dose	Supporting Clinical Pharmacology Data/Information
A4250-001 (Phase 1 SAD/MAD study in healthy adults)	Formulation A (b) (4) capsule)	Part 1 SAD: Placebo, 0.1, 0.3, 1, 3, and 10 mg Part 2 MAD (7 days): 1 mg QD, 1.5 mg BID, 3 mg QD with or without 1 g cholestyramine	PK, PD (bile acids in plasma, C4, FGF-19) with and without cholestyramine
A4250-007 (Mass balance study in healthy adults)	Formulation C (oral capsule with pellets that can be sprinkled)	Single dose of radiolabeled- odevixibat 3 mg	PK
A4250-004 (Food effect study/sprinkle study in healthy adults)	Formulation C (oral capsule with pellets that can be sprinkled)	Single dose of 9.6 mg (8*1200 mcg capsule) as a whole capsule at fasted status, fed status, or sprinkled on applesauce	PK
A4250-013 (Drug-drug interaction study in healthy adults)	Formulation C (oral capsule with pellets that can be sprinkled)	Part A: Multiple doses of 7.2 mg (6*1200 mcg capsule) for 4 days with or without midazolam Part B: Single dose of 7.2 mg (6*1200 mcg capsule) with or without itraconazole	PK
A4250-003 (Phase 2 dose-ranging study in pediatrics with cholestatic liver disease including PFIC)	Formulation B (b) (4) pellets)	10, 30, 60, 100, 200 mcg/kg QD for 4 weeks with food in the morning	PK, PD (serum total bile acids, autotaxin, C4, FGF-19)
A4250-005 (Phase 3 trial in pediatric patients with PFIC)	To-be-marketed formulations (oral pellets and oral capsule with pellets that can be sprinkled)	40 or 120 mcg/kg QD or matching placebo with food in the morning	PK, PD (serum total bile acid, autotaxin, C4)

Source: Section 2.7.2. Summary of Clinical Pharmacology Studies, Section 1.7.2. Summary of Clinical Efficacy (SDN 1)
Abbreviations: BID, twice daily; C4, complement component 4; FGF19, fibroblast growth factor 19; MAD, multiple ascending dose; PD, pharmacodynamic; PFIC, progressive familial intrahepatic cholestasis; PK, pharmacokinetic; QD, once a day; SAD, single ascending dose

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The final, to-be-marketed (TBM) formulations used in the pivotal phase 3 trial (A4250-005) are oral pellets and oral capsules that can be sprinkled on soft food consisted of four different strengths and formulations as shown in [Table 100](#).

Table 100. Final, To-Be-Marketed Formulations, Trial A4250-005

Patient's Body Weight	Dose	
	40 mcg/kg/day	120 mcg/kg
<19.5 kg	200 mcg sprinkle capsule (b) (4) in capsule size 0)	600 mcg sprinkle capsule (b) (4) in capsule size 0)
≥19.5 kg	400 mcg capsule (b) (4) in capsule size 3)	1200 mcg capsule (b) (4) capsule size 3)

Source: Section 2.7.1. Summary of Biopharmaceutic Studies and Associated Analytical Methods (SDN 1)

The two clinical pharmacology trials—A4250-004 and A4250-013—were conducted using one of the TBM formulations: 1200 mcg oral capsule.

No in vivo comparative pharmacokinetic (PK) study was conducted between the TBM formulation and the previously used formulations. Clinical pharmacology information presented in the label is primarily based on the four clinical studies using the TBM formulations given the potential formulation differences.

14.2.1. Phase 1 SAD/MAD Trial (A4250-001)

Title

A Phase 1, Double-Blind Single and Multiple Ascending Dose (MAD) Study to Assess Safety and Pharmacokinetics of A4250 as Monotherapy, and in Combination with Colonic Release Cholestyramine (A3384) or Commercially Available Cholestyramine (Questran™) in Healthy Subjects

Objectives

To evaluate the safety, tolerability, PK and pharmacodynamic (PD) of odevixibat in healthy volunteers after single oral doses in the fasted state (Part 1), multiple oral doses (Part 2a), and multiple oral doses in combination with cholestyramine (Part 2b)

Study Design

This was a single-center, two-part, double-blind, placebo-controlled trial in healthy volunteers. The trial parts consisted of the following:

- Part 1: Single-dose evaluation of odevixibat in five cohorts each with up to eight healthy volunteers (N=2 placebo; N=8 odevixibat), in an escalating scheme (0.1, 0.3, 1, 3, and 10 mg)
- Part 2a: Multiple dosing (7 days) of odevixibat in three cohorts each with up to eight healthy volunteers (N=2 placebo; N=8 odevixibat), with the following dosing regimens (1, 3, and 1.5 mg twice daily (BID))

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- Part 2b: Multiple dosing (7 days) of odevixibat (3 mg once daily [QD]) in combination with cholestyramine 1 g BID as follows in three cohorts each with up to eight healthy volunteers (N=2 placebo; N=8 odevixibat):
 - Questran (a commercial cholestyramine) 1 g BID at 4 hours and 14–15 hours post dose of odevixibat
 - An in-house colonic release formulation of cholestyramine (CRC) 1 g BID at -0.5 hour predose and 8.5 hours postdose of odevixibat
 - CRC 1 g BID at 4 hours and 14–15 hours postdose of odevixibat

Odevixibat was administered 15 minutes before breakfast. Lunch, dinner, and snack were provided at 4 hours, 9 hours, and 14 hours postdose of odevixibat.

PK and PD [i.e., plasma bile acids, plasma 7 α -hydroxy-4-cholensten-3-one (C4), and plasma fibroblast growth factor-19 (FGF-19)] were assessed with plasma samples obtained at following timepoints:

- PK: at predose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 hours postdose in Part 1, and Day 1 and Day 7 in Part 2
- PD: at predose, 4, and 24 hours postdose in Part 1; at predose, 4, and 24 hours postdose on Day 1 and at predose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 hours postdose on Day 7 in Part 2

Fecal bile acids were measured from feces homogenates in Part 2. Cumulative feces were collected before dosing for 48 hours (from Day -2 to Day -1), and after the last administration on Day 7 for 24 hours.

PK Results

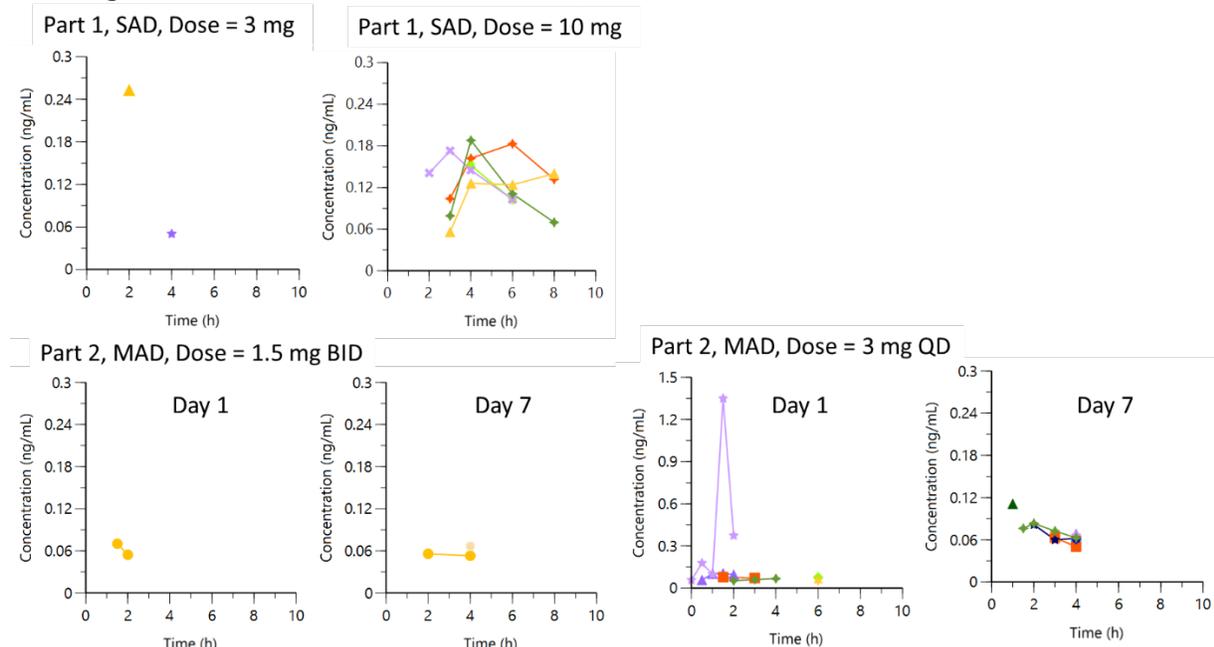
The majority of plasma drug concentrations were below the lower limit of quantification (LLOQ) (0.05 ng/mL) following a single dose of odevixibat 0.1 to 3 mg and multiple dose of 1 to 3 mg/day ([Table 101](#), [Figure 9](#)).

Table 101. Number of Samples With Unquantifiable Concentrations, Trial A4250-001

Odevixibat Dose Levels	N	Number of Plasma Samples With Unquantifiable Concentrations/ Total Number of Samples n (%)	Number of Samples With Quantifiable Concentrations/ Total Number of Samples n (%)
Part 1 (SAD)			
0.1 mg	6	66/66 (100.0)	0/66 (0.0)
0.3 mg	6	66/66 (100.0)	0/66 (0.0)
1 mg	5	55/55 (100.0)	0/55 (0.0)
3 mg	6	64/66 (97.0)	2/66 (3.0)
			(2 healthy volunteers, 0.0501, 0.253 ng/mL)
10 mg	6	48/66 (72.7)	18/66 (27.3)
			(5 healthy volunteers, 0.0559 to 0.581 ng/mL)
Part 2 (MAD)			
1 mg QD	5	110 (100.0)	0/110 (0.0)
1.5 mg BID	6	446/456 (97.8)	10/456 (2.2)
			(2 healthy volunteers, 0.053 to 0.0702 ng/mL)
3 mg QD	24	496/528 (93.9)	32/528 (6.0)
			(12 healthy volunteers, 0.0572 to 1.35 ng/mL)

Source: Reviewer's analysis based on the dataset EX.xpt, PC.xpt of A4250-001
Abbreviations: BID, twice daily; MAD, multiple ascending dose; QD, once a day; SAD, single ascending dose
The limit of quantitation was 0.05 ng/mL

Figure 9. Individual Plasma Odevixibat Concentrations: Time Profiles in Healthy Volunteers Showing Measurable Plasma Concentrations, Trial A4250-001



Source: Reviewer's analysis based on the dataset EX.xpt, PC.xpt of A4250-001
Abbreviations: BID, twice daily; MAD, multiple ascending dose; QD, once a day; SAD, single ascending dose

Due to the limited number of measurable drug concentrations, PK could not be adequately characterized for odevixibat 0.1 to 3 mg. However, at a single dose of odevixibat 10 mg, PK parameters were able to be estimated in five out of six healthy volunteers (83%). Following multiple dose of odevixibat 3 mg QD, 12 out of 24 healthy volunteers (50%) had one or more

measurable concentrations and AUC were able to be derived in two out of 24 healthy volunteers (Table 102).

Table 102. Available Pharmacokinetic Parameters of Odevixibat, Trial A4250-001

Parameter	Day 1		Day 7
	Single Dose 10 mg N=6	Multiple Dose 3 mg QD N=24	Multiple Dose 3 mg QD N=24
C_{max} (ng/mL)			
N	5	6	10
Mean (SD)	0.167 (0.021)	0.289 (0.520)	0.070 (0.018)
Min, Max	0.140, 0.188	0.053, 1.350	0.051, 0.111
T_{max} (h)			
N	5	6	10
Mean (SD)	4.0	2.75	4.0
Min, Max	3.0, 8.0	1.5, 6.0	1.0, 4.0
AUC_{0-t} (ng·h/mL)			
N	5	3	2
Mean (SD)	0.612 (0.184)	0.417 (0.436)	0.228 (0.021)
Min, Max	0.330, 0.845	0.156, 0.920	0.213, 0.243

Source: Reviewer's analysis based on the dataset EX.xpt, PC.xpt of A4250-001

Abbreviations: AUC, area under the concentration-time curve; C_{max} , maximum plasma concentration; QD, once a day; SD, standard deviation; T_{max} , time of maximum concentration observed

AUC was estimated only when there were three or more measurable concentrations per patient.

PD Results

The following PD results were focused on the data in Part 2 following multiple administration of odevixibat because intensive PD sampling was conducted only on Day 7 in Part 2. The AUC_{0-12h} on Day 7 following multiple doses of odevixibat was calculated for each PD marker such as total bile acids (BAs) (i.e., sum of 15 individual bile acids), C4, and FGF-19 in plasma.

In Part 2a, total bile acids and FGF-19 decreased and C4 increased significantly following multiple administration of odevixibat for 7 days compared to placebo even though the extents of changes did not significantly differ between odevixibat dose groups (Table 103).

It is in line with the mechanism of action (MOA) of odevixibat that inhibits reuptake of bile acid via ileal bile acid transporter (IBAT) in the ileum subsequently increasing bile acid clearance and decreasing bile acids in systemic circulation. An increase in plasma C4, an intermediate of bile acids reflects increased bile acids synthesis which is attributed to the reduced bile acid pool in the body. FGF-19 is excreted from ileal enterocytes after bile acid-dependent FXR activation so a decrease in FGF-19 also reflects the reduced bile acid pool.

Table 103. AUC_{0-12h} on Day 7 for Total Bile Acids, C4, and FGF-19 in Plasma Following Multiple Administration of Odevixibat for 7 Days, Trial A4250-001

Part	Treatment For 7 Days	N	AUC_{0-12h} Geometric Mean (Geometric CV%)		
			Total Bile Acid (ng·h/mL)	C4 (ng·h/mL)	FGF-19 (pg·h/mL)
Part 2a					
	Placebo (1 to 3)	6	33456.33 (53.4)	143 (70.4)	2270 (50.5)
	Odevixibat 1 mg QD	5	19827.06 (38.2)	592 (42.4)	530 (55.1)
	Odevixibat 3 mg QD	6	15272.29 (16.1)	882 (79.7)	543 (68.5)
	Odevixibat 1.5 mg BID	6	19493.37 (23.7)	785 (82.6)	383 (434.3)

Part	Treatment For 7 Days	N	AUC _{0-12h} Geometric Mean (Geometric CV%)		
			Total Bile Acid (ng·h/mL)	C4 (ng·h/mL)	FGF-19 (pg·h/mL)
Part 2b					
	Odevixibat 3 mg QD + Questran 1 g BID at 4h	6	16714.33 (27.3)	1420 (17.9)	244 (302.9)
	Odevixibat 3 mg QD + CRC 1 g BID at -0.5 h	6	20575.40 (16.0)	998 (39.6)	616 (117.6)
	Odevixibat 3 mg QD + CRC 1 g BID at 4h	6	19025.79 (32.8)	1130 (40.4)	487 (102.1)
	CRC 1 g BID	6	26633.19 (24.3)	2120 (22.1)	348 (66.5)
	Questran 1 g BID	2	20795.77 (2.0)	720 (88.5)	1750 (139.4)
	Placebo	8	29760.49 (23.8)	201 (69.2)	2740 (49.6)

Source: A4250-001 CSR, Table 30, Table 31

Abbreviations: AUC, area under the concentration-time curve; BID, twice daily; C4, complement component 4; CRC, cholestyramine; CV, coefficient of variation; FGF19, fibroblast growth factor 19; QD, once a day

In Part 2b, compared to placebo, odevixibat with cholestyramine and cholestyramine alone decreased total bile acid levels and FGF-19 and increased C4 as expected. Cholestyramine adsorbs the bile acids in the intestine to form an insoluble complex which is excreted in the feces. Compared to cholestyramine alone (Questran and CRC), coadministration of odevixibat with cholestyramine resulted in greater changes in total bile acid levels, FGF-19, and C4 ([Table 104](#)).

Compared to odevixibat 3 mg QD alone, coadministration of odevixibat with commercial cholestyramine product, Questran 1 g BID, given at 4 hours after odevixibat did not significantly affect the effects of odevixibat on total bile acids ([Table 104](#); geometric mean ratio, 1.094). With in-house colonic release cholestyramine, CRC 1 g BID, the extent of serum bile reduction was decreased approximately 25 to 35% compared to odevixibat alone when CRC was administered 0.5 hours before or 4 hours after odevixibat dosing. These results support the concomitant administration of cholestyramine with odevixibat with at least 4 hours dosing interval, which is aligned with the dosing instruction of Questran in the approved label.

Table 104. Geometric Mean Ratio of AUC_{0-12h} on Day 7 for Total Bile Acids in Plasma Following Multiple Administration of Odevixibat for 7 Days, Trial A4250-001

Treatment	N	Geometric Mean (CV%) AUC _{0-12h} Total Bile Acid (ng·h/mL)	Geometric Mean Ratio (95% CI)	
			Compared to Placebo	Compared to Odevixibat 3 mg QD
Odevixibat 3 mg QD	6	15272.29 (16.1)	0.456 (0.303, 0.689)	-
Odevixibat 3 mg QD + Questran 1 g BID at 4h	6	16714.33 (27.3)	0.562 (0.433, 0.729)	1.094 (0.828, 1.446)
Odevixibat 3 mg QD + CRC 1 g BID at -0.5 h	6	20575.40 (16.0)	0.691 (0.533, 0.897)	1.347 (1.020, 1.780)
Odevixibat 3 mg QD + CRC 1 g BID at 4h	6	19025.79 (32.8)	0.639 (0.493, 0.830)	1.246 (0.943, 1.646)
CRC 1 g BID	6	26633.19 (24.3)	0.895 (0.690, 1.161)	1.745 (1.319, 2.304)
Placebo	8	29760.49 (23.8)	-	-

Source: A4250-001 CSR, Table 14.2.6.2.

Abbreviations: AUC, area under the concentration-time curve; BID, twice daily; CI, confidence interval; CV, coefficient of variation; QD, once a day

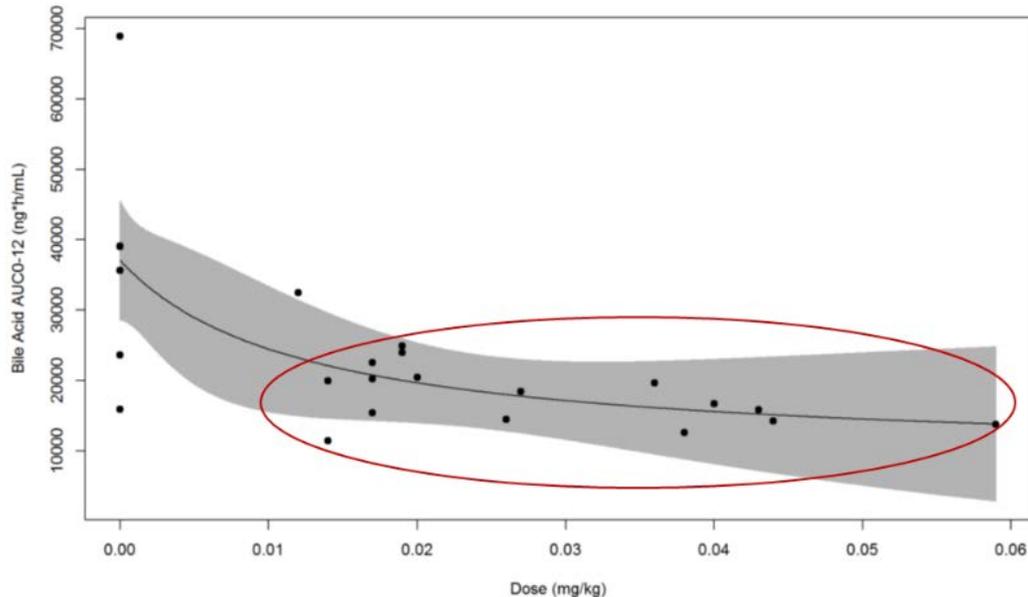
For fecal bile acid levels, the final study report stated that the mean increases from predose to Day 7 were 6- to 13-fold greater in healthy volunteers who received active odevixibat at 1 to 3 mg/day compared to healthy volunteers who received placebo. However, there is limitation that precludes further interpretation on fecal bile acids because its bioanalytical method was not qualified to support the data reliability due to significant matrix interference and interbatch variability above acceptable range (see subsection “Individual bile acids in plasma and feces and C4 in plasma” in Section [14.4.2](#)).

Dose-Response

The Applicant performed dose-response analyses for total bile acids, FGF-19, and C4 (Report No. ALBI-PMX-A4250-1167) obtained on Day 7 in Part 2 following multiple administration of odevixibat 1 mg QD, 3 mg QD, or 1.5 mg BID. The individual AUC_{0-12h} of total bile acids, FGF-19, and C4 were plotted against body weight normalized dose and fitted to an Emax model. The body weight normalized daily dose ranged from approximately 12 to 59 mcg/kg/day ([Figure 10](#), [Figure 11](#), [Figure 12](#)).

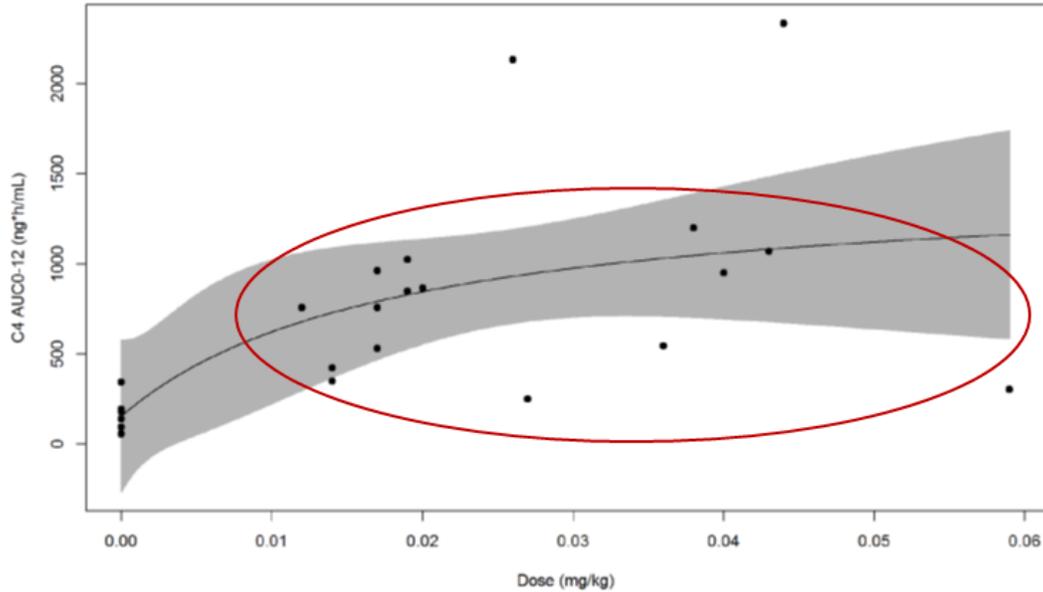
For all of the three PD markers, the dose-response relationship was flat over the 5-fold range from 12 to 59 mcg/kg. While an Emax model fitted the overall dose-response relationship including placebo data, there was little slope by dose among individual healthy volunteers who received active odevixibat and the PD responses were similar between odevixibat dose groups.

Figure 10. Dose-Response Relationship of Odevixibat: Dose (mg/kg) vs. AUC_{0-12h} of Total Bile Acids, Trial A4250-001



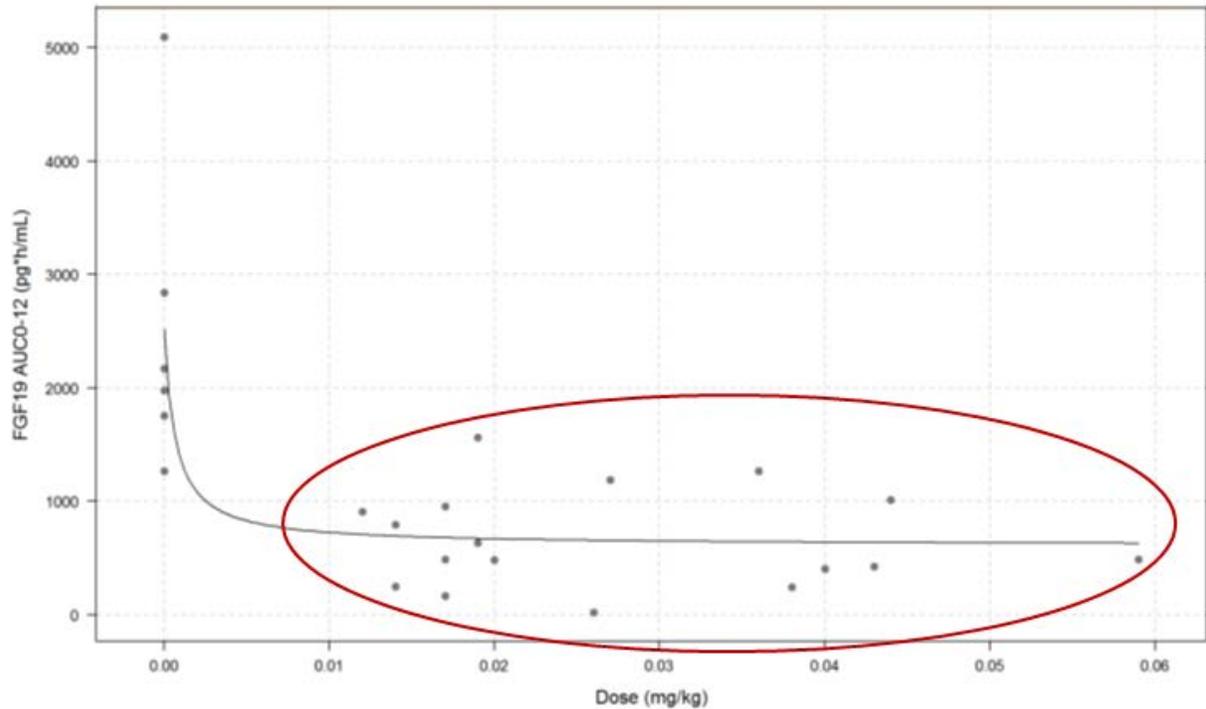
Source: Report ALBI-PMX-A4250-1167, Figure 2
Abbreviations: AUC, area under the concentration-time curve

Figure 11. Dose-Response Relationship of Odevixibat: Dose (mg/kg) vs. AUC_{0-12h} of C4 in Trial A4250-001



Source: Report ALBI-PMX-A4250-1167, Figure 5
Abbreviations: AUC, area under the concentration-time curve; C4, complement component 4

Figure 12. Dose-Response Relationship of Odevixibat: Dose (mg/kg) vs. AUC_{0-12h} of FGF-19 in Trial A4250-001



Source: Report ALBI-PMX-A4250-1167, Figure 3
Abbreviations: AUC, area under the concentration-time curve; FGF19, fibroblast growth factor 19

Per the Applicant's dose-response analysis using the Emax model for total bile acids, the estimated ED₅₀, ED₉₀ and ED₉₅ were 12.3, 111, and 234 mcg/kg, respectively. However, the estimates had high uncertainties with high standard errors, probably due to limited number of observations at low doses.

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The flat dose-response might have been because the drug concentration at the site of action (i.e., the apical lumen of the ileum) at the lowest dose already reached the concentration that leads to the maximum IBAT inhibition. Of note, in an in vitro trial using HEK293 cells expressing the various bile acid transporters, odevixibat was found to inhibit the human IBAT with $IC_{50}=0.13$ nmol/L (=0.097 ng/mL).

14.2.2. Mass Balance Trial (A4250-007)

Title

An Open-Label, Single-Dose, Single Period Study Designed to Assess the Mass Balance Recovery, Metabolite Profile, and Metabolite Identification of [^{14}C]-A4250 in Healthy Male Subjects

Objectives

- To assess the mass balance recovery after a single dose of ^{14}C -odevixibat as a capsule.
- To determine the routes and rates of elimination of ^{14}C -odevixibat
- To identify the chemical structure of each metabolite accounting for more than 10% of circulating total radioactivity

Study Design

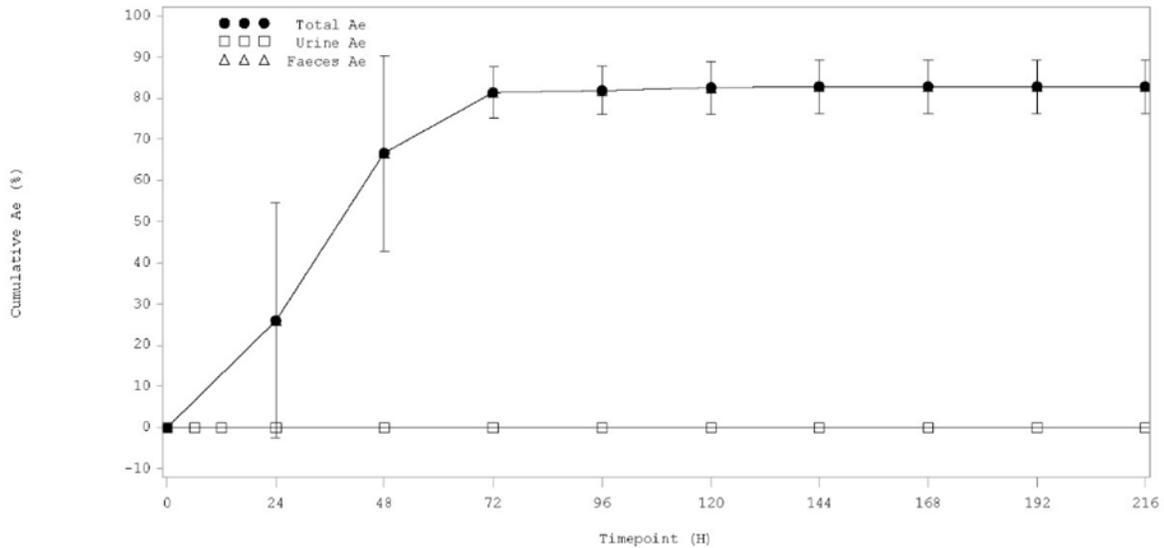
Six healthy volunteers received a single oral administration of 3 mg radiolabeled odevixibat capsule containing not more than 4.3 MBq (116 microCi), in the fasted state. Urine and feces were collected up to 216 hours (9 days) post dose. Whole blood samples were collected up to 96 hours post dose and plasma samples were collected up to 168 hours post dose.

Results of Total Radioactivity

Following a single oral dose of 3 mg ^{14}C -odevixibat, an average of 0.002% of the total radioactivity was recovered from the urine and 82.886% was recovered from the feces (cumulative recovery by 72 hours, 81.429%). The urinary radioactivity was all recovered within the first 24 hours ([Figure 13](#)).

Total radioactivity in both plasma and whole blood were below the limit of detection, i.e., 1 ng equiv/mL for plasma and 1 ng equiv/g for whole blood, respectively. All plasma concentrations of odevixibat was also below the limit of quantitation, i.e., 0.05 ng/mL.

Figure 13. Mean (\pm SD) Cumulative Recovery of Total Radioactivity in Urine and Feces Following a Single 3 mg Oral Dose of [14 C]-Odevixibat



Source: A4250-007 CSR, Figure 2
Abbreviations: SD, standard deviation

Results of Metabolite Profiling

Since radioactivity was primarily detected in feces and virtually undetectable in the urine or plasma samples, only feces samples were assessed. The feces samples were pooled across the 0-72 hours postdose timepoints for each healthy volunteer. No metabolite was found accounting for greater than 10% of circulating radioactivity in counting for greater than 10% of the dose in the pooled feces samples. In all samples, 96.58 to 99.31% of the fecal radioactivity was determined to be unchanged odevixibat. Components consisting of 0.69 to 1.72% of the fecal radioactivity were neither deemed significant nor further characterized.

14.2.3. Food Effect/Sprinkle Trial (A4250-004)

Title

A Phase I, Open-Label, Randomized, Three-Way Crossover Relative Bioavailability of A4250 in Healthy Adult Subjects Under Fasting and Fed Conditions and When Sprinkled on Applesauce

Objectives

- To determine the effect of a high-fat meal on the single-dose PK of odevixibat
- To determine the effect of odevixibat sprinkled on applesauce on the single-dose PK of odevixibat

Study Design

This was an open-label, randomized, three-way crossover, food-effect, and sprinkle trial using one of the TBM formulation (i.e., 1200 mcg oral capsule). On Day 1 of each period, healthy volunteers received a single dose of 9.6 mg odevixibat (8×1200 mcg capsules) under one of the following three conditions:

- Treatment A: 9.6 mg odevixibat, following an overnight fast
- Treatment B: 9.6 mg odevixibat, 30 minutes after the start of high-fat breakfast
- Treatment C: 9.6 mg odevixibat, sprinkled on a weigh boat of unsweetened applesauce following an overnight fast

All study drugs were administered orally with approximately 240 mL of water. Especially, for Treatment C, the healthy volunteers were asked to eat the content completely without chewing, followed by approximately 240 mL of water. PK blood samples were collected following each dose at predose and 0.75, 1.25, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 10, 12, 14, and 18 hours post dose. Washout period was 7 days between doses. The high-fat meal contained approximately 150, 250, and 500-600 calories from protein, carbohydrate, and fat, respectively, to provide about 50% fat of a high caloric meal accounting for approximately 800 to 1000 calories.

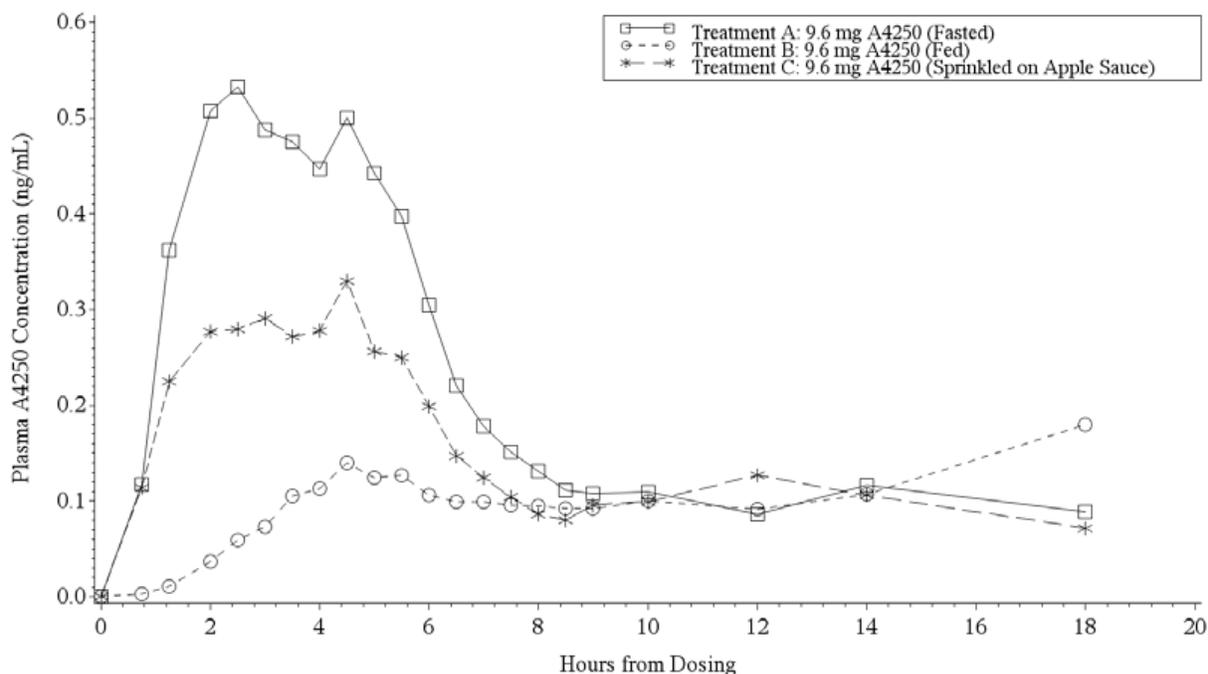
PK Results

Mean plasma odevixibat concentration-time profiles following administration of a single 9.6 mg oral dose of odevixibat under fasted, fed, and sprinkled on applesauce conditions (Treatments A, B, and C, respectively) are presented in [Figure 14](#). Plasma concentrations and systemic exposure to odevixibat were low, regardless of dosing conditions, indicating minimal absorption through the gastrointestinal tract. No mean odevixibat concentration value exceeded 0.6 ng/mL.

The summary of PK parameters is presented in [Table 105](#). Based on geometric mean ratios, odevixibat administered after a high-fat meal exposure reduced exposure to odevixibat, as measured by AUC_{0-t} , AUC_{0-inf} , and C_{max} , 62%, 43%, and 72%, respectively, compared to when it was administered under fasting conditions. Time to reach C_{max} , i.e., T_{max} , (time to maximum concentration) was delayed by 1.5 hours when odevixibat was administered after high-fat breakfast.

In addition, when odevixibat was sprinkled on applesauce, exposure to odevixibat, as measured by AUC_{0-t} , AUC_{0-inf} , and C_{max} , was 35%, 39%, and 39%, respectively, compared to administration of the whole capsule under fasting conditions. T_{max} was delayed by 1.5 hours when sprinkled on applesauce.

Figure 14. Mean Plasma A4250 Concentration Versus Time Profiles Following Administration of Single 9.6 mg Oral Dose of Odevixibat Under Fasted, Fed, and Sprinkled on Applesauce Conditions



Source: A4250-004 CSR, Figure 11-1

Table 105. Summary of Plasma Odevixibat Pharmacokinetic Parameters Following Administration of Single 9.6 mg Oral Dose of Odevixibat Under Fasted, Fed, and Sprinkled on Applesauce Conditions

	Treatment A (Fasted) N=17	Treatment B (Fed) N=15	Treatment C (Sprinkle) N=17
PK Parameters			
AUC _{0-t} (ng·hr/mL)			
Mean (CV%)	2.937 (50.38)	1.146 (65.58)	1.800 (31.58)
GMR (90% CI) to Treatment A	-	0.377 (0.303-0.467)	0.645 (0.525-0.793)
AUC _{0-inf} (ng·hr/mL)			
Mean (CV%)	3.161 (49.15)	1.605 (20.22) ¹	2.002 (33.21)
GMR (90% CI) to Treatment A	-	0.566 (0.470-0.682)	0.644 (0.558-0.742)
C _{max} (ng·hr/mL)			
Mean (CV%)	0.6112 (51.78)	0.1734 (45.36)	0.3521 (35.08)
GMR (90% CI) to Treatment A	-	0.284 (0.234-0.345)	0.608 (0.505-0.732)
T _{max} (hr)			
Median (min, max)	3.0 (1.2, 5.0)	4.5 (2.0, 18.0)	4.5 (2.0, 5.0)
T _{1/2} (hr)			
Mean (CV%)	2.258 (38.83)	6.439 (40.08)	2.083 (70.13)

Source: A4250-004 CSR, Table 14.2.1.4, Table 14.2.1.5, Table 14.2.1.6, Table 14.2.1.9, Table 14.2.1.10

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; CV, coefficient of variation; CI, confidence interval; GMR, geometric mean ratio; T, time of concentration observed

¹ N=9; mean AUC extrapolation was >30%, therefore estimation of AUC_{0-inf} may not be robust and interpretation should be treated with caution.

The site of pharmacologic action for odevixibat is the apical lumen of the ileum so odevixibat is designed to be minimally absorbed. The reduced systemic exposure to odevixibat does not have clinical implications in efficacy. The study results support that odevixibat can be taken with food or sprinkled on soft food without safety concerns associated with systemic exposure because it did not cause any greater systemic exposure compared to when odevixibat was administered at fasted status as a whole capsule.

14.2.4. Drug-Drug Interaction Trial (A4250-013)

Title

A Phase I, Open-Label, Two-Part Study to Evaluate the Interaction of A4250 (Odevixibat) with Midazolam, a Sensitive CYP3A4 Substrate, and with Itraconazole, a P-gp Inhibitor, in Healthy Adult Subjects

Objectives

- Part A: To determine the effect of odevixibat on the PK of midazolam, a sensitive CYP3A4 substrate, in healthy adult volunteers.
- Part B: To determine the effect of a P-gp inhibitor, itraconazole, on the PK of odevixibat in healthy adult volunteers.

Part A: Evaluation of Odevixibat as a CYP3A4 Inhibitor

A total of 22 healthy volunteers were enrolled. A single oral dose of midazolam 2 mg was administered in Period 1. In Period 2, oral doses of odevixibat 7.2 mg (6×1.2 mg capsules) were administered once a day for 4 consecutive days with a single oral dose of midazolam 2 mg coadministered on Day 4. The final formulation developed of odevixibat (oral capsules with pellets that can be sprinkled) was used. Period 2 started at least 24 hours following midazolam dosing in Period 1.

PK samples for midazolam and 1-OH-midazolam were collected at predose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 8, 12, 16, 24 hours after each midazolam dose. PK samples for odevixibat were taken at predose on Days 2 to 4 and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 8, 12, 16, 24 hours postdose on Day 4.

As seen in [Table 106](#), the coadministration of odevixibat once daily for 4 days with a single dose of midazolam decreased AUC of both midazolam and 1-OH-midazolam by approximately 30% and by less than 20%, respectively, compared to when midazolam was administered alone. It indicated that odevixibat does not inhibit CYP3A4 enzyme. The extent of the decrease (20-30%) is not deemed clinically significant. Since odevixibat did not induce CYP3A4 enzyme in vitro, there is no clear evidence to explain a decrease in both midazolam and 1-OH-midazolam exposure by odevixibat.

Table 106. Summary of Midazolam and 1-OH Midazolam Pharmacokinetic Parameters Following Single Dose of Midazolam 2 mg Alone or Coadministered With Odevixibat

Analytes	Midazolam	Midazolam + Odevixibat	GMR (90% CI)
	N=22 Mean (CV%)	N=20 Mean (CV%)	
Midazolam			
AUC _{0-t} (ng·hr/mL)	36.4 (35.6)	25.9 (33.0)	0.711 (0.662-0.764)
AUC _{0-inf} (ng·hr/mL)	38.2 (37.1)	27.3 (32.7)	0.715 (0.666-0.769)
C _{max} (ng/mL)	14.3 (27.6)	10.5 (34.5)	0.712 (0.640-0.792)
T _{max} ^a (hr)	0.5 (0.5-1.5)	0.5 (0.47-2.0)	-
T _½ (hr)	6.01 (24.8)	5.13 (26.7)	-
1-OH-Midazolam			
AUC _{0-t} (ng·hr/mL)	138 (17.0)	121 (17.3)	0.867 (0.837-0.899)
AUC _{0-inf} (ng·hr/mL)	147 (15.8)	127 (17.3)	0.859 (0.825-0.895)
C _{max} (ng/mL)	62.6 (24.9)	57.0 (22.8)	0.900 (0.839-0.966)
T _{max} ¹ (hr)	0.75 (0.5-1.0)	0.75 (0.47-1.0)	-
T _½ (hr)	7.81 (60.7)	7.70 (42.1)	-

Source: A4250-013 CSR, Table 1.1.2, Table 1.1.3, Table 1.2.2, Table 1.2.3

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; CI, confidence interval; CV, coefficient of variation; GMR, geometric mean ratio; T, time of concentration observed

¹ T_{max} is presented as median (min-max)

In Period 2, PK of odevixibat was determined following 4-day dosing of odevixibat 7.2 mg once daily with a single dose of midazolam 2 mg on Day 4 ([Table 107](#)).

Table 107. Summary of Odevixibat Pharmacokinetic Parameters Following Administration of Odevixibat 7.2 mg Once Daily for 4 Days With Single Dose of Midazolam 2 mg

PK Parameters	Mean (CV%) N=20
C _{max} (ng/mL)	0.526 (48.0)
T _{max} ¹ (hr)	2.76 (1.5-5.0)
AUC _t (ng·hr/mL)	2.66 (42.9)
C _{trough} (Day 2) (ng/mL)	All samples were <LLOQ (0.05 ng/mL)
C _{trough} (Day 3) (ng/mL)	All samples were <LLOQ
C _{trough} (Day 4) (ng/mL)	All samples were <LLOQ except one healthy volunteer (0.0937 ng/mL)

Source: A4250-013 CSR, Table 1.3.2

Abbreviations: AUC, area under the concentration-time curve; C, concentration; CV, coefficient of variation; LLOQ, lower limit of quantification; PK, pharmacokinetic; T_{max}, time of maximum concentration observed

¹ T_{max} is presented as median (min-max)

Part B: Evaluation of the Effect of P-gp Inhibition on the Pharmacokinetics of Odevixibat

A total 21 healthy volunteers were enrolled. A single oral dose of odevixibat 7.2 mg (6 × 1.2 mg capsules) was administered in Period 1. In Period 2, oral doses of itraconazole 200 mg were administered QD for 5 consecutive days with a single oral dose of odevixibat 7.2 mg coadministered on Day 5. The final formulation developed of odevixibat (oral capsules with pellets that can be sprinkled) was used. Period 2 started at least 24 hours following odevixibat dosing in Period 1. PK samples for odevixibat were taken at predose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 8, 12, 16, and 24 hours post dose following each odevixibat dosing.

As seen in [Table 108](#), the coadministration of itraconazole once daily for 5 days with a single dose of odevixibat increased C_{max} and AUC of odevixibat by approximately 50% and 65%, respectively, compared to when odevixibat was administered alone. No significant AEs occurred in this single dose DDI trial at odevixibat 7.2 mg with itraconazole compared to odevixibat 7.2 mg alone.

Table 108. Summary of Odevixibat Pharmacokinetic Parameters Following Single Dose of Odevixibat 7.2 mg Alone or Coadministered With Itraconazole

PK Parameters	Odevixibat	Odevixibat + Itraconazole	GMR (90% CI)
	N=21 Mean (CV%)	N=21 Mean (CV%)	
AUC _{0-t} (ng·hr/mL)	2.19 (36.2)	3.87 (76.0)	1.656 (1.382-1.985)
AUC _{0-inf} (ng·hr/mL)	2.55 (28.0)	4.17 (72.2)	1.509 (1.251-1.821)
C _{max} (ng/mL)	0.465 (34.8)	0.744 (65.3)	1.519 (1.252-1.843)
T _{max} ¹ (hr)	2.5 (1.0-5.0)	4.5 (2.0-5.0)	-
T _½ (hr)	2.36 (84.7)	2.96 (46.2)	-

Source: A4250-013 CSR, Table 1.4.2, Table 1.4.3

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; CI, confidence interval; CV, coefficient of variation; GMR, geometric mean ratio; PK, pharmacokinetic; T, time of concentration observed

¹ T_{max} is presented as median (min-max)

The in vivo DDI result with a P-gp inhibitor is consistent with the in vitro DDI indicating that odevixibat is a P-gp substrate. Since odevixibat is locally acting in the gut and the systemic exposures of odevixibat is not related to clinical efficacy, the 50-65% increase in systemic exposure does not have impact on clinical efficacy.

Additionally, based on available safety data taken together, the increased systemic exposure by coadministration with a P-gp inhibitor is not expected to be clinically significant in terms of safety, either. The phase 2 trial in pediatric patients with 4-week treatment of odevixibat up to 200 mcg/kg (i.e., up to 5-fold the recommended 40 mcg/kg dose; 1.7-fold the highest dose available 120 mcg/kg), where the C_{max} above LLOQ ranged from 0.051 to 1.11 ng/mL after a single administration (see Section [14.2.5](#)). Although the sample size is small (four patients per dose), no significant safety concerns were found during the 4-week treatment.

14.2.5. Phase 2 Dose-Ranging Trial (A4250-003)

Title

An Exploratory Phase 2 Study to Demonstrate the Safety and Efficacy of A4250 in Children with Cholestatic Pruritus

Study Design

This phase 2 trial was a single and multiple dosing open-label trial of odevixibat to evaluate the safety and efficacy of odevixibat when administered for 4 weeks in pediatric patients diagnosed with cholestatic pruritus.

Objectives

The primary aims of this exploratory phase 2 trial were:

- To explore changes in total sBA levels after a 4-week treatment period
- To assess the safety and tolerability of odevixibat, orally administered first as a single dose and then during a 4-week treatment period

Twenty patients completed the one-dose cohort (i.e., they received one single dose followed by 4 weeks of daily dosing) and four patients were re-enrolled into a second treatment cohort resulting in a total of 24 exposures. For each exposure, a patient received a single dose, followed by at least a 14-day washout, and then daily doses for 4 weeks approximately 30 minutes before breakfast, as follows:

- Cohort 1 (n=4): 10 mcg/kg/day
- Cohort 2 (n=6): 30 mcg/kg/day
- Cohort 3 (n=4): 60 mcg/kg/day
- Cohort 4 (n=6): 100 mcg/kg/day
- Cohort 5 (n=4): 200 mcg/kg/day

Pediatric patient age ranged from 1 to 17 years with body weight ranged from 7.4 to 57.5 kg. Thirteen of the 20 patients had progressive familial intrahepatic cholestasis (PFIC), including type 1 (n=2), type 2 (n=9), and type 3 (n=2); six patients had Alagille syndrome, three had biliary atresia, and two had intrahepatic cholestasis associated with microvillous atrophy.

The primary efficacy assessment was the change in total serum bile acid levels. As secondary efficacy assessments, pruritus and sleep-related endpoints from patient diary data (including [visual analog scale] VAS-itch score; patient-oriented scoring atopic dermatitis (PO-SCORAD) itching score; Whittington Scale; and PO-SCORAD sleep disturbance score) were evaluated at baseline and after a 4-week treatment period. In addition to total serum bile acid levels as a primary endpoint, autotaxin, C4, and FGF19 were measured as exploratory markers of efficacy at baseline, the start, and the end of 4-week treatment. PK was evaluated based on odevixibat concentration in plasma, which were collected before first dose and at 1, 2, 4, and 8 hours after the first dose during Visit 2 (single-dose administration) and prior to last dose at Visit 5 (end of 4-week treatment).

PK Results

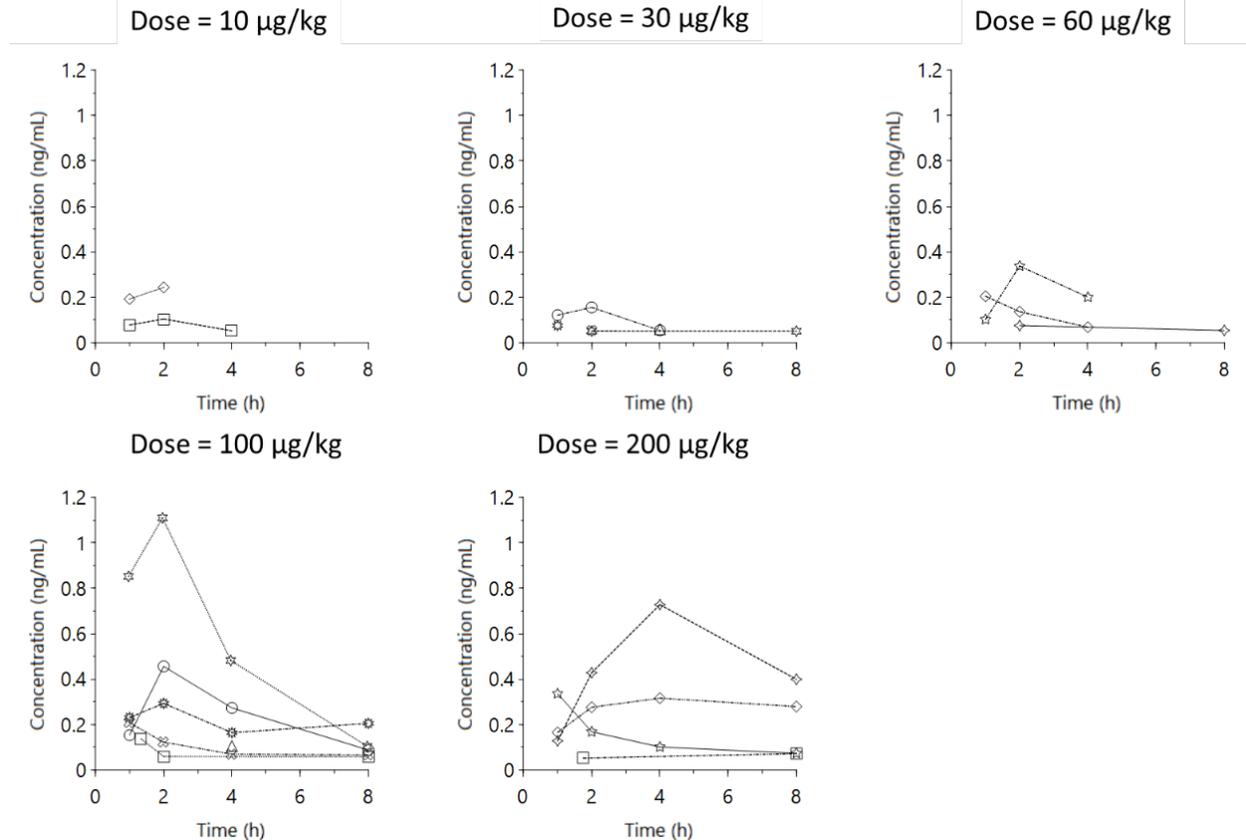
As expected due to the minimal absorption of odevixibat, plasma odevixibat concentrations were below LLOQ (0.05 ng/mL) in >50% samples at 10 to 60 mcg/kg whereas more than 50% drug concentrations were measurable above LLOQ at 100 and 200 mcg/kg ([Table 109](#), [Figure 15](#)).

Table 109. Number of Samples With Unquantifiable Concentrations, Trial A4250-003

Odevixibat Dose Levels	N	Number of Plasma Samples With Unquantifiable Concentrations/ Total Number of Samples	Number of Samples With Quantifiable Concentrations/ Total Number of Samples
		n (%)	n (%)
10 mcg/kg/day	4	18/24 (75.0)	6/24 (25.0)
30 mcg/kg/day	6	26/34 (76.5)	8/34 (23.5)
60 mcg/kg/day	4	15/24 (62.5)	9/24 (37.5)
100 mcg/kg/day	6	13/33 (39.4)	20/33 (60.6)
200 mcg/kg/day	4	8/23 (34.8)	15/23 (65.2)

Source: Reviewer's analysis based on the dataset EX.xpt, PC.xpt of A4250-003
 The limit of quantitation was 0.05 ng/mL.

Figure 15. Individual Plasma Odevixibat Concentrations-Time Profiles Following the First Dose in Patients Showing Measurable Plasma Concentrations, Trial A4250-003



Source: Reviewer's analysis based on the dataset EX.xpt, PC.xpt of A4250-003

In [Table 110](#), PK parameters estimated only based on the measurable concentrations were summarized. While the mean C_{max} and AUC were generally greater at higher doses (100 and 200 mcg/kg) compared to lower doses (10, 30, and 60 mcg/kg), the range of C_{max} and AUC values were much overlapped. Note that the parameter estimates are limited by sparse samples in a small number of patients for each dose group.

Table 110. Summary of Pharmacokinetic Parameters of Odevixibat in Patients With Measurable Plasma Concentrations Following First Dose of 4-Week Treatment, Trial A4250-003

Parameter	Odevixibat Dose Groups				
	10 mcg/kg N=4	30 mcg/kg N=6	60 mcg/kg N=4	100 mcg/kg N=6	200 mcg/kg N=4
C_{max} (ng/mL)					
N	2	5	3	6	4
Mean (SD)	0.174 (0.098)	0.079 (0.044)	0.205 (0.131)	0.385 (0.377)	0.363 (0.271)
Min, max	0.104, 0.243	0.051, 0.155	0.075, 0.337	0.105, 1.11	0.072, 0.728
T_{max} (hr)					
N	2	5	3	6	4
Median	2.0	2.0	1.98	1.98	3.98
Min, max	2.0, 2.0	1.0, 4.0	1.0, 2.0	1.0, 4.0	1.0, 8.0

Parameter	Odevixibat Dose Groups				
	10 mcg/kg N=4	30 mcg/kg N=6	60 mcg/kg N=4	100 mcg/kg N=6	200 mcg/kg N=4
AUC _{0-t} (ng·h/mL)					
N	1	1	2	5	3
Mean (SD)	0.311	0.434	0.684 (0.247)	1.809 (1.455)	2.352 (1.380)
Min, max	-	-	0.462, 0.807	0.517, 1.573	0.432, 3.752
C _{trough} (ng/mL)					
N	1	0	1	0	1
Min, max	0.258	-	0.344	-	0.48

Source: Reviewer's analysis based on the dataset EX.xpt, PC.xpt, and PP.xpt of A4250-003

Abbreviations: AUC, area under the concentration-time curve; C, concentration; SD, standard deviation; T_{max}, time of maximum concentration observed

AUC was estimated only when there were three or more measurable concentrations per patient.

PD Results

Serum Bile Acid Levels

As the bioanalytical method for serum bile acid levels was not adequately validated according to the FDA guidance for industry *Bioanalytical Method Validation* (2018) (see Section 14.4.2), so the reliability of the reported serum bile acids levels was not be sufficiently demonstrated. With such limitation, the results of serum bile acid levels below should only be considered exploratory to read overall trends in a general, qualitative manner.

All patients had elevated total serum bile acid levels at baseline (>2-fold upper limit of normal (ULN) range, i.e., 20 micromole/L), as required by inclusion criteria. Following 4-week treatment of odevixibat, serum bile acid levels substantially decreased from baseline (i.e., mean 50.3% from baseline across dose groups), but the extent of serum bile acid reduction were not significantly different between dose groups from 10 to 200 mcg/kg with large individual variability (Table 111, Figure 16). The largest mean decrease was observed in the 30 mcg/kg dose group.

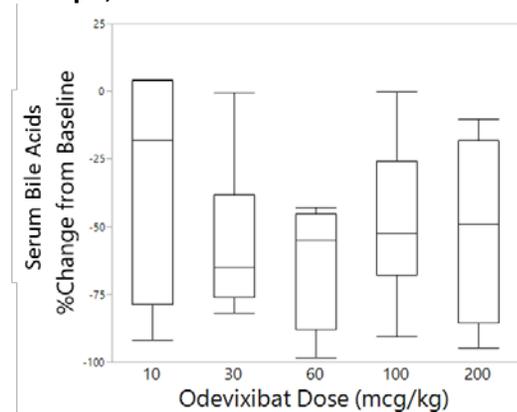
Table 111. Mean (SD) Total Serum Bile Acid Levels, Trial A4250-003

Variable	Odevixibat Dose Groups					Total N=24
	10 mcg/kg N=4	30 mcg/kg N=6	60 mcg/kg N=4	100 mcg/kg N=6	200 mcg/kg N=4	
Baseline (micromole/L)	217.8 (100.67)	216 (177.29)	220.5 (159.99)	288.5 (126.83)	213.2 (236.61)	234.7 (152.65)
At 4 weeks (micromole/L)	151.4 (146.26)	69.4 (42.11)	61.9 (64.22)	163.6 (165.81)	107 (150.01)	111.6 (120.44)
Change from baseline (micromole/L)	-66.4 (118.35)	-146.6 (139.48)	-158.6 (156.06)	-124.8 (105.84)	-106.2 (96.76)	-123.1 (117.74)
Change from baseline (%)	-30.9 (45.43)	-56.2 (29.61)	-62.8 (24.36)	-48.4 (29.94)	-50.9 (35.08)	-50.3 (31.55)

Source: A4250-003 CSR, Table 11-7

Abbreviations: SD, standard deviation

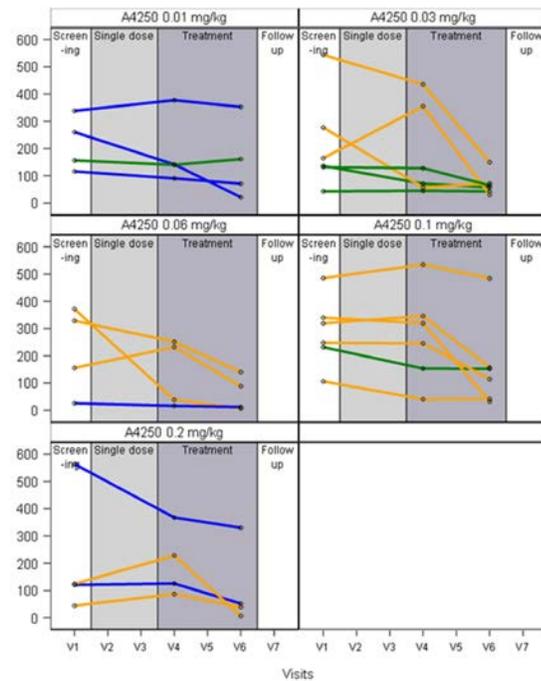
Figure 16. Percent Change From Baseline in Total Serum Bile Acid Levels at Week 4 by Dose Groups, Trial A4250-003



Source: Reviewer's analysis based on ADLB.xpt of Trial A4250-003
 Blue lines (areas) = a linear regression (95% CI); red lines = 0% changes

Individual plots for serum bile acid levels by dose groups and disease types are shown in [Figure 17](#), indicating that the majority of patients with PFIC experienced decreases in serum bile acid levels from baseline following 4-week treatment of odevixibat.

Figure 17. Individual Plots of Total Serum Bile Acid Levels (micromole/L) by Dose Group and Disease Type, Trial A4250-003



Source: A4250-003 CSR, Figure F2.1.1.1.1
 Patients with PFIC are denoted in yellow; Alagille syndrome in blue; and cholestasis or other disease type in green.

Pruritus Assessment

In general, an improvement in pruritus was observed in patients with PFIC as assessed by VAS-itch, POSCORAD-itching, and Whittington scores. An improvement in sleep, as measured by the PO-SCORAD-sleep disturbance score, was also observed ([Table 112](#)). However, given the open-label, uncontrolled design, the pruritus assessment is considered exploratory.

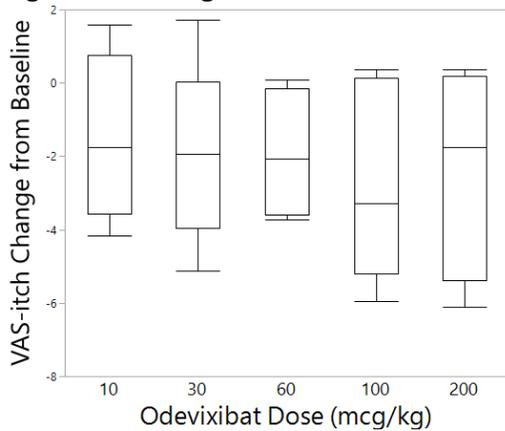
There was no apparent dose-dependent decrease in pruritus scores over the dose range from 10 to 200 mcg/kg with large individual variability and small sample size (4-6 per dose) ([Table 112](#), [Figure 18](#)). The largest mean decrease in VAS-itch score was observed in the 100 mcg/kg dose group.

Table 112. Mean (SD) Change From Baseline to End of Treatment for VAS-Itch, PO-SCORAD Itching, Whittington, and PO-SCORAD Sleep Disturbance Scales

MEASURE (SCALE)	A4250 0.01 MG/KG (N=4)	A4250 0.03 MG/KG (N=6)	A4250 0.06 MG/KG (N=4)	A4250 0.1 MG/KG (N=6)	A4250 0.2 MG/KG (N=4)
VAS-itch (0-10)	-1.5 ± 2.36	-2.0 ± 2.46	-1.9 ± 1.85	-2.8 ± 2.62	-2.3 ± 2.96
PO-SCORAD itching (0-10)	-1.0 ± 1.63	-2.1 ± 2.57	-1.2 ± 1.21	-2.7 ± 2.35	-2.4 ± 3.23
Whittington (0-4)	0 ± 0.72	-0.8 ± 0.71	-1.1 ± 1.0	-1.4 ± 1.24	-0.5 ± 0.8
PO-SCORAD sleep disturbance (0-10)	+0.1 ± 1.32	-1.8 ± 2.51	-1.4 ± 1.5	-2.9 ± 2.3	-2.1 ± 2.74

Source: A4250-003 CSR, Table 11-8
 Abbreviations: PO-SCORAD, patient-oriented scoring atopic dermatitis; VAS, visual analog scale

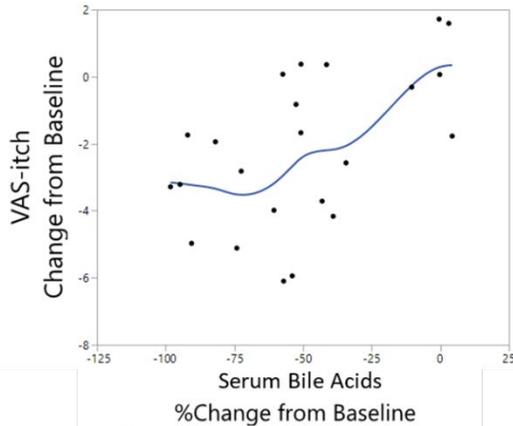
Figure 18. Change From Baseline in VAS-Itch at Week 4 by Dose Groups, Trial A4250-003



Source: Reviewer's analysis based on ADQS.xpt of Trial A4250-003
 Abbreviations: VAS, visual analog scale

A trend was shown that the reduction of serum bile acid levels was generally associated with pruritus improvement ([Figure 19](#)).

Figure 19. Correlation of Change in VAS-Itch Score With Percent Decrease in Serum Bile Acid Levels, Trial A4250-003



Source: Reviewer's analysis based on ADQS.xpt and ADLB.xpt of Trial A4250-003
 Abbreviations: VAS, visual analog scale
 Blue line = a smooth curve through the data.

Other Exploratory Biomarkers Such as C4, FGF-19, and Autotaxin in Plasma

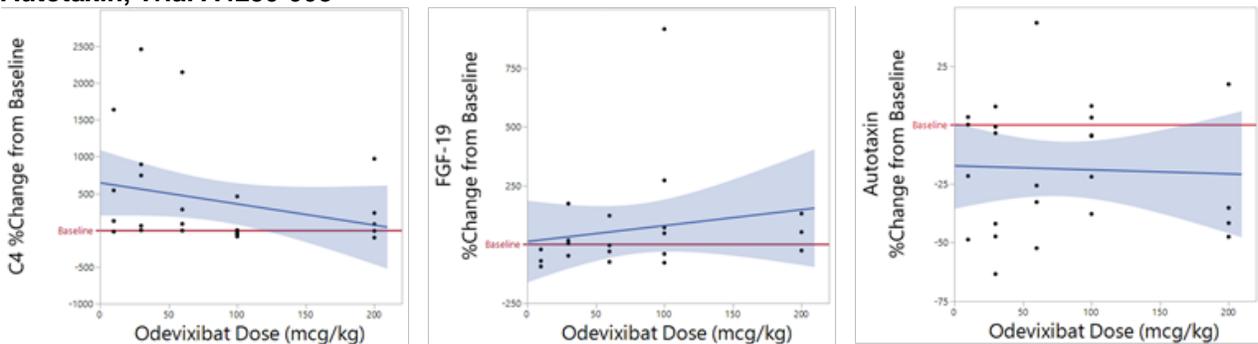
In all dose groups, mean increases in plasma C4 levels were observed and tendencies of mean decreases were seen in both FGF19 and autotaxin (Table 113). However, any apparent dose-dependent changes were not found for C4, FGF19, or autotaxin (Figure 20).

Table 113. Mean (SD) Change From Baseline at Week 4 for C4, FGF-19, and Autotaxin

Variable	Odevixibat Dose Groups					
	10 mcg/kg N=4	30 mcg/kg N=6	60 mcg/kg N=4	100 mcg/kg N=6	200 mcg/kg N=4	Total N=24
C4 (ng/mL)	7.5 (8.18)	13.2 (23.44)	9.9 (11.06)	1.6 (2.02)	5.8 (5.01)	7.7 (13.18)
FGF-19 (pg/mL)	-27.8 (29.42)	-46.8 (102.29)	-48.5 (91.99)	7.1 (97.06)	-60.4 (67.53)	-30.7 (83.34)
Autotaxin (ng/mL)	-196.3 (443.12)	-795.5 (701.24)	-85.3 (865.16)	-336.7 (348.23)	-416 (627.92)	-389.2 (604.83)

Source: A4250-003 CSR, Table 11-19
 Abbreviations: C4, complement component 4; FGF19, fibroblast growth factor 19; SD, standard deviation

Figure 20. Dose-Response Relationship for Percent Change From Baseline of C4, FGF-19, and Autotaxin, Trial A4250-003



Source: Reviewer's analysis based on ADLB.xpt of Trial A4250-003
 Abbreviations: C4, complement component 4; FGF19, fibroblast growth factor 19
 Blue lines (areas) = a linear regression (95% CI); Red lines =0% changes.

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Overall trends of changes in C4 and FGF19 were consistent with the previous observations in healthy volunteers in Trial A4250-001 following multiple dose of odevixibat at 1 to 3 mg/day. Autotaxin is a serum enzyme that converts lysophosphatidylcholine to lysophosphatidic acid. While the exact mechanism of cholestatic pruritus is not clearly understood, the literature (Kremer et al. 2012) reported that elevated levels of autotaxin was associated with cholestatic pruritus. It is unknown if a decrease in autotaxin is associated with pruritus.

Despite no apparent dose-response relationship over 10 to 200 mcg/kg, the largest mean changes in serum bile acid levels and pruritus scores were observed at 30 mcg/kg and 100 mcg/kg, respectively. Based on this phase 2 dose-ranging trial, 40 and 120 mcg/kg doses were selected for the subsequent pivotal phase 3 trial.

14.2.6. Pivotal Phase 3 Trial (A4250-005)

Title

A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC1)

Objectives

The main objective of this pivotal phase 3 trial was to evaluate the efficacy and safety of repeated daily doses of 40 and 120 mcg/kg/day of odevixibat in children with PFIC1 and PFIC2.

Study Design

A total of 62 patients received treatment, including 23 patients who received odevixibat 40 mcg/kg/day, 19 patients who received odevixibat 120 mcg/kg/day, and 20 patients who received placebo.

For PK evaluation, blood samples for analysis of odevixibat concentrations were collected at Visit 4 (corresponding to 4 weeks after the start of treatment) and Visit 9 (corresponding to the end of treatment) for children with body weight >10 kg.

Serum total bile acids were measured as a secondary endpoint at baseline (at randomization), and 4, 8, 12, 18, 22, 24 weeks of the treatment. Patients were required to fast for at least 4 hours prior to the collection of samples for serum bile acid but exceptions could be made for infants <12 months of age, if unable to fast for the full 4 hours. As exploratory biomarkers, plasma autotaxin and C4 were measured at baseline, and 4 and 24 weeks of the treatment.

Patients with Hepatic Impairment

The Applicant assessed the degree of hepatic impairment in the patients in Trial A4250-005 primarily based on hepatic biochemistry according to the Child-Pugh classification and the criteria developed by National Cancer Institute Organ Dysfunction Working Group (the NCI-ODWG) criteria ([Table 114](#)). Based on the Child-Pugh score and NCI-ODWG criteria, most patients were classified to have mild to moderate hepatic impairment. Because of the highly elevated serum total bilirubin in patients with PFIC, some patients were classified to have severe hepatic impairment based on NCI-ODWG (> 3 xULN total bilirubin and any ALT or AST).

Table 114. The Degree of Hepatic Impairment of Patients at Baseline Based on Child-Pugh Classification and the NCI-ODWG Criteria, Trial A4250-005

Parameter	Odevixibat Dose Per Day				Overall N=62 n (%)
	Placebo N=20 n (%)	40	120	All Doses N=42 n (%)	
		mcg/kg N=23 n (%)	mcg/kg N=19 n (%)		
Child-Pugh classification					
A (mild)	12 (60.0)	15 (65.2)	14 (73.7)	29 (69.0)	41 (66.1)
B (moderate)	8 (40.0)	8 (34.8)	5 (26.3)	13 (31.0)	21 (33.9)
C (severe)	0	0	0	0	0
Hepatic impairment classification based on NCI-ODWG					
Normal	2 (10.0)	0	1 (5.3)	1 (2.4)	3 (4.8)
Mild	8 (40.0)	8 (34.8)	9 (47.4)	17 (40.5)	25 (40.3)
Moderate	4 (20.0)	11 (47.8)	5 (26.3)	16 (38.1)	20 (32.3)
Severe	6 (30.0)	4 (17.4)	4 (21.1)	8 (19.0)	14 (22.6)

Source: A4250-005 CSR, Table 15

Abbreviations: NCI-ODWG, National Cancer Institute Organ Dysfunction Working Group

However, neither of Child-Pugh classification and the NCI-ODWG criteria was deemed appropriate to assess hepatic impairment in pediatric patients with PFIC. Child-Pugh classification was originally developed for adult patients with cirrhosis and is not commonly used for pediatric patients. For patient to be categorized under Child Pugh class the prerequisite is to have cirrhosis at baseline. Also, the Child-Pugh scores were primarily determined by the results of the laboratory tests for serum bilirubin, serum albumin and INR without assessment of the presence of cirrhosis. Although a liver biopsy was not obtained before or during the trial, with exception of two patient, who had liver biopsy during the trial, in whom cirrhosis was not detected. It appears that majority of the patients in Trial A4250-005 did not have cirrhosis or evidence of portal hypertension at baseline, based on imaging, laboratory values and clinical findings as noted in Laboratory and Imaging Findings, Section [7.6.1.10](#).

The NCI-ODWG criteria were also used alternatively to grade hepatic impairment based on two parameters (total bilirubin and AST) but may not be applicable to patients with PFIC to reflect the degree of hepatic impairment. Since total bilirubin is elevated in patients with PFIC (even in early stages of disease) due to its cholestatic disease pathophysiology, the NCI-ODWG criteria are likely to overestimate the degree of hepatic dysfunction for patients with PFIC. Different cutoffs for total bilirubin may need to be used to better characterize the degree of hepatic impairment in patients with PFIC.

PK Results

Of all blood samples collected in pediatric patients treated with the 40 and 120 mcg/kg/day, 82.8% and 80.0% of samples, respectively, were below LLOQ (0.05 ng/mL) of odevixibat, confirming the very low bioavailability of odevixibat ([Table 115](#)).

Table 115. Number of Samples With Unquantifiable Concentrations, Trial A4250-005

Odevixibat Dose Levels	N	Number of Plasma Samples With Unquantifiable Concentrations/ Total Number of Samples	Number of Samples With Quantifiable Concentrations/ Total Number of Samples
		n (%)	n (%)
40 mcg/kg	17	24/29 (82.8)	5/29 (17.2)
100 mcg/kg	16	20/25 (80.0)	5/26 (20.0)

Source: Population pharmacokinetic report, ALBI-PMX-A4250-1167, Table 5

The limit of quantitation was 0.05 ng/mL. Plasma samples were only collected in patients weighing >10 kg.

Only ten plasma samples from eight patients were quantifiable above LLOQ ranging from 0.0616 to 0.723 ng/mL, from which any apparent tendencies by the drug dose, age, body weight, or hepatic impairment of patients could not be evaluated given the limited data ([Table 116](#)).

Table 116. Measurable Plasma Odevixibat Concentrations in Patients, Trial A4250-005

Subject ID	Dose (mcg/kg)	Age (years)	Weight (kg)	Hepatic Impairment ¹	Timepoint	Odevixibat Concentration (ng/mL)
(b) (6)	40	3.4	10.82	Moderate	Visit 9 (End of treatment), 22.4 h postdose	0.077
	40	3.8	15.52	Severe	Visit 4 (Week 4), 23.6 h postdose	0.723
					Visit 9 (End of treatment), 25.9 h postdose	0.215
	40	3.1	11.15	Moderate	Visit 9 (End of treatment), 26.0 h postdose	0.0616
	40	16.0	55.2	Severe	Visit 4 (Week 4), 5.4 h postdose	0.511
	120	9.5	34.6	Mild	Visit 4 (Week 4), 2.3 h postdose	0.455
	120	1.9	10.6	Mild	Visit 4 (Week 4), 27.7 h postdose	0.179
	120	10.4	25.54	Severe	Visit 4 (Week 4), 71.9 h postdose	0.176
					Visit 9 (End of treatment), 26.5 h postdose	0.591
	120	1.1	5.63	Severe	Visit 9 (End of treatment), 25.7 h postdose	0.0595

Source: A4250-005, PC.xpt; Population pharmacokinetic report, ALBI-PMX-A4250-1167, FINALDAT.xpt

¹ Hepatic impairment was classified based on National Cancer Institute Organ Dysfunction Working Group (NCI ODWG) based on total bilirubin and aspartate aminotransferase values.

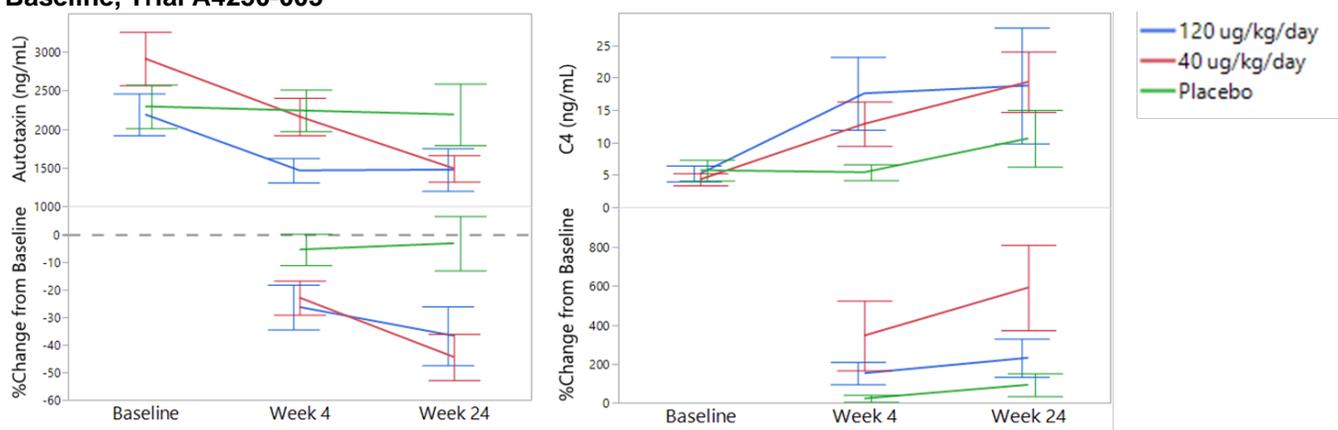
PD Results

See Section [14.2.6.1](#) regarding serum bile acid level results in Trial A4250-005.

The mean values and mean changes of plasma autotaxin and C4 over time are presented in [Figure 21](#). Plasma autotaxin decreased and plasma C4 increased from baseline following odevixibat administration whereas no significant changes were observed for both PD markers in placebo group. Comparing between 40 and 120 mcg/kg dose groups, the extent of changes in autotaxin levels were similar. For C4 levels, the changes observed in the 40 mcg/kg dose group were greater than those of 120 mcg/kg dose group.

The trends observed for autotaxin and C4 following odevixibat treatment are consistent with the observed in the previous phase 2 trial, A4250-003, in line with the expected pharmacologic action of odevixibat increasing bile acid clearance and improving cholestatic pruritus. The concentration of plasma C4 is commonly quantified as a surrogate marker for the amount of bile acid synthesis in the liver that can be up-regulated by increased bile acid clearance in the body. Some literature has reported that elevated autotaxin levels have an association with cholestatic pruritus.

Figure 21. Mean (SE) Plasma C4 and Autotaxin Levels Over Time and Their Percent Changes From Baseline, Trial A4250-005



Source: Reviewer's plot based on dataset ADLBSI.xpt of A4250-005
Abbreviations: C4, complement component 4; SE, standard error

14.2.6.1. Serum Bile Acid Levels

The protocol of Trial A4250-005 specified the primary endpoint for Europe and the rest of the world (ROW) as the proportion of patients with at least a 70% reduction in serum bile acid level from baseline or reaching a level ≤ 70 micromole/L after 24 weeks. Per the inclusion criteria include, patients had to have elevated serum bile acid levels, specifically to be ≥ 100 micromole/L, taken as the average of two samples at least 7 days apart prior to randomization.

Serum bile acid levels were measured at the following timepoints: screening period (Day -56, Day -28), at baseline (at randomization, Day 0) and Weeks 4, 8, 12, 18, 22, and 24. Serum bile acid levels were measured using an enzymatic assay in a clinical laboratory improvement amendments (CLIA)-certified laboratory. However, the bioanalytical method for serum bile acid levels was not adequately validated according to the FDA guidance for industry *Bioanalytical Method Validation* (2018) (see Section 14.4.2) which is especially pivotal when the biomarker plays the important role in evaluating efficacy and supporting a regulatory decision making. Since the inadequate bioanalytical validation cannot assure the reliability of the reported serum bile acid levels, the results of serum bile acid levels below should only be considered exploratory in order to read overall trends in a general, qualitative manner.

The majority of patients (55/62, 88.7%) had elevated serum bile acid levels above 100 micromole/L at baseline (at randomization). Of note, one patient who was randomized to odevixibat 120 mcg/kg/day, failed to show serum bile acid levels ≥ 100 micromole/L, taken as the average of two samples at least 7 days apart prior to randomization. Median levels of serum bile acid levels were elevated at baseline at 228.0 micromole/L, 188.5 micromole/L, and 254.5 micromole/L in the odevixibat 40 mcg/kg/day, odevixibat 120 mcg/kg/day, and placebo groups, respectively.

Per the Applicant's analysis, the proportion of patients with at least a 70% reduction in serum bile acid level from baseline or reaching a level ≤ 70 micromole/L are summarized in Table 117. After 24 weeks of treatment, the proportion of patients with at least a 70% reduction in serum bile acid level from baseline or reaching a level ≤ 70 micromole/L was 33.3% across all patients who received odevixibat, including 43.5% and 21.1% of patients in the odevixibat 40 mcg/kg/day and 120 mcg/kg/day dose groups, respectively; none of the patients in the placebo group met the serum bile acid endpoint.

Table 117. Analysis of the Number (%) of Patients Experiencing at Least a 70% Reduction in Serum Bile Acid Levels From Baseline to End of Treatment or Reaching a Level ≤ 70 micromole/L After 24 Weeks of Treatment (Full Analysis Set)

STATISTIC	PLACEBO N=20	ODEVIXIBAT, ONCE DAILY DOSING		
		40 $\mu\text{g}/\text{kg}$ N=23	120 $\mu\text{g}/\text{kg}$ N=19	ALL DOSES N=42
Responders, n (%)	0	10 (43.5)	4 (21.1)	14 (33.3)
95% CI ^a	(0.00, 16.84)	(23.19, 65.51)	(6.05, 45.57)	(19.57, 49.55)
Proportion Difference without Adjusting for Stratification Factors (Odevixibat - Placebo)		0.435	0.211	0.333
95% CI ^a		(0.2195, 0.6551)	(0.0210, 0.4557)	(0.0861, 0.4955)
Proportion Difference Adjusting for Stratification Factors (Odevixibat - Placebo)		0.441	0.216	0.307
95% CI ^b		(0.2361, 0.6464)	(-0.0050, 0.4380)	(0.1260, 0.4879)
Odds Ratio (Odevixibat/Placebo)		NC	NC	NC
95% CI ^c		(4.228, -)	(1.002, -)	(2.767, -)
1-sided unadjusted p-value ^d		0.0003	0.0174	0.0015
1-sided adjusted p-value ^e		0.0015	0.0174	—

CI: confidence interval; NC: not calculable; PFIC: progressive familial intrahepatic cholestasis.

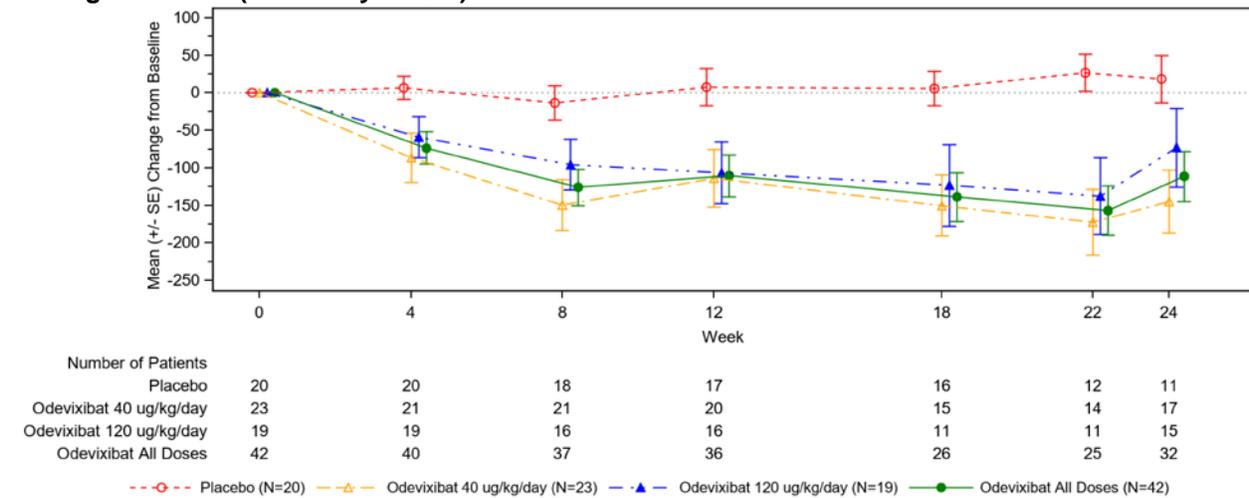
- a. Clopper-Pearson exact CI is reported for the percentage of responders, and the exact unconditional CI is reported for the proportion difference without adjusting for stratification factors.
- b. Miettinen-Nurminen (score) CI is reported adjusting for stratification factors.
- c. The exact CI is reported based on Vollset, Hirji, and Elashoff adjusting for stratification factors.
- d. Based on the Cochran-Mantel-Haenszel test adjusting for stratification factor (PFIC Type).
- e. For an individual dose, the adjusted p-value was calculated as the maximum value of the unadjusted p-value for odevixibat all doses and the unadjusted p-value for the individual dose.

Note: For the primary endpoint, a closed test procedure was used to compare odevixibat all doses combined and placebo, and then odevixibat 40 $\mu\text{g}/\text{kg}$ vs placebo and odevixibat 120 $\mu\text{g}/\text{kg}$ vs placebo.

Source: A4250-005 CSR, Table 19

As shown in [Figure 22](#), the mean decreases from baseline in odevixibat-treated patients were consistently larger than for placebo-treated patients in whom essentially no changes from baseline in serum bile acid levels were observed. The extent of changes from baseline over time on treatment were similar between 40 and 120 mcg/kg dose groups. The reduction in serum bile acid levels observed as early as at Week 4 (the first observation after baseline) with continued improvement through Week 8 and reached a relative plateau thereafter. Mean changes from baseline to Weeks 22/24 in serum bile acid levels were -141.5 micromole/L in the 40 mcg/kg/day group representing a mean decrease of 54.5%, and -83.7 micromole/L in the 120 mcg/kg/day group representing a mean decrease of 19.4%, compared with an increase of 13.1 micromole/L (8.8%) in the placebo group.

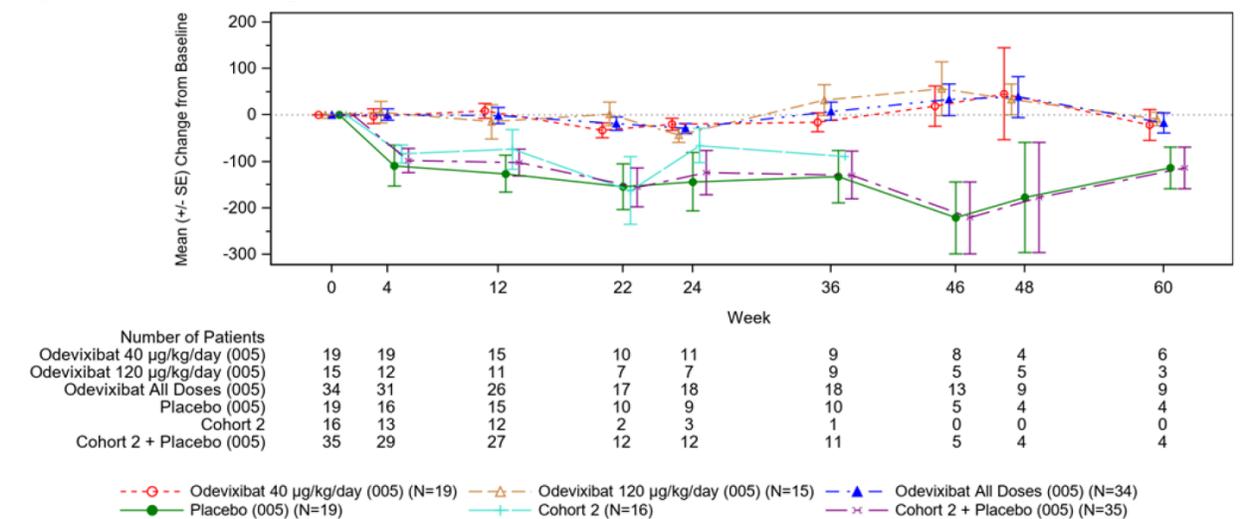
Figure 22. Mean (SE) Change From Baseline in Serum Bile Acid Levels (micromole/L) by Visit Through Week 24 (Full Analysis Set)



Source: A4250-005 CSR, Figure 3.
Abbreviations: SE, standard error

In the open-label, long-term extension trial (A4250-008, currently ongoing), the patients who completed or discontinued Trial A4250-005 were rolled over and received 120 mcg/kg/day; 19 patients each had received placebo or 40 mcg/kg/day in Trial A4250-005 and escalated to 120 mcg/kg/day, whereas 15 patients who had received 120 mcg/kg/day kept receiving 120 mcg/kg/day. Serum bile acid level did not decrease after the dose increase from 40 mcg/kg/day to 120 mcg/kg/day (Figure 23).

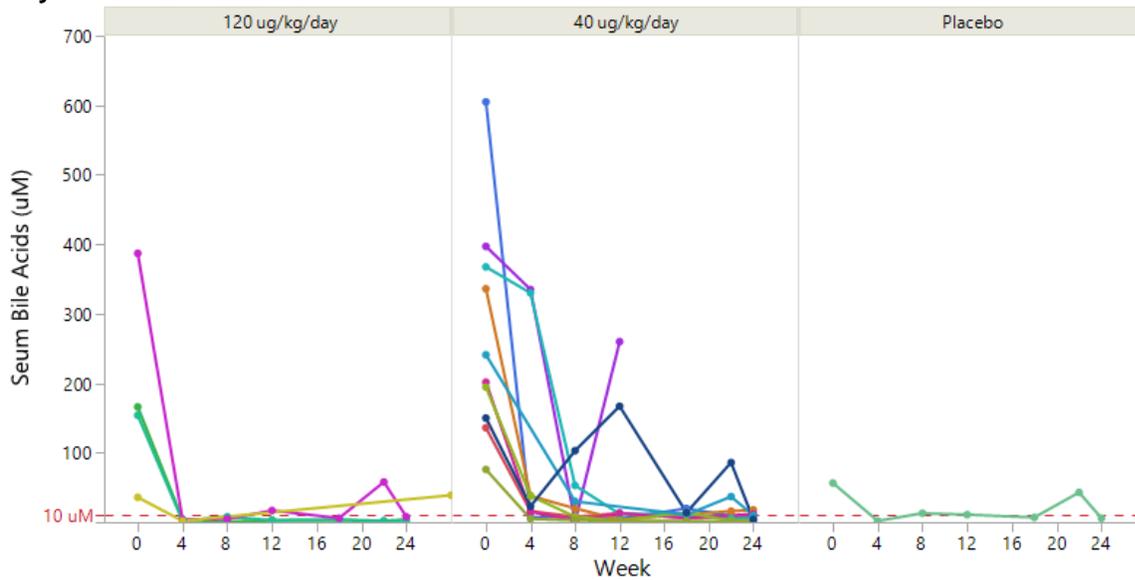
Figure 23. Mean Change From Baseline in Serum Bile Acid Levels (micromole/L), Trial A4250-008



Source: CSR A4250-008, Figure 14.2.1.1.1.
Abbreviations: SE, standard error

In A4250-005, among 62 patients, 15 patients reached below 10 micromole/L (the upper limit of the normal range for serum bile acid levels) once or more times during the trial: N=10 for 40 mcg/kg; N=4 for 120 mcg/kg; N=1 for placebo. Once the serum bile acid levels reached the normal range, in general, it maintained lowered levels for the rest of trial period with fluctuations over time (Figure 24). In only two patients out of the 15 patients (both were in 120 mcg/kg group), serum bile acids levels were constantly below 10 micromole/L until the end of treatment.

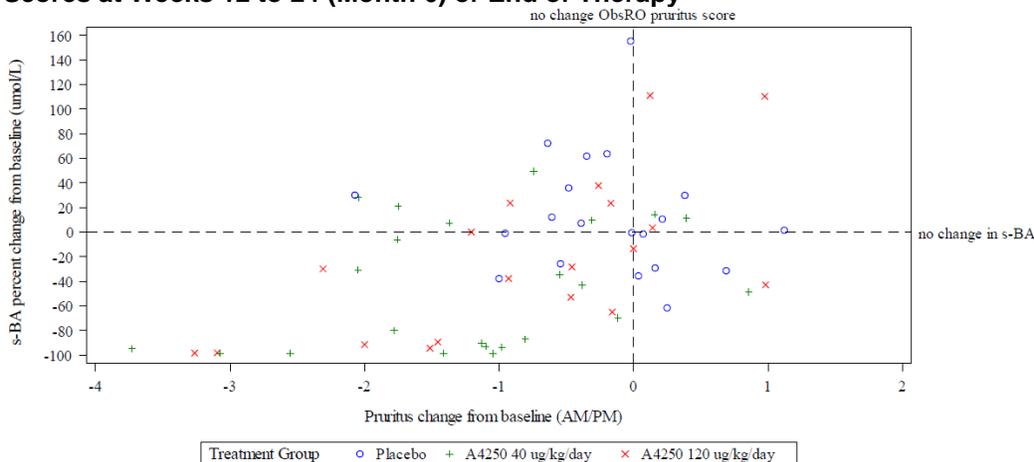
Figure 24. Serum Bile Acid Levels Over Time for Patients Who Achieved Below 10 micromole/L at Any Time After Baseline



Source: Reviewer's analysis based on ADEFF.xpt of A4250-005

The Applicant provided a graphical representation of the reduction of pruritus scores and sBA levels at baseline relative to end-of-treatment (EOT) (Figure 25). On the “x” axis change in pruritus, as assessed by “change from baseline in the average of the AM and PM monthly average pruritus scores at Weeks 12 to 24 (Month 6) or end of therapy” and on the “y” axis reduction in sBA levels are denoted. It depicts a greater number of odevixibat-treated patients achieved reduction in pruritus scores (ObsRO) and sBA levels (percent reduction) simultaneously relative to placebo.

Figure 25. Percent Change From Baseline in Average of Serum Bile Acid Level at Weeks 22/24 or End of Therapy Versus Change From Baseline in Average of AM and PM Monthly Average Pruritus Scores at Weeks 12 to 24 (Month 6) or End of Therapy

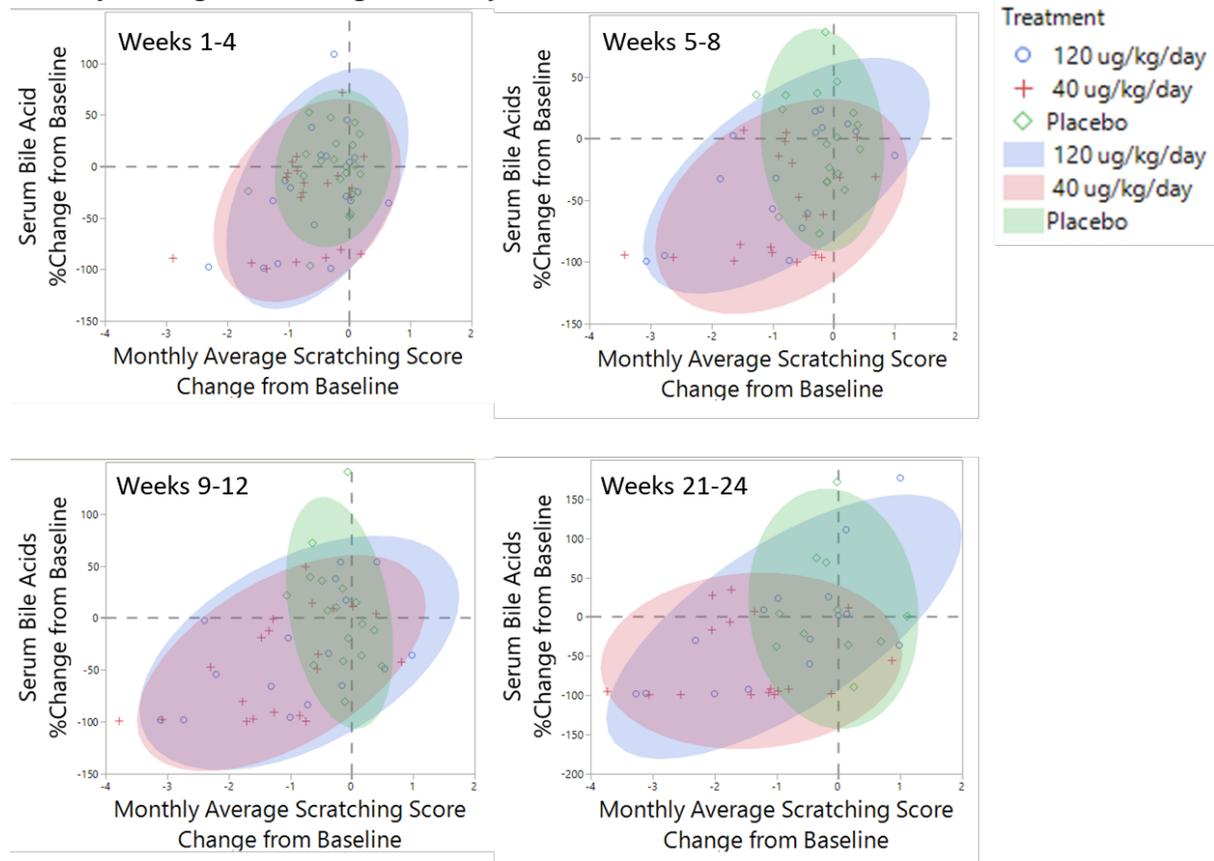


Source: Generated by the Applicant, Information Request response March 1, 2021, SD #0019
 Abbreviations: s-BA, serum bile acid

Clinical pharmacology reviewer also conducted analyses for interpreting relation between change in serum bile acid levels and change in ObsRO, overtime, in patients enrolled to Trial A4250-005.

In [Figure 26](#), “Y” axis represent change in sBA levels (horizontal dashed line denotes movement relative to baseline); “X” axis represents change in monthly averaged ObsRO (scratch score), as prespecified by the Applicant. In the plot on left upper quadrant (Weeks 1 to 4), all three groups started almost at same baseline. The blue ellipse and the pink ellipse continue to move during the treatment period from Weeks 5 to 8 to Weeks 9 to 12 to Weeks 21 to 24. Overall, there appears to be an association between improvement in pruritus and reduction in sBA levels in odevixibat-treated patients, but this is not observed in patients in the placebo group.

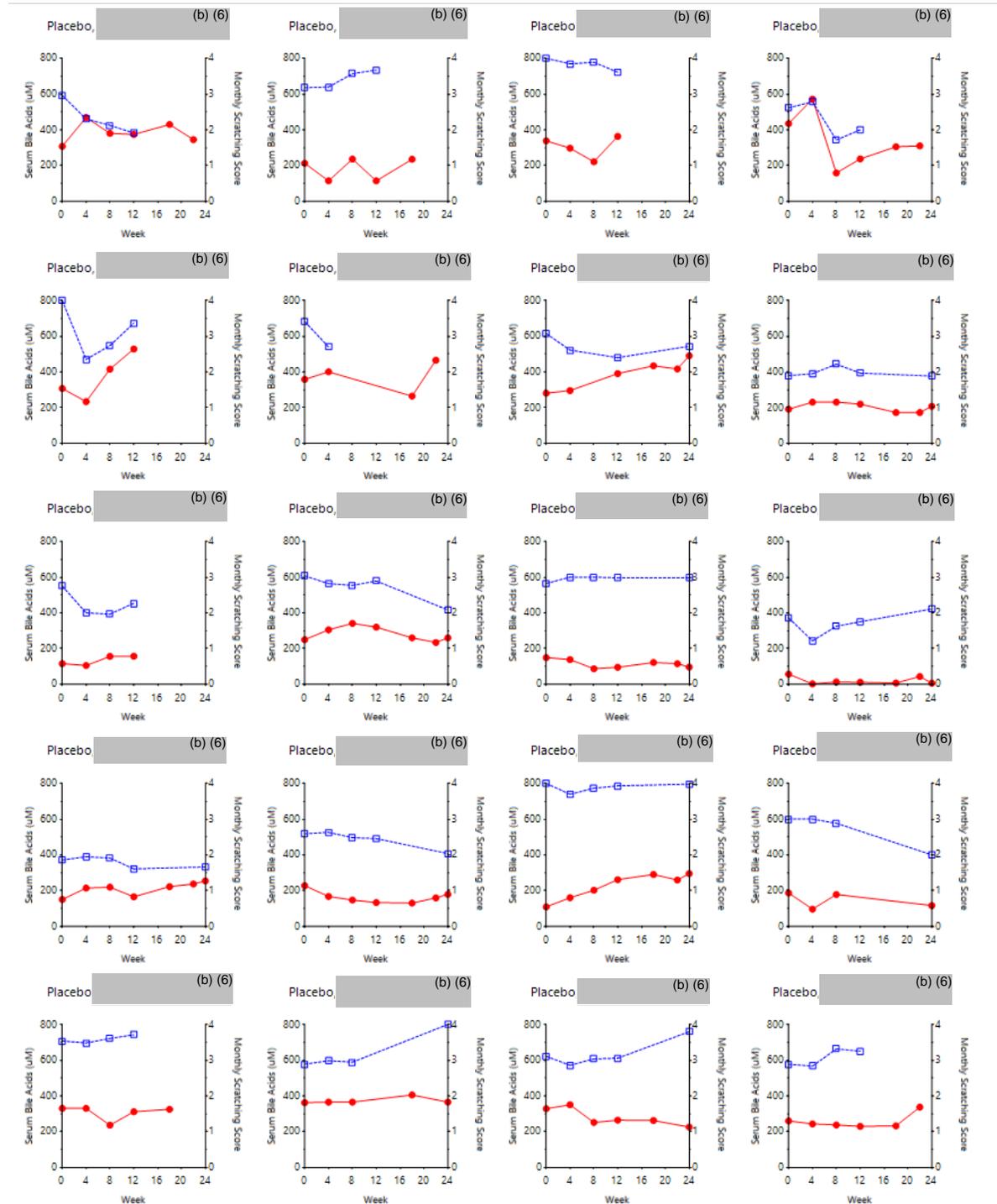
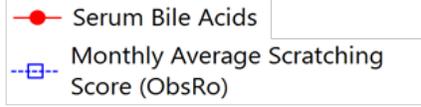
Figure 26. Percent From Baseline in Serum Bile Acid Levels Versus Change From Baseline in Monthly Average Scratching Scores by Time Period, Trial A4250-005



Source: Reviewer's analysis based on ADEFF.xpt, ADQS.xpt of Trial A4250-005
Each area covers 90% of the data

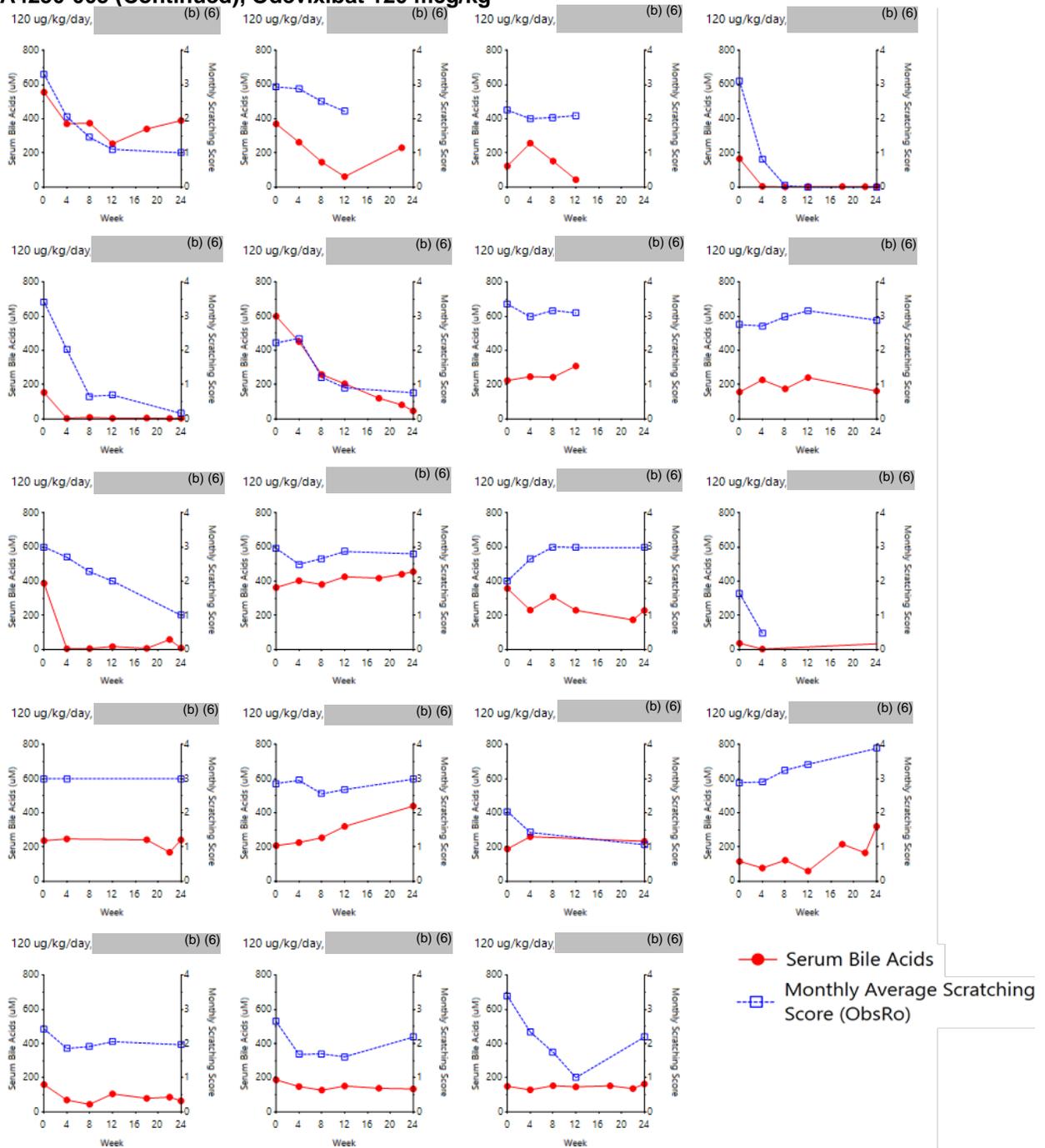
Individual patient data for serum bile acids levels with scratching score over time are provided in [Figure 27](#), [Figure 28](#), and [Figure 29](#).

Figure 27. Individual Plots of Serum Bile Acid Levels and Scratching Score Over Time, Trial A4250-005, Placebo



Source: Reviewer's analysis based on ADEFF.xpt, ADQS.xpt of Trial A4250-005
Abbreviations: ObsRO, observer-reported outcome

Figure 29. Individual Plots of Serum Bile Acid Levels and Scratching Score Over Time, Trial A4250-005 (Continued), Odevixibat 120 mcg/kg



Source: Reviewer's plots based on ADEFF.xpt, ADQS.xpt of Trial A4250-005
 Abbreviations: ObsRO, observer-reported outcome

14.3. Population Pharmacokinetics

A population PK analysis (Population PK Study Report ALBI-PMX-A4250-1167) was performed based on PK data from the clinical studies aforementioned in [Table 99](#) except Trial A4250-007, where all plasma drug concentrations were below LLOQ. For the summary of each study information, see Section [14.2](#).

A total of 158 individuals were included for the population PK analysis, of which 105 were healthy volunteers and 53 were pediatric patients. Given the minimal systemic absorption of odevixibat, many plasma drug concentrations were below LLOQ, especially in the pediatric patients in Trial A4250-003 and Trial A4250-005 ([Table 118](#)).

Table 118. Number of Samples With Unquantifiable Concentrations in Studies Included in Population Pharmacokinetic Analysis

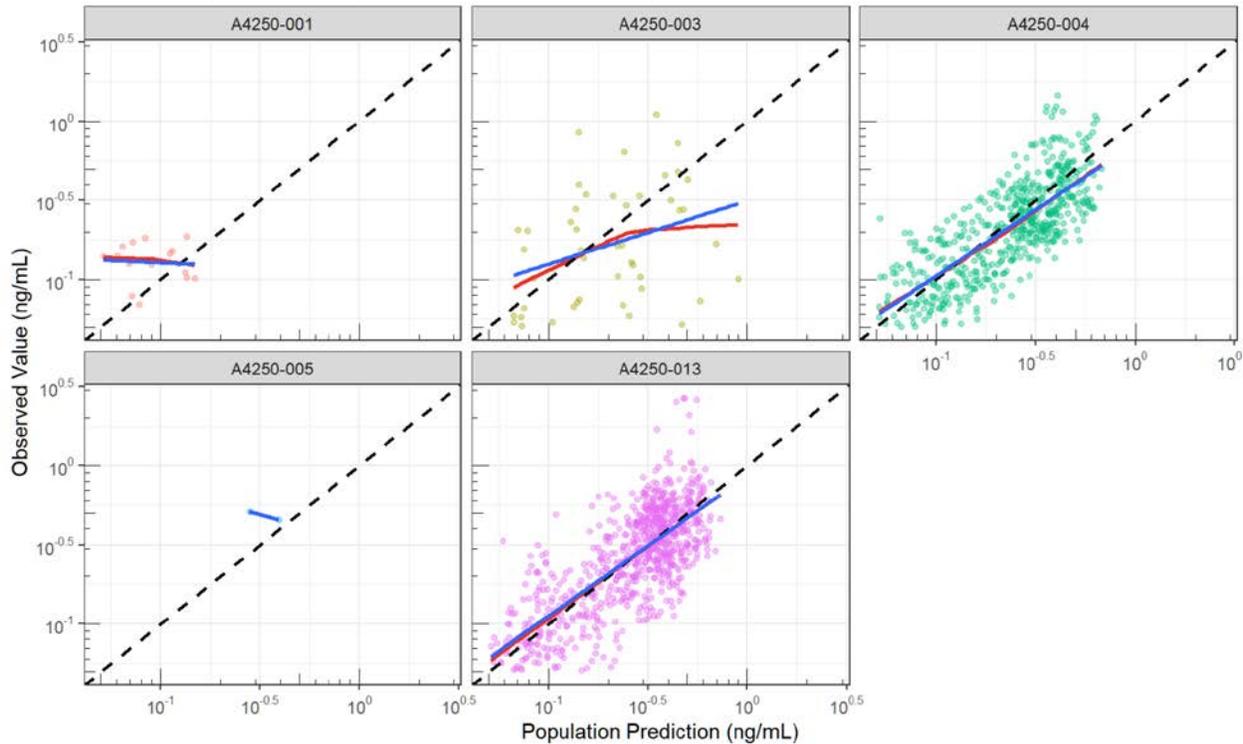
Study	Dose Level	Number of Samples with Undetectable Concentrations / Total Number of Samples (%)	Number of Samples with Detectable Concentrations / Total Number of Samples (%)
A4250-001	0.1 mg	66/66 (100%)	0/66 (0%)
	0.3 mg	66/66 (100%)	0/66 (0%)
	1 mg	165/165 (100%)	0/165 (0%)
	1.5 mg	223/228 (97.8%)	5/228 (2.20%)
	3 mg	189/198 (95.5%)	9/198 (4.5%)
	10 mg	48/66 (72.7%)	18/66 (27.3%)
A4250-003	0.01 mg/kg	18/24 (75.0%)	6/24 (25.0%)
	0.03 mg/kg	27/35 (77.1%)	8/35 (22.9%)
	0.06 mg/kg	18/27 (66.7%)	9/27 (33.3%)
	0.1 mg/kg	10/31 (32.3%)	21/31 (67.7%)
	0.2 mg/kg	9/23 (39.1%)	14/23 (60.9%)
A4250-004	9.6 mg	182/747 (24.4%)	565/747 (75.6%)
A4250-005	0.04 mg/kg	24/29 (82.8%)	5/29 (17.2%)
	0.12 mg/kg	20/25 (80.0%)	5/25 (20.0%)
A4250-013	7.2 mg	423/1223 (34.6%)	800/1223 (65.4%)

Source: Population pharmacokinetic report, ALBI-PMX-A4250-1167, Table 5
The limit of quantitation was 0.05 ng/mL

As the final population PK model, a linear one-compartment model with first-order absorption with an absorption lag was determined to fit the observed data best. However, as shown in the goodness-of-fit plots by study ([Figure 30](#)), the final PK model does not appear to adequately describe the observed data in the pediatric patients (Trial A4250-003 and Trial A4250-005), although PK characterization in pediatric patients is important given the target patient population. This is likely due to the limitation of data quality, i.e., only sparse PK samples in a small number of pediatric patients were available, with ~60% and 80% of plasma samples below LLOQ in Trial A4250-003 and A4250-005, respectively. Also see Sections [14.2.5](#) and [14.2.6](#) for details about PK sample availability in pediatric patients.

With such limitations, the population PK analysis is considered inadequate for estimating PK parameters of odevixibat in pediatric patients. Instead, observed odevixibat concentrations in pediatric patients, and PK parameters in healthy adults estimated by a noncompartmental method were reviewed and used to inform PK of odevixibat in the label.

Figure 30. Goodness-of-Fit Plots for Observed vs. Predicted Concentrations by Clinical Studies, Trials A4250-001, A4250-003, A4250-004, A4250-005, A4250-013



Source: Population pharmacokinetic report, ALBI-PMX-A4250-1167, Appendix 12.3.
Dotted line = the line of unity; blue line = regression; red line = smoothing function

14.4. Bioanalytical Methods

14.4.1. Drug Concentrations

Odevixibat in Human Plasma (Trials A4250-001, A4250-003, A4250-004, A4250-005, and A4250-007)

Plasma concentrations of odevixibat were measured in (b) (4) using a validated bioanalytical method with LC-MS/MS for Trials A4250-001, A4250-003, A4250-004, A4250-005, and A4250-007 (Table 119).

The initial method “MWI3036” was used for Trials A4250-001, A4250-003, A4250-004, and A4250-007. Later, the method was partially modified in terms of the calibration range and the long-term stability and validated for Trial A4250-005 (MWI4155).

The performance and validation parameters of the bioanalytical method “MWI3036” (Validation Report ID: (b) (4)) and the modified method “MWI4155” (Validation Report ID: (b) (4)) are summarized in Table 119.

Table 119. Summary of Performance and Validation Parameters of Bioanalytical Method to Measure Odevixibat in Plasma (Method ID: MWI3036, MWI4155)

Item	Description			
Bioanalytical method review summary	Method validation was adequate to support pharmacokinetic results obtained in Trials A4250-001, A4250-003, A4250-004, A4250-005, A4250-007.			
Method description	Drug extraction from Li-heparinized human plasma by protein precipitation and quantitation with LC-MS/MS method			
Materials used for calibration curve and QCs	Li-heparinized human plasma			
Validated assay range	MWI3036: 0.05 to 50.0 ng/mL; MWI4155: 0.05-5.0 ng/mL			
Regression model and weighting	Linear regression, 1/x ²			
Validation Parameters	Method Validation Summary	MWI3036	MWI4155	Acceptability
Calibration curve	No of standard calibrators	9	8	Yes
performance during accuracy and precision	Cumulative accuracy (%bias)	-1.8 to 3.0	-2.4 to 1.1	Yes
	Cumulative precision (%CV)	≤7.5	≤7.0	Yes
QCs performance during accuracy and precision	Cumulative accuracy (%bias) in 4 QCs	-10.8 to 15.4	-2.2 to 4.0	Yes
	Interbatch %CV	≤15.4	≤7.4	Yes
Selectivity and matrix effect/Interference and specificity	Successfully demonstrated absence of potentially interfering peaks from six batches of control blank human plasma samples (analyte response at LLOQ ≥5 times the average response of blank matrix)			Yes
Hemolysis effect and Lipemic effect	No significant effect observed			Yes
Dilution linearity	MWI3036: Linear within 10-fold dilutions. MWI4155: Linear within 2-fold dilutions.			Yes
Bench-top/process stability	At room temperature for 24 hours in plasma with and without light; At room temperature for 4 hours in blood			Yes
Freeze-thaw stability	4 cycles at -20°C when protected from light			Yes
Long-term storage	MWI3036: 103 days at -20°C			Yes
	MWI4155: 146 days at -20°C			
Carryover	MWI3036: Carryover observed. Recommended that control blank samples are analyzed after high concentration samples to assess carryover throughout each run. MWI4155: No carryover was observed			Yes

Source: Bioanalytical Method Validation Report, (b) (4)
Abbreviations: CV, coefficient of variation; LC-MS/MS, high-performance liquid chromatography with two mass spectrometry detectors; QC, quality control

Based on the bioanalytical reports for Trials A4250-001, A4250-003, A4250-004, A4250-005, and A4250-007 (Report ID: (b) (4) respectively), the in-study performance was all acceptable. All plasma samples were analyzed within the established long-term stability. No carryover effects were observed during the analysis.

Midazolam and 1'-Hydroxymidazolam in Human Plasma (A4250-013)

For Part A of Trial A4250-013, midazolam and 1'-hydroxymidazolam were extracted from sodium heparinized human plasma using a liquid-liquid extraction and analyzed using LC-MS/MS by (b) (4). The validated ranges of quantitation were 0.1 to 40 ng/mL for midazolam and 0.1 to 20 ng/mL for 1'-hydroxymidazolam. Per the bioanalytical report (located in A4250-013 CSR, Appendix 16.2.5.10), the method validations for midazolam and 1'-hydroxymidazolam were adequate to support Trial A4250-013. All samples were

analyzed within the established long-term stability (i.e., stable up to 346 days of collection following storage at -20°C).

14.4.2. Pharmacodynamic Biomarkers

Total Bile Acid Levels in Human Serum (Trials A4250-003, A4250-005, and A4250-008)

Given the mechanism of action of odevixibat as an IBAT inhibition, odevixibat leads to reduction of total serum bile acid levels. Reduction of serum total bile acid levels was the secondary endpoint (the primary endpoint for Europe and the rest of the world) in the pivotal phase 3 trial (A4250-005) and it was constantly monitored in the long-term extension trial (A4250-008). In the phase 2 dose-ranging trial (A4250-003), serum total bile acid levels concentration was the primary endpoint. Serum total bile acid levels is considered an important biomarker throughout the clinical program of odevixibat to support a regulatory decision making, for which an adequate bioanalytical method validation is necessary to demonstrate its reliable assay performance.

Therefore, the Agency recommended the following in a Type C meeting minutes for IND 130591 dated February 20, 2019:

“We recommend that you measure serum bile acids by adequately validated assay methods. The same assay method should be used across the study sites throughout the study duration or a central laboratory(ies) should analyze the samples. If multiple bioanalytical assay methods/ local laboratories will be used, we recommend that cross-comparison among methods be performed to allow comparison of serum bile acids values obtained by different assay methods. Refer to guidance for industry *Bioanalytical Method Validation* (2018) for details (<https://www.fda.gov/media/70858/download>).”

However, given the bioanalysis validation data provided by the Applicant, we concluded that the bioanalytical methods for serum total bile acid levels in Trials A4250-005, A4250-008, and A4250-003 were not adequately validated; therefore, it is unable to support any quantitative interpretation of serum bile acid data in Trials A4250-005, A4250-008, and A4250-003. It may be carefully interpreted with limitation to general and qualitative descriptions.

In Trials A4250-005, A4250-008, and A4250-003, total bile acid levels in human serum samples were determined using a commercial kit (Total Bile Acids Assay Kit) manufactured by Diazyme (Poway, USA) in CLIA-certified laboratories (b) (4) for Trials A4250-005 and A4250-008; (b) (4) for Trial A4250-003]. Per the FDA guidance for industry *Bioanalytical Method Validation* (2018), when a commercial diagnostic kit is repurposed as an analytical method in clinical studies to measure the concentrations of an important biomarker, the performance of diagnostic kits (specificity, accuracy, precision, and stability) should be assessed in the facility conducting the sample analysis under actual conditions of use. Since such commercial kits are generally developed for use as clinical diagnostic tools, their suitability for use in clinical studies should be demonstrated separately and diagnostic kit validation data provided by the kit manufacturer may not ensure that the kit method is reliable for drug development purposes.

According to the FDA guidance, we found the following critical issues in the bioanalytical validation for serum total bile acid levels in Trials A4250-005, A4250-008, and A4250-003, which are also summarized in [Table 120](#).

Accuracy, Precision, Stability, and Specificity

Per the kit manufacturer, intra- and interassay precision at total bile acids 8 micromole/L and 23micromole/L are 1.3 to 3.9% and 2.6 to 2.9%, respectively, but the intra- and interassay accuracy data are not available. Sample stability and specificity data are provided by the kit manufacturer.

However, the assay performance in each facility was not adequately assessed in either of (b) (4) (for A4250-005 and A4250-008) and (b) (4) (for A4250-003). In (b) (4) (b) (4) a partial validation for the serum bile acid assay was performed in 2013 which was 5 years earlier than the clinical studies were started in 2018.

The interassay accuracy was not evaluated, and the intra-assay accuracy was reported (b) (4) (b) (4), which is not acceptable, because intra- and interassay accuracy should be evaluated with quality controls (QCs) at the LLOQ, low, mid, and high concentrations. The intra- and interassay precision were (b) (4)

(b) (4) which is not acceptable, either, because (b) (4)

(b) (4) The validation report did not specify how the standard and QC samples had been prepared during the validation despite the FDA guidance indicating standards and QCs should be prepared in the same matrix as the subject samples (i.e., blank human serum) unless adequately justified. Stability and specificity were not separately tested by the facility.

For (b) (4) for A4250-003, a partial validation was performed in 2014 before the clinical trial started in 2015, and the same issues were identified as mentioned above for the assay validation of (b) (4)

Dilution Linearity

The quantitation range established by the kit manufacturer is 1.0 to 180 micromole/L. The patients with PFIC tend to have elevated serum bile acid levels. In Trial A4250-005, 54% of the reported values were above the upper limit of the quantitation (ULOQ), i.e., 180 micromole/L (the range of reported values: 1 to 627 micromole/L).

Per the FDA guidance, the dilution linearity should be evaluated by diluting QCs above the ULOQ to bring to within quantitation range, and the accuracy and precision of these diluted QCs should be demonstrated. The QCs and clinical samples should be diluted with similar matrix (i.e., blank serum).

In (b) (4) for A4250-005 and A4250-008, when readout values were above 180 micromole/L, the serum samples were diluted 1.5 to 7.5-fold with 9% NaCl solution. Similarly, in (b) (4) for A4250-003, the serum samples were diluted 5-fold with 9% NaCl solution, and it is unclear whether the samples were further diluted if the 5-fold diluted samples were still above 180 micromole/L. The dilution linearity and the matrix effect of dilution with 9% NaCl were not evaluated in either of the two laboratories.

In-Study Assay Performance

No in-study analysis reports were available for Trials A4250-005, A4250-008, or A4250-003. Even though both (b) (4) as CLIA-certified laboratories presumably

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performed quality control procedures daily, those procedures do not necessarily fulfill the FDA's regulatory standards of bioanalysis for clinical studies.

Per the provided assay summary of [REDACTED]^{(b) (4)}, it appears that the assay was calibrated based on two points at two levels were tested in each assay run. The concentrations of standard samples used for the daily calibration were not reported. Two QCs whose exact nominal concentrations were unknown were used. The assay acceptance criterion was that all QCs are within ± 2 SD of the established mean. However, per the FDA guidance, a calibration curve per each assay run should include a blank, a zero, and at least six standards covering the calibration range even when commercial kits use sparse calibration standards (e.g., one- or two-point calibration curves). Also, QCs with known nominal concentrations should be used and individual batches include at least three QC levels. FDA-recommended assay acceptance criteria are that $\geq 67\%$ of QCs are within $\pm 20\%$ of the nominal, and $\geq 50\%$ of QCs per level should be $\pm 20\%$ of their nominal.

In addition, multiple kit lots may have been used within each clinical study, but between-lot variability and comparability were not addressed.

Table 120. Summary of Performance and Validation Parameters of Bioanalytical Method to Measure Total Bile Acid Levels in Human Serum, Trials A4250-005, A4250-008, and A4250-003

Item	Description				
Bioanalytical method review summary	Method validation was inadequate to support the quantitative interpretation of serum bile acid data in Trials A4250-005, A4250-008, and A4250-003				
Method description	an enzymatic cycling method that employs absorbance detection using the commercial kit named 'Total Bile Acids Assay Kit' manufactured by Diazyme (Poway, US) to quantify total bile acids in human serum collected using serum separation tubes				
Materials used for calibration curve, QCs, and concentration	Not clearly specified but appeared to be the reagents included in the commercial kit				
Assay range	1.0 to 180 micromole/L established by the kit manufacturer.				
	(b) (4)			(b) (4)	
Validation Parameters	Method Validation Summary (A4250-005/A4250-008)	Acceptability (A4250-005)	Acceptability (A4250-008)	Acceptability (A4250-003)	Acceptability (A4250-005)
Calibration curve	Range	1.0 to 180 micromole/L	Yes	1.0 to 180 micromole/L	Yes
performance during accuracy and precision	No of standard calibrators	5	Yes	Not available	No
	Cumulative accuracy (%bias)	(b) (4)	No (absence of interassay accuracy)	Not available	No
QCs performance during accuracy and precision	Cumulative precision (%CV)	Not evaluated	No	Not available	No
	Cumulative accuracy (%bias)	Not evaluated	No	Not evaluated	No
	Interbatch %CV	(b) (4)	No (exact QC concentrations are unknown, no high QC)	(b) (4)	No (exact QC concentrations are unknown)
Dilution linearity	Not established despite 1.5-fold to 7.5-fold dilution in >50% samples	No	Not established	Despite 5-fold dilution	No
Selectivity & matrix effect	Not evaluated	No	Not evaluated	No	
Interference & specificity ¹	Not evaluated	No	Not evaluated	No	
Stability ²	Not evaluated	No	Not evaluated	No	
Carry over ³	Not evaluated	No	Not evaluated	No	
In-study Performance	Not available	No	Not available	No	

Source: Total Bile Acids Validation Summary by (b) (4) and Validation of Total Biliary Acids by (b) (4) under 5.3.1.4. Reports of Bioanalytical and Analytical Methods for Human Studies; Information Request response dated March 2, 2021 (SDN 12)

Abbreviations: CV, coefficient of variation; QC, quality control

¹ Per the kit manufacturer, <10% deviation in the presence of 750 mg/dL triglycerides, 50 mg/dL ascorbic acid, 50 mg/dL bilirubin, and 500 mg/dL hemoglobin

² Per the kit manufacturer, serum samples are stable for a week at 4°C and for 3 months at -20°C; reconstituted samples and lyophilized samples and are stable for 7 days at 4°C and for 9 months at 37°C, respectively.

³ Per the kit manufacturer, no evidence of carryover

Autotaxin in Human Plasma, Trials A4250-003 and A4250-005

Autotaxin was measured in human plasma in Trials A4250-003 and A4250-005 using ELISA with at (b) (4) Human ENPP-2/Autotaxin kits manufacture by R&D Systems (Abingdon, UK) was used. The analytical method validation (Report ID: (b) (4)) and the assay performance during analysis (Report ID: (b) (4)) and (b) (4), respectively) were acceptable to support C4 data obtained in Trials A4250-003 and A4250-005.

The quantitation range was from 0.781 to 50 ng/mL. Accuracy of dilution was demonstrated for up to 16-fold dilutions. The precision and accuracy of the method was found to be $\leq 7.7\%CV$ and $\leq \pm 8.7\%RE$. C4 was found to be stable in human plasma for up to 24 hours at room temperature, after four freeze-thaw cycles stored at nominally $-80^{\circ}C$ and after storage for 37 days at nominally $-80^{\circ}C$.

C4 in Human Plasma, Trials A4250-003 and A4250-005

7 α -hydroxy-4-cholensten-3-one (C4) was measured in human plasma collected in Trials A4250-003 and A4250-005 at (b) (4). C4 was extracted from plasma using a solid-liquid extraction method and analyzed by LC-MS/MS. The analytical method validation (Report ID: (b) (4)) and the assay performance during analysis in Trials A4250-003 and A4250-005 (Report ID: (b) (4) and (b) (4)), respectively) were acceptable to support autotaxin data obtained in the clinical studies.

The quantitation range was from 2.0 to 500 ng/mL. Accuracy of dilution was demonstrated for up to 10-fold dilutions. The precision and accuracy of the method was found to be $\leq 12.4\%CV$ and $\leq \pm 14.5\%RE$. Autotaxin was found to be stable in human plasma for up to 24 hours at room temperature, after three freeze-thaw cycles stored at nominally $-80^{\circ}C$ and after storage for 27 and 181 days at nominally $-80^{\circ}C$.

Individual Bile Acid Levels in Plasma and Feces and C4 in Plasma, Trial A4250-001

In Trial A4250-001, a phase 1 trial in healthy volunteers, C4 in plasma and 15 individual bile acid levels in conjugated and unconjugated forms were measured in human plasma and feces at (b) (4). Analytes were extracted from plasma and feces using a solid-liquid extraction method and analyzed by LC-MS/MS. Since blank human plasma and feces were likely to include endogenous bile acids, phosphate buffered saline (PBS) was used as a surrogate matrix for preparation of calibration standards and surrogate QC samples, as well as a dilution medium for study samples.

The quantitation range for C4 and 15 individual bile acid levels in plasma are as follows:

- C4, 2.0 to 500 ng/mL
- CA, Cholic acid: 0.500 to 500 ng/mL
- CDCA, Chenodeoxycholic acid: 1.00 to 1000 ng/mL
- DCA, Deoxycholic acid: 1.00 to 1000 ng/mL
- GCA, Glycocholic acid: 0.500 to 500 ng/mL
- GCDCA, Glycochenodeoxycholic acid: 1.00 to 1000 ng/mL

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- GDCA, Glycodeoxycholic acid: 1.00 to 1000 ng/mL
- GLCA, Glycolithocholic acid: 0.500 to 500 ng/mL
- GUDCA, Glycoursodeoxycholic acid: 1.00 to 1000 ng/mL
- LCA, Lithocholic acid: 0.500 to 500 ng/mL
- TCA, Taurocholic acid: 0.500 to 500 ng/mL
- TCDCA, Taurochenodeoxycholic acid: 0.500 to 500 ng/mL
- TDCA, Taurodeoxycholic acid: 0.500 to 500 ng/mL
- TLCA, Taurolithocholic acid: 0.500 to 500 ng/mL
- TUDCA, Tauroursodeoxycholic acid: 0.500 to 500 ng/mL
- UDCA, Ursodeoxycholic acid: 1.00 to 1000 ng/mL

The assay validation was performed for each analyte separately. Per the analytical method validation and analysis report (Report ID: (b) (4)), the analytical method for human plasma was not entirely validated for all of each analyte due to insufficient stability while the method may have fitted the exploratory purpose of the phase 1 trial in healthy volunteers to see relative changes from baseline in total bile acid levels (i.e., sum of 15 individual bile acid levels) and C4. On the other hand, the method for human feces was not qualified due to significant matrix interference for CDCA and LCA and interbatch variability above acceptable range.

The precision and accuracy of the method for human plasma was found to be $\leq 23.4\%CV$ and -13.0 $19.4\%RE$. There was no significant matrix effect observed for undiluted plasma samples. The selectivity was found to be satisfactory except for LCA where interference was observed, which was later found to be consistent in each sample. Stability was demonstrated for most bile acid levels, but not all due to the potential for interconversion of bile acids in matrix. Individual bile acid levels were stable in human plasma at room temperature for 12 hours (UDCA, GCDCA, and LCA) or 24 hours (the rest of bile acids). Long-term stability was not demonstrated for GLCA but GCA and CDCA were stable for 7 days and the rest of bile acid levels were stable for 30 days after storage at nominally $-20^{\circ}C$. Stability of C4 in plasma samples at room temperature was not established.

FGF-19 in Human Plasma, Trials A4250-001 and A4250-003

Fibroblast growth factor 19 (FGF-19) was measured in human plasma collected in Trial A4250-001 using ELISA at (b) (4) FGF-19 ELISA kits manufacture by R&D Systems (Abingdon, UK) was used. The analytical method validation and the assay performance during analysis (Report ID: (b) (4)) were acceptable to support FGF-19 data obtained in Trial A4250-001.

The quantitation range was from 17.3 to 1000 pg/mL. The precision across the reported batches for all the QC levels was 5.3 to 13.6%CV. Accuracy ranged from -8 to 4%RE. FGF-19 was found to be stable in human plasma after storage for 195 days at nominally $-80^{\circ}C$.

Of note, bioanalytical method validation of plasma FGF-19 in Trial A4250-003 was not provided while FGF-19 results were presented in the study reports of A4250-003.

14.4.3. Pharmacogenomics

The detailed description of variants identified in *ABCB11* and *ATP8B1* genes and pathogenicity determination in Trial A4250-005 are summarized below.

Table 121. *ABCB11* Variants, Trial A4250-005

<i>ABCB11</i> Variants	Type	Frequency	Percent (%)	Pathogenicity Reference ¹
c.890A > G p.Glu297Gly ²	Missense	11	12.2	Database/Literature
c.1445A > G p.Asp482Gly	Missense	7	7.8	Database/Literature
c.3457C > T p.Arg1153Cys	Missense	6	6.7	Database/Literature
c.1409G > A p.Arg470Gln	Missense	4	4.4	Database/Literature
c.154C > T p.Arg52Trp	Missense	4	4.4	In silico prediction tool
c.3892G > A p.Gly1298Arg	Missense	4	4.4	In silico prediction tool
c.1160G > A p.Arg387His	Missense	3	3.3	Database/Literature
c.2012-8T > G	Splicing	3	3.3	Database/Literature
c.1415A > G p.Tyr472Cys	Missense	2	2.2	Database/Literature
c.149T > G p.Leu50Trp	Missense	2	2.2	In silico prediction tool
c.1621A > C p.Ile541Leu	Missense	2	2.2	Database/Literature
c.1698G > T p.Arg566Ser	Missense	2	2.2	In silico prediction tool
c.2495G > A p.Arg832His	Missense	2	2.2	Database/Literature
c.2702G > T p.S901I ³	Missense	2	2.2	Database/Literature
c.3352G > A p. Gly1118Se	Missense	2	2.2	In silico prediction tool
c.3904G > T p.Glu1302Ter	Nonsense	2	2.2	Database/Literature
c.677C > T p.Ser226Leu	Missense	2	2.2	Database/Literature
c.-27-789_99-48del p.?	Deletion	1	1.1	Database/Literature
c.1064c > Tp.Thr355Ile	Missense	1	1.1	In silico prediction tool
c.1146_1166del p.Phe383	Deletion	1	1.1	Database/Literature
c.1273G > A p.Val425Met	Missense	1	1.1	In silico prediction tool
c.1384A > C p. Ser462Arg	Missense	1	1.1	In silico prediction too
c.1460G > C p.Arg487Pro	Missense	1	1.1	In silico prediction tool
c.1723C > T p.Arg575Ter	Nonsense	1	1.1	Database/Literature
c.192G > A p.=	Silent	1	1.1	Database/Literature
c.1966_1967delTT p.Leu6	Deletion	1	1.1	Database/Literature
c.2296G > A p.Gly766Arg	Missense	1	1.1	Database/Literature
c.2343+1G > T p.?	Splicing	1	1.1	Database/Literature
c.2492C > T p.Arg832Cys ⁴	Missense	1	1.1	Database/Literature
c.2636T > G p. Ile879Arg	Missense	1	1.1	In silico prediction tool
c.2703C > G p.Ser901Arg	Missense	1	1.1	Database/Literature
c.2783_2787dupGAGATc.As	Frameshift	1	1.1	Database/Literature
c.2944G > A p.Gly982Arg	Missense	1	1.1	Database/Literature
c.3083C > Tp.Ala1028Val	Missense	1	1.1	In silico prediction tool
c.3130G > C p.Ala1044Cys	Missense	1	1.1	In silico prediction tool
c.3148C > t p.Arg1050Cys	Missense	1	1.1	Database/Literature
c.3343A > G p.Ser1115Gly	Missense	1	1.1	In silico prediction tool
c.3383G > A p.Arg1128His	Missense	1	1.1	In silico prediction tool
c.3458G > A p. Arg1153His	Missense	1	1.1	In silico prediction tool
c.3692G > A p.Arg1231Gin	Missense	1	1.1	In silico prediction tool
c.3722T > C p.Leu1241Pro	Missense	1	1.1	In silico prediction tool
c.3767C > G p.Thr1256Arg	Missense	1	1.1	In silico prediction tool
c.3945delC p.Thr1316Leu	Missense	1	1.1	In silico prediction tool

ABCB11 Variants	Type	Frequency	Percent (%) Pathogenicity Reference¹
c.640G > A p.Ala214Thr ⁵	Missense	1	1.1 In silico prediction tool
c.763G > Cp.Gly255Arg	Missense	1	1.1 In silico prediction tool
c.922T > T p.Leu308Phe	Missense	1	1.1 In silico prediction tool
c.936G > T p.Gln312His	Missense	1	1.1 In silico prediction tool
Total		90	100.0

Source: Reviewer's table. The reference sequence used for variant description for ABCB11 is NM_003742.2.

¹ Databases/Literature: Variants were defined as pathogenic or likely pathogenic in at least one database with human variants, or the variants had been observed in previously reported patients with PFIC2 in literature. Databases include Clinvar (<https://www.ncbi.nlm.nih.gov/clinvar/>), Global Variome shared LOVD (<https://databases.lovd.nl/shared/genes>), and (b) (4). If pathogenicity support was not found based on databases/literature, in silico prediction tool based on polyphen-2 tool (<http://genetics.bwh.harvard.edu/pph2/>) was used to assess pathogenicity.

² The variant for one patient was submitted as c.809A > G p.Glu297Gly in Trial A4250-005.

³ The variant was submitted as c.2702G > Tp in Trial A4250-005.

⁴ The variant was recorded c.2494C > T (p.Arg832Cys) in the Clinvar database.

⁵ The variant was submitted as c.640G > A p.Ala241Thr in Trial A4250-005.

Table 122. ATP8B1 Variants, Trial A4250-005

ATP8B1 Variants	Type	Frequency	Percent Pathogenicity (%) Reference¹
c.3622_3628delGCCTACG p.Ala1208ProFsTer	Deletion	6	17.7 Null variant
c.923G > T p.Gly308Val	Missense	6	17.7 Database/Literature
c.1660G > A p.Asp554Asn	Missense	2	8.8 Database/Literature
c.2150C > Ap.Thr717Asn	Missense	3	5.9 Database/Literature
c.2742_2743delinsT p.Gly914AspfsTer32	Nonsense	2	5.9 Null variant
c.300C > G p.Pro100Ter	Nonsense	2	5.9 Null variant
c.811C > T p.Arg271Ter	Nonsense	2	5.9 Null variant
Deletion of the gene 5' to exon 18	Deletion	1	2.9 Null variant
c.1039_1041delc.Val347del	Deletion	1	2.9 Null variant
c.1573C > T; p.Arg525Ter	Nonsense	1	2.9 Null variant
c.1587_1589delCTT p.Phe529del	Deletion	1	2.9 Null variant
c.1962_1967del; p.Leu656_Cy657del_	Deletion	1	2.9 Null variant
c.2122G > C p.Asp708His	Missense	1	2.9 In silico prediction tool
c.2600G > Ap.Arg867His	Missense	1	2.9 In silico prediction tool
c.2821C > T p.Arg941Ter	Nonsense	1	2.9 Null variant
c.2854C > Tp.Arg952Ter	Nonsense	1	2.9 Null variant
c.3531G > A p.? ²	Splicing	1	2.9 Database/Literature
c.958_967del p.Met320Valfs	Frame Shift	1	2.9 Null variant
Total		34	100.0

Source: Reviewer's table. The reference sequences used for variant description for ATP8B1 is NM_005603.4.

¹ Databases/Literature: Variants were defined as pathogenic or likely pathogenic in at least one database with human variants, or the variants had been observed in previously reported patients with PFIC2 in literature. Databases include Clinvar (<https://www.ncbi.nlm.nih.gov/clinvar/>), Global Variome shared LOVD (<https://databases.lovd.nl/shared/genes>), or (b) (4). Certain types of null variants including nonsense, frameshift, single exon or multi-exon deletion) can often be assumed to disrupt gene function based on the American College of Medical Genetics and Genomics criteria (Richards et al. 2015). If pathogenicity support was not found based on databases/literature or the type of variant was not a null variant, in silico prediction tool based on polyphen-2 tool (<http://genetics.bwh.harvard.edu/pph2/>) was used to assess pathogenicity.

² The variant was reported as c.3531+1G > A in (b) (4)

15. Trial Design: Additional Information and Assessment

[Table 123](#) and [Table 124](#) summarize the key trial design of clinical protocol for Trials A4250-005 and A4250-008. Both trials were conducted under IND 130591, i.e., under the odevixibat development program. The data from both trials (completed Trial A4250-005 and ongoing Trial A4250-008 data cut-off December 4, 2020) were submitted to support this new drug application (NDA). Trial A4250-005 provides the key data for efficacy and safety. Trial A4250-008, an open-label extension, is an ongoing trial that provided limited data as supportive data for long-term safety and durability. A total of six protocol amendments were submitted for A4250-005 and five for Trial A4250-008, but, final amendments were reviewed for writing the trial summaries. Section [14.2.5](#) summarizes key trial design as well as safety and efficacy findings for Trial A4250-003.

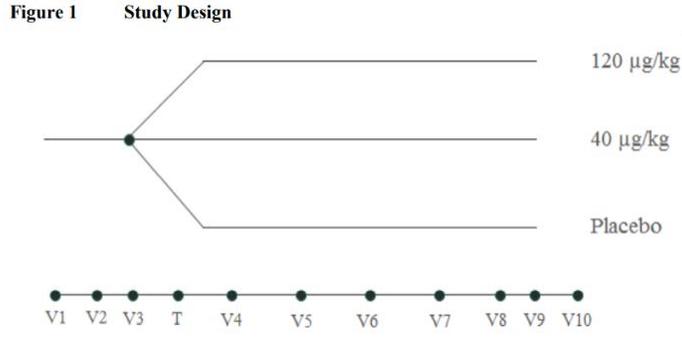
Table 123. Protocol Summary, Trial A4250-005

Protocol Section	Protocol Summary
Protocol number	A4250-005
Phase	Phase 3
Study center	This was a multicenter trial; patients were enrolled at 33 study centers, including 17 in Europe (Belgium, France, Germany, Italy, Netherlands, Poland, Sweden, the United Kingdom), 8 in the United States, and 8 in the rest of world (RoW) (Australia, Canada, Israel, Saudi Arabia, Turkey).
Study title	A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children With Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC1)
Principal investigator	Richard Thompson, MD, PhD
Study objectives	<p>Primary</p> <ul style="list-style-type: none"> • U.S.: Proportion of positive pruritus assessment at the subject level at 24 weeks • RoW: Proportion of > a 70% reduction in fasting serum bile acid levels at 24 weeks <p>Secondary objective: To evaluate the effect of odevixibat treatment on the following:</p> <ul style="list-style-type: none"> • ALT • Growth • Sleep disturbance • Need for surgical treatment (biliary diversion or liver transplantation) • Safety and tolerability
Endpoints	<p>Primary efficacy endpoint</p> <ul style="list-style-type: none"> • Proportion of positive pruritus assessments at the subject level at 24 weeks <p>Secondary efficacy endpoint</p> <ul style="list-style-type: none"> • Proportion of at least 70% reduction in fasting sBA levels at 24 weeks <p>Safety endpoint</p> <ul style="list-style-type: none"> • Treatment-emergent adverse events • Treatment-emergent serious adverse events • Trends on physical exam, concomitant medications, vital signs, vitamins A, E, INR, 25-hydroxy vitamin D

Protocol Section	Protocol Summary
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Study design	<ul style="list-style-type: none"> • Double-blind, randomized, placebo-controlled, phase 3 trial • Randomization ratio: 1:1:1 to 40 mcg/kg/day, 120 mcg/kg/day or matching placebo
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Figure 31. Study Design, Trial A4250-005 (IND 130591)



Source: Applicant's Protocol Amendment 6; page 24 of 1064

Study population	Pediatric patients with PFIC1 and PFIC2
Inclusion and Exclusion criteria	<p>Key inclusion criteria</p> <ul style="list-style-type: none"> • Clinical diagnosis of PFIC types 1 or 2 • Age: >6 months and <18 years; with body weight above 5 kg • Genetic confirmation of PFIC1 or PFIC2 of biallelic pathogenic variants in ATP8B1 or ABCB11 genes • Serum bile acid level >100 micromole/L with average of two samples 7 days apart prior to randomization • Significant pruritus and a caregiver-reported observed scratching average of >2 (on 0 to 4 scale) in the 2 weeks prior to randomization <p>Key exclusion criteria</p> <ul style="list-style-type: none"> • Pathologic variations of the ABCB11 gene that predict complete absence of the BSEP protein • Liver diseases such as biliary atresia, benign recurrent intrahepatic cholestasis, liver cancer, or other cancer metastasis to liver • Disease conditions known to interfere with absorption, distribution and metabolism or excretion of drugs in the intestine • Ongoing diarrhea >3months requiring IV fluid or nutritional intervention • HIV, confirmed cancers • GFR <70 mL/min/1.73 m²; INR >1.4, ALT or total bilirubin >10 x ULN; ALT >15 x ULN any time in past 6 months • Liver transplant or planned within 6 months of randomization • Decompensated liver diseases • Administration of bile acid or lipid binding resins or medication • Pregnancy or lactation
Sample size	N=60
Dose and dosing regimen	<ul style="list-style-type: none"> • Product: A4250; selective inhibitor of the IBAT • Treatment: two dose arms: 40 mcg/kg/day or 120 mcg/kg/day; placebo arm • Route: oral
Duration	24 weeks

Protocol Section	Protocol Summary
Safety monitoring	<ul style="list-style-type: none"> • AE, concomitant medication, vital signs, height and weight, pregnancy test: all visits (Day 0, Weeks 4, 8, 12, 18, 22, 24) • Physical exam: Day 0, Week 12 and 24 • Skin exam: Day 0, Weeks 4, 12, 24 • Clinical chemistry, hematology: Day 0, Weeks 4, 8, 12, 18, 24 • Urinalysis: screening, Week 12 and 24 • INR: screening, Weeks 4, 12, 22 • Vitamin A, E, D: screening or Day 0, Week 12 and 24 • Assessment with abdominal ultrasound, Fibroscan, alpha-fetoprotein: Day 0, Week 24
Exploratory efficacy assessment	<ul style="list-style-type: none"> • Autotaxin, p-C4, PK: Day 0, Week 4 and 24 • QoL questionnaire (PedsQL): Day 0, week 24 • Patient/caregiver/clinician global impression of change: Weeks 4, 12, 24 • Patient/caregiver/clinician global impression of symptoms: Day 0, Weeks 4, 12, 24
Treatment-interruption criteria	<p>Liver chemistry</p> <ol style="list-style-type: none"> 1. ALT or AST >3 x baseline or 800 U/L AND total bilirubin >2 x ULN 2. ALT or AST >10 X ULN or 5 x baseline or absolute threshold of 800 in presence of normal LDH or CPK 3. Total bilirubin doubling if baseline <3 or increase by >3 mg/dL if TB was >3 mg/dL at baseline 4. INR increase refractory to vitamin K administration: INR >1.5 (if normal baseline) or increase by >0.4 (if abnormal baseline) 5. Any increase in total bilirubin or AST/ALT accompanied by either a symptom of clinical hepatitis or immunological reactions <p>Diarrhea, with at least one of following</p> <ul style="list-style-type: none"> • Grossly bloody stools • Vomiting • Dehydration requiring treatment with oral or intravenous rehydration and/or electrolyte imbalances • Fever ($\geq 38^{\circ}$ C) • Diarrhea persists for 7 or more days

Source: Adapted from Protocol Amendment 06

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSEP, bile salt export pump; CPK, creatine phosphokinase; GFR, glomerular filtration rate; INR, international normalized ratio; LDH, lactate dehydrogenase; PFIC, progressive familial intrahepatic cholestasis; PK, pharmacokinetic; TB, total bilirubin; ULN, upper limit of normal; sBA, serum bile acid; IBAT, ileal bile acid transporter

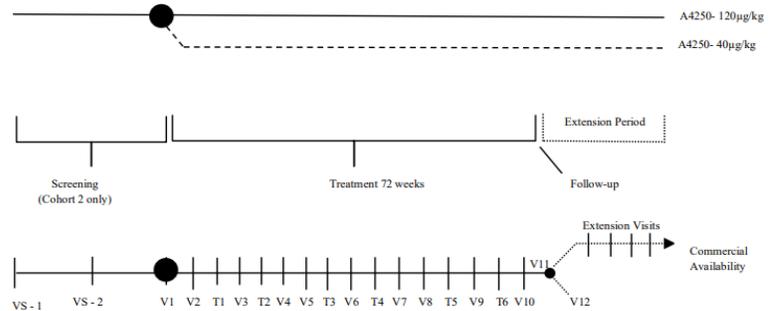
Table 124. Protocol Summary, Trial A4250-008

Protocol Section	Protocol Summary
Protocol number	A4250-008
Development phase	Phase 3
Study center	A total of 33 study centers, including 18 in Europe (Belgium, France, Germany, Italy, Netherlands, Poland, Spain, United Kingdom), 6 in the United States (US), and 9 in the rest of world (RoW) (Australia, Canada, Israel, and Turkey).
Study title	An Open-label Extension Study to Evaluate Long-term Efficacy and Safety of A4250 in Children With Progressive Familial Intrahepatic Cholestasis Types 1 and 2 (PEDFIC2)

Protocol Section	Protocol Summary
Study objectives	<p>Primary objective U.S.: To demonstrate sustained effect on improvement in pruritus RoW: To demonstrate a sustained effect of odevixibat on serum bile acid levels</p> <p>Secondary objective To evaluate the following: Long-term safety and tolerability Effect on growth Biliary diversion and/or liver transplantation Biochemical markers of cholestasis and liver diseases</p>
Endpoints	<p>Primary efficacy endpoint Proportion of positive pruritus assessments at the patient level over 72 weeks</p> <p>Secondary efficacy endpoint Change from baseline in serum bile acid levels after 72 weeks of treatment</p> <p>Safety endpoint Treatment-emergent AEs Treatment-emergent SAEs Trends in physical exam, concomitant medications, vital signs, lab test (chemistry, hematology, urinalysis, alfa-fetoprotein, vitamins A, E, D, abdominal ultrasound)</p>
Study design	<p>Multicenter, open-label extension phase 3 Cohort 1: Participants who completed A4250-005 or withdrew from A4250-005 due to intolerable symptoms. Cohort 2: Patients with PFIC in whom diagnosis was genetically confirmed, weight ≥ 5 kg, and sBA levels ≥ 100 micromole/L, and caregiver-reported average scratching score in the eDiary of ≥ 2 (on a 0 to 4 scale) in the 2 weeks prior to Visit 1. 12 site visits (screening, Weeks 4, 12, 22, 24, 36, 46, 48, 60, 70, 72, 76); 6 telephone contacts: Weeks 8, 18, 30, 42, 54, 66)</p>

Figure 32. Study Design, Trial A4250-008 (IND 130591)

Figure 1 Study Design



T: telephone contact; V: study visit; VS: Visit Screening; W: study week

Note: Visit 1 coincides with Visit 9 of Study A4250-005. Patients who are not tolerating the 120 µg/kg/day dose may be down-titrated to the 40 µg/kg/day dose following a minimum of 1 week of treatment. The patient can return to the higher dose as soon as deemed appropriate.

Source: Applicant A4250-008 Protocol Amendment Submission, page 26 of 1488

Study population	Pediatric patients with PFIC1, PFIC2, PFIC3
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Protocol Section	Protocol Summary
Inclusion and Exclusion criteria	<p>Key inclusion criteria: See study design</p> <p>Key exclusion criteria: Pathologic variations of the <i>ABCB11</i> gene that predict complete absence of the BSEP protein Liver diseases: such as biliary atresia, benign recurrent intrahepatic cholestasis; liver cancer or other cancer metastasis to liver Disease conditions known to interfere with absorption, distribution and metabolism or excretion of drugs in the intestine Ongoing diarrhea >3 months requiring IV fluid or nutritional intervention HIV, confirmed cancers, alcohol, or substance abuse INR >1.4, ALT or total bilirubin >10 x ULN; ALT >15 x ULN any time in past 6 months Has received a liver transplant or if liver transplant is planned within 6 months of randomization Decompensated liver disease Administration of bile acid or lipid binding resins or medication Pregnancy or lactation</p>
Sample size	N=120
Dose	120 mcg/kg/day
Treatment duration	72 weeks
Safety monitoring	<p>AE or concomitant medications, vital signs, pregnancy test: all visits Physical exam, skin exam: screening, Weeks 12, 24, 48, 72, 76 Clinical chemistry. Hematology: Day 1, Weeks 4, 12, 24, 36, 48, 60, 72 Urinalysis: Day 1, Weeks 24, 48, 72 INR: screening, Weeks 4, 12, 22, 36, 46, 60, 70, 76 Alfa-fetoprotein: Day 1, weeks 24, 48, 72 Vitamins A, E, D: Day 1, Weeks 12, 22, 36, 46, 60, 70 Abdominal ultrasound, Fibroscan: Day 1, Weeks 24, 48, 72</p>
Efficacy assessment	<p>Primary: PRO/ObsRO eDiary: daily Secondary: Serum bile acid levels: all visits</p> <p>Exploratory efficacy assessment Autotaxin, p-C4: Day 1, Weeks 4, 24, 48, 72 QoL questionnaire (PedsQL): Day 1, weeks 24, 48, 72 Patient/caregiver/clinician patient global impression of change & patient/caregiver/clinician patient global impression of symptoms: Day 1, Weeks 4, 12, 24, 48, 72 Study drug compliance: Weeks 4, 12, 24, 36, 48, 60, 72</p>
Dose reduction	<ul style="list-style-type: none"> • Down-titrate to a lower dose i.e., 40 mcg/kg/day, if patient is unable to tolerate odevixibat 120 mcg/kg/day • Titrate to higher dose as deemed appropriate by the investigator • More than one upward dose titration (from 40 mcg/kg/day to 120 mcg/kg/day) for the same event was not recommended

Protocol Section	Protocol Summary
Treatment-interruption criteria	<p>Liver chemistry</p> <p>ALT or AST >3 x baseline or 800 U/L AND total bilirubin >2 x ULN</p> <p>ALT or AST >10 X ULN or 5 x baseline or absolute threshold of 800 in presence of normal LDH or CPK</p> <p>Total bilirubin doubling if baseline <3 or increase by >3 mg/dL if TB was >3 mg/dL at baseline</p> <p>INR increase refractory to vitamin K administration: INR >1.5 (if normal baseline) or increase by >0.4 (if abnormal baseline)</p> <p>Any increase in total bilirubin or AST/ALT accompanied by either a symptom of clinical hepatitis or immunological reactions</p> <p>Diarrhea, with at least one of following</p> <p>Grossly bloody stools</p> <p>Vomiting</p> <p>Dehydration requiring treatment with oral or intravenous rehydration and/or electrolyte imbalances</p> <p>Fever ($\geq 38^{\circ}\text{C}$)</p> <p>Diarrhea persists for 7 or more days</p>

Source Adapted from Protocol Amendment 05

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSEP, bile salt export pump; CPK, creatine phosphokinase; INR, international normalized ratio; LDH, lactate dehydrogenase; ObsRO, observer-reported outcome; PedsQL, Pediatric Quality of Life Inventory; PFIC, progressive familial intrahepatic cholestasis; PRO, patient-reported outcome; QoL, quality of life; SAE, serious adverse event; sBA, serum bile acid; TB, total bilirubin; ULN, upper limit of normal

16. Efficacy: Additional Information and Assessment

16.1. Clinical Outcome Assessments Review

Note: Unless otherwise specified, all analyses discussed in this section were conducted using the full analysis set (FAS) of Trial A4250-005 (N=62) with patients pooled across study arms and with no imputation of missing data.

16.1.1. Study COAs Used to Construct or Interpret Primary and Key Secondary Efficacy Endpoints

This review focuses on the Applicant's patient-reported outcome (PRO) and observer-reported outcome (ObsRO) measures of pruritus.

Table 125. Proposed COAs Included in Study

COA Name (COA Type)	Concept(s)	Endpoint Position	Assessment Frequency
Pruritus measure item (ObsRO)	Scratch	Primary	Twice daily
Pruritus measure item (PRO)	Itch	Secondary	Twice daily

Source: DCOA reviewer's table.

Abbreviations: COA, clinical outcome assessment; ObsRO, observer-reported outcome; PRO, patient-reported outcome

Clinical Outcome Assessment Description

The ObsRO Measure of Scratching Severity

The ObsRO item 1 is designed to assess scratching severity on a 5-point numeric rating scale that includes correlating facial expressions ranging from 0 (“no scratching”) to 4 (“the worst possible scratching”). The recall period is over the previous half day (e.g., since he/she went to bed last night, since he/she woke up this morning).

The PRO Measure of Itching Severity

The PRO item 1 is designed to assess itch intensity on a 5-point numeric rating scale that includes correlating facial expressions ranging from 0 (“no itching”) to 4 (“the worst itching”). The recall period is over the previous half day (e.g., since you went to bed last night, since you woke up this morning).

Conceptual Framework

Table 126. Conceptual Framework of Observer-Reported Outcome Pruritus Item

Item	Domain	General Concept
How bad was your child’s worst scratching since he/she went to bed last night?	Nighttime scratching	Daily scratching
How bad was your child’s worst scratching since he/she woke up this morning?	Daytime scratching	

Source: Page 25/89 Table 9 of COA Pruritus Evidence Dossier

Table 127. Conceptual Framework of Patient-Reported Pruritus Item

Item	Domain	General Concept
How bad was your worst itching since you went to bed last night?	Nighttime itching	Daily itching
How bad was your worst itching since you woke up this morning?	Daytime itching	

Source: Page 23/89 Table 6 of COA Pruritus Evidence Dossier

Content Validity

Pruritus Measure Items (ObsRO and PRO)

Literature review

The Applicant completed a targeted literature search using public databases to identify the signs, symptoms and impacts of PFIC with a focus on cholestatic pruritus. Pruritus was the most common symptom identified in the literature (Bergasa et al. 2000). We agree that pruritus is an important and relevant concept in cholestatic pruritus diseases. This conclusion is also supported by patient interview data, and pruritus is a debilitating symptom brought up in past public patient focused drug development meetings.

Documentation of expert input

Expert input was gathered from five clinicians with PFIC expertise. Three clinicians’ interviews focused on PFIC symptoms, and the other two interviews focused on sleep disturbance. Clinicians confirmed the literature review findings and the importance of pruritus in patients with PFIC. They also emphasized that pruritus is most troublesome when a child goes to bed.

Patient and caregiver interviews

The Applicant conducted two sets of interviews (stage I and stage II) with a total number of 36 interviews with 28 patients and caregivers across the stages, and eight patients and caregivers interviewed in both stages I and II. Each Stage I interview comprised of a concept elicitation and a cognitive debriefing segment.

The objectives of stage I and II interviews were to:

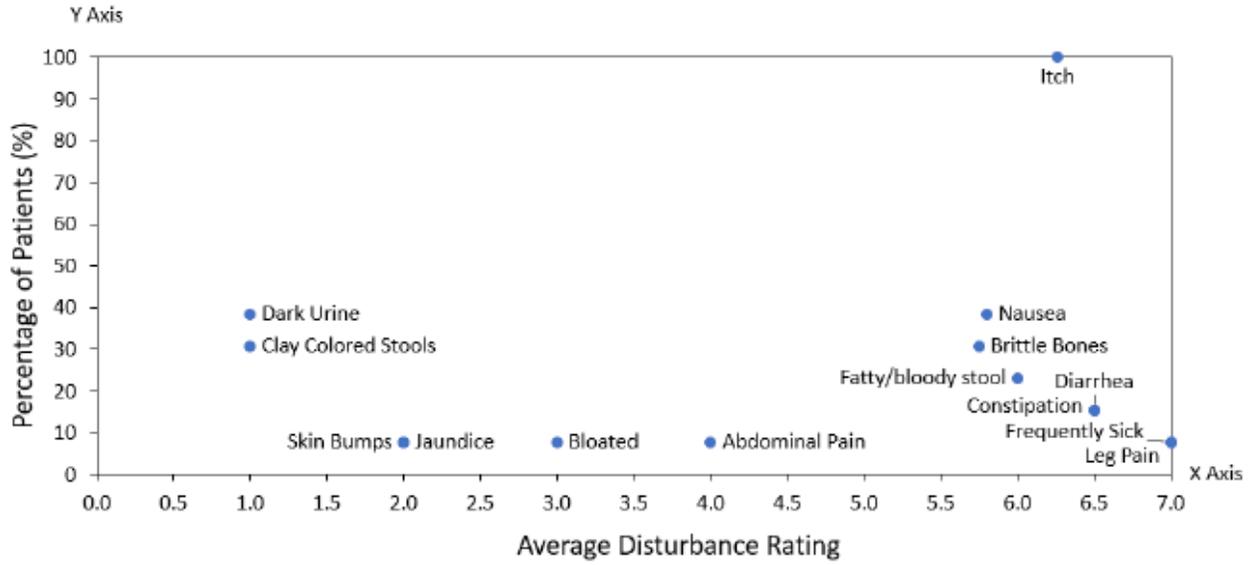
- Identify the most relevant pediatric PFIC and cholestatic liver disease signs, symptoms and impacts for patients
- Evaluate patient/caregiver understanding of the draft instrument instructions, items, and response scales and to refine the draft PRO and ObsRO measures
- Determine the appropriate age at which pediatric patients with PFIC and other cholestatic liver diseases can begin to self-report

Patient selection inclusion and exclusion criteria for interviews:

- Inclusion criteria
 - Diagnosis of PFIC, Alagille Syndrome, Biliary Atresia or Primary Sclerosing Cholangitis
 - Diagnosis of cholestatic pruritus
 - Patient or caregiver report of pruritus “Sometimes,” “Most of the time,” or “All of the time”
 - Age \leq 18 years
 - Resides in the United States, Canada, United Kingdom, or Australia
 - Any education level
- Exclusion criteria
 - Previous liver transplantation
 - Has a family member currently affiliated with the FDA or a government agency that approves medications, advertising agency, marketing research company, or pharmaceutical or biotechnology company

The frequency and average disturbance of each symptom reported from the patient/caregiver interviews are displayed below.

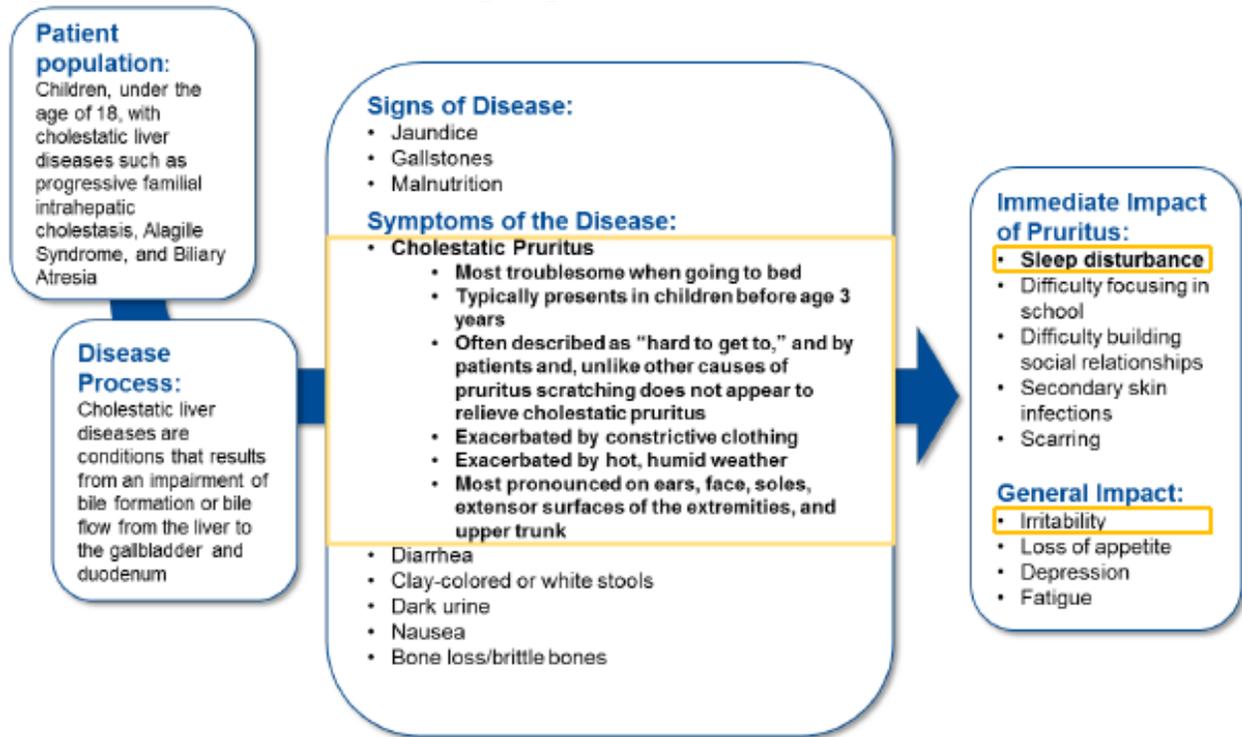
Figure 33. Sign and Symptom Frequency Versus Average Disturbance, Trial A4250-005



Source: Page 45/89 Figure 2 of COA Pruritus Evidence Dossier
 Based on sample of N=13 (disturbance ratings not obtained during two patient interviews).

The Applicant updated their conceptual model after the concept elicitation interview findings. The impact that pruritus had on sleep was also considered to be highly disruptive because it often interfered with both the sleep of the child and of the family. Subsequently, many caregivers reported that their children were highly irritable as a result of fatigue and constant pruritus. This irritability was therefore added to the conceptual model as a general impact.

Figure 34. Conceptual Model Following Stage I Interviews, Trial A4250-005



Source: Page 47/89 Figure 4 of COA Pruritus Evidence Dossier

A summary of the results is described below:

- The stage I concept elicitation interview results confirmed that pruritus is the most frequent symptom that is experienced by patients with PFIC and other cholestatic liver diseases. Itch was reported in 100% of patients.
- Itch was the only symptom to be reported by more than 50% of respondents and it received an average disturbance rating of 7.3. The other most frequently reported signs and symptoms were dark urine (38%), nausea (38%), clay colored stools (30%), and bone loss/brittle bones (30%).
- Itch most frequently occurred at night. Parents also reported that their children verbally tell them they are itchy or ask to be scratched and that they observe visible signs of scratching such as marks on the child's skin or blood on their bedsheets. Clinicians also agreed that itch fluctuations can be worse at nighttime.
- Stage I and II cognitive interviews resulted in retaining faces in the pictorial scale, and card sorting exercises were done by pediatric patients (>6 years of age) who were able to correctly sort the severity of the response options in order from "feeling ok" to "feeling the worst." The Applicant demonstrated that children 6 years and older may have the ability to reliably differentiate the scratching severity according to the pictorial scale. However, the administration of the PRO measure was decided to be administered in patients 8 years of age and older due to some of the 7- and 6-year-old patients having difficulty responding to the PRO measure. We agree with the PRO age of administration due to other pediatric data in drug development programs and recommendations from literature guidelines.
- We acknowledge and agree that itch is the most frequent and important symptom to assess for improvement in patients with cholestatic liver diseases and item 1 of the itch instruments (PRO and ObsRO) are reasonable tools to assess the severity of pruritus in these patients.

16.1.2. Study COAs Used as Reference Measures in Psychometric Analyses

16.1.2.1. Single-Item Global Measures

Symptom Severity

- Patient Global Impression of Symptoms (PGIS)
- Caregiver Global Impression of Symptoms (CaGIS)
- Clinician Global Impression of Symptoms (CGIS)

The symptom severity global measures are a single item instrument that assesses the overall symptom severity in the patient's itching or scratching at the time of assessment with reference to the recall period. Note that the recall period is "over the past week." There are five response options for the verbal rating scale (none, mild, moderate, severe, very severe). Screenshots of the global measures are available in clinical outcome assessment (COA) Appendix 6.

Symptom Change

- Patient Global Impression of Change (PGIC)
- Caregiver Global Impression of Change (CaGIC)
- Clinician Global Impression of Change (CGIC)

The symptom change global measures are a single item instrument that assesses the overall change in the patient’s itching or scratching at the time of assessment compared to since starting the study. There are seven response options for the verbal rating scale (very much better, much better, a little better, no change, a little worse, much worse, very much worse). Screenshots of the global measures are available in COA Appendix 6.

16.1.2.2. Pediatric Quality of Life Inventory (PedsQL)

The Pediatric Quality of Life Inventory (PedsQL) is a measure of the quality of life of an individual younger than 18 years of age (COA Appendix G, Appendix A, Section 2.4.5). The PedsQL has four content domains: physical functioning, emotional functioning, social functioning, and school functioning. There are different versions of the PedsQL depending on the age of the child. The PedsQL Family Impact Module is designed to measure the impact of pediatric chronic health conditions on parents and the family. The PedsQL Family Impact Module was developed as a parent-report instrument. For all versions, respondents were asked to rate each problem on a 5-point scale (0 = never a problem, 1 = almost never a problem, 2 = sometimes a problem, 3 = often a problem, 4 = almost always a problem), with the exception of the 23-item young child (ages 5 to 7 years) report, where an interviewer asks the child to rate each problem on a three-point scale (0 = not at all, 2 = sometimes, 4 = a lot).

[Table 128](#) describes the PedsQL versions broken down into different developmental age groups and descriptions of the relevant questionnaire.

Table 128. PedsQL Versions

Age Group	Questionnaire Description
Toddlers 2-4 years	21-item parent report questionnaire where respondents are asked to rate each problem on a 5-point scale
Children 5-7 years	23-item young child report where an interviewer asks the child to rate each problem on a 3-point scale; parent report version where parents are asked to rate each problem on a 5-point scale
Children 8-12 years	23-item child report that instructs the child to rate each problem on a 5-point scale; parent report version where parents are asked to rate each problem on a 5-point scale
Children 13-18 years	23-item teen report that instructs the teen to rate each problem on a 5-point scale; parent report version where parents are asked to rate each problem on a 5-point scale
Family Impact Module	36-item parent report that instructs a parent to rate on a 5-point scale how much of each problem within eight domains (physical functioning, emotional functioning, social functioning, cognitive functioning, communication, worry, daily activities, family relationships) they have had, as a result of their child’s health

Source: Pruritus Clinical Outcome Assessments Dossier Appendix G Table 1
Patient-reported outcome: PedsQL Generic Core Scales 4.0 (21 to 23 items depending on age of patient)
Observer-reported outcome: PedsQL Generic Core Scales 4.0 (21 to 23 items depending on age of patient)
Family functioning as measured by observer-reported outcome: PedsQL Family Module 2.0 (18 items)

“For self-report and parent-report separately, items are reverse-scored and linearly transformed to a 0–100 scale (0=100, 1=75, 2=50, 3=25, 4=0), so that higher scores indicate better health related quality of life (HRQOL).” (COA Appendix G, Appendix A, Section 2.4.5)

16.1.3. Patient-Reported Outcome and Observer-Reported Outcome Pruritus Scores

The computation of PRO and ObsRO pruritus scores pertinent to psychometric evaluation, construction of focal efficacy endpoints, and/or interpretation of efficacy results in Trial A4250-005 is defined in [Table 129](#). Computational algorithms described in [Table 129](#) are defined for each patient at each salient measurement occasion or assessment period. Missing data are not imputed in any of the computations defined in [Table 129](#). The PRO and ObsRO pruritus scores enumerated in [Table 129](#) are derived from select items included in the PRO and ObsRO morning (AM) and evening (PM) electronic diaries (see [Figure 40](#), [Figure 41](#), [Figure 42](#), [Figure 43](#), respectively) administered daily between Visit 1 (screening; Study Days -56 ±2 to -35 ±2) and Visit 9 (end-of-treatment [EOT]; Week 24; Study Days 168 ±3). According to Section 4.3 of the Pruritus COA Evidence Dossier, trial participants were provided with training materials to facilitate proper completion of the electronic diaries and: “Patients or caregivers could set alarms on the device, so that the eDiary would audibly signal the user to complete the morning and bedtime assessments at the prespecified times. Patients and caregivers could call a help desk if they experienced any problems with the electronic diaries (e.g., device crash, hardware problem).”

Table 129. Scores Computed From Applicant’s PRO and ObsRO Measures of Pruritus

Score	Derivation
Daytime Itching Score ¹	Scored response to PM PRO pruritus measure (i.e., item 1 / screen 2 in the PRO daily evening e-diary; see Figure 41): 0 = “No itching”; 1 = “A little itching”; 2 = “Medium itching”; 3 = “A lot of itching”; 4 = “The worst itching.” Note that a higher score indicates a higher symptom burden.
Daytime Scratching Score ¹	Scored response to PM ObsRO pruritus measure (i.e., item 4 / screen 5 in the ObsRO daily evening e-diary; see Figure 43): 0 = “No scratching”; 1 = “A little scratching”; 2 = “Medium scratching”; 3 = “A lot of scratching”; 4 = “Worst possible scratching.” Note that a higher score indicates a higher symptom burden.
Nighttime Itching Score ¹	Scored response to AM PRO pruritus measure (i.e., item 1 / screen 2 in the PRO daily morning e-diary; see Figure 40): 0 = “No itching”; 1 = “A little itching”; 2 = “Medium itching”; 3 = “A lot of itching”; 4 = “The worst itching.” Note that a higher score indicates higher symptom burden.
Nighttime Scratching Score ¹	Scored response to AM ObsRO pruritus measure (i.e., item 1 / screen 2 in the ObsRO daily morning e-diary; see Figure 42): 0 = “No scratching”; 1 = “A little scratching”; 2 = “Medium scratching”; 3 = “A lot of scratching”; 4 = “Worst possible scratching.” Note that a higher score indicates higher symptom burden.
Daily Itching Score ^{1,2}	The Daily Itching Score for an assessment day is computed as the average of the Daytime and Nighttime Itching Scores that day. The Daily Itching Score is considered to be missing if <u>both</u> the Daytime and Nighttime Itching Scores for that day are missing.
Daily Scratching Score ^{1,2}	The Daily Scratching Score for an assessment day is computed as the average of the Daytime and Nighttime Scratching Scores that day. The Daily Scratching Score is considered to be missing if <u>both</u> the Daytime and Nighttime Scratching Scores for that day are missing.
Worst Daily Itching Score ³	The Worst Daily Itching Score for an assessment day is computed as the maximum of the Daytime and Nighttime Itching Scores that day. The Worst Daily Itching Score is considered to be missing if <u>both</u> the Daytime and Nighttime Itching Scores for that day are missing.
Worst Daily Scratching Score ³	The Worst Daily Scratching Score for an assessment day is computed as the maximum of the Daytime and Nighttime Scratching Scores that day. The Worst Daily Scratching Score is considered to be missing if <u>both</u> the Daytime and Nighttime Scratching Scores for that day are missing.

Score	Derivation
Weekly Itching Score ^{1,2}	The Weekly Itching Score for an assessment week is computed as the average of the Daytime and Nighttime Itching Scores across the 7 days (14 assessments) comprising the week. The Weekly Itching Score is considered to be missing if ≥ 8 of the 14 assessments are missing. Note: <i>If only Daytime Itching Scores or only Nighttime Itching Scores are available for a given patient, then the construct being measured (the score interpretation) for that patient is narrowed to either “daytime itching severity” or “nighttime itching severity,” respectively.</i>
Weekly Scratching Score ^{1,2}	The Weekly Scratching Score for an assessment week is computed as the average of the Daytime and Nighttime Scratching Scores across the 7 days (14 assessments) comprising the week. The Weekly Scratching Score is considered to be missing if ≥ 8 of the 14 assessments are missing. Note: <i>If only Daytime Scratching Scores or only Nighttime Scratching Scores are available for a given patient, then the construct being measured (the score interpretation) for that patient is narrowed to either “daytime scratching severity” or “nighttime scratching severity,” respectively.</i>
Weekly Daytime Itching Score ¹	The Weekly Daytime Itching Score for an assessment week is computed as the average of the Daytime Itching Scores across the 7 days (7 assessments) comprising the week. The Weekly Daytime Itching Score is considered to be missing if ≥ 4 of the 7 assessments are missing.
Weekly Daytime Scratching Score ¹	The Weekly Daytime Scratching Score for an assessment week is computed as the average of the Daytime Scratching Scores across the 7 days (7 assessments) comprising the week. The Weekly Daytime Scratching Score is considered to be missing if ≥ 4 of the 7 assessments are missing.
Weekly Nighttime Itching Score ¹	The Weekly Nighttime Itching Score for an assessment week is computed as the average of the Nighttime Itching Scores across the 7 days (7 assessments) comprising the week. The Weekly Nighttime Itching Score is considered to be missing if ≥ 4 of the 7 assessments are missing.
Weekly Nighttime Scratching Score ¹	The Weekly Nighttime Scratching Score for an assessment week is computed as the average of the Nighttime Scratching Scores across the 7 days (7 assessments) comprising the week. The Weekly Nighttime Scratching Score is considered to be missing if ≥ 4 of the 7 assessments are missing.
Biweekly Itching Score ^{1,2}	For an assessment period of 2 consecutive weeks, the Biweekly Itching Score is computed as the average of the Daytime and Nighttime Itching Scores across the 14 days (28 assessments) comprising the 2-week period. The Biweekly Itching Score is considered to missing if ≥ 15 of the 28 assessments are missing. Note: <i>If only Daytime Itching Scores or only Nighttime Itching Scores are available for a given patient, then the construct being measured (the score interpretation) for that patient is narrowed to either “daytime itching severity” or “nighttime itching severity,” respectively.</i>
Biweekly Scratching Score ^{1,2}	For an assessment period of 2 consecutive weeks, the Biweekly Scratching Score is computed as the average of the Daytime and Nighttime Scratching Scores across the 14 days (28 assessments) comprising the 2-week period. The Biweekly Scratching Score is considered to missing if ≥ 15 of the 28 assessments are missing. Note: <i>If only Daytime Scratching Scores or only Nighttime Scratching Scores are available for a given patient, then the construct being measured (the score interpretation) for that patient is narrowed to either “daytime scratching severity” or “nighttime scratching severity,” respectively.</i>
Biweekly Daytime Itching Score ¹	For an assessment period of 2 consecutive weeks, the Biweekly Daytime Itching Score is computed as the average of the Daytime Itching Scores across the 14 days (14 assessments) comprising the 2-week period. The Biweekly Daytime Itching Score is considered to missing if ≥ 8 of the 14 assessments are missing.
Biweekly Daytime Scratching Score ¹	For an assessment period of 2 consecutive weeks, the Biweekly Daytime Scratching Score is computed as the average of the Daytime Scratching Scores across the 14 days (14 assessments) comprising the 2-week period. The Biweekly Daytime Scratching Score is considered to missing if ≥ 8 of the 14 assessments are missing.

Score	Derivation
Biweekly Nighttime Itching Score ¹	For an assessment period of 2 consecutive weeks, the Biweekly Nighttime Itching Score is computed as the average of the Nighttime Itching Scores across the 14 days (14 assessments) comprising the 2-week period. The Biweekly Nighttime Itching Score is considered to missing if ≥ 8 of the 14 assessments are missing.
Biweekly Nighttime Scratching Score ¹	For an assessment period of 2 consecutive weeks, the Biweekly Nighttime Scratching Score is computed as the average of the Nighttime Scratching Scores across the 14 days (14 assessments) comprising the 2-week period. The Biweekly Nighttime Scratching Score is considered to missing if ≥ 8 of the 14 assessments are missing.
Monthly Itching Score ^{1,2}	For an assessment period of 4 consecutive weeks, the Monthly Itching Score is computed as the average of the Daytime and Nighttime Itching Scores across the 28 days (56 assessments) comprising the 4-week assessment period. The Monthly Itching Score is considered to be missing if ≥ 29 of the 56 assessments are missing. Note: <i>If only Daytime Itching Scores or only Nighttime Itching Scores are available for a given patient, then the construct being measured (the score interpretation) for that patient is narrowed to either "daytime itching severity" or "nighttime itching severity," respectively.</i>
Monthly Scratching Score ^{1, 2}	For an assessment period of 4 consecutive weeks, the Monthly Scratching Score is computed as the average of the Daytime and Nighttime Scratching Scores across the 28 days (56 assessments) comprising the 4-week assessment period. The Monthly Scratching Score is considered to be missing if ≥ 29 of the 56 assessments are missing. Note: <i>If only Daytime Scratching Scores or only Nighttime Scratching Scores are available for a given patient, then the construct being measured (the score interpretation) for that patient is narrowed to either "daytime scratching severity" or "nighttime scratching severity," respectively. The Monthly Scratching Score is referred to as the Monthly Average of the Scratching Scores in all sections other than Section 16.1.</i>
Monthly Daytime Itching Score ¹	For an assessment period of 4 consecutive weeks, the Monthly Daytime Itching Score is computed as the average of the Daytime Itching Scores across the 28 days (28 assessments) comprising the 4-week period. The Monthly Daytime Itching Score is considered to be missing if ≥ 15 of the 28 assessments are missing.
Monthly Daytime Scratching Score ¹	For an assessment period of 4 consecutive weeks, the Monthly Daytime Scratching Score is computed as the average of the Daytime Scratching Scores across the 28 days (28 assessments) comprising the 4-week period. The Monthly Daytime Scratching Score is considered to be missing if ≥ 15 of the 28 assessments are missing. Note: <i>The Monthly Daytime Scratching Score is referred to as the Monthly Average of the Daytime Scratching Scores in all sections other than Section 16.1.</i>
Monthly Nighttime Itching Score ¹	For an assessment period of 4 consecutive weeks, the Monthly Nighttime Itching Score is computed as the average of the Nighttime Itching Scores across the 28 days (28 assessments) comprising the 4-week period. The Monthly Nighttime Itching Score is considered to be missing if ≥ 15 of the 28 assessments are missing.
Monthly Nighttime Scratching Score ¹	For an assessment period of 4 consecutive weeks, the Monthly Nighttime Scratching Score is computed as the average of the Nighttime Scratching Scores across the 28 days (28 assessments) comprising the 4-week period. The Monthly Nighttime Scratching Score is considered to be missing if ≥ 15 of the 28 assessments are missing. Note: <i>The Monthly Nighttime Scratching Score is referred to as the Monthly Average of the Nighttime Scratching Scores in all sections other than Section 16.1.</i>
Monthly Average of the Worst Daily Scratching Scores ³	For an assessment period of 4 consecutive weeks, the Monthly Average Worst Scratching Score is computed as the average of the Worst Daily Scratching Scores across the 28 days comprising the 4-week assessment period. The Monthly Average of the Worst Daily Scratching Scores is considered to be missing if ≥ 15 of the 28 Worst Daily Scratching Scores are missing.

Score	Derivation
Worst Weekly Itching Score ³	<p>For an assessment period of 4 consecutive weeks, the Worst Weekly Itching Score is computed as follows.</p> <p>Step 1: For each pertinent assessment week, compute the Weekly Average Worst Itching Score as the average of the Worst Daily Itching Scores across the 7 days comprising the week. The Weekly Average Worst Itching Score is considered to be missing if \geq four Worst Daily Itching Scores are missing.</p> <p>Step 2: For each pertinent assessment month (28-day period), compute the Worst Weekly Itching Score as the maximum Weekly Average Worst Daily Itching Score within the 4-week interval (month). The Worst Weekly Itching Score for a given 28-day period is considered to be missing if all four Weekly Average Worst Itching Scores are missing.</p>
Worst Weekly Scratching Score ³	<p>For an assessment period of 4 consecutive weeks, the Worst Weekly Scratching Score is computed as follows.</p> <p>Step 1: For each pertinent assessment week, compute the Weekly Average Worst Scratching Score as the average of the Worst Daily Scratching Scores across the 7 days comprising the week. The Weekly Average Worst Scratching Score is considered to be missing if \geq four Worst Daily Scratching Scores are missing.</p> <p>Step 2: For each pertinent assessment month (28-day period), compute the Worst Weekly Scratching Score as the maximum Weekly Average Worst Scratching Score within the 4-week interval (month). The Worst Weekly Scratching Score for a given 28-day period is considered to be missing if all four Weekly Average Worst Scratching Scores are missing.</p>

Source: PFSS Reviewer's table

Abbreviations: ObsRO, observer-reported outcome; PRO, patient-reported outcome

¹ Source: COA Appendix G \ Appendix A \ Section 5.1 \ Table 3.

² Source: Pruritus COA Evidence Dossier \ Table 7.

³ Source: Review team

16.1.4. Comparability of Daytime and Nighttime Scratching Scores and Stability of Scratching Scores Over Time

The comparability of Daytime and Nighttime Scratching Scores within a given Study Day and the stability of scratching scores over time were evaluated to inform, respectively, selection of (1) an appropriate daily summary of scratching severity and (2) an appropriate aggregate summary score (including an appropriate time interval over which to aggregate Daily Scratching Scores) to both (a) construct and interpret efficacy endpoints and (b) evaluate score-dependent measurement properties (e.g., construct validity, test-retest reliability).

16.1.4.1. Methods

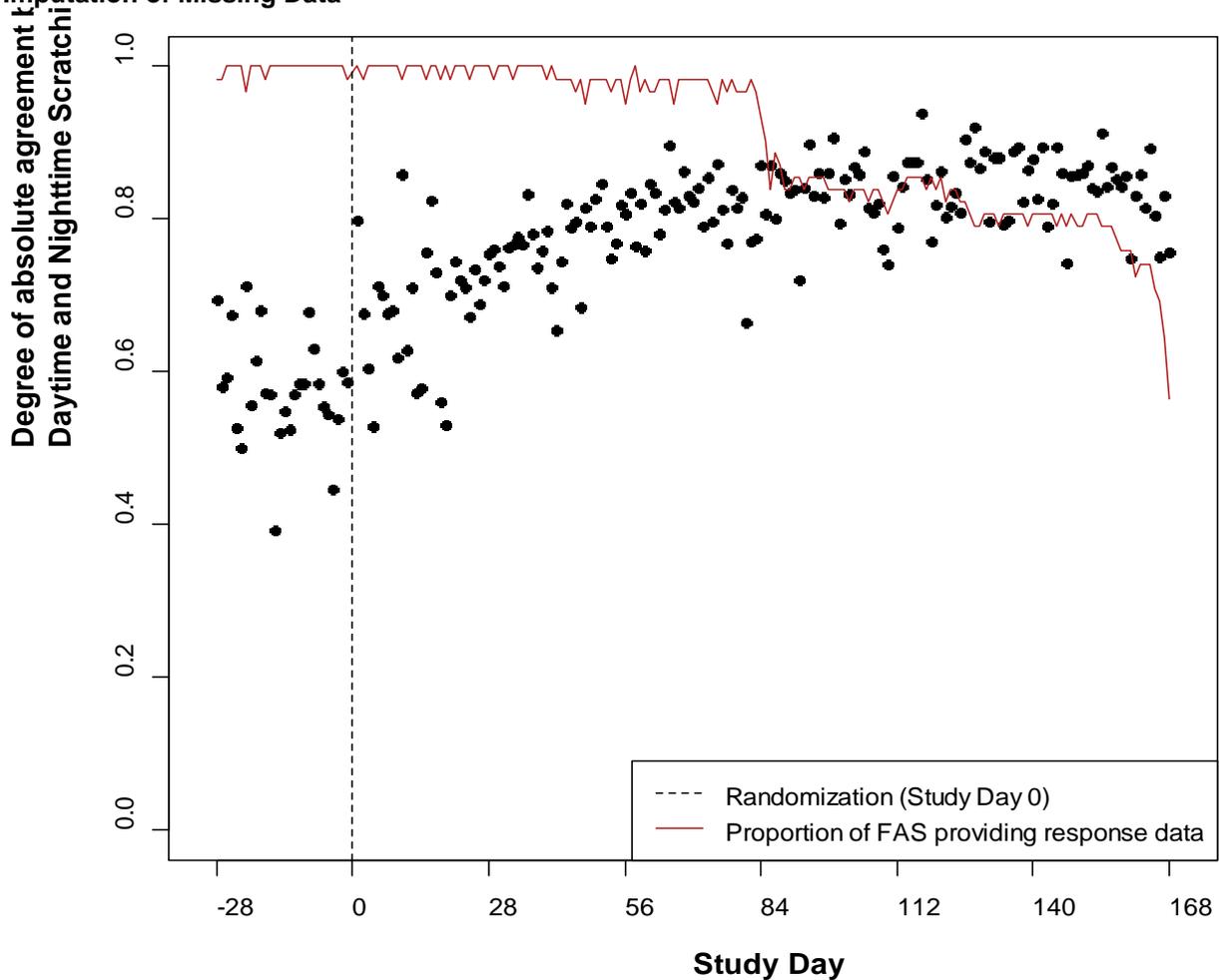
Post hoc analyses conducted by the Agency are listed below.

- A scatterplot of the intraclass correlation between Daytime and Nighttime Scratching Scores on a given Study Day between Days -28 and 168, inclusive (in other words, between 4 weeks prerandomization and Week 24—the end of blinded treatment [EOT]), was visually examined. For each Study Day, the ICC(1) based on absolute agreement between Daytime and Nighttime Scratching Scores that day was computed per (McGraw and Wong 1996) based on a mixed model with no interaction term with scratching score as the response variable, patients (individuals) included as a random effect, and item (i.e., the AM and PM ObsRO measures of pruritus) included as a fixed effect.
- Second, a series of Generalizability Studies (G-studies) were conducted to quantify the exchangeability of Daytime and Nighttime Scratching Scores within a given day and the stability of Daytime and Nighttime Scratching Scores across the 1-, 2-, 3-, and 4-week periods immediately prior to randomization (Study Day 0). Specifically:
 - The four separate G-studies conducted to evaluate the stability of Daytime Scratching Scores over the 1-, 2-, 3-, and 4-week periods immediately prior to randomization each utilized a two-way mixed effects model with interaction, with Daytime Scratching Score as the response variable, patients included as a random effect, and measurement occasion (study day) included as a fixed effect.
 - The four separate G-studies conducted to evaluate the stability of Nighttime Scratching Scores over the 1-, 2-, 3-, and 4-week periods immediately prior to randomization each utilized a two-way mixed effects model with interaction, with Nighttime Scratching Score as the response variable, patients included as a random effect, and measurement occasion (study day) included as a fixed effect.
 - The four separate G-studies conducted to evaluate the exchangeability of Daytime and Nighttime Scratching Scores within a given day over the 1-, 2-, 3-, and 4-week periods immediately prior to randomization each utilized a three-way mixed effects model with all possible interactions, with scratching score as the response variable, item (i.e., the AM and PM ObsRO measures of pruritus) included as a fixed effect, measurement occasion (study day) included as a fixed effect, and patients included as a random effect.

16.1.4.2. Results

[Figure 35](#) shows the degree of absolute agreement between patients' Daytime and Nighttime Scratching Scores within a given study day between Days -28 and 168, inclusive. Each black dot represents the degree of absolute agreement (quantified as ICC(1), computed per (McGraw and Wong 1996) between Daytime and Nighttime Scratching Scores on the indicated study day among patients in the FAS of Trial A4250-005 providing responses that day to both the AM and PM ObsRO measures of pruritus.

Figure 35. Degree of Absolute Agreement Between Daytime and Nighttime Scratching Scores Per Day for Study Days [-28, 168], Trial A4250-005 FAS (N=62), With Study Arms Pooled and No Imputation of Missing Data



Source: PFSS Reviewer's figure
Abbreviations: FAS, full analysis set

Visual inspection of [Figure 35](#) suggests that during the 4 weeks immediately prior to randomization (i.e., Study Days [-28, -1]) there is:

- Only moderate agreement between Daytime and Nighttime Scratching Scores on a given study day; and
- A high level of day-to-day variability in the degree of absolute agreement between Daytime and Nighttime Scratching Scores.

Collectively, these results suggest:

- Daytime and nighttime scratching severity notably differ during the 4 weeks immediately prior to randomization; and
- The degree to which daytime and nighttime scratching severity differ on a given day varies notably over time.

These findings are further supported by the results of the G-studies conducted to quantify (a) the exchangeability of Daytime and Nighttime Scratching Scores within a given day and (b) the

stability of Daytime and Nighttime Scratching Scores over various lengths of time during Study Days [-28, -1]. Specifically:

- The high percentage of Daytime and Nighttime Scratching Score variance attributable to the interaction between patients and measurement occasions noted in the univariate G-studies (see the last column in [Table 130](#) and [Table 131](#), respectively) suggests notable day-to-day variation in Daytime and Nighttime Scratching Scores, respectively.
- The notable level of score variance attributable to items (i.e., AM and PM ObsRO measures of pruritus) and the interaction between patients and items noted in the multivariate G-study (rows 2 and 4 of [Table 132](#)) suggests patients' Daytime and Nighttime Scratching Scores notably differ on a given study day.
- The high level of error variance attributable to the interaction between patients, items (i.e., AM and PM ObsRO measures of pruritus), and measurement occasion (study day) noted in the multivariate G-study (row 7 of [Table 132](#)) suggests notable variability over time in the degree of alignment between patients' Daytime and Nighttime Scratching Scores.

Table 130. Percentage of Daytime Scratching Score Variance Attributable to Patients and Facets of the Measurement Procedure, During Study Days [-28, -1], Trial A4250-005 FAS (N=62)

Visit(s)	Study Days	Source of Variation		
		Patient	Occasion (Study Day)	Patient x Occasion
Screening + Baseline	[-28, -1]	52.20	1.60	46.20
	[-21, -1]	54.20	0.90	44.90
Baseline	[-14, -1]	59.60	0.00	40.40
	[-7, -1]	59.20	0.00	40.80

Source: PFSS Reviewer's table
Abbreviations: FAS, full analysis set

Table 131. Percentage of Nighttime Scratching Score Variance Attributable to Patients and Facets of the Measurement Procedure in Trial A4250-005 FAS (N=62) During Study Days [-28, -1]

Visit(s)	Study Days	Source of Variation		
		Patient	Occasion (Study Day)	Patient x Occasion
Screening + Baseline	[-28, -1]	46.20	0.80	53.00
	[-21, -1]	48.80	0.50	50.70
Baseline	[-14, -1]	50.30	0.20	49.40
	[-7, -1]	53.50	0.50	46.00

Source: PFSS Reviewer's table
Abbreviations: FAS, full analysis set

Table 132. Percentage of Score Variance Attributable to Patients and Facets of the Measurement Procedure, During Study Days [-28, -1], Trial A4250-005 FAS (N=62)

Source of Variation	Study Days	Study Days	Study Days	Study Days
	[-28, -1]	[-21, -1]	[-14, -1]	[-7, -1]
Patient	41.00	43.50	46.90	48.50
Item (AM and PM ObsRO pruritus measures)	2.60	2.30	1.90	1.90
Occasion (study day)	1.30	0.80	0.10	0.30
Patient x Item	7.20	7.00	7.40	7.10
Patient x Occasion	15.00	11.80	9.10	6.60
Item x Occasion	0.00	0.00	0.00	0.00
Error (Patient x Item x Occasion)	32.90	34.50	34.60	35.60

Source: PFSS Reviewer's table
Abbreviations: FAS, full analysis set; ObsRO, observer-reported outcome
The last row in the table above represents error variance arising from the interaction between patient, item (AM and PM ObsRO measures of scratching), and measurement occasion (study day).

16.1.4.3. Conclusions

Collectively, the results of analyses conducted to evaluate the comparability of Daytime and Nighttime Scratching Scores within a given study day and the stability of scratching scores over time imply:

- It may be most informative to look at a patient's worst scratching severity within a day;
- An appropriate time interval over which to aggregate Daily Scratching Scores would be at least a week; and
- It may be challenging to evaluate the test-retest reliability of scratching scores due to the lack of a stable period prerandomization (see Section [16.1.6.2](#) for additional discussion on test-retest reliability).

16.1.5. Comparability of Patient and Caregiver Pruritus Assessments

Due to the young age of patients enrolled in Trial A4250-005, pruritus severity was assessed through caregiver report for all patients. Pruritus severity was additionally assessed through self-report for those patients aged 8 years and older (see Section [6.2.1](#)). ObsRO pruritus scores were used to construct the Applicant's prespecified primary efficacy endpoint and other focal efficacy endpoints considered by the Agency in its evaluation of treatment benefit (see Section [6.2.1.1](#)). However, given the first time use of an ObsRO to support evaluation of a drug product intended to treat a pediatric cholestatic liver disease (e.g., PFIC) and a patient's itching severity is known only to the patient himself/herself, it was important to understand the relationship (i.e., comparability) between itching severity as reported by patients directly and scratching severity as observed by the caregiver.

16.1.5.1. Methods

The Agency conducted post hoc descriptive analyses to evaluate the relationship between patient and caregiver assessments of pruritus severity among those patients (n=9) in FAS of Trial A4250-005 with both PRO and ObsRO score data during the 4 weeks immediately prior to randomization (i.e., during Study Days [-28, -1]). The Agency evaluated the relationship between (a) patient and caregiver pruritus assessments on a given study day, and (b) the Worst Weekly Itching Score and Worst Weekly Scratching Score (both defined in [Table 129](#)) as specified below.

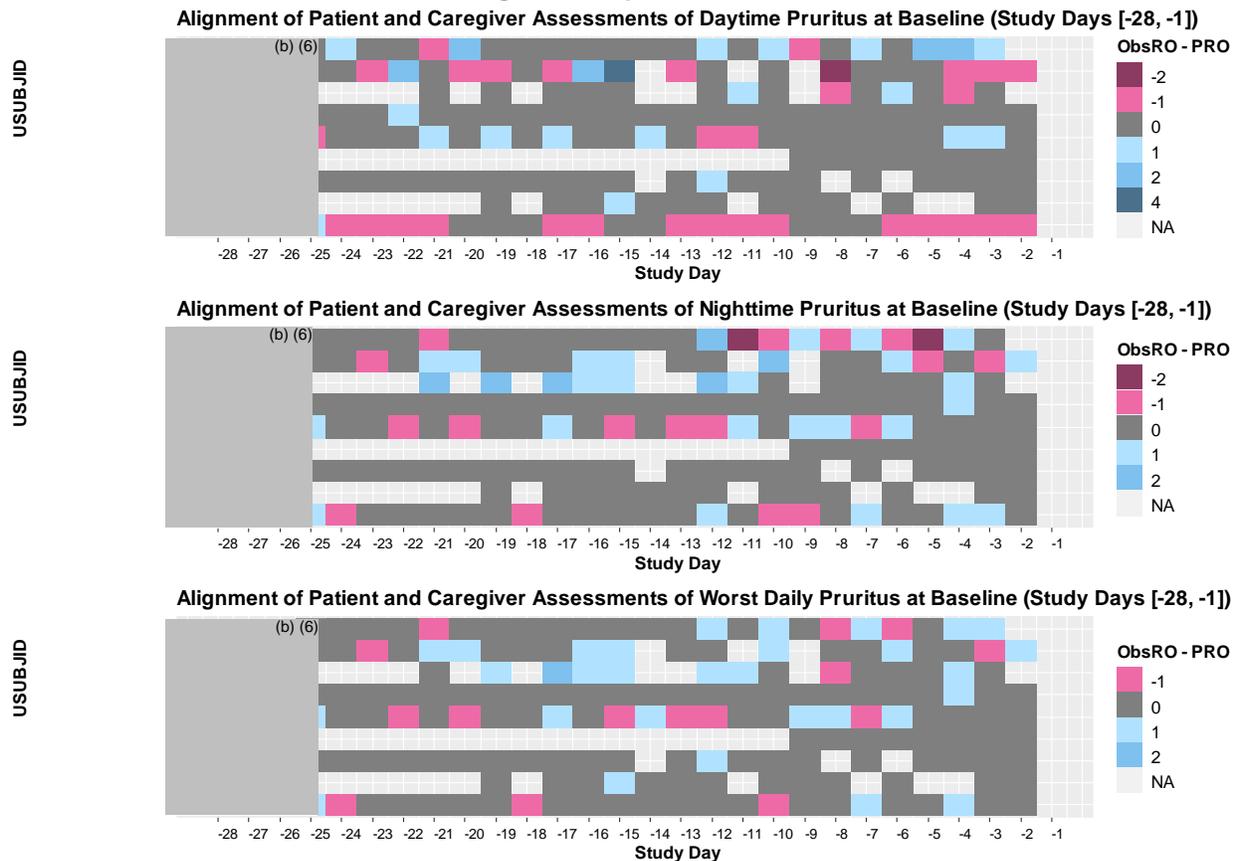
- The relationship between patients' Worst Weekly Itching Scores and Worst Weekly Scratching Scores computed over Study Days [-28, -1] were graphically evaluated via scatterplot.
- Heatmaps were used to graphically evaluate the relationship between (a) Daytime Itching and Daytime Scratching Scores, (b) Nighttime Itching and Nighttime Scratching Scores, and (c) Worst Daily Itching and Worst Daily Scratching Scores on a given day during Study Days [-28, -1].

All pruritus scores used in this analysis are defined in [Table 129](#). Worst Daily Itching Scores and Worst Daily Scratching Scores were analyzed instead of the Daily Itching Scores and Daily Scratching Scores proposed by the Applicant for reasons discussed in Section [16.1.4](#).

16.1.5.2. Results

Discussion of the scatterplot results are presented in Section [6.2.1.4](#). The heatmaps in [Figure 36](#), below, indicate general alignment between patient and caregiver assessments of daytime, nighttime, and worst daily pruritus severity (where agreement between itching and scratching scores is denoted by the dark gray cells in [Figure 36](#)), with particularly strong alignment noted for the worst daily pruritus scores upon which the worst weekly pruritus scores are based (see the bottom panel in [Figure 36](#)). Moreover, of those itching and scratching scores that differed, the majority differed by a single response category (the light pink and light blue cells in [Figure 36](#)).

Figure 36. Heatmap Showing Difference Between Patient and Caregiver Assessments of Daytime, Nighttime, and Worst Daily Pruritus at Baseline (Study Days [-28, -1]) for Nine Patients for Whom Scores From Both the Patient and Caregiver Perspectives Were Available, Trial A4250-005 FAS



Source: PFSS Reviewer's figure
 Abbreviations: FAS, full analysis set; ObsRO, observer-reported outcome; PRO, patient-reported outcome
 No difference between itching and scratching scores is denoted in dark gray. Missing data were not imputed in the computation of itching and scratching scores.

16.1.5.3. Conclusions

Although some uncertainty surrounds the findings reported in Section [16.1.5](#) due to the very small number of patients ($n = 9$) with both PRO and ObsRO score data available during Study Days [-28, -1], these results suggest that caregiver report of scratching severity may align with patient self-report of itching severity. As such, these results support the use of ObsRO scratching scores to evaluate treatment effects in Trial A4250-005.

16.1.6. Evaluation of Quantitative Validity and Reliability Evidence

Quantitative validity and reliability evidence submitted by the Applicant and/or generated by the Agency is summarized in [Table 133](#).

Table 133. Quantitative Validity and Reliability Evidence Submitted by Applicant and/or Generated by the Agency Using Data Collected From the FAS in Trial A4250-005 (N=62) With Patients Pooled Across Study Arms and No Imputation of Missing Data

Quantitative Evidence Type Pruritus Score(s) ¹	Analysis	Pertinent Section	Analysis Conducted by	Replicated by PFSS Reviewer?
Concurrent convergent validity (construct validity)				
Biweekly Daytime Itching Scores	Spearman correlations and corresponding scatterplots	16.1.6.1	Applicant	Yes ²
Biweekly Nighttime Itching Scores				
Biweekly Itching Scores				
Biweekly Daytime Scratching Scores				
Biweekly Nighttime Scratching Scores				
Biweekly Scratching Scores				
Test-retest reliability				
Weekly Daytime Itching Scores	ICC(2,1) based on two-way mixed effects models (McGraw and Wong 1996)	16.1.6.2	Applicant	No
Weekly Nighttime Itching Scores				
Weekly Itching Scores				
Weekly Daytime Scratching Scores				
Weekly Nighttime Scratching Scores				
Weekly Scratching Scores				
Biweekly Daytime Itching Scores				
Biweekly Nighttime Itching Scores				
Biweekly Itching Scores				
Biweekly Daytime Scratching Scores				
Biweekly Nighttime Scratching Scores				
Biweekly Scratching Scores				
Monthly Daytime Itching Scores				
Monthly Nighttime Itching Scores				
Monthly Itching Scores				
Monthly Daytime Scratching Scores				
Monthly Nighttime Scratching Scores				
Monthly Scratching Scores				

Source: PFSS Reviewer's table

Abbreviations: FAS, full analysis set; ICC, intraclass correlation coefficient

All pruritus scores are defined in [Table 129](#).

¹ See [Table 129](#)

² Review team replicated the Applicant's results but disagreed with the Applicant's selection of which patients to include in the analysis. Refer to Section [16.1.6.1](#) for discussion

16.1.6.1. Quantitative Validity Evidence

The construct validity of a COA refers to the degree to which the instrument measures the intended construct (e.g., disease severity; or a specific clinical sign, symptom, function, or impact)—in this case, pruritus severity. In a clinical trial, sufficient construct validity evidence—in conjunction with sufficient reliability evidence—is needed to interpret COA scores and efficacy results based on COA scores (i.e., to help establish evidence and confidence that observed differences between study arms reflect a true treatment benefit).

16.1.6.1.1. Methods

The Applicant submitted quantitative (construct) validity evidence from the FAS of Trial A4250-005 (N=62) to support evaluation of the validity of both PRO and ObsRO pruritus scores. However, the review team's evaluation of quantitative validity evidence focused exclusively on scores generated by the AM and PM ObsRO pruritus measures (provided in [Figure 42](#) and [Figure 43](#), respectively) as only ObsRO pruritus scores are used to construct efficacy endpoints considered by the Agency in its evaluation of treatment effects (see Section [6.2.1.1](#)). The Applicant evaluated the concurrent convergent validity of Biweekly Daytime Scratching Scores, Biweekly Nighttime Scratching Scores, and Biweekly Scratching Scores computed per [Table 129](#) over Study Days [-14, -1]. Missing data were not imputed in any of the Applicant's quantitative validity analyses.

The Applicant evaluated the concurrent convergent validity of Biweekly Daytime Scratching Scores, Biweekly Nighttime Scratching Scores, and Biweekly Scratching Scores by comparing Spearman rank correlations between the pairs of scores enumerated in [Table 134](#) with prespecified thresholds. Scatterplots showing the graphical relationship between (a) baseline CaGIS scores and (b) baseline Biweekly Daytime Scratching Scores, Biweekly Nighttime Scratching Scores, and Biweekly Scratching Scores, respectively, were additionally provided by the Applicant (COA Evidence Dossier Appendix G, Figure 11).

Although there is currently no consensus as to what minimum magnitude of a correlation provides evidence of convergent validity, it should be noted that the thresholds prespecified by the Applicant (see [Table 134](#)) are lower than what is currently recommended by the Agency for use in establishing evidence of convergent validity. The Agency's current position is that a correlation coefficient with an absolute value < 0.5 indicates a weak relationship, $[0.5, 0.7)$ indicates a moderate relationship, $[0.7, 0.9)$ indicates a strong relationship, and ≥ 0.9 indicates a very strong relationship (Hinkle et al. 2003). Our rationale is based on the coefficient of determination, for which a correlation coefficient of 0.3 corresponds to 9% of variance explained, while correlation coefficients of 0.7 and 0.9 correspond to 49% and 81% of variance explained, respectively. With that said, the thresholds prespecified by the Applicant (see [Table 134](#)) are not unreasonable given the differences between the biweekly ObsRO pruritus scores and the reference measures in terms of recall period and construct(s) being measured.

Table 134. Scores Compared in Quantitative Evaluation of ObsRO Pruritus Score Validity

ObsRO Pruritus Score	Reference Measure Score	Prespecified Threshold¹
Biweekly Daytime Scratching Score	Biweekly Daytime Itching Score	≥0.3
	CaGIS score	≥0.3
	PedsQL v4.0 total and functional domain scores	(-0.5, -0.1]
Biweekly Nighttime Scratching Score	Biweekly Nighttime Itching Score	≥0.3
	CaGIS Score	≥0.3
	PedsQL v4.0 total and functional domain scores	(-0.5, -0.1]
Biweekly Scratching Score	Biweekly Itching Score	≥0.3
	CaGIS score	≥0.3
	PedsQL v4.0 total and functional domain scores	(-0.5, -0.1]

Source: PFSS Reviewer's table

Abbreviations: CaGIS, Caregiver Global Impression of Symptoms; ObsRO, observer-reported outcome; PedsQL, Pediatric Quality of Life Inventory

CaGIS scores used in the evaluation of convergent validity were collected at Visit 3 (Study Day -2 or -1, depending on the patient).

¹ In the assessment of convergent validity, the Applicant defined a small correlation as having magnitude [0.1, 0.3), a moderate correlation as having an absolute value of [0.3, 0.5), and a strong correlation as having an absolute value ≥0.5 (COA Appendix G [Psychometric Analysis Report], Section 7.5.1, page 33/163).

16.1.6.1.2. Results

The Applicant and review team's results are collectively presented in [Table 135](#). The review team was unable to exactly replicate the Applicant's results due to differences in which patients were included in the analyses. Specifically, the Applicant included baseline measurements taken after randomization (Study Day 0) and initiation of study therapy while the review team excluded baseline measurements taken after randomization. With that said, the Applicant and review team's results otherwise generally aligned. The Applicant also provided scatterplots of scratching scores versus CaGIS baseline scores (COA Evidence Dossier Appendix G \ Appendix B \ Figures 3.3.1, 3.3.2, 3.3.4).

The pattern of correlations noted in [Table 135](#) is as expected given the differences between measures in target construct, recall period, response scale(s), and frequency of data collection. This alignment between empirical results and theory constitutes supportive construct validity evidence for the biweekly scratching scores despite the lack of a criterion measure of pruritus. High correlations are noted between biweekly itching and scratching scores. Moderate positive monotonic relationships are noted between all three biweekly scratching scores and baseline CaGIS scores, which is to be expected given these are measures of similar constructs as reported by caregivers (same reporter) but utilize different recall periods and contributing data are collected at different frequencies. Small negative correlations are noted between PedsQL scores and biweekly scratching scores, which is to be expected given differences in recall period, reverse score interpretation, and all the many factors contributing to a patient's level of physical, emotional, social, cognitive, and school functioning that are not proximal to pruritus or the underlying disease PFIC.

Table 135. Scores Compared in Quantitative Evaluation of Observer-Reported Outcome Pruritus Score Validity

ObsRO Pruritus Score	Reference Measure Score	Prespecified Threshold for Supportive Evidence of Construct Validity ⁴	Applicant's Results		Review Team's Results		
			Spearman Correlation	N	Spearman Correlation	N	
Biweekly Daytime Scratching Score	Biweekly Daytime Itching Score	≥0.3	NR	NR	0.72	9	
	CaGIS Score	≥0.3	0.498 ¹	58 ^a	0.47	55	
	PedsQL v4.0 parent-report total score	(-0.5, -0.1]	-0.134 ²	38 ²	-0.07	37	
	PedsQL v4.0 parent-report physical functioning score		-0.168 ²	38 ²	-0.10	37	
	PedsQL v4.0 parent-report emotional functioning score		-0.084 ²	38 ²	-0.12	37	
	PedsQL v4.0 parent-report social functioning score		-0.013 ²	38 ²	-0.04	37	
	PedsQL v4.0 parent-report school functioning score		-0.199 ²	25 ²	-0.11	24	
	PedsQL v4.0 family impact module total score		-0.323 ³	58 ³	-0.32	55	
	PedsQL v4.0 family impact module physical functioning score		-0.128 ³	58 ³	-0.09	55	
	PedsQL v4.0 family impact module emotional functioning score		-0.378 ³	58 ³	-0.40	55	
	PedsQL v4.0 family impact module social functioning score		-0.143 ³	58 ³	-0.15	55	
	PedsQL v4.0 family impact module cognitive functioning score		-0.286 ³	58 ³	-0.30	55	
	Biweekly Nighttime Scratching Score	Biweekly Nighttime Itching Score	≥0.3	NR	NR	0.93	9
		CaGIS Score	≥0.3	0.595 ^a	58 ^a	0.57	55
PedsQL v4.0 parent-report total score		(-0.5, -0.1]	-0.300 ²	38 ²	-0.22	37	
PedsQL v4.0 parent-report physical functioning score			-0.247 ²	38 ²	-0.17	37	
PedsQL v4.0 parent-report emotional functioning score			-0.298 ²	38 ²	-0.32	37	
PedsQL v4.0 parent-report social functioning score			-0.123 ²	38 ²	-0.08	37	
PedsQL v4.0 parent-report school functioning score			-0.404 ²	25 ²	-0.34	24	
PedsQL v4.0 family impact module total score			-0.361 ³	58 ³	-0.31	55	
PedsQL v4.0 family impact module physical functioning score			-0.214 ³	58 ³	-0.17	55	
PedsQL v4.0 family impact module emotional functioning score			-0.473 ³	58 ³	-0.46	55	
PedsQL v4.0 family impact module social functioning score			-0.237 ³	58 ³	-0.21	55	
PedsQL v4.0 family impact module cognitive functioning score			-0.220 ³	58 ³	-0.21	55	

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ObsRO Pruritus Score	Reference Measure Score	Prespecified Threshold for Supportive Evidence of Construct Validity ⁴	Applicant's Results		Review Team's Results	
			Spearman Correlation	N	Spearman Correlation	N
	Biweekly Itching Score	≥0.3	NR	NR	0.95	9
	CaGIS Score	≥0.3	0.548 ¹	58 ¹	0.50	55
Biweekly Scratching Score	PedsQL v4.0 parent-report total score	(-0.5, -0.1]	-0.223 ²	38 ²	-0.14	37
	PedsQL v4.0 parent-report physical functioning score		-0.223 ²	38 ²	-0.15	37
	PedsQL v4.0 parent-report emotional functioning score		-0.158 ²	38 ²	-0.18	37
	PedsQL v4.0 parent-report social functioning score		-0.078 ²	38 ²	-0.07	37
	PedsQL v4.0 parent-report school functioning score		-0.236 ²	25 ²	-0.13	24
	PedsQL v4.0 family impact module total score		-0.350 ³	58 ³	-0.32	55
	PedsQL v4.0 family impact module physical functioning score		-0.174 ³	58 ³	-0.12	55
	PedsQL v4.0 family impact module emotional functioning score		-0.450 ³	58 ³	-0.45	55
	PedsQL v4.0 family impact module social functioning score		-0.195 ³	58 ³	-0.19	55
	PedsQL v4.0 family impact module cognitive functioning score		-0.244 ³	58 ³	-0.25	55

Source: PFSS Reviewer's table

Abbreviations: CaGIS, Caregiver Global Impression of Symptoms; NR, not reported; ObsRO, observer-reported outcome; PedsQL, Pediatric Quality of Life Inventory

CaGIS scores used in the evaluation of convergent validity were collected at Visit 3 (Study Day -2 or -1, depending on the patient).

¹ Source: COA Evidence Dossier Appendix G (Psychometric Analysis Report) \ Appendix B \ Table 10.1.

² Source: COA Evidence Dossier Appendix G (Psychometric Analysis Report) \ Appendix B \ Table 10.2.2.

³ Source: COA Evidence Dossier Appendix G (Psychometric Analysis Report) \ Appendix B \ Table 10.2.3.

⁴ In the assessment of convergent validity, the Applicant defined a small correlation as having magnitude [0.1, 0.3), a moderate correlation as having an absolute value of [0.3, 0.5), and a strong correlation as having an absolute value ≥0.5 (COA Appendix G [Psychometric Analysis Report], Section 7.5.1, page 33/163).

16.1.6.1.3. Limitations

Limitations of the Applicant's quantitative validity analyses are enumerated below.

- Neither the prespecified primary efficacy endpoint nor additional endpoints considered by the Agency (see Section [6.2.1](#)) are based on Biweekly Daytime Scratching Scores, Biweekly Nighttime Scratching Scores, or Biweekly Scratching Scores.
- Misalignment between (a) the 2-week assessment period by Biweekly Scratching Scores, Biweekly Daytime Scratching Scores, and Biweekly Nighttime Scratching Scores, respectively, and (b) the 1-week recall period of the CaGIS
- Convergent validity:
 - Correlations between patient-reported outcome (PRO) and observer-reported outcome (ObsRO) pruritus scores were not reported by the Applicant despite prespecified plans.
 - Inclusion of baseline measurements taken after randomization (Study Day 0) and initiation of study therapy

16.1.6.2. Test-Retest Reliability

The Applicant conducted two analyses to evaluate the test-retest reliability of PRO and ObsRO pruritus scores.

- First, the degree of absolute agreement between daytime, nighttime, and daily PRO and ObsRO pruritus scores, respectively, averaged over (a) Study Days [-14, -8] and (b) Study Days [-7, -1] was evaluated using ICC(2,1) generated by fitting a two-way mixed-effects model to the salient weekly average score, with patients included as a random effect and measurement occasions included as a fixed effect (COA Evidence Dossier Appendix G, Section 7.4.2). All patients in the FAS were assumed to be stable with respect to pruritus during Study Days [-14, -1].
- Second, the degree of absolute agreement between daytime, nighttime, and daily PRO and ObsRO pruritus scores, respectively, averaged over (a) Study Days [-14, -1] and (b) Study Days [1, 28] was evaluated in "stable" patients using ICC(2,1) generated by fitting a two-way mixed-effects model to the salient weekly average score, with patients included as a random effect and measurement occasions included as a fixed effect (COA Evidence Dossier Appendix G, Section 7.4.2). The Applicant conducted separate analyses under each of the following definitions of "stable" patients:
 - Patients in the FAS who experienced "no change" according to the PGIC and/or CaGIC at Week 4 (Visit 4).
 - Patients in the FAS who experienced no change in PGIS and/or CaGIS score between baseline (Visit 3) and Week 4 (Visit 4).

The first approach is problematic due to the lack of a mechanism for identifying stable patients during Study Days [-14, -1] as (a) the PGIS, CaGIS, and CGIS were not administered any earlier than 1 to 2 days immediately preceding randomization (i.e., Study Day -2 or -1, depending on the patient) and (b) the PGIC, CaGIC, and CGIC were not administered until Visit 4 (Week 4; Study Days 28 ±3). Results from the second approach are inadmissible due to the comparison of scores

aggregated over different time intervals (i.e., 2 weeks versus 4 weeks). The severity of these limitations to the Applicant's analytic approach preclude discussion of the Applicant's results and replication by the Agency.

16.1.6.2.1. Conclusions

The test-retest reliability of PRO and ObsRO pruritus scores is challenging to evaluate (a) at the daily and weekly levels due to the lack of a stable period prerandomization (see Section [16.1.4](#)) and (b) at the monthly level due to the lack of sufficient data. The evaluation of test-retest reliability of PRO pruritus scores is additionally impeded by sample size limitations (n=9). Of particular relevance to the focal efficacy endpoints:

- The test-retest reliability of Daytime, Nighttime, and Worst Daily Scratching Scores is challenging to evaluate due to the lack of a period of stable scratching severity prerandomization (see Section [16.1.4](#)); and
- The test-retest reliability of Worst Weekly Scratching Scores is challenging to evaluate due to both (a) limited availability of Daytime and Nighttime Scratching Score data prior to Study Day -28 and (b) the lack of a mechanism for identifying stable patients during Study Days [-56, -1] as (a) the PGIS, CaGIS, and CGIS were not administered any earlier than 1 to 2 days immediately preceding randomization (i.e., Study Day -2 or -1, depending on the patient) and (b) the PGIC, CaGIC, and CGIC were not administered until Visit 4 (Week 4; Study Days 28 ±3).

Due to the insurmountable challenges in evaluating the test-retest reliability of PRO and ObsRO pruritus scores in Trial A4250-005 the test-retest reliability of these scores remains unknown. Demonstration that ObsRO and PRO scores have sufficient test-retest reliability is used needed to help establish evidence and confidence that observed differences between study arms are not notably attributable to construct-irrelevant variance in observed scores. Specifically, demonstration of test-retest reliability supports the absence of practice effects (a potential source of bias). However, despite the absence of such evidence, the review team did not view the Applicant's PRO and ObsRO measures of pruritus as being particularly prone to practice effects due to the nature of the construct being measured. Therefore, the lack of sufficient test-retest reliability evidence was considered to be a review issue but not an approvability issue.

16.1.7. Clinically Meaningful Within-Patient Change

The Applicant submitted both (a) quantitative evidence generated from anchor-based analyses conducted in Trial A4250-005 (Section [16.1.7.1](#)) and (b) qualitative evidence generated from exit interviews with patients and/or their caregivers who participated in Trial A4250-005 (Section [16.1.7.2](#)) to support the selection of a range of thresholds for clinically meaningful within-patient change in scratching severity from baseline. However, limitations of the qualitative and quantitative evidence submitted by the Applicant necessitated the Agency's exclusive reliance upon post hoc anchor-based analyses conducted by the review team (Section [16.1.7.1](#)) to support the interpretation of clinically meaningful within-patient change in pruritus.

Note: Meaningful change analyses should not be construed to represent evaluation of the statistical significance of group-level differences in treatment effects.

16.1.7.1. Anchor-Based Analyses

Interpretation of the Applicant's anchor-based analyses was collectively precluded by:

- The uninterpretability of the prespecified primary efficacy endpoint (see Sections [6.2.1.2](#) and [16.1.4](#));
- The inherent interpretability and lack of reference to within-patient change in the alternative primary efficacy endpoint proposed by the review team (see Sections [6.2.1.2](#) and [6.3.2](#)); and
- Misalignment between the assessment periods of prespecified secondary and exploratory efficacy endpoints constructed from ObsRO pruritus scores (enumerated in Trial A4250-005 CSR Section 9.7.1.1.1) and the recall periods implied by available anchor measures (i.e., the PGIS, PGIC, CaGIS, CaGIC, CGIS, and CGIC; see Figure 42).

To facilitate evaluation of clinically meaningful within-patient change in pruritus, the review team proposed the efficacy endpoint of “change in Worst Weekly Scratching Score from baseline (Study Days [-28, -1]) to Weeks 21–24 (Study Days [141, 168]),” where the Worst Weekly Scratching Score is defined in [Table 129](#). This proposed endpoint facilitates evaluation of clinically meaningful within-patient change in pruritus by:

Utilizing an assessment period (1 week) that aligns with the recall period implied by the CaGIS ([Figure 44](#)).

- The anchor measure administered in Trial A4250-005 with the least potential for recall bias that aligns with the caregiver perspective captured by the ObsRO pruritus measures used to construct the focal efficacy endpoints; and
- Being constructed from a daily summary of pruritus (the Worst Daily Scratching Score; defined in [Table 129](#)) that accommodates differences between daytime and nighttime scratching severity within a given day (see Section [16.1.4](#)) while also capturing the patient's worst pruritus experience that day.

Note that the Agency's statistical evaluation of treatment efficacy based on the endpoint “change in Worst Weekly Scratching Score from baseline (Study Days [-28, -1]) to Weeks 21–24 (Study Days [141, 168])” was strictly exploratory (e.g., this endpoint was not included in the testing hierarchy). Meaningful change analyses should not be construed to represent evaluation of the statistical significance of group-level differences in treatment effects.

16.1.7.1.1. Methods

The review team conducted post hoc anchor-based analyses enumerated in [Table 136](#), below, to evaluate clinically meaningful within-patient change in Worst Weekly Scratching Score from baseline (Study Days [-28, -1]) to Weeks 21–24 (Study Days [141, 168]) using patients in the FAS of Trial A4250-005 (N=62), with patients pooled across study arms and no imputation of missing data. The CaGIS ([Figure 44](#)) was used as the anchor measure in all post hoc anchor-based analyses conducted by the Agency, where change in CaGIS score from baseline to Week 24 was used to construct anchor change categories. The following post hoc anchor-based analyses were conducted by the Agency:

Table 136. Post Hoc Anchor-Based Analyses Conducted by the Agency

Analysis	Purpose	Analysis Set	Sample Size
Empirical cumulative distribution function (eCDF) plots of the distribution of change in Worst Weekly Scratching Score from baseline to Weeks 21-24 by change in CaGIS (anchor) score from baseline to Week 24, with anchor change categories collapsed as appropriate to accommodate small sample sizes	To inform selection of a range of thresholds for meaningful improvement in Worst Weekly Scratching Score from baseline (Study Days [-28, -1]) to Weeks 21–24 (Study Days [141, 168])	Patients in the FAS of Trial A4250-005 with complete Worst Weekly Scratching Score data and complete CaGIS score data at both baseline (Study Days [-28, -1]) and Weeks 21–24 (Study Days [141, 168])	40
Cross-tabulation of change in CaGIS score from baseline to Week 24 by baseline CaGIS score	To evaluate the distribution of change patterns in CaGIS severity between baseline and Week 24	Trial A4250-005 FAS	62

Source: PFSS Reviewer's table

Abbreviations: CaGIS, Caregiver Global Impression of Symptoms; FAS, full analysis set

16.1.7.1.2. Results

Of the 58 patients in the FAS of Trial A4250-005 for whom baseline CaGIS score data were available, 12 (20.69%) had “moderate” scratching at baseline, 32 (55.17%) had “severe” scratching, and 14 (24.14%) had “very severe” scratching. Of the 41 patients in the FAS of Trial A4250-005 for whom CaGIS score data were available at both baseline and Week 24, most patients (60.00%) with “moderate” scratching at baseline experienced no change or worsening by Week 24 while most (69.57% and 75.00%, respectively) patients with “severe” or “very severe” scratching at baseline improved by Week 24 ([Table 137](#)).

Table 137. Change in CaGIS Score From Baseline to Week 24 by Baseline CaGIS Score, Trial A4250-005 (N=62), FAS

Baseline CaGIS Score	Change in CaGIS Score From Baseline to Week 24						
	Improved 4 Categories n (%)	Improved 3 Categories n (%)	Improved 2 Categories n (%)	Improved 1 Category n (%)	No Change n (%)	Worsened 1 Category n (%)	Missing n (%)
None	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Mild	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Moderate	0 (0.00)	0 (0.00)	1 (8.33)	3 (25.00)	2 (16.67)	4 (33.33)	2 (16.67)
Severe	0 (0.00)	4 (12.50)	4 (12.50)	8 (25.00)	4 (12.50)	3 (9.38)	9 (28.12)
Very severe	1 (7.14)	2 (14.29)	1 (7.14)	2 (14.29)	2 (14.29)	0 (0.00)	6 (42.86)
Missing	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	4 (100.00)

Source: Review team's table

Abbreviations: CaGIS, Caregiver Global Impression of Symptoms; FAS, full analysis set

Percentages are given out of the total number of patients in the FAS endorsing the indicated CaGIS response category at baseline.

Visual inspection of the empirical cumulative distribution function plots of change in Worst Weekly Scratching Score from baseline to Weeks 21–24 by anchor change category in [Figure 37](#) and [Figure 38](#) suggests:

- Notable overlap between patients who experienced a 2-category improvement, 1-category improvement, no change, and 1-category worsening on the CaGIS (see [Figure 37](#)); but
- Clear separation between patients who experienced a 3+ ([Figure 37](#)) or 2+ category ([Figure 38](#)) improvement on the CaGIS and patients who experienced a 1-category improvement, no change, or 1-category worsening, respectively (except in the upper tail of the empirical cumulative distribution of change scores among patients who experienced a 2+ category improvement on the CaGIS; see [Figure 38](#)).

Based on these results, the review team selected a 2+ category improvement on the CaGIS as the target anchor change category for use in deriving a range of thresholds for meaningful within-patient improvement in scratching severity. Per [Table 137](#), above, note that:

- 8.33% of patients in the FAS with “moderate” scratching at baseline experienced a 2+ category improvement on the CaGIS;
- 25.00% of patients in the FAS with “severe” scratching at baseline experienced a 2+ category improvement on the CaGIS; and
- 28.57% of patients in the FAS with “very severe” scratching at baseline experienced a 2+ category improvement on the CaGIS.

Looking at [Figure 38](#), the threshold for meaningful improvement in Worst Weekly Scratching Score from baseline to Weeks 21–24 appears to lie (approximately) within $[-2.14, -1]$, where:

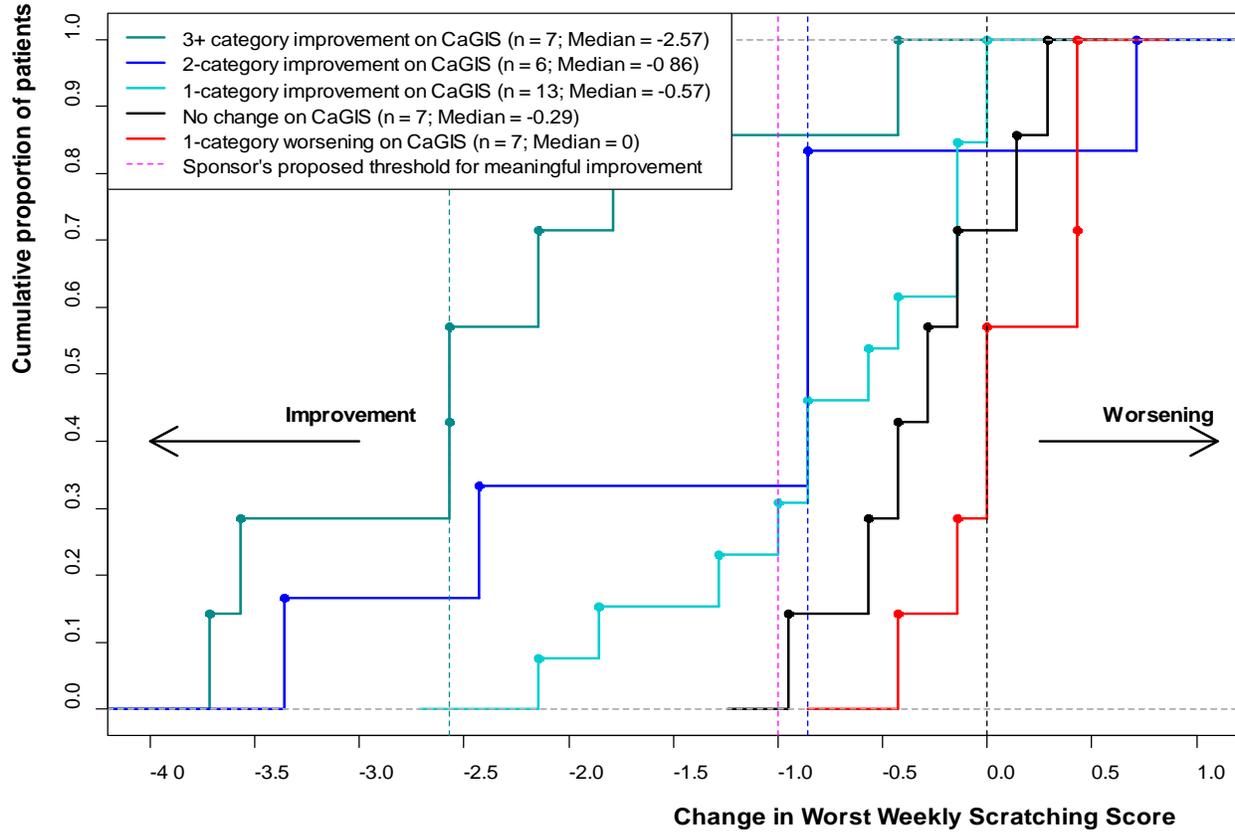
- -2.14 is the median change in Worst Weekly Scratching Score from baseline to Weeks 21–24 among patients who experienced a 2+ category improvement on the CaGIS between baseline and Week 24; and
- -1 is the threshold for meaningful within-patient change proposed by the Applicant for use in interpreting other endpoints constructed from the AM and PM ObsRO measures of pruritus (provided in [Figure 40](#) and [Figure 41](#), respectively).

A threshold of -1 or less (corresponding to a reduction in Worst Weekly Scratching Score of 1 point or more) would result in:

- No misclassification of patients who experienced no change or worsening as quantified by change in CaGIS score from baseline to Week 24; but
- 30.77% of patients who experienced only a 1-category improvement on the CaGIS being misclassified as having experienced a 2+ category improvement.

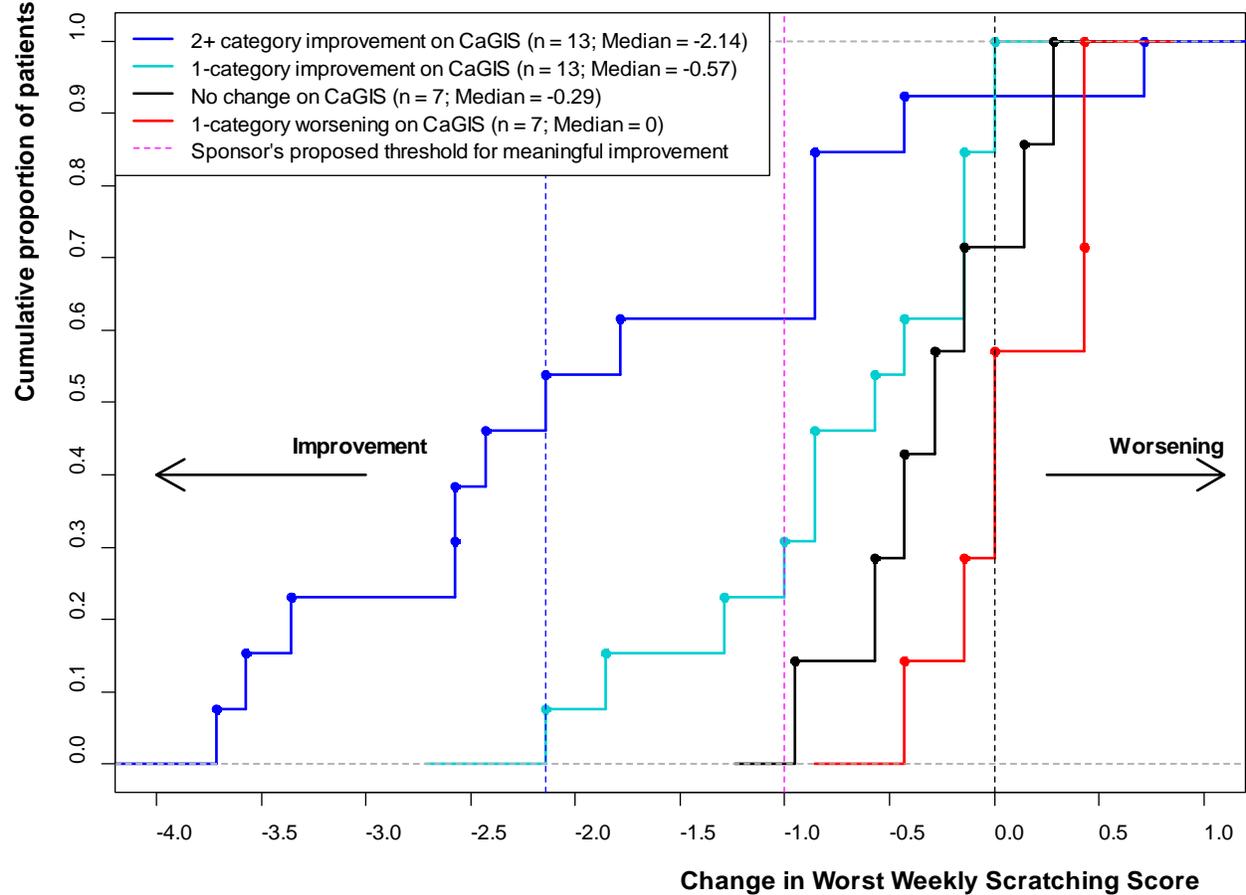
Alternatively, thresholds of -1.3 and -1.9, respectively, would result in only 15.38% and 7.69% of patients who experienced a 1-category improvement on the CaGIS being misclassified as having experienced a 2+ category improvement.

Figure 37. eCDF Plot of Change in Worst Weekly Scratching Score From Baseline (Study Days [-28, -1]) to Weeks 21–24 (Study Days [141, 168]) by Change in CaGIS Score From Baseline to Week 24, Collapsing Anchor Change Categories Representing a 3+ Category Improvement on CaGIS



Source: PFSS Reviewer's figure
Abbreviations: CaGIS, Caregiver Global Impression of Symptoms; eCDF, empirical Cumulative Distribution Function; FAS, full analysis set

Figure 38. eCDF Plot of Change in Worst Weekly Scratching Score From Baseline (Study Days [-28, -1]) to Weeks 21–24 (Study Days [141, 168]) by Change in CaGIS Score From Baseline to Week 24, Collapsing Anchor Change Categories Representing a 2+ Category Improvement on CaGIS



Source: PFSS Reviewer's figure

Abbreviations: CaGIS, Caregiver Global Impression of Symptoms; eCDF, empirical Cumulative Distribution Function; FAS, full analysis set

16.1.7.1.3. Conclusions

Based on the results of post hoc anchor-based analyses conducted by the review team, the Agency ultimately selected a range of thresholds of (-2.14, -1) for meaningful improvement in Worst Weekly Scratching Score from baseline to Weeks 21–24.

16.1.7.2. Exit Interviews With Patients and Caregivers

16.1.7.2.1. Methods

Per the Agency's request from the Written Responses Only communication dated February 20, 2019, the Applicant conducted an exit survey with patients in the FAS of Trial A4250-005 (N=62) and their caregivers on September 5, 2019 (Trial A4250-005 CSR Sections 9.7.1.13.5 and 11.4.1.2.10). Page 86/248 of the clinical study report (CSR) states:

“The exit survey was taken by patients ≥ 8 years old and caregivers separately and had the following 3 questions:

5. Have you or your child experienced change from the study drug at the end of the study? Yes/No;
6. Was the change meaningful? Yes/No;
7. In what way was the change meaningful?

“The answers of Yes in Questions 1 and 2 were verified with Question 3 for a positive and meaningful change. Answer to Question 3 was recorded verbatim. ... Available by-patient exit survey data are presented in a data listing.”

Although not explicitly stated by the Applicant, it appears that exit survey Question 2 was only administered if the response provided to Question 1 was “Yes,” and likewise the exit survey Question 3 was only administered if the response provided to Question 2 was “Yes.” Patient and caregiver exit survey responses were submitted on November 20, 2020, as data listing 16.2.6.11 in Trial A4250-005 CSR Appendix 16.2.6. Patient and caregiver responses to exit survey Questions 1 and 2 were additionally submitted on November 20, 2020, in Trial A4250-005 ADaM dataset *adefx.xpt*. Exit survey responses were “recorded in the last 2 weeks patients were on the study medication” (A4250-005 CSR Section 9.7.1.13.5, page 86/248). Missing exit survey responses were not imputed except in sensitivity analyses conducted by the Applicant.

16.1.7.2.2. Results

A total of 42 patients (67.74% of Trial A4250-005 FAS) contributed data to the exit survey through self-report (six patients; 9.68% of Trial A4250-005 FAS; 14.29% of patients contributing exit survey data) and/or report provided by caregivers (42 patients; 67.74% of Trial A4250-005 FAS; 100.00% of patients contributing exit survey data). Of these 42 patients, 14 (33.33%) had been randomized to and received 120 mcg/kg/day of odevixibat (A4250), 16 (38.10%) had been randomized to and received 40 mcg/kg/day of odevixibat, and the remaining 12 (28.57%) had been randomized to and received the placebo.

Descriptive statistics for the last day of study therapy (here, a proxy for approximate timing of survey response collection) for the 42 patients contributing exit survey responses are provided both overall and by study arm in [Table 138](#). Of note, recalling that exit survey responses were “recorded in the last 2 weeks patients were on the study medication” (A4250-005 CSR Section 9.7.1.13.5, page 86/248), visual inspection of the right-most column in [Table 138](#), suggests most but not all exit survey responses were collected within a window of time that aligns with administration of the CaGIS and evaluation of the efficacy endpoint “change in Worst Weekly

Scratching Score from baseline (Study Days [-28, -1]) to Weeks 21–24 (Study Days [141, 168])” proposed by the Agency to facilitate interpretation of meaningful within-patient change in pruritus (see Sections [6.2.1.1](#), [16.1.4](#), and [16.1.7.1](#)).

Table 138. Distribution of Last Day of Study Therapy Among Patients Contributing Exit Survey Responses (N=42) by Study Arm and Overall (Pooling Across Study Arms), Trial A4250-005 FAS

Study Arm	N	Min	Mean	SD	Median	Max	Weeks 23-24 Study Days [155, 168]	
							N ¹	% ²
Placebo	12	120	160.50	21.65	167	204	8	66.67
Odevixibat 40 mcg/kg/day	16	75	157.75	31.47	167	180	10	62.50
Odevixibat 120 mcg/kg/day	14	164	171.07	8.77	167	193	10	71.43
Overall (study arms pooled)	42	75	162.98	23.40	167	204	28	66.67

Source: PFSS Reviewer’s table, based on Trial A4250-005 ADaM dataset *adeff.xpt* submitted by the Applicant on 11/20/2020.

Abbreviations: FAS, full analysis set; SD, standard deviation

¹ Number (N) of patients whose last day of study therapy occurred during Weeks 23–24, Study Days [155, 168]

² Percentage (%) of patients whose last day of study therapy occurred during Weeks 23–24, Study Days [155, 168]

Patient and caregiver exit survey responses are summarized by study arm in [Table 140](#) and [Table 141](#), and overall (pooling across study arms) in [Table 139](#). A meaningful change from baseline to end of study therapy (i.e., a response of “Yes” to exit survey Questions 1 and 2) was reported for a total of 25 patients (40.32% of Trial A4250-005 FAS; 59.52% of patients contributing exit survey data). Of the six patients providing self-reported exit survey responses, patient responses to Questions 1 and 2 fully aligned with those provided by their caregivers (Trial A4250-005 CSR Appendix 16.2.6, data listing 16.2.6.11).

Table 139. Patient and Caregiver Exit Survey Responses (N=42), With Patients Pooled Across Study Arms

Evaluator	Q1 Response	Q2 Response	Q1 and Q2 Response Pattern Frequency	Q3 Response (note that each free response is unique to a particular reporter)
Patient	No	N/A	2	N/A
Caregiver	No	N/A	16	N/A
Caregiver	Yes	No	1	N/A
Patient	Yes	Yes	4	Pruritus and sleeping problem is much better The drug reduced patient’s itching. Patient has started back itching since stopping the study drug. I almost don’t scratch myself anymore, I manage to sleep better, I feel less tired, I feel better at school Stop scratching, better sleep, hungrier
Caregiver	Yes	Yes	25	Patient is sleeping significantly better at night since study drug treatment start. Relief of pruritus, better sleep Pruritus and sleeping problem is much better than the beginning. Improved pruritus, sleeping and quality of life. Subject is sleeping through the night. Not as itchy no itching, no scratching, child’s physical and mental development have improved Yes but improvable A little better scratching. No change

Evaluator	Q1 Response	Q2 Response	Q1 and Q2 Response Pattern Frequency	Q3 Response (note that each free response is unique to a particular reporter)
				Reduction in pruritus
				Pruritus is decreased and the color of skin is much more normal.
				Reduction in pruritus and much better sleeping.
				Child sleeping well and not itching in impression from parents.
				Sleeping more 70% better
				Happy to see him not suffer from itch.
				Better weight gain and growth, slight improvement in itching and jaundice
				The drug helped stop the patient's itching. However, since stopping the study drug, the patient is visibly more itchy.
				She doesn't wake up at night anymore because of scratching, she doesn't rub herself anymore against walls, she stopped pulling off her hair. Skin is less hurt; hairs and eyebrows are growing back.
				Itching improved immensely
				The change was meaningful - improvement in itching and sleeping
				Recovery from pruritus, no sleeping problems
				Pruritus is little better than the beginning. Sleeping is much better than the beginning
				Improved in every way
				Slept better, not as much scratching, overall, more satisfied
				Gained weight, appetite improved, somewhat less scratching

Source: PFSS Reviewer's table, aggregating information provided by the Applicant in data listing 16.2.6.11 included in Trial A4250-005 CSR Appendix 16.2.6.

Table 140. Applicant's Summary of Exit Survey Responses Collected From Caregivers, Trial A4250-005 (N=42) FAS

PARAMETER	PLACEBO N=20 n (%)	ODEVIXIBAT, ONCE DAILY DOSING		
		40 µg/kg N=23 n (%)	120 µg/kg N=19 n (%)	ALL DOSES N=42 n (%)
Number of Caregivers answering Exit Survey ^a	12	16	14	30
Q1 and Q2 = Yes: Change experienced and considered meaningful	4 (33.3)	13 (81.3)	8 (57.1)	21 (70.0)
Q1 = Yes and Q2 = No: Change experienced and not considered meaningful	1 (8.3)	0	0	0
Q1 = No: No change experienced	7 (58.3)	3 (18.8)	6 (42.9)	9 (30.0)

^a The percentage is calculated based on the number of caregivers/patients with the exit survey answered.

Q1: Have you or your child experienced change from the study drug at the end of the study?

Q2: Was the change meaningful?

Source: Table 14.2.3.11.

Source: A4250-005 CSR Table 31

Abbreviations: FAS, full analysis set

Table 141. Number and Percentage of Patients in Each Study Arm for Whom a Meaningful Change Was Reported in Exit Survey (N=25), Trial A4250-005

Study Arm	Number of Patients With Exit Survey Response Data	Number of Patients for Whom Meaningful Change Was Reported	Percentage of Patients for Whom Meaningful Change Was Reported Out of Number of Patients in Indicated Study Arm Who Provided Exit Survey Response Data	Percentage of Patients for Whom Meaningful Change Was Reported Out of Total Number of Patients Across Study Arms for Whom Meaningful Change Was Reported
Placebo	12	4	33.33	16.00
Odevixibat 40 mcg/kg/day	16	13	81.25	52.00
Odevixibat 120 mcg/kg/day	14	8	57.14	32.00
Overall (study arms pooled)	42	25	59.52	100.00

Source: PFSS Reviewer's table, based on patient and caregiver responses to exit survey Questions 1 and 2 included in Trial A4250-005 ADaM dataset adef.xpt submitted by the Applicant on November 20, 2020.

16.1.7.2.3. Limitations and Conclusions

Interpretation of the exit survey results is limited by (a) the focus of the survey questions on change, which may be either improvement or worsening, and (b) the misalignment of response collection timeframe with timing of CaGIS administration and evaluation of efficacy endpoint “change in Worst Weekly Scratching Score from baseline (Study Days [-28, -1]) to Weeks 21–24 (Study Days [141, 168])” proposed by the Agency to facilitate interpretation of meaningful within-patient change in pruritus (see Sections [6.2.1.1](#), [16.1.4](#), and [16.1.7.1](#)). Further, interpretation is precluded by concerns surrounding response data quality. Although examination of patient and caregiver responses to exit survey Question 3 (provided in [Table 139](#)) suggests patients and caregivers interpreted meaningful “change” in Questions 1 to 3 as meaningful improvement, it is unclear in many cases who is actually providing the responses. For example, many responses to Question 3 appear to be provided by a clinician rather than by the patient or a caregiver. Moreover, some responses to Question 3 (e.g., “No change”) are inconsistent with corresponding responses provided to Questions 1 and 2 indicating the patient had experienced a meaningful change. As such, exit interview results cannot yield trustworthy information and cannot be used to help inform evaluation of either meaningful improvement or meaningful decline.

16.1.7.3. Interpretation of Efficacy

As discussed in Section [16.1.7](#), the Agency’s evaluation of meaningful within-patient change and subsequent interpretation of efficacy relied solely upon the quantitative evidence generated by post hoc anchor-based analyses conducted by the review team. Based on the results of post hoc anchor-based analyses conducted by the review team, the Agency ultimately selected a range of thresholds of [-2.14, -1] for meaningful improvement in Worst Weekly Scratching Score from baseline (Study Days [-28, -1]) to Weeks 21–24 (Study Days [141, 168]) (see Section [16.1.7.1](#)). Note that to allow for comparative analyses with the data generated from the change in CaGIS anchor categories (i.e., anchor-based methods), the review team generated an empirical cumulative distribution function plot ([Figure 39](#)) of the distribution of raw change in Worst

Weekly Scratching Score from baseline (Study Days [-28, -1]) to Weeks 21–24 (Study Days [141, 168]) by study arm using all patients in the FAS of Trial A4250-005 with complete Worst Weekly Scratching Score data at both baseline and Weeks 21–24 (n=50). Note that the empirical cumulative distribution function plot shown in [Figure 39](#) was replicated by the Applicant in its response (Figure Q4 in Clinical Information Amendment 1.11.3 received on March 12, 2021) to the Agency’s Information Request sent late in the review cycle to conduct analyses to support interpretation of the Agency’s proposed efficacy endpoint “change in Worst Weekly Scratching Score from baseline (Study Days [-28, -1]) to Weeks 21–24 (Study Days [141, 168]).”

Examination of [Figure 39](#) indicates

- Clear separation between the two odevixibat treatment arms on the one hand and the placebo arm on the other for decreases (improvement) in Worst Weekly Scratching Score of 0 or more, with clear and notable separation within the range of thresholds for meaningful improvement of (-2.14, -1); with
- Notable overlap between the low and high doses of odevixibat, especially among patients who experienced a decrease (improvement) in Worst Weekly Scratching Score of 1 point or more

These findings are echoed by the rows reporting “overall” differences between the three study arms in percentage of patients achieving various thresholds of meaningful within-patient improvement in [Table 142](#). Overall, in both odevixibat arms, gains in meaningful within-patient improvement in scratching severity relative to placebo appear to decrease as the magnitude of threshold for meaningful improvement increases ([Figure 39](#); [Table 142](#)).

Collectively, these results suggest that overall:

- Both the low and high doses of odevixibat yield meaningful within-patient improvement in scratching severity for a notably higher proportion of patients than the placebo; and
- The low and high doses of odevixibat yield comparable levels of meaningful within-patient improvement in scratching severity from baseline to Weeks 21–24.

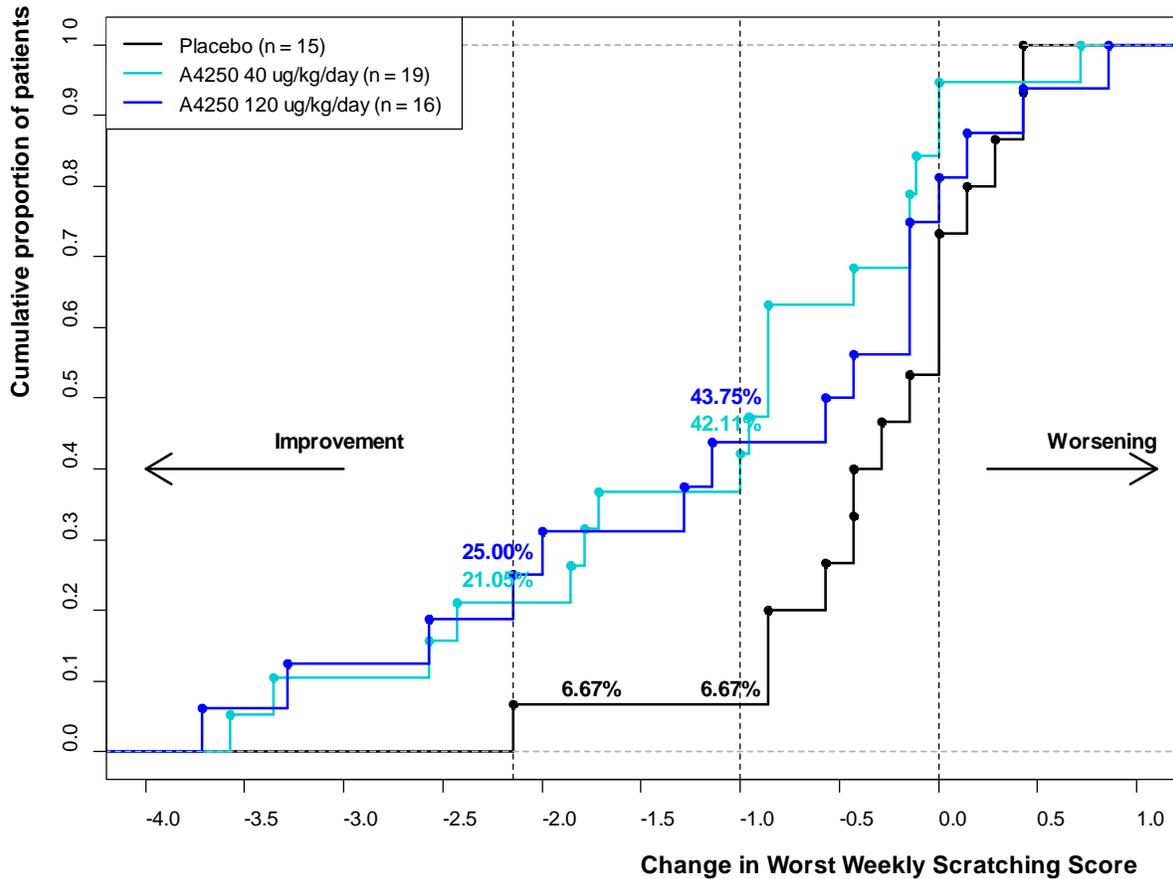
However, some potentially substantive differences between odevixibat arms in meaningful within-patient improvement in scratching severity are noted for different levels of scratching severity at baseline. Specifically, examination of [Table 142](#) suggests:

- The low dose (40 mcg/kg/day) of odevixibat yields the highest level of meaningful within-patient improvement in scratching severity for patients with “moderate” scratching severity at baseline; and
- The high dose (120 mcg/kg/day) of odevixibat yields the highest level of meaningful within-patient improvement in scratching severity for patients with “severe” or “very severe” scratching severity at baseline, with gains in meaningful improvement appearing to increase as baseline scratching severity and/or threshold magnitude increases.

Collectively, these findings imply both the low and high doses of odevixibat confer a meaningful improvement in scratching severity relative to placebo.

Note: Meaningful change analyses should not be construed to represent evaluation of the statistical significance of group-level differences in treatment effects.

Figure 39. eCDF Plot of Change in Worst Weekly Scratching Score From Baseline (Study Days [-28, -1]) to Weeks 21–24 (Study Days [141, 168]) by Study Arm Among Patients With Complete Worst Weekly Scratching Score Data at Both Baseline and Weeks 21–24 (N=50), Trial A4250-005 FAS



Source: Review team's figure
 Abbreviations: CaGIS, Caregiver Global Impression of Symptoms; eCDF, empirical cumulative distribution function; FAS, full analysis set

Table 142. Differences Between Study Arms in Percentage of Patients Who Achieved Various Thresholds for Meaningful Within-Patient Improvement in Scratching Severity (Worst Weekly Scratching Score) by Baseline Scratching Severity (Baseline CaGIS Score) Among Patients With Complete Worst Weekly Scratching Score Data at Both Baseline and Weeks 21–24 (N=50), Trial A4250-005-FAS

Baseline Scratching Severity (Baseline CaGIS Score)	Threshold for Meaningful Within-Patient Improvement in Scratching Severity (Worst Weekly Scratching Score)	
	-2.14	-1
Low dose vs. placebo (low dose % – placebo %)		
Moderate	25.00	50.00
Severe	12.22	34.44
Very Severe	16.67	33.33
Overall ¹	14.38	35.44

Baseline Scratching Severity (Baseline CaGIS Score)	Threshold for Meaningful Within-Patient Improvement in Scratching Severity (Worst Weekly Scratching Score)	
	-2.14	-1
High dose vs. placebo (high dose % – placebo %)		
Moderate	0.00	0.00
Severe	18.57	47.14
Very Severe	50.00	50.00
Overall ¹	18.33	37.08
High dose vs. low dose (high dose % – low dose %)		
Moderate	-25.00	-50.00
Severe	6.35	12.70
Very Severe	33.33	16.67
Overall ¹	3.95	1.64

Source: Review team's table

Abbreviations: CaGIS, Caregiver Global Impression of Symptoms; FAS, full analysis set

The low dose study arm is the odevixibat 40 mcg/kg/day study arm, and the high dose study arm is the odevixibat 120 mcg/kg/day study arm.

¹ Using all patients in the FAS of Trial A4250-005 with complete Worst Weekly Scratching Score data both at baseline (Study Days [-28, -1]) and Weeks 21–24 (Study Days [141, 168]) (n=50) in the indicated study arms being compared.

16.1.8. COA Appendices

Table 143. Documents Submitted Under NDA 215498 Reviewed in COA-Focused Evaluation

Document	Date Received
Trial A4250-005 Clinical Study Report (CSR) (dated 10/30/2020), entitled Odevixibat: A Double-Blind, Randomized, Placebo-Controlled, Phase 3 Study to Demonstrate Efficacy and Safety of A4250 in Children with Progressive Familial Intrahepatic Cholestasis types 1 and 2 (PEDFIC1)	11/20/2020
Trial A4250-005 CSR Appendix 16.1.1: Protocol and Protocol Amendments: Protocol Final Amendment 06 (dated 6/24/2019)	11/20/2020
Trial A4250-005 CSR Appendix 16.1.2: Sample Case Report Form (Version 5.0; dated 8/8/2019)	11/20/2020
Annotated Draft Labelling Text	11/20/2020
Clinical Outcome Assessment Evidence Dossier for Patient- and Observer-Reported Outcome Pruritus Measures (Draft Version 1.0; dated 10/19/2020)	11/20/2020
Clinical Outcome Assessment Evidence Dossier Appendices <ul style="list-style-type: none"> • Appendix A: PRO and ObsRO pruritus item screenshots • Appendix B: User and site training materials for PRO and ObsRO items • Appendix C: Translation and linguistic validation report • Appendix F: Qualitative interview study protocol • Appendix G: Psychometric analysis report 	11/20/2020
Clinical Information Amendment submitted in response to FDA Information Request	12/31/2020
Clinical Information Amendment submitted in response to FDA Information Request	3/12/2021

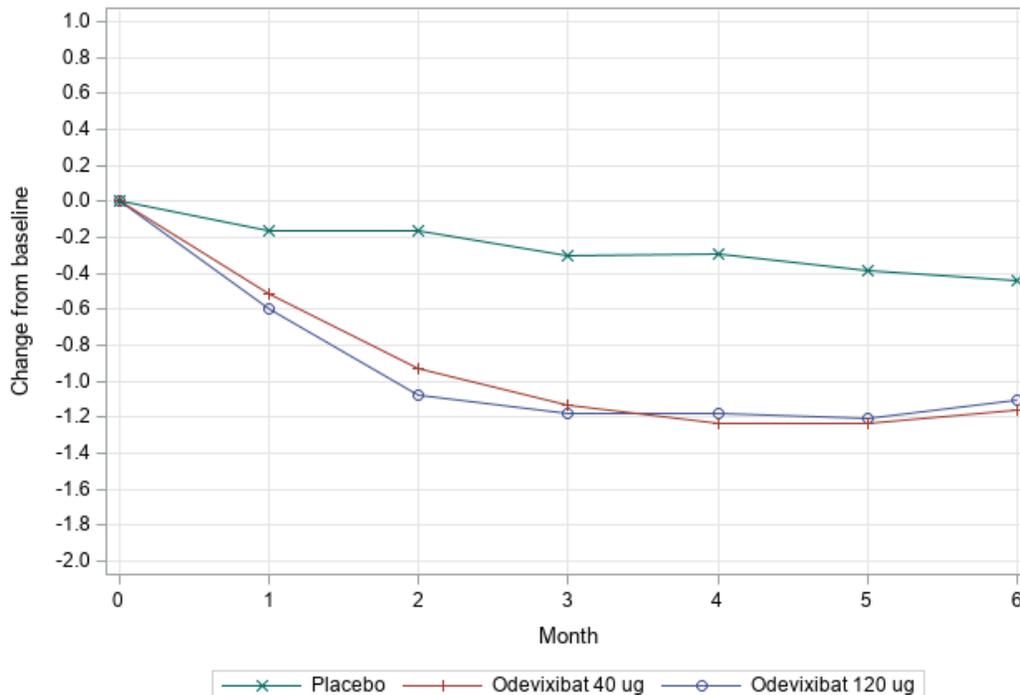
Source: PFSS Reviewer's table

Abbreviations: COA, clinical outcome assessment; ObsRO, observer-reported outcome; PRO, patient-reported outcome; FDA, Food and Drug Administration

16.2. Additional Efficacy Analyses

Figure 45 shows the least squares (LS) mean of the change from baseline in Worst Weekly Scratching Score, where missing data were handled using the Applicant's prespecified multiple imputation (MI) approach under the Missing Not at Random (MNAR) assumption. Both odevixibat arms show larger decrease in LS mean compared to placebo beginning from Month 1 through Month 6, and the magnitudes of the changes in pruritus severity appears similar between the 40 mcg/kg/day odevixibat and 120 mcg/kg/day odevixibat groups across the 6-month treatment period.

Figure 45. LS Mean Change From Baseline in the Worst Weekly Scratching Scores for Each Month^{1,2,3}



Source: Statistical Reviewer's Analysis

Abbreviations: LS, least squares

¹ Results presented for the randomized set as the intent-to-treat (ITT) population.

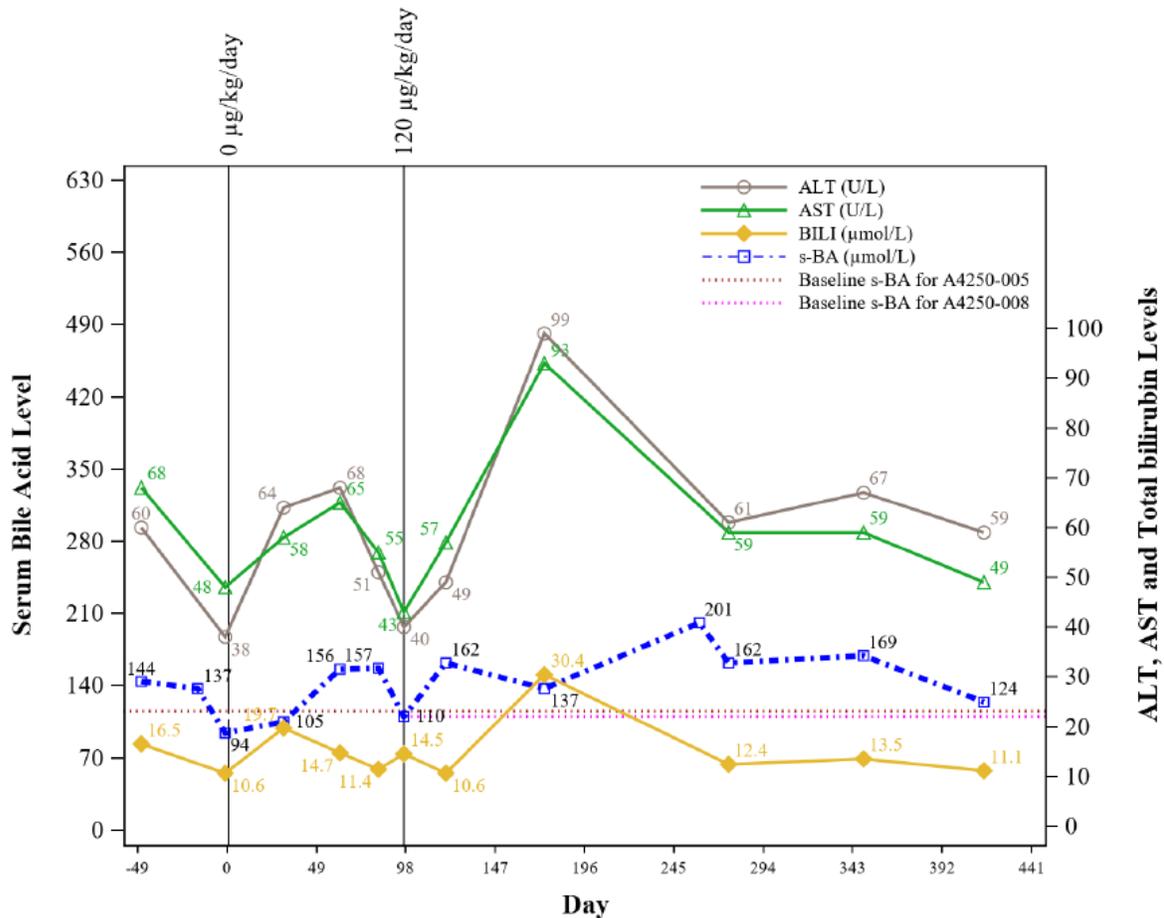
² The analysis was based on a mixed model for repeated measures (MMRM) with baseline score as a covariate, and treatment group, time (in months), treatment-by-time interaction, treatment-by-baseline interaction, and stratification factors (PFIC type and age category) as fixed effects.

³ A placebo-based multiple imputation approach under a MNAR assumption was conducted as specified by the Applicant (refer to Section 6.2.1.3).

17. Clinical Safety: Additional Information and Assessment

Patient (b) (6) who required biliary diversion.

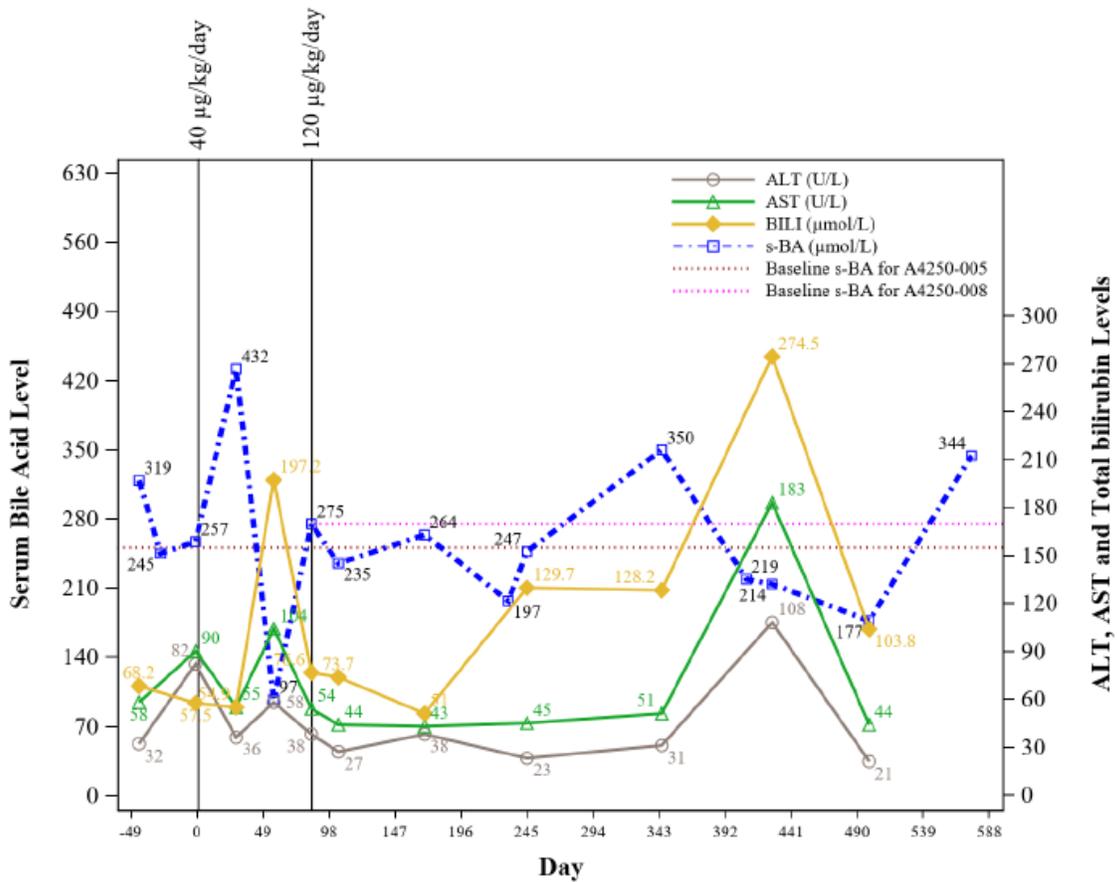
Figure 46. Overtime Graphical Patient Profile for PT ID (b) (6)



Source: Patient Journey, pages 510-1429
 Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BILI, total bilirubin; sBA, serum bile acid

Patient experienced worsening of cholestasis, and presented with elevations in total bilirubin, ALT, and AST while receiving odevixibat. On day 196 the patient experienced elevation in liver enzymes and bilirubin, however the drug was not discontinued. On day 253, the drug was discontinued permanently, due to AE of worsening of cholestasis and underwent surgical biliary diversion.

Figure 47. Patient ID (b) (6) Graphical Profile of Serum Bile Acid Levels Versus Duration of Treatment Versus Liver Tests



ALT = alanine aminotransferase, AST = aspartate aminotransferase; BILI = total bilirubin; s-BA = serum bile acid; ULN = upper limit of normal

Note: Day 0 is the baseline visit for Study A4250-005; Day 85 is the baseline visit for Study A4250-008. The baseline serum bile acids level is 251 µmol/L (ULN: 10 µmol/L) for A4250-005 and 275 µmol/L (ULN: 10 µmol/L) for A4250-008. Note, patient had a dose reduction (not shown on Figure) during Study A4250-008 as stated in the Exposure section.

Source: Copied and electronically reproduced from Applicant's Patient Journeys pdf; page 253 of 1429

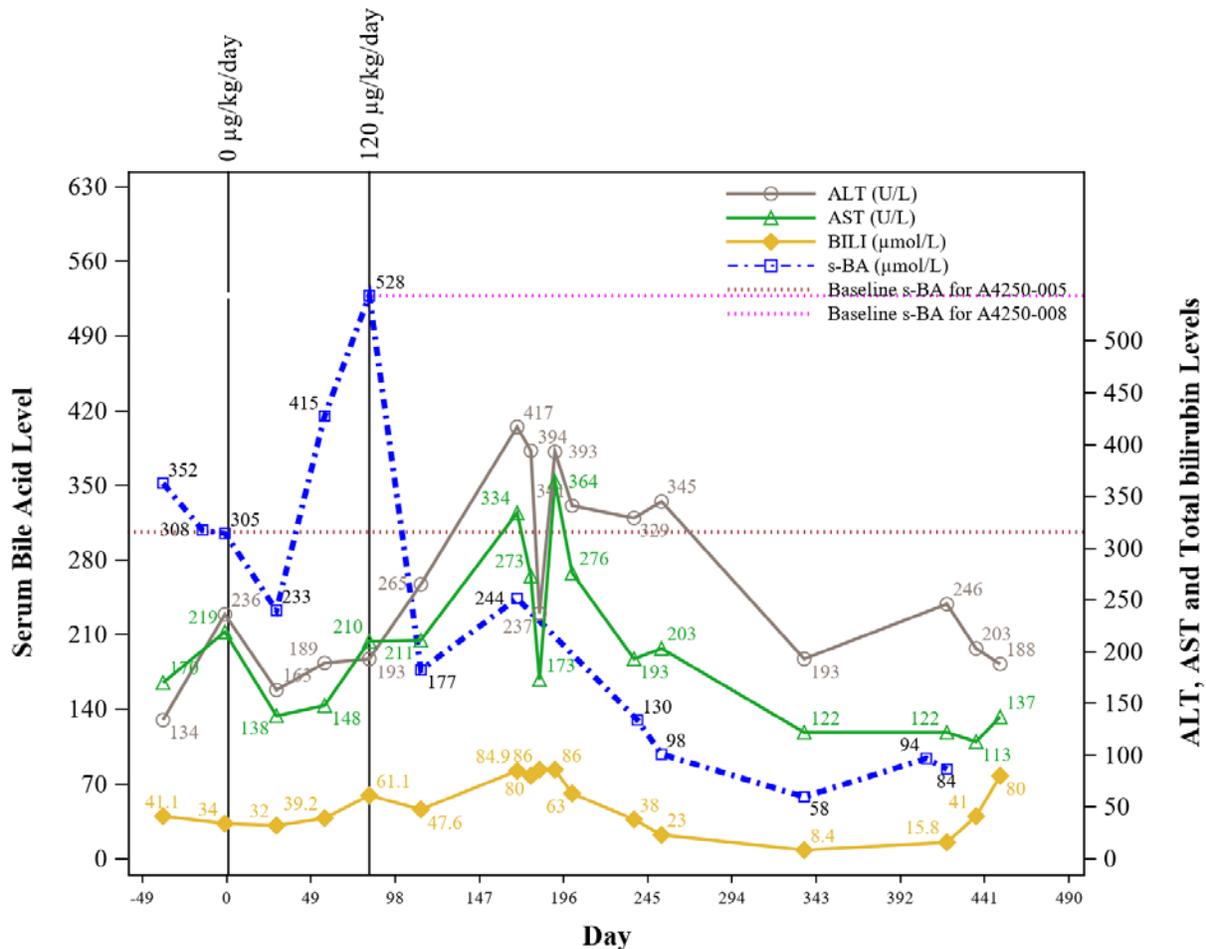
The patient had to discontinue treatment from

1. Day 168 to Day 261 (93 days), due to elevation in total bilirubin (TB)
2. Day 339 to Day 342 (3 days), due to diarrhea (possibly gastroenteritis)

The patient had elevation of total bilirubin, aspartate aminotransferase, and alanine aminotransferase on Day 441 as well, and the Applicant has not provided whether the treatment was discontinued; both aminotransferases and total bilirubin were elevated. Patient continues to be on odevixibat and continues to have elevations in liver enzymes.

This patient's liver enzymes and bilirubin trends are concerning. The patient now has a new baseline, which is worse than patient's baseline liver test fluctuations. Whether the worsening of cholestasis presenting as new worsened baseline would impact progression of disease is unknown, but logically one could infer, that the liver is now being injured at a faster rate as indicated by elevation in AST, ALT, and TB, the progression to liver transplant may occur sooner.

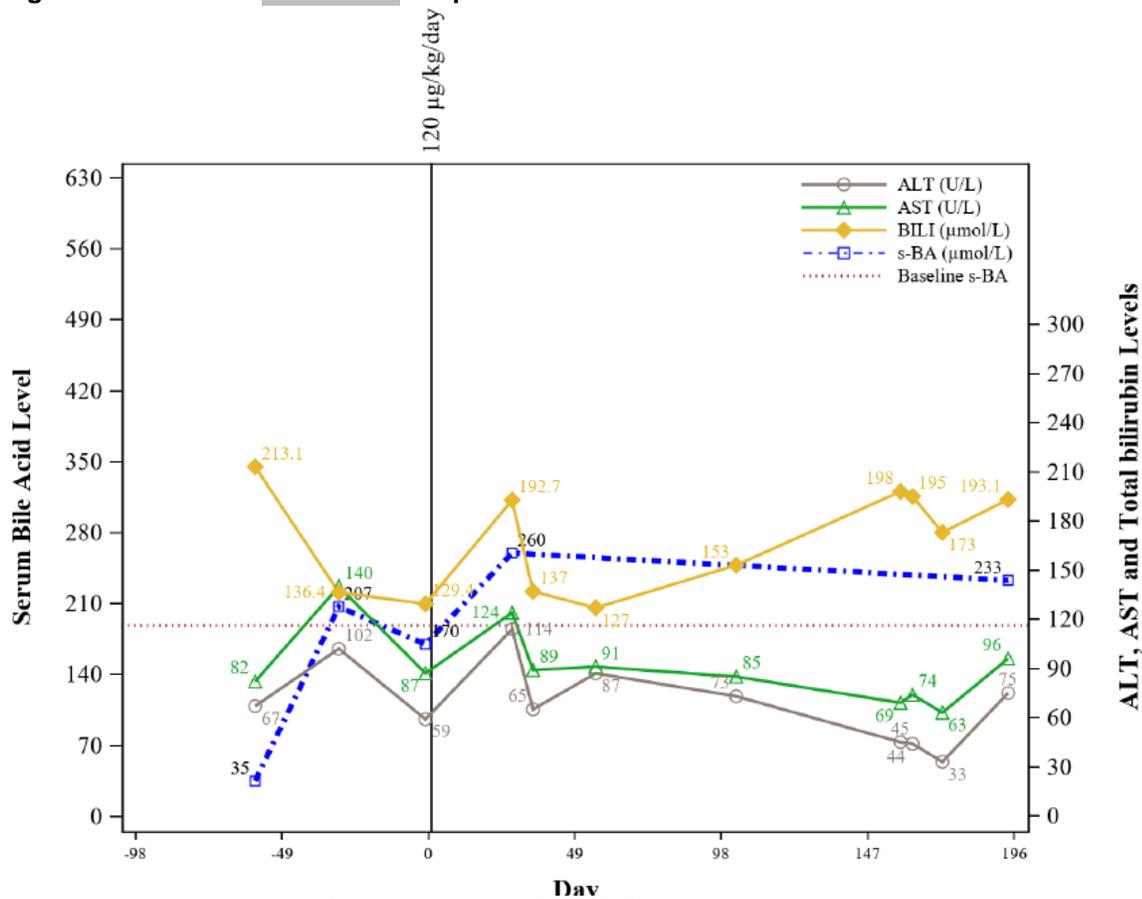
Figure 48. Patient ID (b) (6) Overtime Graphical Patient Profile Serum Bile Acid, ALT, AST, and Total Bilirubin Levels



Source: Copied and electronically reproduced from Applicant's Patient Journeys pdf; page 253 of 1429
 Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BILI, total bilirubin; sBA, serum bile acid

It is not clear why the Applicant did not discontinue the treatment when the aminotransferases and total bilirubin elevated around Day 150 and seemed to have met the criteria for suspected liver injury. The new fluctuations are relatively higher than pretreatment fluctuations. Whether these higher fluctuations would portend poor outcomes is not known and if possible, should be studied in a long-term trial.

Figure 49. Patient ID (b) (6) Graphical Profile

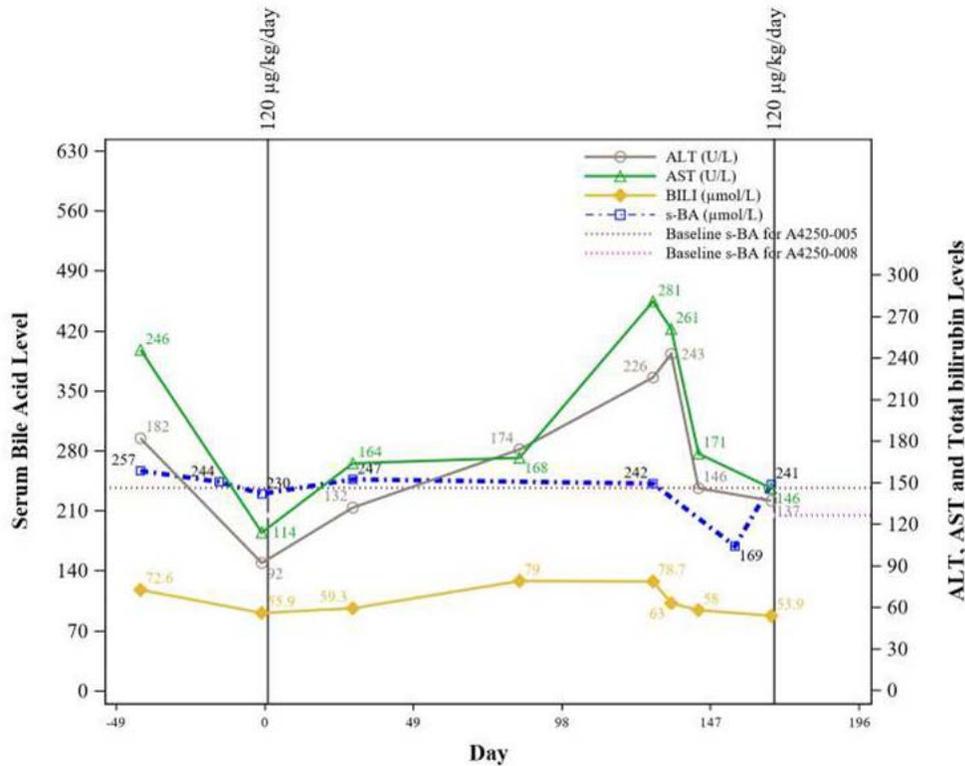


Source: Applicants submission of Patient Journey, page 950 of 1429
 Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BILI, total bilirubin; sBA, serum bile acid

Liver enzymes, total bilirubin, serum bile acid levels all fluctuated after starting odevixibat. Although it might seem that the patient did have fluctuations prior to enrollment in Trial A4250-005, however, the cause for these fluctuations were not provided in the narrative. Patients with PFIC generally experience elevations in liver enzymes secondary to intercurrent illness, and whether these elevations were also related to intercurrent illness was not specified. It is difficult to ascertain treatment related injury as patients baseline fluctuations, however, a possibility of odevixibat treatment causing these fluctuations cannot be ruled out.

Figure 50. Patient ID (b) (6) Who Had Concurrent Elevation of Transaminases and Bilirubin At Week 12

Figure 1: Serum Bile Acid (s-BA), ALT, AST and Total Bilirubin Levels



ALT = alanine aminotransferase; AST = aspartate aminotransferase; BILI = bilirubin; s-BA = serum bile acid; ULN = upper limit of normal

Note: Day 0 is the baseline visit for Study A4250-005; Day 168 is the baseline visit for Study A4250-008.

The baseline serum bile acids level is 237 µmol/L (ULN: 10 µmol/L) for A4250-005 and 205 µmol/L (ULN: 10 µmol/L) for A4250-008.

Source: Applicant's submission of patient journey

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BILI, total bilirubin; sBA, serum bile acid

17.1. Narratives of Patients Who Were Found to Have Splenomegaly

Placebo

Patient (b) (6), a 11.3-year-old male with PFIC2, with a time from diagnosis of 9.9 years, completed 24 weeks of treatment with placebo. Abdominal ultrasound at baseline showed hepatomegaly and splenomegaly with continued hepatomegaly and splenomegaly (actual size note reported) worsened at Week 24. No AEs of acute viral or other infections were reported. The patient's hepatic biochemical markers at baseline and Week 24 were: ALT of 52 and 60 U/L, AST of 65 and 88 U/L and total bilirubin of 27.4 and 51.7 micromole/L, respectively. Platelet counts were 161 and 160 × 10⁹/L at baseline and Week 24, respectively, and leukocyte count was 7.79 and 6.73 × 10⁹/L, respectively.

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Patient (b) (6), a 0.6-year-old male with PFIC2, with a time from diagnosis of 0.3 years, completed 24 weeks of treatment with placebo. Abdominal ultrasound at baseline showed abnormal liver with normal spleen size; liver size was reported as normal and splenomegaly was noted at Week 24. The patient experienced influenzae at approximately Week 6. ALT, AST, and total bilirubin improved over time on treatment. Platelet and leukocyte counts were not reported at baseline and were 601 and $13.1 \times 10^9/L$, respectively, at Week 22.

Odevixibat 40 mcg/kg/day

Patient (b) (6), a 5-year-old female with PFIC2, with a time from diagnosis of 0.3 years, and jaundice for 20 months of age, completed 24 weeks of treatment with odevixibat 40 mcg/kg/day. Abdominal ultrasound reported normal spleen size at baseline; spleen was reported as enlarged at Week 24. No TEAEs of acute viral infection or other infections were reported at the time of examination. ALT, AST, and total bilirubin values improved from baseline to Week 24. Platelet counts were 487 and $405 \times 10^9/L$ at baseline and Week 24, respectively, and leukocyte count was 8.42 and $8.88 \times 10^9/L$, respectively.

Odevixibat 120 mcg/kg/day

Patient (b) (6), a 1.2-year-old male with PFIC2, with a time from diagnosis of 0.7 years, completed 24 weeks of dosing with odevixibat 120 mcg/kg/day. Abdominal ultrasound at baseline showed normal liver and spleen size; the liver remained of normal size at the end of treatment and the spleen was reported as enlarged. A treatment-emergent adverse event (TEAE) of splenomegaly was reported at Week 24; the event was mild in severity and considered by the investigator as unrelated to study drug. No TEAEs of acute viral infection or other infections were reported. ALT, AST, and total bilirubin improved during treatment. Platelet counts were 415 and $327 \times 10^9/L$ at baseline and Week 24, respectively, and leukocyte count was 24.4 and $10.5 \times 10^9/L$, respectively.

Patient (b) (6), 2.7-year-old male with PFIC2, with a time from diagnosis of 2.4 years, completed 24 weeks of treatment with odevixibat 120 mcg/kg/day. Abdominal ultrasound results demonstrated hepatomegaly and splenomegaly at baseline, with hepatomegaly unchanged and splenomegaly worsened at Week 24. The patient experienced seven events of viral infections or infection (viral infection, otitis media or upper respiratory tract infection), all were assessed as mild and unrelated to study drug. ALT, AST, and total bilirubin improved during treatment. Platelet counts were 451 and $279 \times 10^9/L$ at baseline and Week 24, respectively, and leukocyte count was 14.0 and $12.1 \times 10^9/L$, respectively.

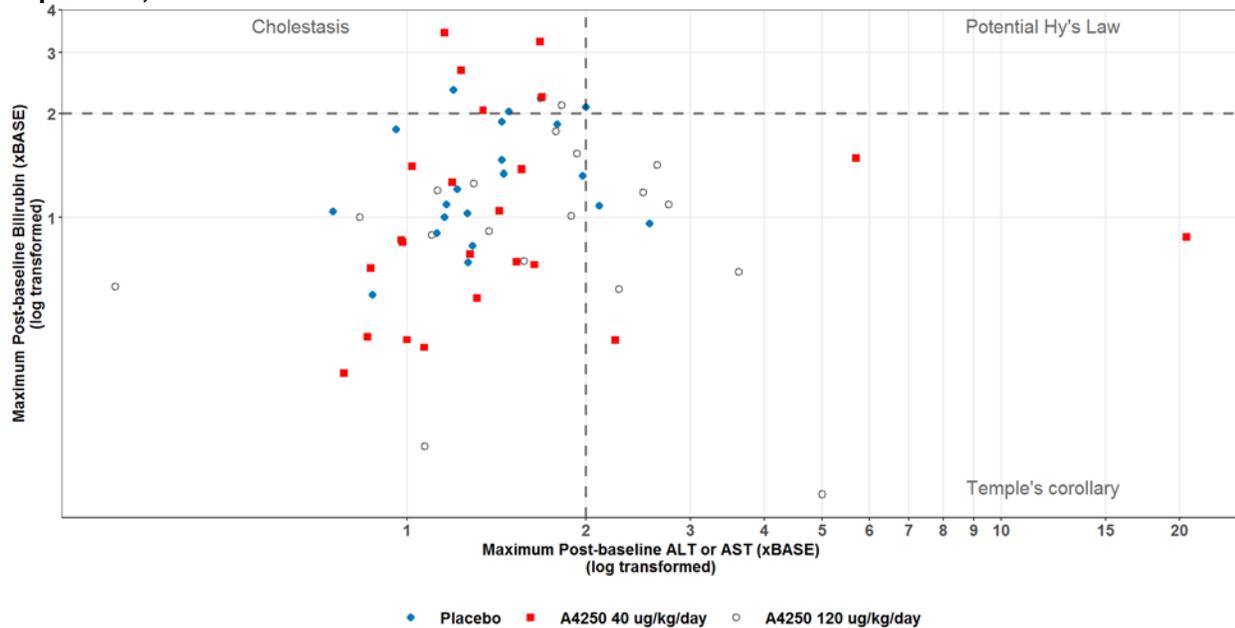
Patient (b) (6), 5-year-old male with PFIC2, with a time from diagnosis of 4.1 years, completed 24 weeks of treatment with odevixibat 120 mcg/kg/day. Abdominal ultrasound results demonstrated hepatomegaly and splenomegaly at baseline, with continued hepatomegaly and splenomegaly worsened at Week 24. The patient experienced mild nasopharyngitis (common cold) at Week 22 assessed as unrelated to study drug. The patient's hepatic biochemical markers at baseline and Week 24 were: ALT of 92 and 137 U/L, AST of 114 and 146 U/L and total bilirubin of 55.9 and 53.9 micromole/L, respectively. Hematology samples were not obtained.

17.2. Liver Test Abnormalities

FDA’s review team and clinical data scientists (CDSs) assessed the liver enzyme using modified eDISH plots to accommodate baseline elevations in liver tests.

Figure 51 shows a greater percentage of odevixibat-treated patients had elevations in TB and transaminases; the number and percentages are presented in Table 29.

Figure 51. DILI Case Screening Plot With Adjusted Reference Limits (2X Baseline), Safety Population, Trial A4250-005



Source: adlbsi.xpt; Software: R
 Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DILI, drug-induced liver injury; ULN, upper limit of normal
 Each data point represents at least one visit (from a patient) with both ALT/AST and total bilirubin values in the postbaseline period. A potential Hy's Law case was defined as having a postbaseline total bilirubin equal to or exceeding 2X baseline on or within 30 days after postbaseline ALT or AST elevation equal to or exceeding 2X baseline, without findings of cholestasis (defined as ALP <2X baseline) on or within 30 days after ALT or AST elevation. A cholestatic injury case was defined as total bilirubin equal to or exceeding 2X baseline on or within 30 days after alkaline phosphatase elevation ($\geq 2X$ baseline).

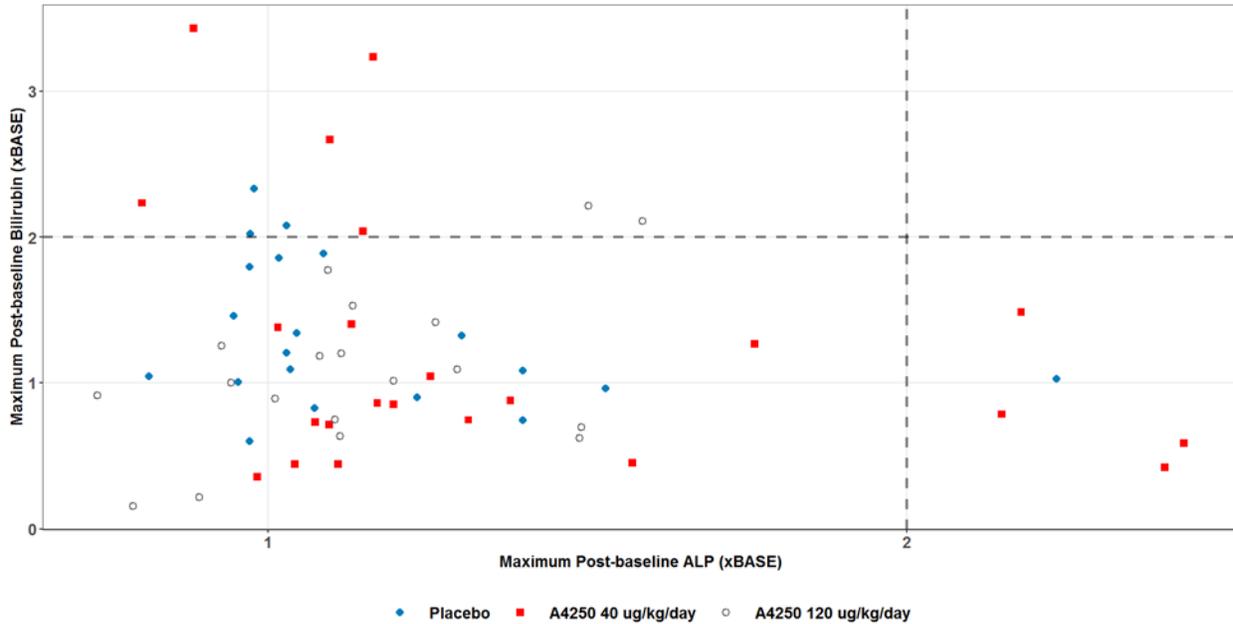
Table 144. Potential DILI With Adjusted Reference Limits (2X Baseline), Safety Population, Trial A4250-005

	Placebo N=20 n (%)	Odevixibat 40 mcg/kg/day N=23 n (%)	Odevixibat 120 mcg/kg/day N=19 n (%)
Quadrant			
Potential Hy's Law (right upper)	1 (5)	0	0
Cholestasis (left upper)	2 (10)	5 (21.7)	2 (10.5)
Temple's corollary* (right lower)	2 (10)	3 (13)	6 (31.6)
Total	5 (25)	8 (34.8)	8 (42.1)

Source: adlbsi.xpt; Software: R
 Abbreviations: AT, aminotransferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DILI, drug-induced liver injury; ULN, upper limit of normal
 A potential Hy's Law case was defined as having a postbaseline total bilirubin equal to or exceeding 2X baseline on or within 30 days after postbaseline ALT or AST elevation equal to or exceeding 2X baseline, without findings of cholestasis (defined as ALP <2X baseline) on or within 30 days after ALT or AST elevation. A cholestatic injury case was defined as total bilirubin equal to or exceeding 2X baseline on or within 30 days after alkaline phosphatase elevation ($\geq 2X$ baseline).

When cholestasis screening was performed with modified eDISH plots, a higher percentage of odevixibat-treated patients had elevations in TB and alkaline phosphatase (ALP), which means that patients' cholestasis worsened. A more quantitative assessment (individual narratives assessment) shows that a greater percentage of patients treated with odevixibat experienced elevations in TB (Section 7.7.1). A greater percentage of patients treated with odevixibat experienced cholestatic injury (Table 145 and Table 146). This injury would be difficult to ascertain without a comparator arm, as the disease presents with cholestasis.

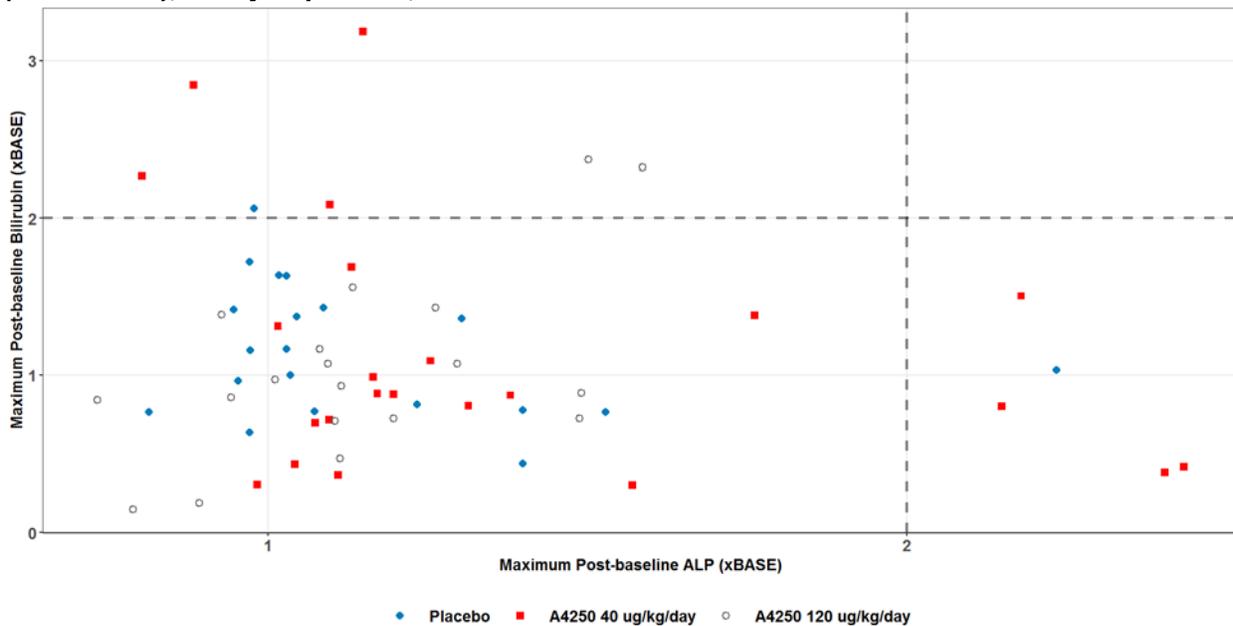
Figure 52. Cholestasis Screening Plot With Total Bilirubin and Adjusted Reference Limits (2X Baseline), Safety Population, Trial A4250-005



Source: adlbsi.xpt; Software: R
Abbreviations: ALP, alkaline phosphatase; BASE, baseline
Each data point represents at least one visit (from a patient) with both ALP and total bilirubin values in the postbaseline period. A cholestatic injury case was defined as total bilirubin equal to or exceeding 2X baseline on or within 30 days after a alkaline phosphatase elevation ($\geq 2X$ baseline).

Figure 53 is plotted to review elevations in direct bilirubin and ALP.

Figure 53. Cholestasis Screening Plot With Direct Bilirubin and Adjusted Reference Limits (2X Baseline), Safety Population, Trial A4250-005



Source: adlbsi.xpt; Software: R
 Abbreviations: ALP, alkaline phosphatase; BASE, baseline
 Each data point represents at least one visit (from a patient) with both ALP and direct bilirubin values in the postbaseline period. A cholestatic injury case was defined as direct bilirubin equal to or exceeding 2X baseline on or within 30 days after alkaline phosphatase elevation ($\geq 2X$ baseline).

When the liver injury was assessed using upper limit of normal values for transaminases and bilirubin, the liver injury signal was not clear (see Table 145 and Table 146).

Table 145. Liver Enzyme Elevations, Safety Population, Trial A4250-005

	Placebo N=20 n (%)	Odevixibat 40 mcg/kg/day N=23 n (%)	Odevixibat 120 mcg/kg/day N=19 n (%)
Liver Enzyme Elevations			
ALT/AST $\geq 3X$ ULN	9 (45)	14 (60.9)	14 (73.7)
ALT/AST $\geq 3X$ ULN and TB $\geq 2X$ ULN	7 (35)	7 (30)	6 (31.6)
ALT/AST $\geq 3X$ ULN and DB $\geq 2X$ ULN	7 (35)	9 (39)	9 (47.4)
ALT/AST $\geq 3X$ ULN and INR $\geq 1.5X$ ULN	0	1 (4.3)	0
ALT/AST $\geq 5X$ ULN	5 (25)	12 (52.2)	4 (21.1)
ALT/AST $\geq 10X$ ULN	0	2 (8.7)	3 (15.8)

Source: adlbsi.xpt; Software: R
 Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; INR, prothrombin international normalized ratio; TB, total bilirubin; ULN, upper limit of normal
 ALT/AST elevation categories are cumulative incidence based.

When the liver injury was assessed as values above “X times” baseline elevation, then also the signal for liver injury was not clear. Patients enrolled in the trial had different baseline transaminases and bilirubin levels. If elevations in transaminases were the only laboratory abnormality at baseline, then values could have been assessed using different categories i.e., assess in three buckets: ALT and AST - normal, <250 U/L, or >250 U/L, and a different “X times” for each category could be employed to ascertain liver injury. However, concurrent

elevations of aminotransferases and total and direct bilirubin made it difficult to interpret liver injury using “X times” baseline elevation.

Therefore, the clinical reviewer decided to assess liver enzymes and bilirubin increase over the absolute value of transaminases and total/direct bilirubin, similar to how a clinician would generally assess in clinical practice, when patients present with abnormally elevated transaminase is and bilirubin (see [Table 27](#), Section [7.7.1](#)).

Table 146. Liver Enzyme Elevations With Adjusted Reference Limits (Baseline), Safety Population, Trial A4250-005

	Placebo N=20 n (%)	Odevixibat 40 mcg/kg/day N=23 n (%)	Odevixibat 120 mcg/kg/day N=19 n (%)
Liver Enzyme Elevations			
ALT/AST ≥3X baseline	0	2 (8.7)	2 (10.5)
ALT/AST ≥3X baseline and TB ≥2X baseline	0	0	0
ALT/AST ≥3X baseline and DB ≥2X baseline	0	0	0
ALT/AST ≥3X baseline and INR ≥1.5X baseline	0	0	0
ALT/AST ≥5X baseline	0	2 (8.7)	1 (5.3)
ALT/AST ≥10X baseline	0	1 (4.3)	0

Source: adlbsi.xpt; Software: R

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; INR, prothrombin international normalized ratio; TB, total bilirubin

ALT/AST elevation categories are cumulative incidence based.

17.3. Liver Test Changes, Trial A4250-005

The following laboratory tests (sBA, AST, ALT, TB, DB, INR) are presented as trellis plots, i.e., for patients overtime laboratory tests evaluation. The laboratory tests are stacked one top of each other for ease of presenting overtime data in individual patient and viewing them concurrently:

- If overtime graphical presentation for laboratory test value is in **blue**, that signifies either “no change” or “worsening” of liver laboratory tests.
- If the overtime graphical presentation for laboratory test value is in **purple**, that indicates reduction in sBA level $\geq 70\%$ AND reduction of liver tests at least one-time postbaseline as well as no worsening of the liver test values, overtime during the trial.
- If the overtime graphical presentation for laboratory test value is in **red**, that would indicate sBA level normalization as well as liver test normalization, **none of the patients achieved liver test normalization.**
- If the overtime graphical presentation for the laboratory test value is in **green**, that indicates, at least one post baseline value demonstrated reduction in sBA levels $\geq 70\%$ and liver test values. After achieving reduction, the liver tests worsened or fluctuated. One patient dosed with odevixibat 120 mcg/kg/day achieved this threshold.

Patient (ID (b) (6)) are excluded from these graphs and plotted on a separate graph as the liver test elevations were significantly elevated.

The Applicant stated that of the patients who met the criteria of reduction in sBA level $\geq 70\%$ few patients also had reduction of liver enzymes, i.e., presented in **purple**:

- Placebo treatment arm (N=20) = one (5%) patient

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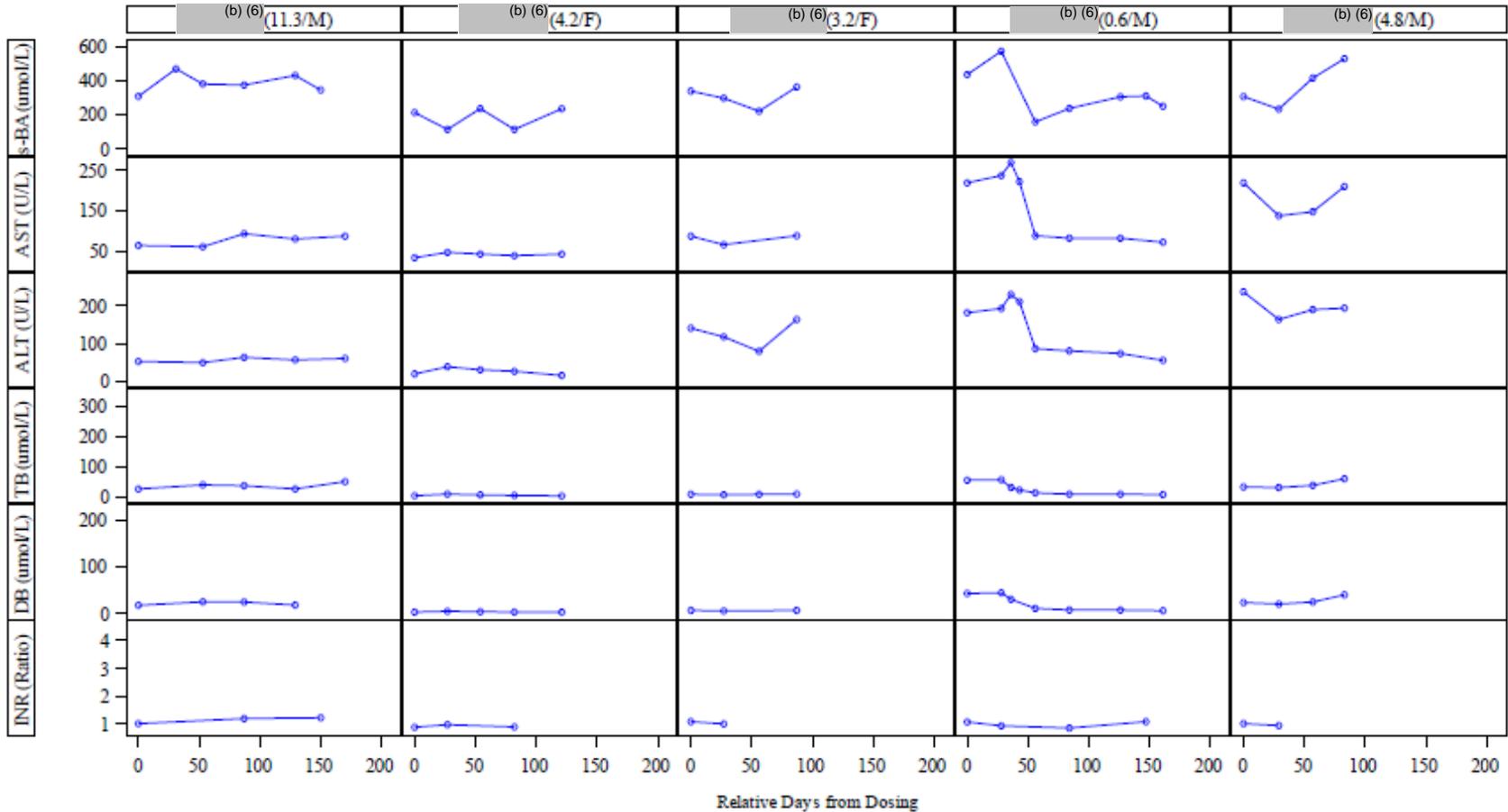
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- Odevixibat 40 mcg/kg/day treatment arm (N23) = six (26%) patients
- Odevixibat 120 mcg/kg/day treatment arm (N=19) = two (10.5%) patients

However, it is difficult to interpret and agree with Applicant's statement. It appears that most patients had stabilization i.e., no worsening of liver tests. Moreover, the scale on trellis plots ranged from 0 to 300 for ALT and AST, and minor reductions were difficult to visualize and appreciate. Therefore reduction, worsening, or normalization were not easy to interpret. But overall, according to the trellis plots, many patients who received placebo also did not have worsening of liver tests overtime.

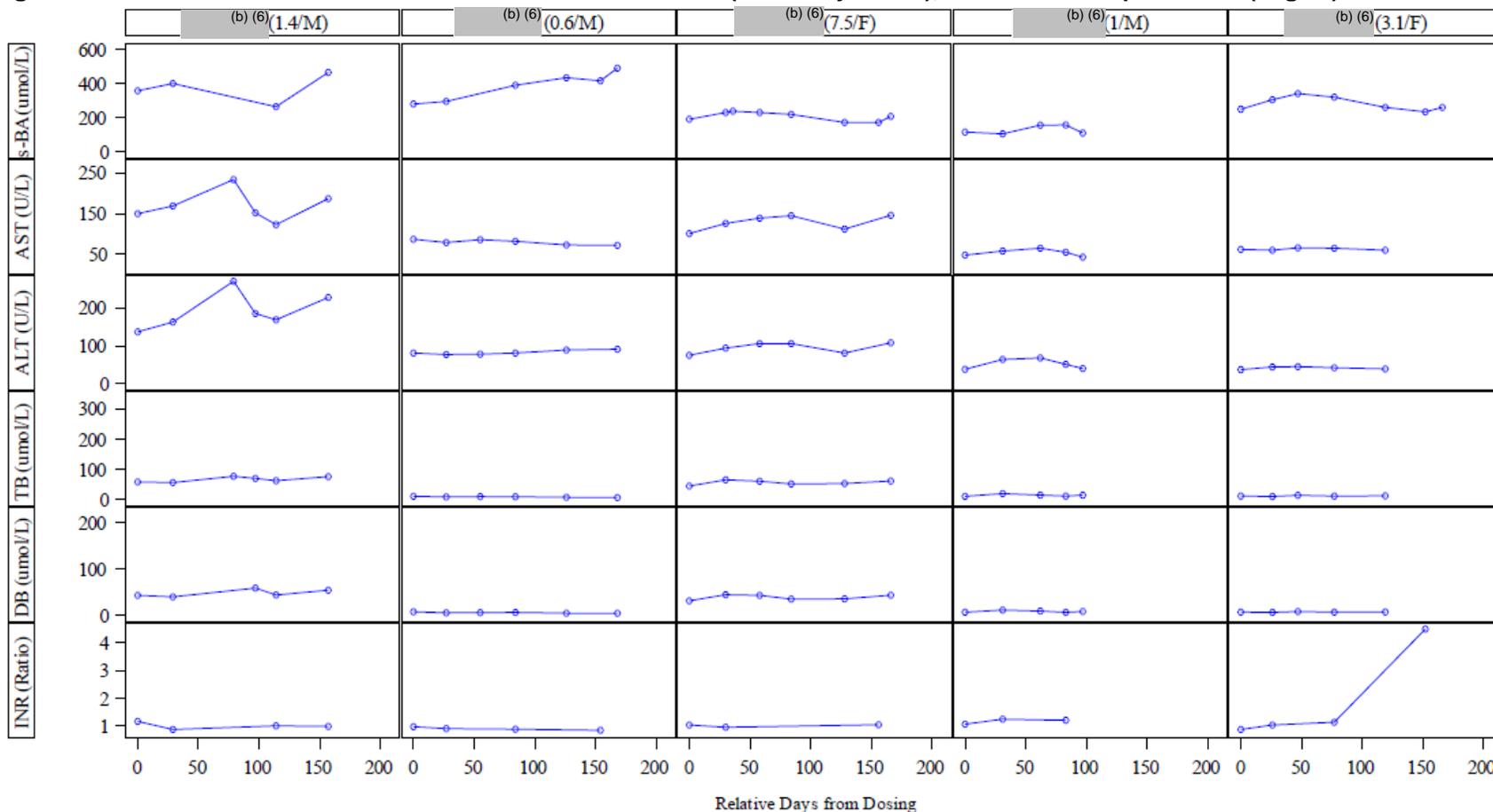
Individual patient Trellis plots overtime for liver tests are presented below for placebo ([Figure 54](#), [Figure 55](#), [Figure 56](#), and [Figure 57](#)), for odevixibat 40 mcg/kg/day ([Figure 58](#), [Figure 59](#), [Figure 60](#), [Figure 61](#), [Figure 62](#), and [Figure 67](#)) and for odevixibat 120 mcg/kg/day ([Figure 63](#), [Figure 64](#), [Figure 65](#), and [Figure 66](#)).

Figure 54. Individual Patient Trellis Plot Over Time for Liver Tests (Full Analysis Set), Treatment Group: Placebo (Page 1)



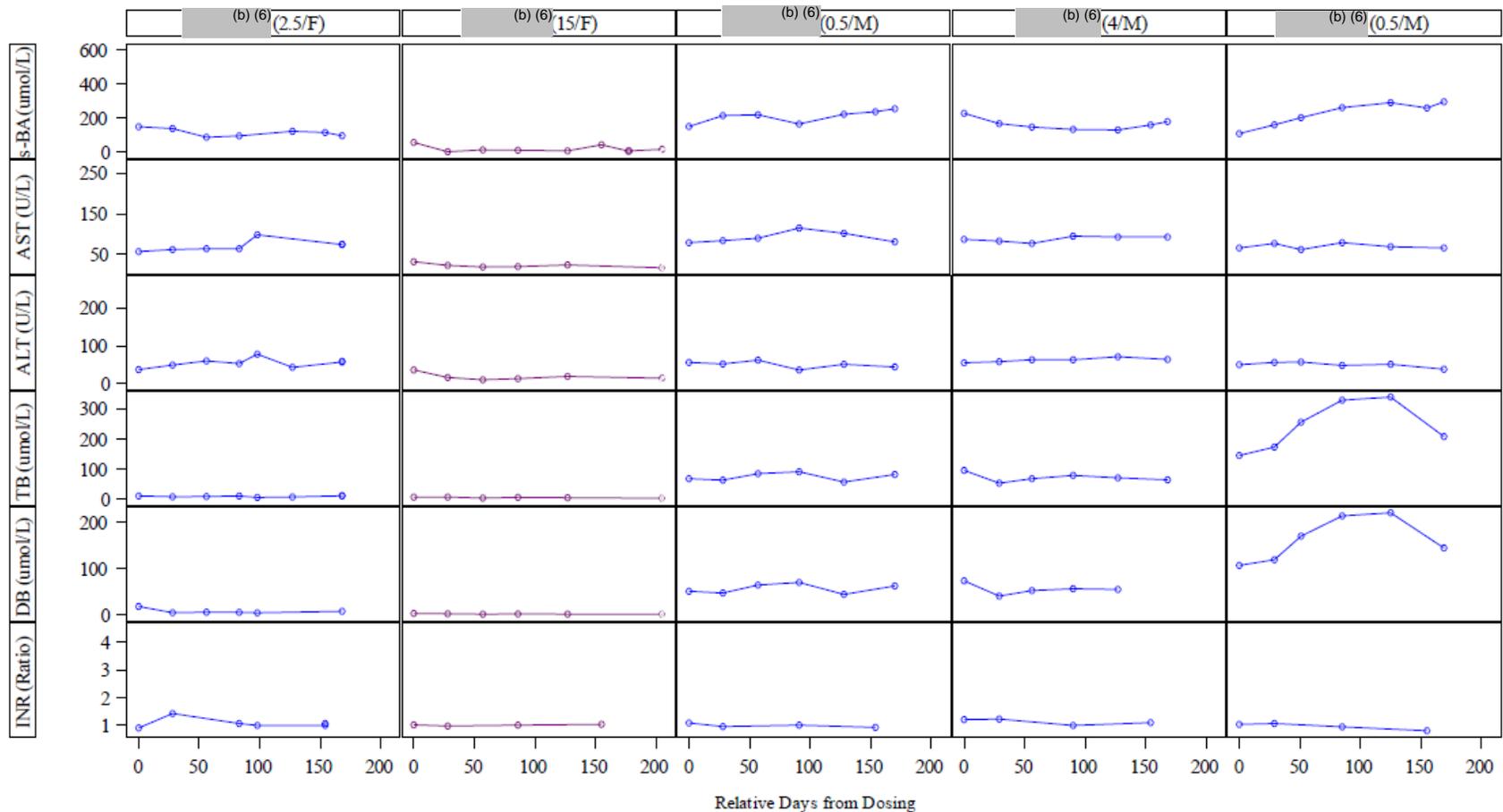
Source: Copied and electronically reproduced from the Applicant's IR Response Submission (March 1, 2021, SD#19)
 Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; F, female; INR, international normalized ratio; M, male; sBA, serum bile acid; TB, total bilirubin

Figure 55. Individual Patient Trellis Plot Over Time for Liver Tests (Full Analysis Set), Treatment Group: Placebo (Page 2)



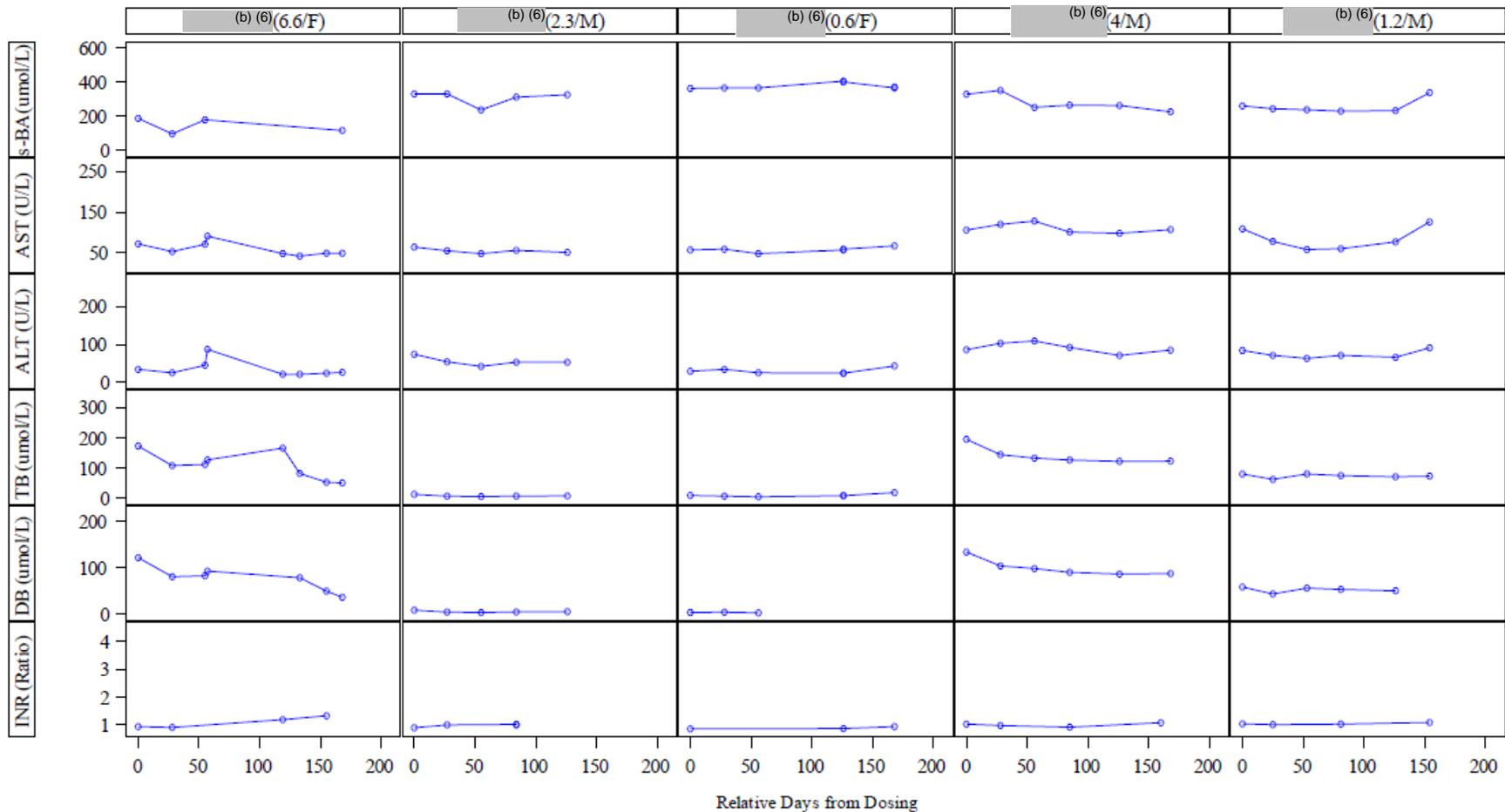
Source: Copied and electronically reproduced from the Applicant's IR Response Submission (March 1, 2021, SD#19)
 Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; F, female; INR, international normalized ratio; M, male; sBA, serum bile acid; TB, total bilirubin

Figure 56. Individual Patient Trellis Plot Over Time for Liver Tests (Full Analysis Set), Treatment Group: Placebo (Page 3)



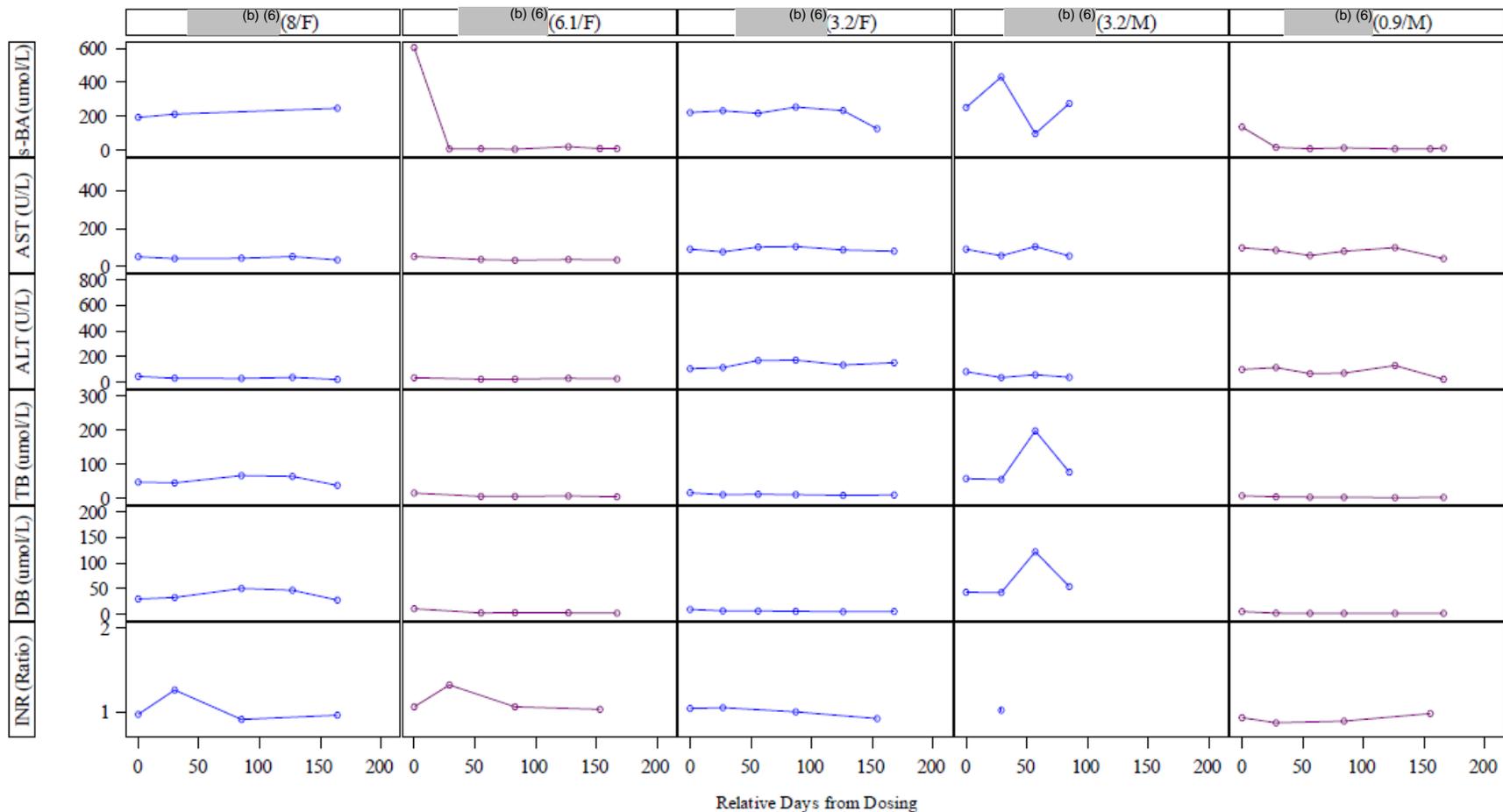
Source: Copied and electronically reproduced from the Applicant's IR Response Submission (March 1, 2021, SD#19)
 Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; F, female; INR, international normalized ratio; M, male; sBA, serum bile acid; TB, total bilirubin

Figure 57. Individual Patient Trellis Plot Over Time for Liver Tests (Full Analysis Set), Treatment Group: Placebo (Page 4)



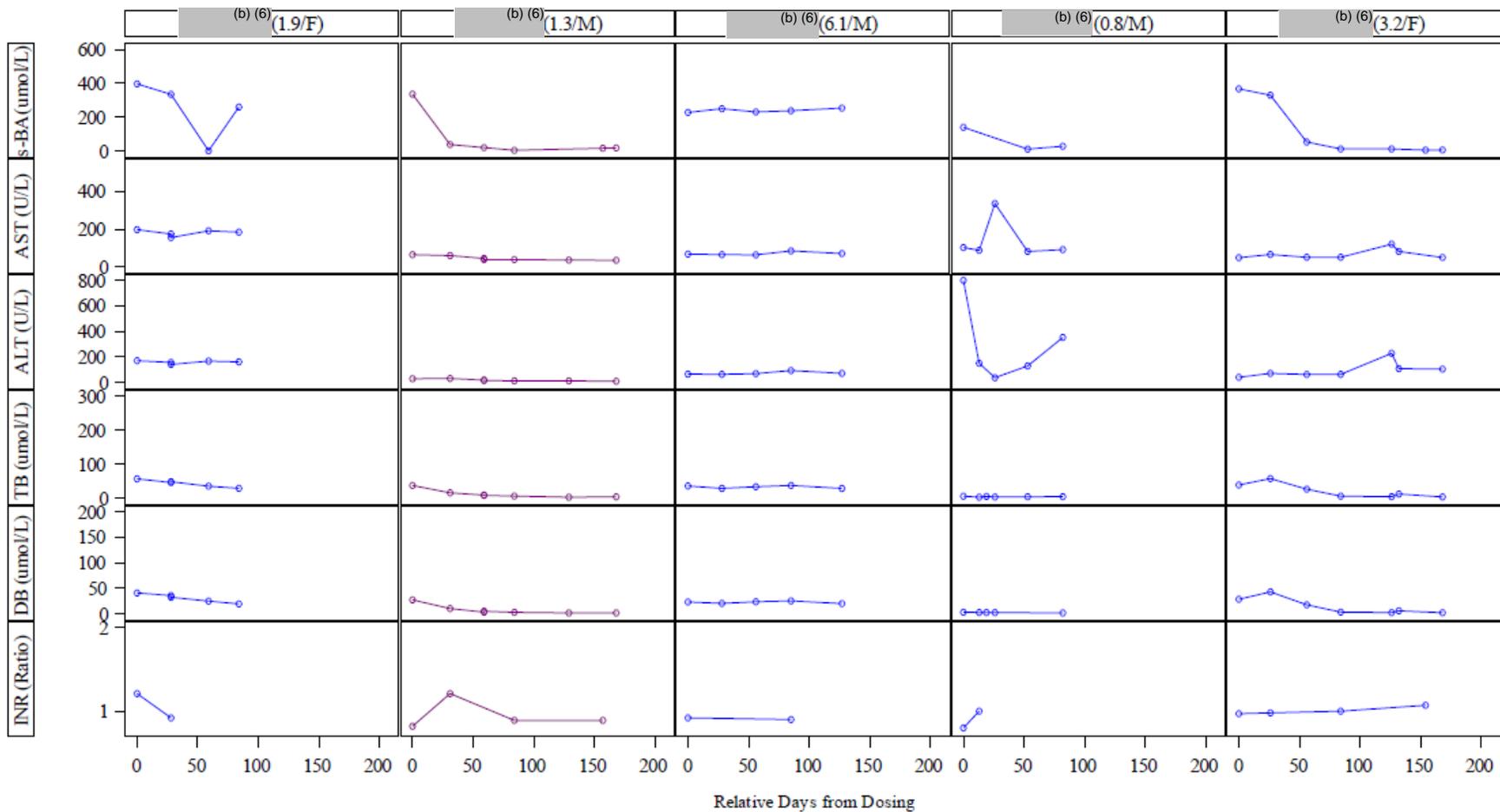
Source: Copied and electronically reproduced from the Applicant's IR Response Submission (March 1, 2021, SD#19)
 Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; F, female; INR, international normalized ratio; M, male; sBA, serum bile acid; TB, total bilirubin

Figure 58. Individual Patient Trellis Plot Over Time for Liver Tests (Full Analysis Set), Treatment Group: Odevixibat 40 mcg/kg/day (Page 1)



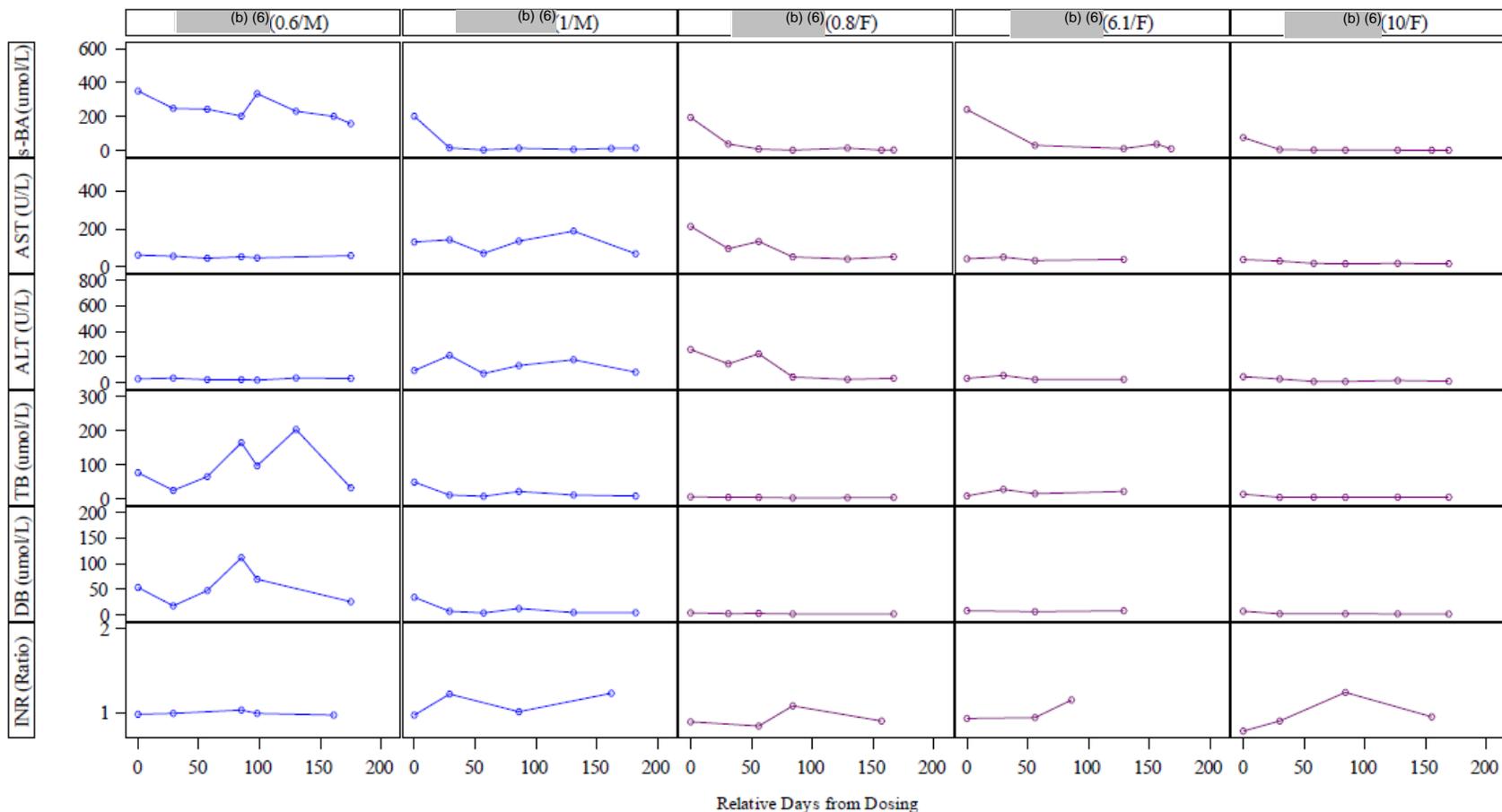
Source: Copied and electronically reproduced from the Applicant's IR Response Submission (March 1, 2021, SD#19)
 Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; F, female; INR, international normalized ratio; M, male; sBA, serum bile acid; TB, total bilirubin

Figure 59. Individual Patient Trellis Plot Over Time for Liver Tests (Full Analysis Set), Treatment Group: Odevixibat 40 mcg/kg/day (Page 2)



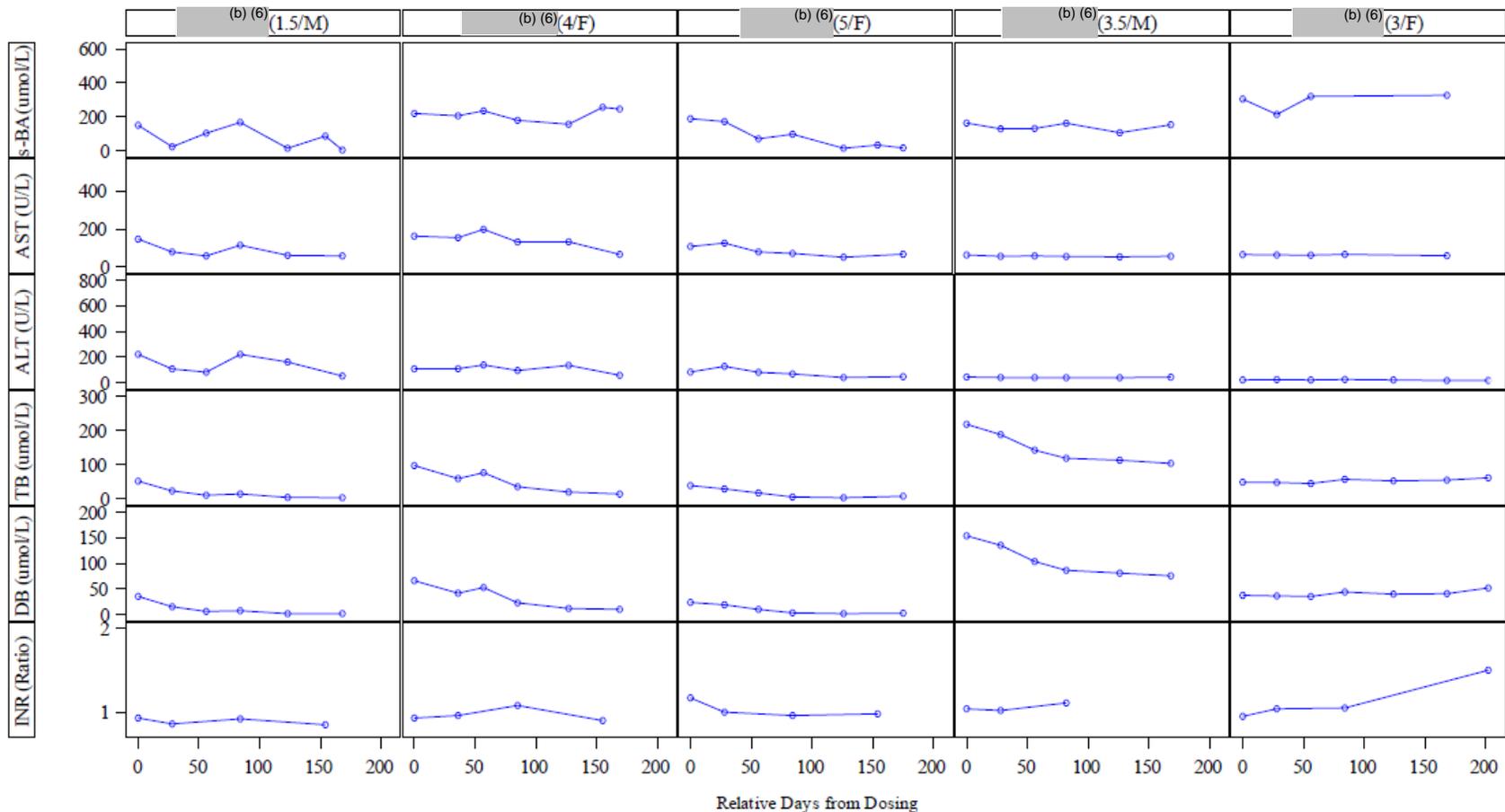
Source: Copied and electronically reproduced from the Applicant's IR Response Submission (March 1, 2021, SD#19)
 Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; F, female; INR, international normalized ratio; M, male; sBA, serum bile acid; TB, total bilirubin

Figure 60. Individual Patient Trellis Plot Over Time for Liver Tests (Full Analysis Set), Treatment Group: Odevixibat 40 mcg/kg/day (Page 3)



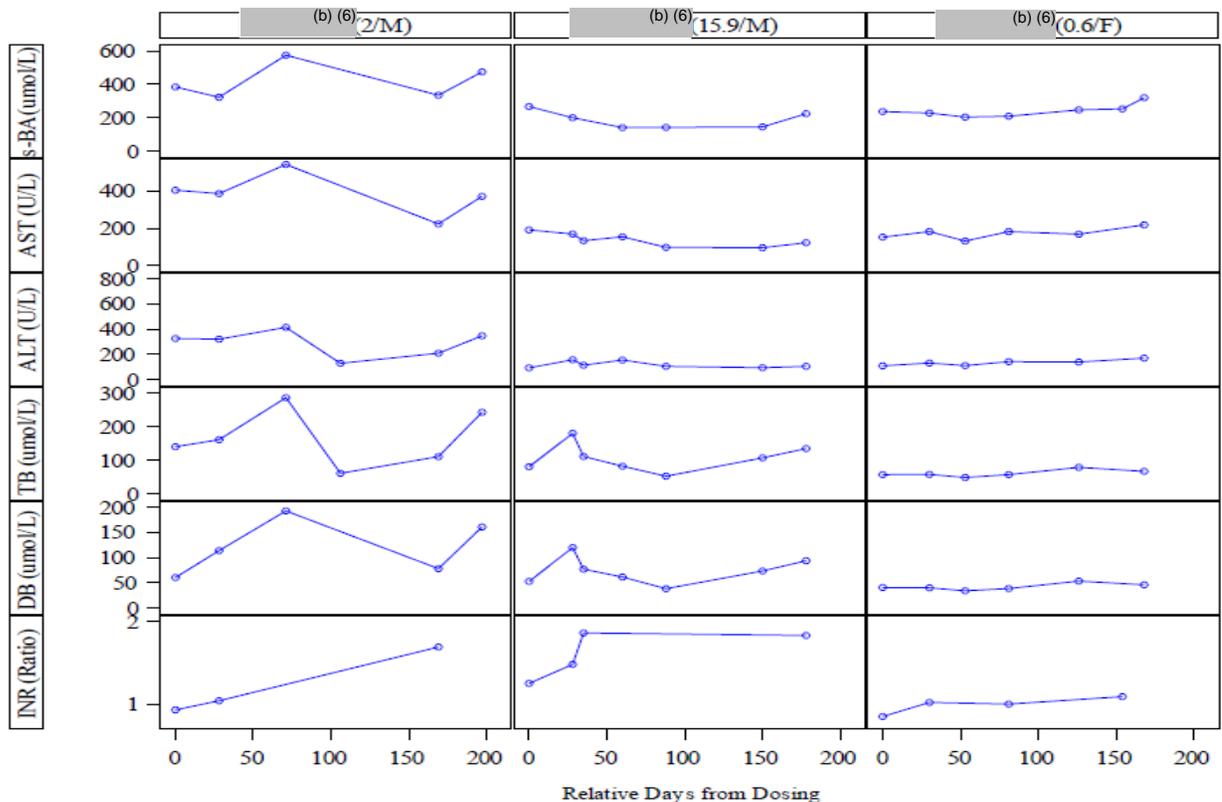
Source: Copied and electronically reproduced from the Applicant's IR Response Submission (March 1, 2021, SD#19)
 Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; F, female; INR, international normalized ratio; M, male; sBA, serum bile acid; TB, total bilirubin

Figure 61. Individual Patient Trellis Plot Over Time for Liver Tests (Full Analysis Set), Treatment Group: Odevixibat 40 mcg/kg/day (Page 4)



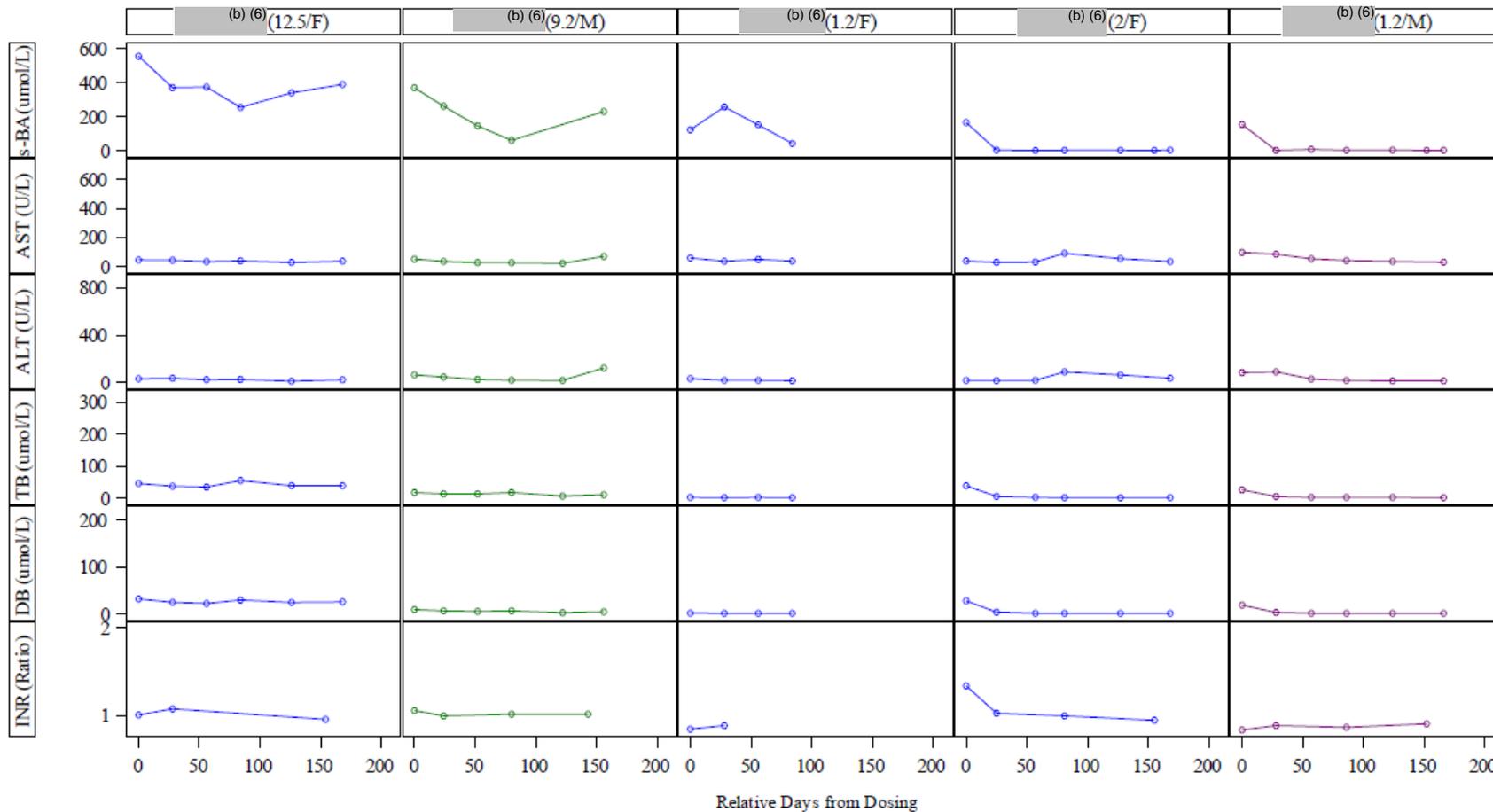
Source: Copied and electronically reproduced from the Applicant's IR Response Submission (March 1, 2021, SD#19)
 Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; F, female; INR, international normalized ratio; M, male; sBA, serum bile acid; TB, total bilirubin

Figure 62. Individual Patient Trellis Plot Over Time for Liver Tests (Full Analysis Set), Treatment Group: Odevixibat 40 mcg/kg/day (Page 5)



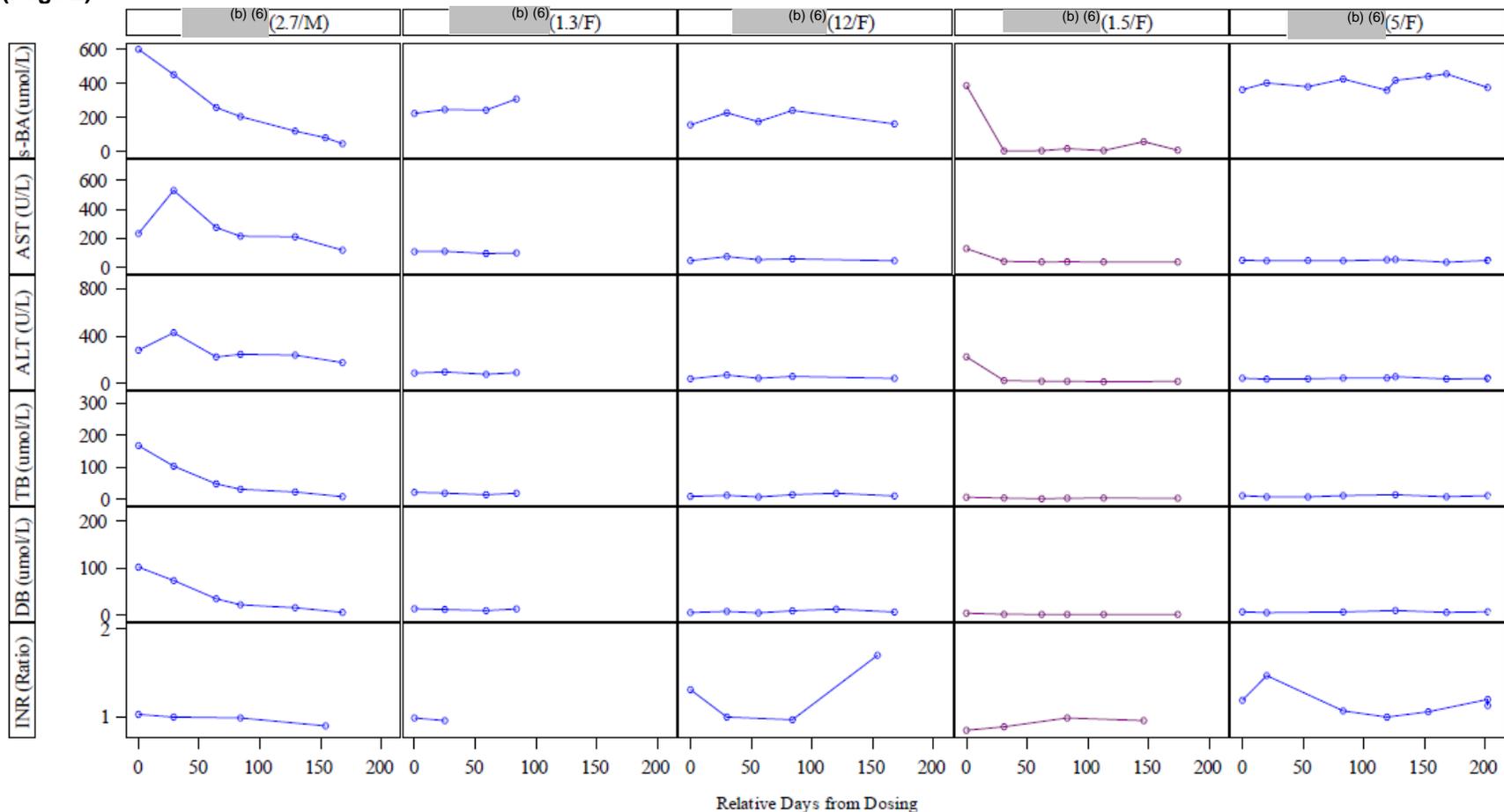
Source: Copied and electronically reproduced from the Applicant's IR Response Submission (March 1, 2021, SD#19)
 Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; F, female; INR, international normalized ratio; M, male; sBA, serum bile acid; TB, total bilirubin

Figure 63. Individual Patient Trellis Plot Over Time for Liver Tests (Full Analysis Set), Treatment Group: Odevixibat 120 mcg/kg/day (Page 1)



Source: Copied and electronically reproduced from the Applicant's IR Response Submission (March 1, 2021, SD#19)
 Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; F, female; INR, international normalized ratio; M, male; sBA, serum bile acid; TB, total bilirubin

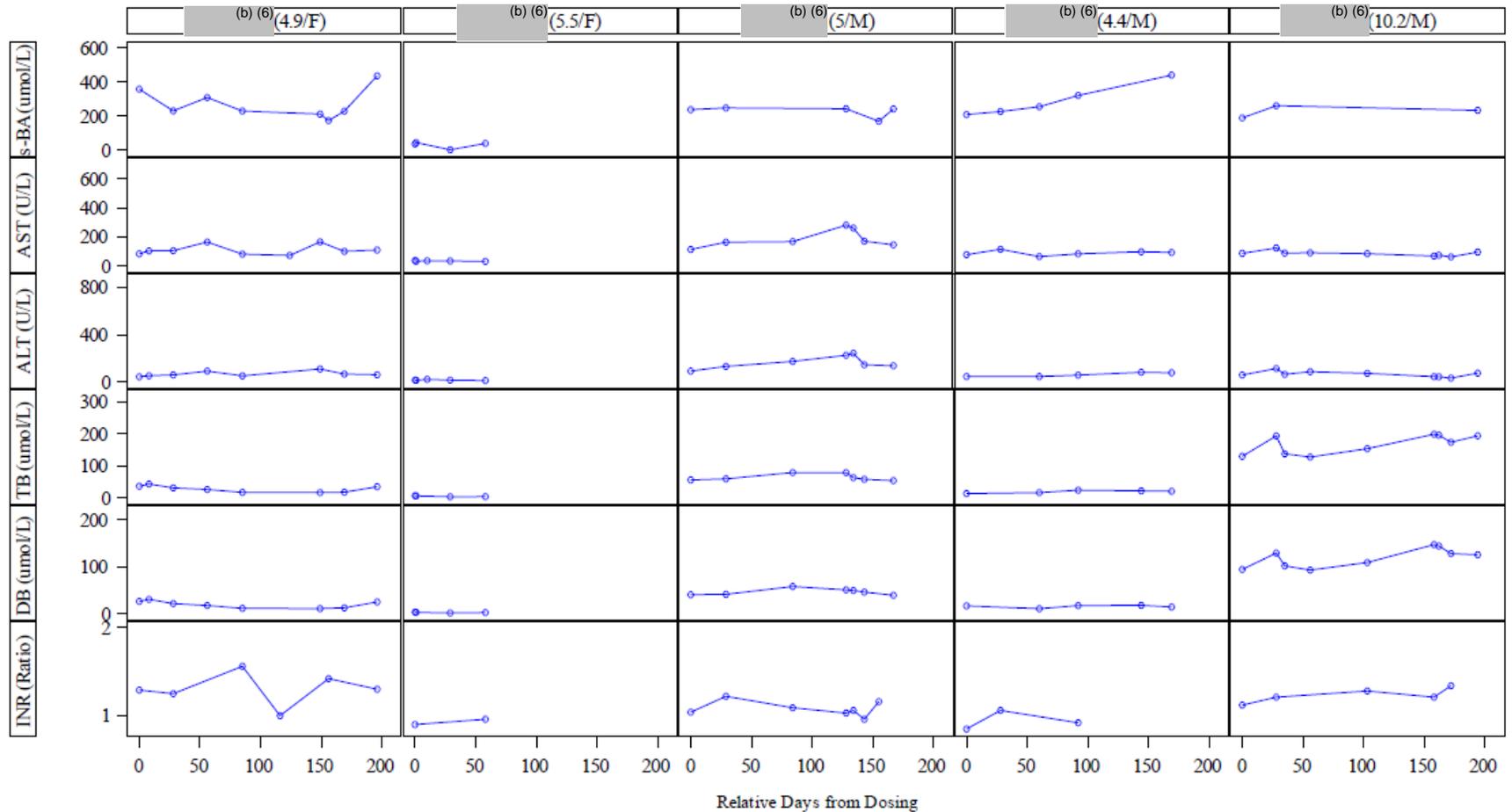
Figure 64. Individual Patient Trellis Plot Over Time for Liver Tests (Full Analysis Set), Treatment Group: Odevixibat 120 mcg/kg/day (Page 2)



Source: Copied and electronically reproduced from the Applicant's IR Response Submission (March 1, 2021, SD#19)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; F, female; INR, international normalized ratio; M, male; sBA, serum bile acid; TB, total bilirubin

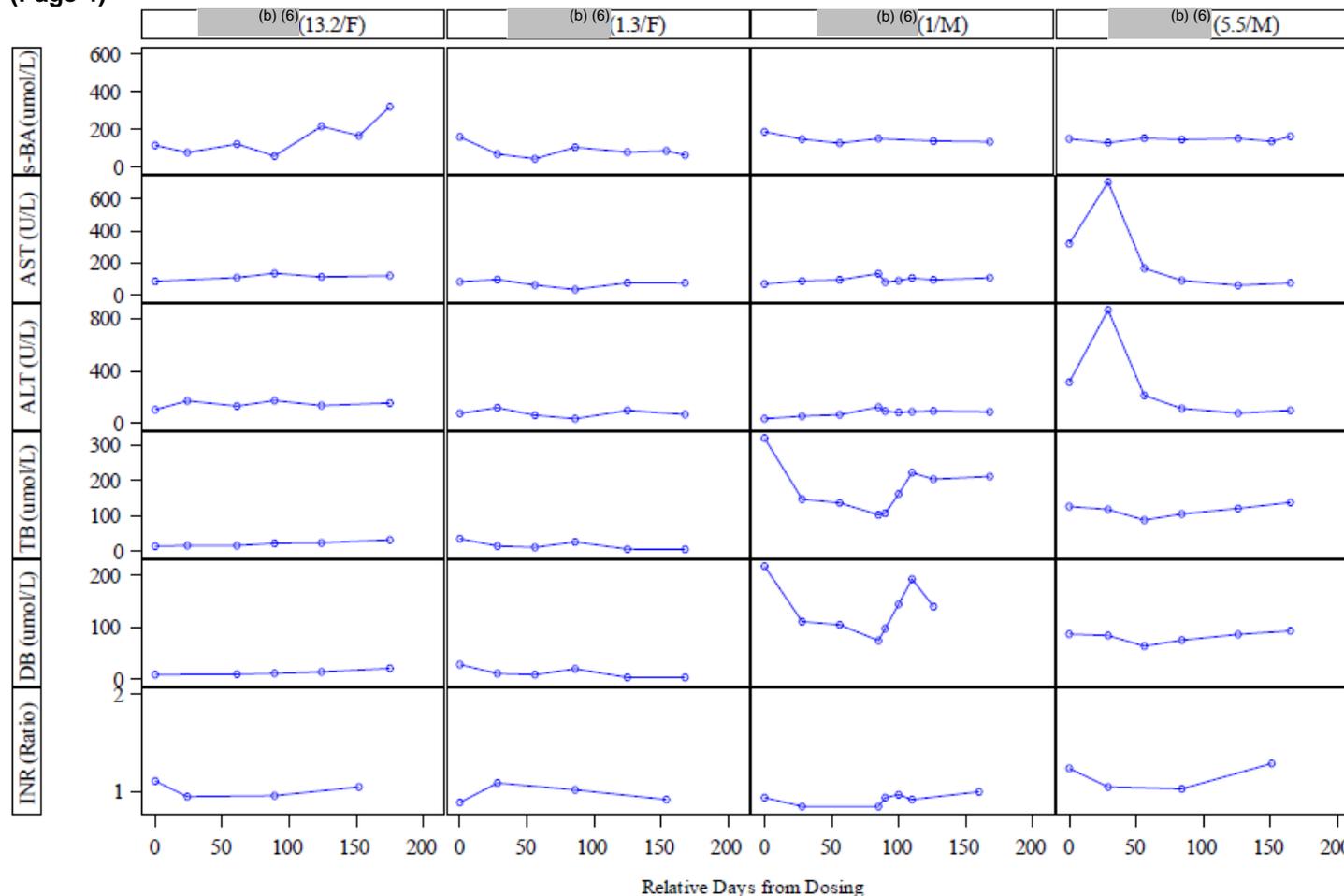
Figure 65. Individual Patient Trellis Plot Over Time for Liver Tests (Full Analysis Set), Treatment Group: Odevixibat 120 mcg/kg/day (Page 3)



Source: Copied and electronically reproduced from the Applicant's IR Response Submission (March 1, 2021, SD#19)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; F, female; INR, international normalized ratio; M, male; sBA, serum bile acid; TB, total bilirubin

Figure 66. Individual Patient Trellis Plot Over Time for Liver Tests (Full Analysis Set), Treatment Group: Odevixibat 120 mcg/kg/day (Page 4)

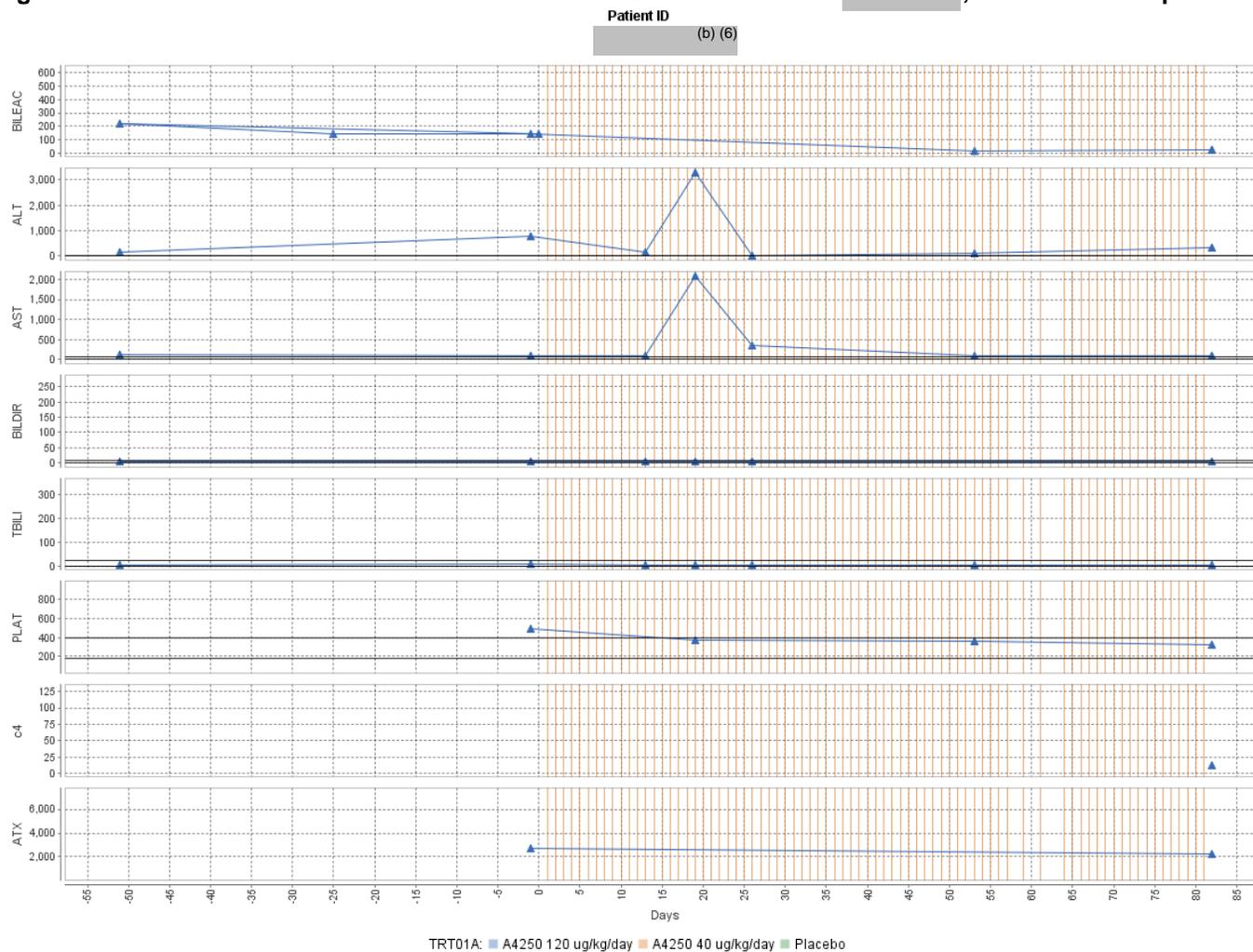


Source: Copied and electronically reproduced from the Applicant's IR Response Submission (March 1, 2021, SD#19)
 Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DB, direct bilirubin; F, female; INR, international normalized ratio; M, male; sBA, serum bile acid; TB, total bilirubin

Patient (ID (b) (6)) dosed with odevixibat 40 mcg/kg/day was not presented in above noted [Figure 66](#) as the elevation of transaminases elevation was too high and would have skewed interpretation of laboratory tests for other patients. Notably, the days on “x” axis range prerandomization values as well as Day 0 (enrollment day) and post baseline values.

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Figure 67. Individual Patient Trellis Plot Over Time for Liver Tests for PT (b) (6), Treatment Group: Odevixibat 40 mcg/kg/day



Source: Copied and electronically reproduced from the Applicant's IR Response Submission (March 1, 2021, SD#19)
 Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATX, autotaxin; BILDIR, direct bilirubin; BILEAC, serum bile acids; c4, plasma 7 α -hydroxy-4-cholesten-3-one concentration (or p-C4); PLAT, platelet; TBILI, total bilirubin

18. Mechanism of Action/Drug Resistance: Additional Information and Assessment

Not applicable.

18.1. Change in sBA Level (micromole/L), Pruritus (as Prespecified in Protocol), Autotaxin (ng/mL) and Plasma (p)-C4 (ng/mL) From Baseline to End-of-Treatment

In patients who achieved normalization of sBA levels (≤ 10 micromole/L) at any time post baseline to end-of-treatment (EOT) were evaluated relative to reduction in pruritus (proportion of positive pruritus assessments at the patient level over the 24-week treatment period, where a positive pruritus assessment was defined as a scratching score of ≤ 1 or at least a 1-point drop from baseline on the Albireo ObsRO instrument), change in autotaxin from baseline to week 12 and week 24 or EOT (if discontinued prior to EOT), and change in p-C4 from baseline to week 12 and week 24 or EOT (if discontinued prior to EOT).

There was a lot of missing data (see [Figure 68](#), [Figure 69](#), and [Figure 70](#)), especially, week 12 data were missing in majority of the patients. Autotaxin is enzyme in serum that converts lysophosphatidylcholine to lysophosphatidic acid (LPA). Elevated levels of autotaxin have been correlated with cholestatic pruritus (Kremer et al. 2010). Plasma 7 α -hydroxy-4-cholesten-3-one concentration (p-C4) is a biomarker for bile acid biosynthesis (Camilleri et al. 2009), as well as bile acid malabsorption (Battat et al. 2019). C4 estimates the rate of hepatic bile acid synthesis rate and correlates with the activity of cholesterol 7-hydroxylase (CYP7A1) (Pattni et al. 2012), which is the rate limiting enzyme of bile acid synthesis. The Applicant did not measure FGF19, which has autocrine function (Chiang 2009), i.e., increase or decrease liver bile acid production by via signaling pathway. pC4 increases with odevixibat treatment, indicating increase bile acid synthesis in liver. If a parallel increase of bile acid secretion into the bile does not occur, then the bile acid l in liver would increase eventually leading to liver injury (see Section [14.2.6](#), [Figure 21](#)).

The data presented in [Figure 68](#), [Figure 69](#), and [Figure 70](#) demonstrate that most patients who achieved normalization of sBA levels, experienced reduction in autotaxin, and increase in p-C4.

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Figure 68. Change in sBA Levels, Pruritus, Autotaxin, and p-C4 From Baseline in Patients Who Achieved Normalization in sBA Levels at Any-Time Postbaseline, Placebo Arm

Treatment group: Placebo

Patient ID/Age (yrs)/Sex	Serum Bile Acids (umol/L)			Change in pruritus from baseline to month 6 or EOT			Autotaxin (ng/mL)			C4 (ng/mL)		
	Baseline	Week 12	Week 22/24[1]	Baseline	Weeks 9-12	Weeks 21-24 or EOT*[2]	Baseline	Week 12	Week 24	Baseline	Week 12	Week 24
(b) (6)/15/F	56.5	11	21.7	1.9	-0.1	0.3	1550		582	8.19		57.1

Source code: ...\\fda-request\info-request-20210301\programs\l_15_sba_norm.sas (15MAR2021 10:48)

Patients may achieve normalization in s-BA at any post-baseline visit.

[1]For treatment completers, the average values of s-BA at Week 22 and Week 24 are used. For early rollover/discontinued patients, sBA result at last visit is used.

[2]For treatment completers/early rollover patients, the final monthly pruritus score is used. For two discontinued patients (b) (6), Weeks 5-8 and Weeks 9-12 pruritus score is used.

EOT*: end of treatment.

s-BA normal range: 0-10 umol/L

Assay ranges: C4: 2-500 ng/mL, Autotaxin: 0.781-50 ng/mL

Source: Copied and Electronically Reproduced from the Applicant's IR Response Submission (March 1, 2021, SD#19)

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Figure 69. Change in sBA Levels, Pruritus, Autotaxin, and p-C4 From Baseline in Patients Who Achieved Normalization in sBA Levels at Any-Time Postbaseline, Odevixibat 40 mcg/kg/day Arm

Treatment group: A4250 40 ug/kg/day

Patient ID/Age (yrs)/Sex	Serum Bile Acids (umol/L)			Change in pruritus from baseline to month 6 or EOT			Autotaxin (ng/mL)			C4 (ng/mL)		
	Baseline	Week 12	Week 22/24[1]	Baseline	Weeks 9-12	Weeks 21-24 or EOT*[2]	Baseline	Week 12	Week 24	Baseline	Week 12	Week 24
(b) (6) /6.1/F	605	5	8.5	3.0	-1.7	-1.4	2500		1600	2		3.48
/0.9/M	136	13	9.5	2	-1.3	-1.1	2740		706	2		4.42
/1.9/F	397	260	260	3.1	-0.6	-0.6	1650	1880		15.5	5.04	
/1.3/M	336	4	17	3.8	-3.8	-3.7	1930			2		34.2
/3.2/F	367.5	12	5.5	2.6	-1.6	-2.6	7440		813	2		37.5
/1/M	201.5	13	13	3	-0.9	-1.0			876			37.7
/0.8/F	194.5	2	2.5	2.5	-0.8	-1.0	3930		1570	2.92		5.04
/6.1/F	241		23.5	3.0	-1.0	-1.1	3070		1680	2		19.9
/10/F	76	2	1	3.1	-3.1	-3.1	4190		428	2		59.7
/1.5/M	150	167	45	2.9	0.0	-0.1	2260		818	2		6.52

Source code: ...\\fda-request\info-request-20210301\programs\1_15_sba_norm.sas (15MAR2021 10:48)

Patients may achieve normalization in s-BA at any post-baseline visit.

[1] For treatment completers, the average values of s-BA at Week 22 and Week 24 are used. For early rollover/discontinued patients, sBA result at last visit is used.

[2] For treatment completers/early rollover patients, the final monthly pruritus score is used. For two discontinued patients (b) (6), Weeks 5-8 and Weeks 9-12 pruritus score is used.

EOT*: end of treatment.

s-BA normal range: 0-10 umol/L

Assay ranges: C4: 2-500 ng/mL, Autotaxin: 0.781-50 ng/mL

Source: Copied and Electronically Reproduced from the Applicant's IR Response Submission (March 1, 2021, SD#19)

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Figure 70. Change in sBA Levels, Pruritus, Autotaxin, and p-C4 From Baseline in Patients Who Achieved Normalization in sBA Levels at Any-Time Postbaseline, Odevixibat 120 mcg/kg/day Arm

Treatment group: A4250 120 ug/kg/day

Patient ID/Age (yrs)/Sex	Serum Bile Acids (umol/L)			Change in pruritus from baseline to month 6 or EOT			Autotaxin (ng/mL)			C4 (ng/mL)		
	Baseline	Week 12	Week	Baseline	Weeks 9-12	Weeks 21-24 or EOT*[2]	Baseline	Week 12	Week 24	Baseline	Week 12	Week 24
			22/24[1]									
(b) (6) /2/F	166	3	3	3.1	-3.1	-3.1	3890		634	16.4		129
/1.2/M	154	3	2.5	3.4	-2.7	-3.3	2800		687	6.27		29.9
/1.5/F	387	17	33	3	-1	-2	2200		696	12.8		31.1
/5.5/F	36		2	1.6		-1.5						

Source code: ...\\fda-request\info-request-20210301\programs\1 15 sba norm.sas (15MAR2021 10:48)

Patients may achieve normalization in s-BA at any post-baseline visit.

[1]For treatment completers, the average values of s-BA at Week 22 and Week 24 are used. For early rollover/discontinued patients, sBA result at last visit is used.

[2]For treatment completers/early rollover patients, the final monthly pruritus score is used. For two discontinued patients (b) (6), Weeks 5-8 and Weeks 9-12 pruritus score is used.

EOT*: end of treatment.

s-BA normal range: 0-10 umol/L

Assay ranges: C4: 2-500 ng/mL, Autotaxin: 0.781-50 ng/mL

Source: Copied and electronically reproduced from the Applicant's IR Response Submission (March 1, 2021, SD#19)

19. Other Drug Development Considerations: Additional Information and Assessment

19.1. Pediatrics Consult Summary and Conclusions

After the pediatric consult was completed, discussions regarding postmarketing requirement and postmarketing commitment were held, including late cycle meeting with the Applicant (May 20, 2021), communications are not captured in this consult. Pediatrics team agreed with PMR and PMC that were issued and labeling changes (for maximum dose), meetings for these discussions were held at later time point during the review cycle (after the review was entered in DARRTS). Summary and conclusions of the Pediatric team consult, entered in DARRTs on April 12, 2021, are noted below.

Applicability of the data submitted in NDA 215498 to the U.S. PFIC population:

8. Applicability of the data submitted in NDA 215498 to the U.S. PFIC population: There is no current published literature to suggest that findings of safety and effectiveness of odevixibat use for the treatment of pruritus in PFIC derived from studies in a population outside the United States would not be applicable to U.S. patients with PFIC.
9. Understanding that there is limited enrollment of patients with BSEP1 (12 of 45) among the PFIC2 cohort in Trial A4250-005, an analysis of pruritus improvement should be undertaken comparing patients with BSEP1 and BSEP2 to ensure there are no obvious differences in efficacy that would suggest clinically meaningful differences between the U.S. and non-U.S. population where the relative proportion of BSEP subtypes may differ.

For assessment of growth in a safety PMR/PMC for odevixibat in pediatric patients with PFIC, DPMH has the following recommendations:

Evaluate whether growth data collected over the 72-week open-label extension trial (Trial A4250-008) is sufficient to assess growth as a safety outcome.

1. A PMR trial assessing growth should be at least 12 months in duration to allow for adequate and interpretable assessment of growth.
2. The primary safety endpoint should focus on assessment of both the absolute change in height/length and changes from baseline in the height/length z-score.
3. The Applicant should submit a draft protocol for Agency review and agreement before initiating the trial.

4. Protocol level comments to consider include the following:

(b) (4)

For assessment of neurocognitive outcomes in a safety PMR for odevixibat, DPMH has the following recommendations:

1. Evaluate whether fat-soluble vitamin (FSV) deficiency data collected over the 72-week open-label extension trial (Trial A4250-008) is sufficient to address the potential impact of long-term odevixibat treatment on neurocognition in pediatric patients with PFIC.
2. PMR assessing neurocognitive outcomes should include patients 4 years of age and older.
3. PMR assessing neurocognition should be at least 24 months in duration and should account for expected neurodevelopmental changes in growing pediatric patients to allow for adequate and interpretable results.
4. Neurocognitive assessments should be performed at a minimum of every 6 months in the study cohort.

5. The Applicant should engage with the Division of Clinical Outcome Assessment early to identify validated neurocognitive measurement tools which could be used to test neurocognitive outcomes across the age range of potential neurodevelopmental susceptibility from odevixibat exposure.
6. The Applicant should submit a draft protocol for Agency review and agreement before initiating the trial.
7. Protocol level comments to consider include the following:

(b) (4)

19.2. Division of Pediatric and Maternal Health (DPMH)

Consult findings from the Division of Pediatric and Maternal Health (DPMH) are summarized below. Consult entered in DARRTS on April 14, 2021, and an addendum was entered in DARRTS on July 1, 2021.

Pregnancy

There are no available data on odevixibat use in pregnant persons. In embryofetal studies, pregnant rabbits exposed to oral odevixibat during organogenesis had a corresponding increase in fetal cardiac malformations, including at low maternal systemic exposures (AUC of 6.28 ng•h/mL). A NOAEL could not be established in the rabbit embryofetal studies.

Clinical pharmacology studies indicate that at the standard treatment dose of 40 mcg/kg/day, odevixibat is minimally absorbed in some participants though the majority of participants do not have measurable systemic absorption. Due to the lack of systemic absorption in most participants, a human AUC cannot be determined at this dose and a direct comparison between human and animal doses is not possible.

Based on the cardiac malformations in rabbits even at low systemic exposures, the lack of establishment of a NOAEL, and the finding that odevixibat has minimal systemic absorption in some participants at the standard treatment dose of 40 mcg/kg, DPMH proposes to add a statement that based on animal studies, odevixibat may lead to cardiac malformations in Subsection 8.1 Risk Summary of labeling.

Pregnancy has rarely been reported in patients with PFIC since most patients will undergo liver transplantation by the age of 10 and will not require additional treatment. Given that pregnancy is unlikely in this rare disease pediatric population, a postmarketing pregnancy safety study is not recommended at this time.

Lactation

Odevixibat is minimally absorbed following oral administration, and breastfeeding is not expected to result in exposure of the infant to odevixibat. There are no data on the presence of odevixibat in animal or human milk, the effects on the breastfed infant, or the effects on milk production. Subsection 8.2 will include standard benefit/risk statement.

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Pregnancy has rarely been reported in patients with PFIC; therefore, a lactation study is not recommended at this time.

Females and Males of Reproductive Potential Animal data in rats did not show effects on fertility. There are no human fertility data with odevixibat use in female and males of reproductive potential.

Labeling Recommendations

DPMH revised subsections 8.1 and 8.2 of labeling for compliance with the PLLR. DPMH discussed our labeling recommendations with the Agency on April 8, 2021. DPMH recommendations are below and reflect the discussions with DHN. DPMH refers to the final NDA action for final labeling.

Addendum to DPMH Consult Entered in DARRTS on July 1, 2021

While pregnancy in PFIC is rare, it is possible for patients with PFIC to become pregnant. Based on the fetal cardiac malformations observed in animal reproduction studies, there may be risks to the fetus from exposure to odevixibat during pregnancy. DPMH agrees that collecting postmarketing information to assess maternal and fetal outcomes in patients with PFIC who become pregnant while undergoing treatment with odevixibat is reasonable because there are currently no available human data to inform the safety of odevixibat during pregnancy.

Conclusion

DPMH agrees with DHN's plan to issue a PMR.

19.3. Summary and Conclusion from Neurology Consult

Division of Neurology-1 consult review entered in DARRTS on May 5, 2021, is summarized below.

The neurology reviewer concludes that it would be very challenging to demonstrate an impact of treatment on neurocognitive outcomes, especially in this small, heterogenous patient population that is already considered high risk for neurodevelopmental delays secondary to their underlying condition, and without any available baseline assessments. An uncontrolled, long-term follow-up study would not be adequate to detect a safety signal for cognition or development in this patient population.

20. Data Integrity-Related Consults (Office of Scientific Investigations, Other Inspections)

Key Findings Noted in OSI Review

Clinical data from Trial A4250-005 and A4250-008 (an extension trial) were submitted to the Agency in support of this New Drug Application (NDA 215498) for the use of odevixibat for the treatment of pruritus in patients with progressive familial intrahepatic cholestasis. The extension trial was reviewed for safety only. Study drug approval will be based on the results of Trial A4250-005. Two clinical investigators who participated in Trial A4250-005 were selected for inspection: Dr. Patrick McKiernan [Site 25111] and Dr. Buket Dalgic [Site 23102] and the Applicant Albireo Pharma, Inc. were inspected. The inspections found no significant regulatory violations at neither of the two investigator sites nor the Applicant Albireo Pharma, Inc. The Applicant's submitted clinical data listings were verifiable against source records, with no discrepancies identified. The clinical data generated by the inspected investigators appear reliable.

Based on the results of these inspections, Trial A4250-005 appears to have been conducted adequately, and the clinical data generated from inspected sites appear to be reliable in support of this NDA.

21. Labeling Summary of Considerations and Key Additional Information

For the final labeling, please see finalized Prescribing Information (PI) submitted to 1.14 of the docuBridge. The changes in PI are summarized below.

DRM and DHN agree that the potential risks of odevixibat can be adequately managed in the postmarket setting through labeling alone, with the exception of a pregnancy registry that will be a PMR, and that a REMS is not necessary to ensure that the drug's benefit will outweigh the risks.

[Table 147](#) summarizes major changes (additions, deletions, or modifications) made by the FDA to the PI proposed by the Applicant. The rationale for the FDA recommendation is provided in the table based on the evidence from Trials A4250-005 and A4250-008, regulatory principles, literature review, and interactive review with the Applicant. Refer to Sections [6](#) and [7](#) for specific information. Another labeling document, Instruction for Use, was drafted during the review of the NDA to assist patients and caretakes to take the oral pellets and capsules.

Table 147. FDA Recommendations to the Prescribing Information

Section and Title	FDA Recommendation	Rationale
1. Indication and Usage	<ul style="list-style-type: none"> Specified the age range as “3 months and older” Deleted (b) (4) Added Limitation of Use: PFIC type 2 patients with ABCB11 variants resulting in nonfunctional or complete absence of BSEP protein type 3 (BSEP3) 	<ul style="list-style-type: none"> Approve across all age groups in patients with PFIC (b) (4) Patient with nonfunctional protein expression, drug may not have efficacy
2. Dosage and Administration	<ul style="list-style-type: none"> Changed the recommended dose to 40 mcg/kg/day, if no response is observed then increase to 80 mcg/kg/day; and if no response is observed then further increases to 120 mcg/kg/day Cap the maximum daily dose to 6 mg Modified daily dosage table and administration procedures Modified Dose Modification to manage liver test abnormality and diarrhea Added precaution of not mixing oral pellets with fluids Instruction for Use added 	<ul style="list-style-type: none"> Option for both doses provided to physicians, even though, 120 mcg/kg dose did not perform better than 40 mcg/kg in efficacy and safety Clarify daily dose and number of Oral Pellets and Capsules given Liver test abnormality are common AE, which need monitoring and dose modification Infants who are 3 months of age may only consume milk as food and solids might not be introduced, There was no compatibility data for mixing odevixibat in liquids for administration to infants who are on liquid diet
3. Dosage Forms and Strengths	<ul style="list-style-type: none"> Changed name (b) (4) to Oral Pellets and (b) (4) to Capsule 	<ul style="list-style-type: none"> Avoid medical errors
4. Contraindications	<ul style="list-style-type: none"> No change 	<ul style="list-style-type: none"> No contraindications were identified during the review
5. Warnings and Precautions	<p>Added three key risks observed during the trial:</p> <ol style="list-style-type: none"> Liver test abnormalities Diarrhea Fat-soluble vitamin deficiency 	<ul style="list-style-type: none"> These adverse reactions were commonly observed in the trials. These could lead to significant medical consequences. The three risks should be monitored and treatment to be interrupted or discontinued based on severity of clinical condition.
6. Adverse Reactions	<ul style="list-style-type: none"> Modified the adverse reaction table based on FDA analyses Added description of AE from the ongoing Trial A4250-008 Deleted (b) (4) 	<ul style="list-style-type: none"> To reflect occurrence of AE that occurred in >2% patients treated with odevixibat relative to placebo To provide safety finding from additional open-label trial To avoid redundancy with Warnings and Precautions
7. Drug Interactions	<ul style="list-style-type: none"> Added potential interaction with bile acid binding resins 	<ul style="list-style-type: none"> To avoid drug-drug interactions in gut lumen
8. Use in Specific Populations	<ul style="list-style-type: none"> Added risk of cardiac malformations from animal studies to Highlights under Pregnancy Modified Pediatric Use Modified hepatic impairment section Deleted (b) (4) 	<ul style="list-style-type: none"> Emphasize the animal data of pregnancy risk To be consistent with other parts of the PI for pediatric use Specify information on hepatic impairment

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Section and Title	FDA Recommendation	Rationale
9. Drug Abuse and Dependence	<ul style="list-style-type: none"> • Omitted by Applicant 	<ul style="list-style-type: none"> • No data
10. Overdosage	<ul style="list-style-type: none"> • Omitted by Applicant 	<ul style="list-style-type: none"> • No data
11. Description	<ul style="list-style-type: none"> • Changed the names of dosage forms 	<ul style="list-style-type: none"> • To be consistent with the names in Dosage forms and strength
12. Clinical Pharmacology	<ul style="list-style-type: none"> • Modified the Mechanism of Action • Specified results of serum bile acid levels in Pharmacodynamics section 	<ul style="list-style-type: none"> • Provide data from the trials
13. Nonclinical Toxicology	<ul style="list-style-type: none"> • Created subtitles for each section • Described nonclinical findings 	<ul style="list-style-type: none"> • To improve readability • Added information on nonclinical findings in rodent and rabbit studies
14. Clinical Studies	<ul style="list-style-type: none"> • Revised the text for description of Trial 1 • Changed Efficacy Table and Figure to FDA approved analyses • Deleted serum bile acid results from this section and moved it to section 12 	<ul style="list-style-type: none"> • To provide key trial information and increase readability • FDA did not agree (b) (4) • (b) (4) sBA level is considered as pharmacodynamic marker, therefore moved to Section 12.
15. References	<ul style="list-style-type: none"> • Omitted by Applicant 	<ul style="list-style-type: none"> • No need for references
16. How Supplied/ Storage and Handling	<ul style="list-style-type: none"> • Changed names of dosage forms 	<ul style="list-style-type: none"> • To be consistent other parts of PI
17. Patient Counseling Information	<ul style="list-style-type: none"> • Deleted redundant information of administration procedures • Added information for risks 	<ul style="list-style-type: none"> • To be consistent with modified PI
18. Instruction for Use (IFU)	<ul style="list-style-type: none"> • Requested the Applicant to generate this separate labeling document during the review to direct patients and caregivers how to use BYLVAY Capsule and Oral Pellets and to avoid medical errors. 	<ul style="list-style-type: none"> • This IFU is needed to clarify a rather complicated administration process involving two dosage forms (capsule versus pellets) for specifying accurate instructions for use and so as to avoid medical errors while administering odevixibat.

Source: Generated from Original PI proposal and FDA final PI
 Abbreviations: AE, adverse event; IFU, Instruction For Use; PFIC, progressive familial intrahepatic cholestasis; PI, Prescribing Information; FDA, Food and Drug Administration; BSEP, bile salt export pump

22. Postmarketing Requirements and Commitments

With the approval of odevixibat, the Division is issuing four postmarketing commitments (PMCs) and one postmarketing requirement (PMR).

Safety PMR (4109-1)

“Conduct a worldwide descriptive study that collects prospective and retrospective data in women exposed to BYLVAY (odevixibat) during pregnancy and/or lactation to assess risk of pregnancy and maternal complications, adverse effects on the developing fetus and neonate, and adverse effects on the infant. Infant outcomes will be assessed through at least the first year of life. The study will collect information for a minimum of 10 years.”

Rationale

In animal reproduction studies, fetal cardiac malformations were observed in rabbits. There may be risks to the fetus from exposure to odevixibat during pregnancy. The goal of this study is to evaluate the long-term safety of odevixibat in women exposed during pregnancy, including assessing risks of pregnancy complications and adverse effects on the developing fetus and neonate. Data are needed on the safe use of odevixibat during pregnancy as there is currently no available human data to inform the safety of odevixibat during pregnancy.

We discussed with the Division of Epidemiology whether the Sentinel Distributed Database (SDD) would be a suitable data source in which to conduct this PMR. Based on this discussion the SDD will not be suitable for the following reasons:

8. DHN requests a study population defined as females of child-bearing age exposed to odevixibat during pregnancy and/or lactation. The SDD does not permit accurate identification of lactating women.
9. PFIC 1 and 2 are uncommon genetic conditions. Therefore, DHN requests a worldwide study to provide an adequate number of exposed patients for meaningful risk assessment. The SDD covers only a portion of the U.S. population.
10. A descriptive pregnancy safety study requires targeted questionnaires for collection of detailed and specific information about important confounders (e.g., body mass index and illicit drug use) and the timing of drug exposures in relation to well-defined pregnancy outcomes. The requirement for targeted questionnaires necessitates data collection not possible in SDD.

PMC #1 (4109-02)

“A prospective, long-term, observational study of patients aged 3 months or older with progressive familial intrahepatic cholestasis PFIC intended to assess the long-term safety of treatment with BYLVAY (odevixibat) over a 72-weeks treatment period.”

Because the odevixibat safety database is small, we seek additional long-term safety data to ensure that we have identified the important safety concerns.

Rationale

Safety of long-term odevixibat use (>6 months) has not been established in a double-blind, placebo-controlled trial. Data collected under this PMC may help characterizing safety and identifying if there is (are) increases in morbidity or mortality relative to natural progression of disease.

PMC #2 (4109-3)

Conduct a 5-year registry-based study to collect data on the course of liver disease in patients chronically treated with BYLVAY (odevixibat). Report yearly on the incidence of biliary diversion surgery, liver transplantation, and death (all-cause mortality; liver disease-related mortality) in patients chronically treated with odevixibat. Compare the incidence of these safety outcomes to a control (non-odevixibat-treated) group.

Rationale

IBAT inhibitors such as odevixibat increase the concentration of bile acids in the liver, potentially promoting a more rapid deterioration of liver function. Because the odevixibat safety database is small, we seek additional long-term safety data to assess whether treatment of pruritus with odevixibat hastens time to liver failure or its related complications, necessitating

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biliary diversion or liver transplant sooner than anticipated in patients with PFIC. A 5-year registry study will provide data to help assess this risk.

PMC #3 (4109-4)

Conduct an in vitro compatibility study with the drug product and each of the following liquids: water, juice, breast milk, infant formulas (most commonly used formulas in this population) to assess for degradation of the drug product in these vehicles. The compatibility study should test for description, assay, and degradation products.

Rationale

Compatibility with soft foods (i.e., apple sauce, baby food, yogurt) was supported by in vitro studies. However, compatibility with liquids such as baby formula, breast milk, or water for pediatric patients who have not started solid foods was not studied. Refer to the OPQ review for more details. Because the indication will go down to 3 month old infants, and not all infants of that age are expected to have started solid foods, it is particularly important to determine the compatibility of the odevixibat oral pellets with liquids typically ingested by 3 to 6 month old infants.

PMC #4 (4109-5)

Dissolution Method

Optimize the dissolution method for quality control to address the following:

- Provide additional justification (b) (4)

[Redacted text block]

[Large redacted text block] (b) (4)

23. Financial Disclosure

Table 148. Covered Clinical Studies, Trials A4250-003, A4250-005, and A4250-008

Was a list of clinical investigators provided	Yes <input checked="" type="checkbox"/>	No <input type="checkbox"/> (Request list from Applicant)
Total number of investigators identified: 81		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 2		
(b) (6)		
<p>If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c), and (f)):</p> <p>Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0</p> <p>Significant payments of other sorts: 2</p> <p>Proprietary interest in the product tested held by investigator: 0</p> <p>Significant equity interest held by investigator: 0</p> <p>Sponsor of covered study: 0</p>		
Reviewer's Comment:		
(b) (6)		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes <input checked="" type="checkbox"/>	No <input type="checkbox"/> (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes <input checked="" type="checkbox"/>	No <input type="checkbox"/> (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3): 0		
Is an attachment provided with the reason:	Yes <input checked="" type="checkbox"/>	No <input type="checkbox"/> (Request explanation from Applicant)

24. References

- Agarwal, S, BB Lal, D Rawat, A Rastogi, KG Bharathy, and S Alam, 2016, Progressive Familial Intrahepatic Cholestasis (PFIC) in Indian Children: Clinical Spectrum and Outcome, *J Clin Exp Hepatol*, 6(3):203-208.
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25. Review Team

Table 149. Reviewers of Integrated Assessment

Role	Name(s)
Regulatory project manager	Ayanna Augustus Bryant, PhD, RAC
Nonclinical reviewer	Fresnida Ramos and Ke Zhang
Nonclinical team leader	David Joseph, PhD
OCP reviewer(s)	Sojeong Yi, PhD Jielin (Jillian) Sun, PhD (Pharmacogenomics)
OCP team leader(s)	Christian Grimstein, PhD (Pharmacogenomics TL) Lian Ma, PhD (Pharmacometrics TL, DPM) Insook Kim, PhD
Clinical reviewer	Yao-Yao Zhu, MD, PhD
Clinical team leader	Ruby Mehta, MD
Statistical reviewer	Hyoyoung Choo-Wosoba and Rebecca Hager
Statistical team leader	George Kordzakhia
Cross-disciplinary team leader	Ruby Mehta, MD
Division director (pharm/tox)	Sushanta Chakder PhD
Division director (OCP)	Suresh Doddapaneni, PhD, Acting Division Director, DIIP
Division director (OB)	Laura Lee Johnson, PhD, Division Director
Division director (clinical)	Joseph Toerner, MD
Office director (or designated signatory authority)	Julie Beitz, MD

Abbreviations: OB, Office of Biostatistics; OCP, Office of Clinical Pharmacology

Table 150. Additional Reviewers of Application

Office or Discipline	Name(s)
OPQ	Zhengfang Ge, Ph.D., Drug Product reviewer Hitesh Shroff, Ph.D., CMC Lead Wendy Wilson, Ph.D., Branch Chief Gaetan Ladouceur, Ph.D., API Reviewer, Donna Christner, Ph.D., API Branch Chief Sachinkumar Patel, MS, Reviewer Yubing Tang, Ph.D., Review Lead, OPMA, Jia Yin, Ph.D., Biopharmaceutics Reviewer Tapash Ghosh, Ph.D., Review Lead
Microbiology	N/A
OPDP	Meeta Patel, PharmD, Reviewer Kathleen Klemm, Team Leader
OSI	Zana Marks, MD, MPh Min Lu, MD, MPh, Team Leader Kassa Ayalew, MD, MPh, Branch Chief
OSE/DEPI	N/A
OSE/DMEPA	Sherly Abraham, RPh, Reviewer Idalia Rychlik, PharmD, Team Leader
OSE/DRISK	Carlisha Gentles, Reviewer Jacqueline Sheppard, Team Leader
DCOA	Yujin Chung, PharmD, COA Reviewer Onyeka Illoh, OD, MPH, COA Team Leader (Acting) David Reasner, PhD, Division Director
PFSS	Marian Strazzeri, MS, PFSS Reviewer Lili Garrard, PhD, PFSS Team Leader Laura Lee Johnson, PhD, Division Director
Other	

Abbreviations: DCOA, Division of Clinical Outcome Assessment; DEPI, Division of Epidemiology; DMEPA, Division of Medication Error Prevention and Analysis; DRISK, Division of Risk Management; OPDP, Office of Prescription Drug Promotion; OPQ, Office of Pharmaceutical Quality; OSE, Office of Surveillance and Epidemiology; OSI, Office of Scientific Investigations; PFSS, Patient-Focused Statistical Support

Table 151. Signatures of Reviewers

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Clinical	Yao-Yao Zhu	OND/DHN	3, 4, 7.4, 7.5, 7.6, 7.7, 15, 21, 23 <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Contributed <input type="checkbox"/> Approved
Primary Reviewer	Signature: Yao-yao Zhu -S <small>Digitally signed by Yao-yao Zhu -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Yao-yao Zhu -S, 0.9.2342.19200300.100.1.1=2000429369 Date: 2021.07.16 13:27:21 -04'00'</small>		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Regulatory Project Management	Ayanna Augustus Bryant	OND/DHN	Section 12 <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Contributed <input checked="" type="checkbox"/> Approved
CPMS	Signature: Ayanna Augustus -S <small>Digitally signed by Ayanna Augustus -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000342669, cn=Ayanna Augustus -S Date: 2021.07.16 11:18:13 -04'00'</small>		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Statistical (Pharmacology/Toxicology)	Malick Mbodj	OB/DBVI	13.2.1 <input type="checkbox"/> Authored <input checked="" type="checkbox"/> Contributed <input type="checkbox"/> Approved
Reviewer	Signature: on behalf of Karl K. Lin -S <small>Digitally signed by Karl K. Lin -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Karl K. Lin -S, 0.9.2342.19200300.100.1.1=1300041229 Date: 2021.07.16 15:03:02 -04'00'</small>		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Statistical (Pharmacology/Toxicology)	Karl Lin	OB/DBVI	13.2.1 <input type="checkbox"/> Authored <input checked="" type="checkbox"/> Contributed <input type="checkbox"/> Approved
Team Leader	Signature: Karl K. Lin -S <small>Digitally signed by Karl K. Lin -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Karl K. Lin -S, 0.9.2342.19200300.100.1.1=1300041229 Date: 2021.07.16 15:01:31 -04'00'</small>		

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 Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Pharmacology/Toxicology	Fresnida Ramos	OND/Division of Pharmacology/ Toxicology for Immunology and Inflammation	5.1, 7.1, 7.7.5, 8.4, 13.1.1, 13.1.2, 13.1.3, 13.1.4, 13.2.1 <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Contributed <input type="checkbox"/> Approved
Reviewer	Signature: Fresnida J. Ramos -S <small>Digitally signed by Fresnida J. Ramos -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2002068926, cn=Fresnida J. Ramos -S Date: 2021.07.16 15:37:29 -04'00'</small>		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Pharmacology/Toxicology	Ke Zhang	OND/Division of Pharmacology/ Toxicology for Immunology and Inflammation	13.2.1 <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Contributed <input type="checkbox"/> Approved
Reviewer	Signature: Ke Zhang -S <small>Digitally signed by Ke Zhang -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Ke Zhang -S, 0.9.2342.19200300.100.1.1=1300087224 Date: 2021.07.16 12:52:29 -04'00'</small>		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Pharmacology/Toxicology	David Joseph	OND/Division of Pharmacology/ Toxicology for Immunology and Inflammation	5.1, 7.1, 7.7.5, 8.4, 13.1.1, 13.1.2, 13.1.3, 13.1.4, 13.2.1 <input type="checkbox"/> Authored <input type="checkbox"/> Contributed <input checked="" type="checkbox"/> Approved
Secondary Reviewer	Signature: David B. Joseph -S <small>Digitally signed by David B. Joseph -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300134835, cn=David B. Joseph -S Date: 2021.07.16 14:19:26 -04'00'</small>		

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Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Pharmacology/Toxicology	Carmen Booker	OND/Division of Pharmacology/ Toxicology for Immunology and Inflammation	5.1, 7.1, 7.7.5, 8.4, 13.1.1, 13.1.2, 13.1.3, 13.1.4, 13.2.1 <input type="checkbox"/> Authored <input type="checkbox"/> Contributed <input checked="" type="checkbox"/> Approved
Tertiary Reviewer	Signature: Carmen D. Booker -S <small>Digitally signed by Carmen D. Booker -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300171107, cn=Carmen D. Booker -S Date: 2021.07.16 15:31:29 -04'00'</small>		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Statistical (Hepatology and Nutrition)	Hyoyoung Choo-Wosoba	OB/DBIII	6.2, 6.3.2, 16.2 <input checked="" type="checkbox"/> Authored <input checked="" type="checkbox"/> Contributed <input type="checkbox"/> Approved
Reviewer	Signature: Hyoyoung Choo-wosoba -S <small>Digitally signed by Hyoyoung Choo-wosoba -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2002109190, cn=Hyoyoung Choo-wosoba -S Date: 2021.07.16 13:11:27 -04'00'</small>		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Statistical (Hepatology and Nutrition)	Rebecca Hager	OB/DBIII	6.2, 6.3.2, 16.2 <input checked="" type="checkbox"/> Authored <input checked="" type="checkbox"/> Contributed <input type="checkbox"/> Approved
Reviewer	Signature: Rebecca S. Hager -S <small>Digitally signed by Rebecca S. Hager -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2002201387, cn=Rebecca S. Hager -S Date: 2021.07.16 11:53:24 -04'00'</small>		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Statistical (Hepatology and Nutrition)	George Kordzakhia	OB/DBIII	6.2, 6.3.2, 16.2 <input type="checkbox"/> Authored <input checked="" type="checkbox"/> Contributed <input checked="" type="checkbox"/> Approved
Team Leader	Signature: George Kordzakhia -S <small>Digitally signed by George Kordzakhia -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300390764, cn=George Kordzakhia -S Date: 2021.07.16 11:44:59 -04'00'</small>		

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Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Statistical (Analytics and Informatics Staff)	Wei Tan	OB/AIS	6.2, 6.3.2, 16.2 <input type="checkbox"/> Authored <input checked="" type="checkbox"/> Contributed <input type="checkbox"/> Approved
Reviewer	Digitally signed by Wei Tan -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Wei Tan -S, 0.9.2342.19200300.100.1.1=2002819990 Date: 2021.07.16 15:44:51 -04'00'		
Statistical (Patient Focused Statistical Support)	Marian Strazzeri	OB/DBIII	6.2, 6.3.2, 16.1 <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Contributed <input type="checkbox"/> Approved
Reviewer	Digitally signed by Marian Strazzeri -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000435448, cn=Marian Strazzeri -S Date: 2021.07.16 12:03:09 -04'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Statistical (Patient Focused Statistical Support)	Lili Garrard	OB/DBIII	6.2, 6.3.2, 16.1 <input type="checkbox"/> Authored <input checked="" type="checkbox"/> Contributed <input checked="" type="checkbox"/> Approved
Team Leader	Digitally signed by Lili Garrard -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Lili Garrard -S, 0.9.2342.19200300.100.1.1=2001916551 Date: 2021.07.16 11:30:40 -04'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Statistical	Laura Lee Johnson	OB/DBIII	6.2, 6.3.2, 16.1, 16.2 <input type="checkbox"/> Authored <input type="checkbox"/> Contributed <input checked="" type="checkbox"/> Approved
Division Director	Digitally signed by Laura L. Johnson -S Date: 2021.07.19 09:44:15 -04'00'		

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Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Clinical Pharmacology	Sojeong Yi	OTS/OCP/DIIP	5, 6.1, 6.3.3, 6.3.4, 8.1.2 to 8.1.4, 8.2, 14 <input checked="" type="checkbox"/> Authored <input checked="" type="checkbox"/> Contributed <input type="checkbox"/> Approved
Reviewer	Signature: Sojeong Yi -S <small>Digitally signed by Sojeong Yi -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Sojeong Yi -S, 0.9.2342.19200300.100.1.1=2002075079 Date: 2021.07.16 17:09:35 -04'00'</small>		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Clinical Pharmacology	Insook Kim	OTS/OCP/DIIP	5, 6.1, 6.3.3, 6.3.4, 7.7.4, 8.1.2 to 8.1.4, 8.2, 14 <input type="checkbox"/> Authored <input checked="" type="checkbox"/> Contributed <input checked="" type="checkbox"/> Approved
Team Leader	Signature: Insook Kim -S <small>Digitally signed by Insook Kim -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Insook Kim -S, 0.9.2342.19200300.100.1.1=1300416436 Date: 2021.07.16 19:00:23 -04'00'</small>		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Clinical Pharmacology	Suresh Doddapaneni	OTS/OCP/DIIP	5, 6.1, 6.3.3, 6.3.4, 8.1.2 to 8.1.4, 8.2, 14 <input type="checkbox"/> Authored <input type="checkbox"/> Contributed <input checked="" type="checkbox"/> Approved
Deputy Director	Signature: Suresh N. Doddapaneni -S <small>Digitally signed by Suresh N. Doddapaneni -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300101327, cn=Suresh N. Doddapaneni -S Date: 2021.07.19 08:58:49 -04'00'</small>		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Clinical Pharmacology/Pharmacogenomics	Jielin (Jillian) Sun	OTS/OCP/DTPM	Section 8.1.1, Section 14.4.3 <input checked="" type="checkbox"/> Authored <input checked="" type="checkbox"/> Contributed <input type="checkbox"/> Approved
Reviewer	Signature: Jielin Sun -S <small>Digitally signed by Jielin Sun -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Jielin Sun -S, 0.9.2342.19200300.100.1.1=2002164921 Date: 2021.07.16 15:52:36 -04'00'</small>		

¹ Include "IA" for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Clinical Pharmacology/Pharmacogenomics	Christian Grimstein	OTS/OCP/DTPM	Section 8.1.1, Section 14.4.3 <input type="checkbox"/> Authored <input checked="" type="checkbox"/> Contributed <input checked="" type="checkbox"/> Approved
Team Leader	Signature: Christian Grimstein -S <small>Digitally signed by Christian Grimstein -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000357917, cn=Christian Grimstein -S Date: 2021.07.16 21:45:43 -04'00'</small>		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Clinical Pharmacology/Pharmacogenomics	Lian Ma	OTS/OCP/DPM	6.1, 6.3.3, 14.3 <input type="checkbox"/> Authored <input type="checkbox"/> Contributed <input checked="" type="checkbox"/> Approved
Team Leader	Signature: Lian Ma -S <small>Digitally signed by Lian Ma -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Lian Ma -S, 0.9.2342.19200300.100.1.1=2000825336 Date: 2021.07.19 12:55:33 -04'00'</small>		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Product Quality	Hitesh Shroff	OPQ/ONDP	Section 9 <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Contributed <input type="checkbox"/> Approved
Team Leader	Signature: Hitesh N. Shroff -S <small>Digitally signed by Hitesh N. Shroff -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000348333, cn=Hitesh N. Shroff -S Date: 2021.07.16 12:31:10 -04'00'</small>		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Clinical	Yujin Chung	OND/DCOA	Section 16.1 <input checked="" type="checkbox"/> Authored <input checked="" type="checkbox"/> Contributed <input type="checkbox"/> Approved
Reviewer	Signature: Yujin Chung -S <small>Digitally signed by Yujin Chung -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Yujin Chung -S, 0.9.2342.19200300.100.1.1=2002349706 Date: 2021.07.19 08:36:48 -04'00'</small>		

¹ Include "IA" for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Clinical	Onyeka Illoh	OND/DCOA	Section 16.1 <input type="checkbox"/> Authored <input checked="" type="checkbox"/> Contributed <input type="checkbox"/> Approved
Team Leader	Signature: David Reasner -S <small>Digitally signed by David Reasner -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=David Reasner -S, 0.9.2342.19200300.100.1.1=2003187028 Date: 2021.07.16 17:27:02 -04'00'</small>		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Clinical	David Reasner	OND/DCOA	Section 16.1 <input type="checkbox"/> Authored <input type="checkbox"/> Contributed <input checked="" type="checkbox"/> Approved
Division Director	Signature: David Reasner -S <small>Digitally signed by David Reasner -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=David Reasner -S, 0.9.2342.19200300.100.1.1=2003187028 Date: 2021.07.16 17:15:12 -04'00'</small>		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Decision Support	Graham Thompson	OSP/DSAS	Section I.2. <input type="checkbox"/> Authored <input checked="" type="checkbox"/> Contributed <input type="checkbox"/> Approved
Consult	Signature: Graham Thompson -S <small>Digitally signed by Graham Thompson -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2001140756, cn=Graham Thompson -S Date: 2021.07.16 15:18:05 -04'00'</small>		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved ¹
Decision Support	Leila Lackey	OSP/DSAS	Section I.2. <input type="checkbox"/> Authored <input checked="" type="checkbox"/> Contributed <input type="checkbox"/> Approved
Consult	Signature: Leila G. Lackey -S <small>Digitally signed by Leila G. Lackey -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2001026181, cn=Leila G. Lackey -S Date: 2021.07.16 15:14:15 -04'00'</small>		

¹ Include "IA" for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

RUBY MEHTA
07/19/2021 03:02:51 PM

JOSEPH G TOERNER
07/19/2021 03:12:31 PM

JULIE G BEITZ
07/19/2021 03:13:34 PM



U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Science
Office of Biostatistics

Statistical Review and Evaluation
CARCINOGENICITY STUDY

IND/NDA Number:	NDA 215498
Drug Name:	odevixibat, A4250
Indication(s):	Treatment of pruritus in patients with progressive familial intrahepatic cholestasis.
Studies	One Two Year Oral Gavage Carcinogenicity Study in Rats and Mice.
Applicant:	Sponsor: Albireo AB C/O Albireo Pharma Inc. 10 Post Office Square Boston, Massachusetts 02109 USA
Test facility:	 (b) (4)
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1. Background

In this submission, the sponsor included reports of two animal carcinogenicity studies, one in regular rats and one in mice. These studies were intended to assess the carcinogenic potential of A4250, an inhibitor of the ileal bile acid transporter (IBAT), intended for the treatment of cholestatic liver disease, in rats and mice when administered orally by gavage at appropriate drug levels for about 104 weeks. Results of this review have been discussed with the reviewing pharmacologist Dr. Zhang.

In this review, the phrase "dose response relationship" (trend) refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor incidence rate as dose increases.

2. Rat Study

In this study two separate experiments were conducted, one in male rats and one in female rats. In each of these two experiments there were three treated groups, one water control group and one vehicle control group. Two hundred and fifty CrI:WI(Han) rats of each sex were assigned to three treated groups, one water control group, and one vehicle control group by a stratified randomization scheme designed to achieve similar group mean body weights in equal size of 50 animals, as indicated in Table 1. The dose levels for the three treated groups were 10, 30, and 100 mg/kg/day for males and females, for up to 104 weeks. In this review, these dose groups were referred to as the low, medium, and high dose group, respectively. The water control group received distilled water, the vehicle control group received the vehicle (20% v/v propylene glycol in purified water), administered orally by gavage for about 104 weeks in the same manner as the treated groups.

Table 1: Experimental Design in Rat Study

Group Name	Group N0.	Dose Level (mg/kg/day)		Number of Animal	
		Male	Female	Males	Females
Water Control	1	0	0	50	50
Vehicle Control	2	0	0	50	50
Low	3	10	10	50	50
Medium	4	30	30	50	50
High	5	100	100	50	50

During the administration period, all animals were examined twice daily for mortality and morbidity, daily for clinical signs of toxicity with detailed clinical examinations being conducted weekly from the start of dosing. The animals were removed from the cage, and detailed observations were conducted for each animal prior to randomization on Day -2 and for each surviving main study animal weekly during the study. On occasion, clinical observations were recorded at unscheduled intervals. Detailed examinations for palpable masses were done every 4 weeks during Weeks 28 to 52 and weekly thereafter, the time of onset, location, size, appearance, and progression of each grossly visible or palpable mass, observed in carcinogenicity rats, were recorded weekly animals with significant and/or persistent clinical signs, notable weight loss and/or masses, which were considered to impact their overall well-being, and if determined to be moribund or suffering excessively were killed for humane reasons. Histopathological examinations were performed on all animals found dead or killed moribund or sacrificed at the end of the experiment. Body weights for all animals were measured and recorded at receipt, prior to randomization and once weekly for the first 16 weeks of treatment and once every 4 weeks thereafter. Additional body weights were also recorded to aid monitoring of individuals where body weight loss was noted.

2.1. Sponsor's analyses

For the survival data and statistical analysis of the neoplastic and non-neoplastic lesions, data for the Control groups (vehicle and water Controls from Groups 1 and 2) were combined as the recommendation of the statistician and compared against the test item dosed groups

2.1.1. Survival analysis

The probability of dying before scheduled kill is compared using the Peto et al method for fatal conditions, which is equivalent to the method of Cox in that it conditions on the numbers of survivors in each group at each time point. Kaplan-Meier survival plots are also shown, as are Kruskal-Wallis one-way analyses of variance by ranks (based on the actual week of death) or its stratified version. The Log Rank Test was used for group comparisons, the analyses are presented separately for males and females.

Any animal with accidental injury that causes its death, or its unscheduled sacrifice was censored in the estimation. In addition, all animals still alive at the end of the experimental period were censored at the following day.

Sponsor's findings:

Sponsor's analysis showed the numbers of rats surviving to their terminal necropsy were 33 (66%), 34 (68%), 25 (50%), 32 (64%), and 32 (64%) in the water control, vehicle control group, low, medium, and high dose groups, in male rats, respectively, and 28 (56%), 36 (72%), 32 (64%), 35 (70%), and 33 (66%) in water control, vehicle control, low, medium, and high dose groups, in female rats, respectively. The sponsor's report concluded that, survival was good in all the groups, confirming that the test item had no effect on survival with 156 males and 164 females surviving to the terminal sacrifice. The survival rates were very similar in all the groups, there was no statistically significant findings in survival in either sex of rats.

Also, the sponsor's report showed that there was no significant difference between the two control groups for either males or females.

2.1.2. Tumor data analysis

Since there was no significant difference between the vehicle and water Control groups, the data for the Controls was combined before comparison against the test item dosed groups in order to increase the power of the statistical analysis.

The statistical method was based upon that described by Peto et al., with extensions to provide exact tests where numbers of tumor bearing animals were low. The method was used to compare incidences in each specific group with that of the defined control group(s), to test for overall between-group variation and to test for dose-related trend, taking account of between-group differences in survival and whether the condition being analyzed was considered to have contributed to the death or not. The following groupings for weeks of death were used for males and females: Week 1-52, 53-74, 75-87, 88-95, 96-106 with terminal kill in weeks 105 to 106

As the treatment doses given to the two sexes were similar for the course of the study, an analysis of sexes combined was performed in the tumor analyses and for the analyses of non-neoplastic lesions. Where incidences are low enough, results of exact tests (ExP is the corresponding exact coded p-value)

are shown, conditional on all the marginal totals in all the time periods where a tumor occurs. For analyses of tumors, results are presented for any type that occurs at least 3 in three tumor bearing animals when combined over sexes.

Adjustment for the multiplicity:

For multiplicity adjustment, the sponsor used significance levels of 0.005 and 0.025 for common and rare tumors, respectively in dose response relationship (trend) tests, however, no detailed information was provided in the sponsor's report for a pairwise test comparison. Site-specific background historical control database was used to determine whether the tumors should be designated as rare or common.

Sponsor's findings:

The sponsor's analysis concluded that there was a significant positive effect for fibroadenomas in the Mammary gland (non-protocol) (Ex $P < 0.05$). However, there was an even more significant negative effect for fibromas and fibrosarcomas at any site (Ex $P < 0.01$) so this could well just be due to chance.

2.2 Reviewer's analyses

To verify sponsor's analysis and to perform additional analyses suggested by the reviewing pharmacologist, this reviewer independently performed the survival and tumor data analyses. Data used in this reviewer's analyses were provided by the sponsor electronically on January 06, 2021 via SN0007.

2.2.1 Survival analysis

In the reviewer's analysis, intercurrent mortality data were analyzed using the Kaplan-Meier product limit method. The Kaplan-Meier's curves were presented graphically for male and female rats separately. The dose response relationship and homogeneity of survival distributions were tested for the treatment groups using the Likelihood Ratio test and the Log-Rank test. The intercurrent mortality data are given in Tables 1A and 1B in the appendix for male and female rats, respectively. The Kaplan-Meier curves for survival rate are given in Figures 1A and 1B in the appendix for male and female rats, respectively. Results of the tests for dose response relationship and homogeneity of survivals, are given in Tables 2A and 2B in the appendix for male and female rats, respectively.

Reviewer's findings:

This reviewer's analysis showed the numbers of rats surviving to their terminal necropsy were 33 (66%), 34 (68%), 25 (50%), 32 (64%), and 32 (64%) in the water control, vehicle control group, low, medium, and high dose groups, in male rats, respectively, and 28 (56%), 36 (72%), 32 (64%), 35 (70%), and 33 (66%) in water control, vehicle control, low, medium, and high dose groups, in female rats, respectively. This reviewer's analysis showed no statistically significant increase in mortality across the vehicle control group and the three treated groups in either sex of rats. The pairwise comparisons showed no statistically significant increase or decrease in mortality between each of the treated groups and the vehicle control group in either sex of rats.

2.2.2. Tumor data analysis

In the reviewer's analysis, the tumor data were analyzed for dose response relationship across vehicle control group and the treated groups without including the water control group, as well as the pairwise

comparisons of vehicle control group with each of the treated groups using the Poly-k method described in the paper of Bailer and Portier (1988) and Bieler and Williams (1993). In this method, an animal that lives the full study period (w_{\max}) or dies before the terminal sacrifice with development of the tumor type being tested gets a score of $s_h = 1$. An animal that dies at Week w_h without development of the given tumor

type before the end of the study gets a score of $s_h = \left(\frac{w_h}{w_{\max}} \right)^k < 1$. The adjusted group size is defined as $\sum s_h$.

As an interpretation, an animal with score $s_h = 1$ can be considered as a whole animal, while an animal with score $s_h < 1$ can be considered as a partial animal. The adjusted group size $\sum s_h$ is equal to N (the original group size) if all animals live up to the end of the study or if each animal develops the given tumor being tested, otherwise the adjusted group size is less than N. These adjusted group sizes are then used for the dose response relationship (or the pairwise comparison) tests using the Cochran-Armitage test. One critical point for Poly-k test is the choice of the appropriate value of k. For long term 104-week standard rat and mouse studies, a value of k=3 is suggested in the literature [Gebregziabher and Hoel (2009), Moon et al. (2003), Portier, et al. (1986)]. Hence, this reviewer used k=3 for the analysis of the data. Based on the intent to treat (ITT) principle Wmax was considered as 104 for both male and female rats.

For the calculation of p-values, if there were less than 10 tumor bearing animals across all treatment groups for a given tumor type, the exact tests based on the discrete permutation distribution were used, with dose levels (0, 10, 30, and 100 for both male and female rats) as scores, and asymptotic tests were used for tumor types with higher incidences. The tumor rates and the p-values of the tested tumor types are listed in Tables 3A and 3B in the appendix for male rats and female rats, respectively.

Multiple testing adjustments:

Following the FDA draft guidance for the carcinogenicity study design and data analysis 2001, for the two-year rat study this reviewer used significance levels of 0.005 and 0.025 for common and rare tumors, respectively in dose response relationship (trend) tests and significance levels of 0.01 and 0.05 for common and rare tumors, respectively in pairwise comparisons.

A tumor is defined as a rare tumor if the published spontaneous rate or the spontaneous rate of the vehicle control of the tumor is less than 1%, and a common tumor is defined as one with tumor rate greater than or equal to 1%.

Reviewer's findings:

Reviewer's comment on the sponsor's tumor data of Rat study:

For the tumor data of the rat study, this reviewer noticed that, two different tumor codes were associated with the same tumor name adenoma in the adrenals, fibroadenoma in the non-protocol mammary gland, and fibroma in the non-protocol skin for both male and female rats (49500 and 39700, 39700 and 49500, and 50200 and 76500 respectively), therefore this reviewer combined them

Table 2: Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or the pairwise Comparisons Treated Groups and Vehicle Control Group in Rats

Sex	Organ Name	Tumor Name	0 mg Water (N=50) P - VC vs. W	0 mg Vehicle Cont (N=50) P - Trend	10 mg Low (N=50) P - VC vs. L	30 mg Med (N=50) P - VC vs. M	100 mg High (N=50) P - VC vs. H
Male	Pituitary Gland	Adenoma	14/50 (44) 0.4078	12/48 (44) 0.9558	22/50 (46) 0.0361 [@]	15/49 (43) 0.2963	9/48 (42) 0.8108
	Non-Protocol Skin	Papilloma, Sessile	0/50 (42) NC	0/50 (44) 0.0229*	0/50 (41) NC	2/50 (43) 0.2414	3/50 (42) 0.1122
Female	Non-Protocol Mammary Gland	Fibroadenoma	10/50 (42) 0.7629	13/50 (46) 0.0142 [@]	9/50 (44) 0.8659	9/50 (43) 0.8525	19/50 (44) 0.1041

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;

NC = Not calculable.

*: Statistically significant at 0.005 and 0.025 level for common and rare tumor or 0.01 and 0.05 level for common and rare tumors for tests of dose response relationship and pairwise comparison, respectively.

[@] = Not statistically significant in rare tumor at 0.025 level for test of dose response relationship and at 0.05 level for test of pairwise comparisons, or in common tumor at 0.005 level for test of dose response relationship and at 0.01 level for test of pairwise comparisons

Following the multiple testing adjustment method described above, this reviewer's analysis showed statistically significant increasing dose response relationships across the vehicle control and the treated groups for the incidence of papilloma, sessile in non-protocol skin in of male rats (p-value =0.0229). The pairwise comparisons showed no tumor types with a statistically significant increase in tumor incidences in A4250 treated groups, when compare to the vehicle control group in either sex of rats.

Reviewer's comment on the tumor types of non-protocol tissues in skin and mammary gland

For the sponsor's electronic tumor dataset, the numbers of animals examined for the tumor types of non-protocol tissues in the skin and in the mammary gland of rats were all 50 in each dose group. However, in the sponsor's study report, the numbers of animals that were examined in the non-protocol tissues in the skin were 18, 25, 16, 13, and 20, in the water control, vehicle control group, low, medium, and high dose groups, in male rats, respectively, and 16, 17, 17, 12, and 17 in female rats, respectively. In the mammary gland the sponsor's study reports shows that 17, 17, 14, 17, and 16 in female rats were examined. It has been advised by the pharm/tox reviewers and the ECAC that those discrepancies do not need to be resolved since the overall conclusions about drug-related tumorigenicity will not be impacted.

3. Mouse Study

Two separate experiments were conducted, one in male mice and one in female mice. In each of these two experiments there were three treated groups, one water control group and one vehicle control group. Two hundred and seventy Crl:CD-1 (ICR) mice of each sex were assigned to three treated groups one water control group and one vehicle control group by a stratified randomization scheme designed to achieve similar group mean body weights in equal size of 54 animals, as indicated in Table 3. The dose levels for the three treated groups were 10, 30, and 100 mg/kg/day for males and females, for up to 104 weeks. In this review, these dose groups were referred to as the low, medium, and high dose group, respectively. The water control group received distilled water, the vehicle control group received the vehicle (20% v/v propylene glycol in purified water), administered orally by gavage for about 104 weeks in the same manner as the treated groups

Table 3: Experimental Design in Mouse Study

Group Name	Group N0.	Dose Level (mg/kg/day)		Number of Animal	
		Male	Female	Males	Females
Water Control	1	0	0	54	54
Vehicle Control	2	0	0	54	54
Low	3	10	10	54	54
Medium	4	30	30	54	54
High	5	100	100	54	54

During the administration period, all animals were examined twice daily for mortality and morbidity, daily for clinical signs of toxicity with detailed clinical examinations being conducted weekly from the start of dosing. The animals were removed from the cage, and detailed observations were conducted for each animal prior to randomization on Day -2 and for each surviving main study animal weekly during the study. On occasion, clinical observations were recorded at unscheduled intervals. Detailed examinations for palpable masses were done every 4 weeks during Weeks 28 to 52 and weekly thereafter, the time of onset, location, size, appearance, and progression of each grossly visible or palpable mass, observed in carcinogenicity rats, was recorded weekly animals with significant and/or persistent clinical signs, notable weight loss and/or masses, which were considered to impact their overall well-being, and if determined to be moribund or suffering excessively were killed for humane reasons. Histopathological examinations were performed on all animals found dead or killed moribund or sacrificed at the end of the experiment. Body weights for all animals were measured and recorded at receipt, prior to randomization and once weekly for the first 16 weeks of treatment and once every 4 weeks thereafter. Additional body weights were also recorded to aid monitoring of individuals where body weight loss was noted.

3.1. Sponsor's analyses

For the survival data and statistical analysis of the neoplastic and non-neoplastic lesions, data for the Control groups (vehicle and water Controls from Groups 1 and 2) were combined as the recommendation of the statistician and compared against the test item dosed groups

3.1.1 Survival analysis

The sponsor used similar methodologies to analyze the mouse survival data as those used to analyze the rat survival data.

Sponsor's findings:

Sponsor's analysis showed the numbers of mice surviving to their terminal necropsy were 25 (46%), 24 (44%), 28 (52%), 32 (59%), and 25 (46%), in water control, vehicle control, low, medium, and high dose groups in male mice, respectively, and 25 (46%), 22 (41%), 21 (39%), 25 (46%), and 22 (41%), in water control, vehicle control, low, medium, medium-high, and high dose groups in female mice, respectively. The sponsor's report concluded that, survival was good in all the groups with 134 males and 115 females surviving to the terminal sacrifice. The survival rates were very similar in all the groups, the test item had no effect on survival in the A4250 treated groups, nor was there any evidence of survival differences between the two control groups.

3.1.2 Tumor data analysis

The sponsor used similar methodologies to analyze the mouse tumor data as those used to analyze the rat tumor data.

The statistical method was based upon that described by Peto et al., with extensions to provide exact tests where incidences were low. The method was used to compare incidences in each specific group with that of the defined control group(s), to test for overall between-group variation and to test for dose-related trend, taking account of between-group differences in survival and whether the condition being analyzed was considered to have contributed to the death or not. The following groupings for weeks of death were used for males and females: Week 1-52, 53-74, 75-87, 88-95, 96-106 with terminal kill in weeks 105 to 106.

As the treatment doses given to the two sexes were similar for the course of the study, an analysis of sexes combined was performed in the tumor analyses and for the analyses of non-neoplastic lesions. Statistical significance was reported at the $P < 0.05$, $P < 0.01$ and $P < 0.001$ levels. Reference to statistical significance at the $P < 0.1$ level was also included for reference purposes where considered appropriate. For analyses of tumors, results are presented for any type that occurs at least 3 animals when combined over sexes.

Multiple testing adjustment:

For multiplicity adjustment testing, sponsor used similar testing adjustment as those used in the rat study.

Sponsor's findings:

The sponsor's analysis concluded that, there was very little evidence of any increases in tumors caused by the treatment. Small effects seen for adenomas or adenocarcinomas in the Pituitary gland and for fibrosarcomas in the Skin/subcutis (non-protocol) could well just be due to chance.

3.2 Reviewer's analyses

Similar to the rat study, this reviewer independently performed the survival and tumor data analyses of the mouse study. For the analysis of the survival data and the tumor data of the mouse study, this reviewer used similar methodologies that were used for the analyses of the survival and tumor data of the rat study. Data used in this reviewer's analyses were provided by the sponsor electronically.

3.2.1 Survival analysis

The intercurrent mortality data are given in Tables 4A and 4B in the appendix for male and female mice, respectively. The Kaplan-Meier curves for death rate are given in Figures 2A and 2B in the appendix for male and female mice, respectively. Results for test of dose response relationship and homogeneity of survivals among treatment groups are given in Tables 5A and 5B in the appendix for male and female mice, respectively.

Reviewer's findings:

This reviewer's analysis showed the numbers of mice surviving to their terminal necropsy were 25 (46%), 24 (44%), 28 (52%), 32 (59%), and 25 (46%), in water control, vehicle control, low, medium, and high dose groups in male mice, respectively, and 25 (46%), 22 (41%), 21 (39%), 25 (46%), and 22 (41%), in water control, vehicle control, low, medium, medium-high, and high dose groups in female mice. This reviewer's analysis showed no statistically significant increase in mortality across the vehicle control group and the three

treated groups in either sex of mice. The pairwise comparisons showed no statistically significant increase or decrease in mortality between each of the treated groups and the vehicle control group in either sex of mice.

3.2.2 Tumor data analysis

The tumor rates and the p-values of the tumor types tested for dose response relationship and the pairwise comparisons of vehicle control and treated groups are given in Table 6A and 6B in the appendix for male and female mice, respectively.

Multiple testing adjustment:

Following the same FDA draft guidance for the carcinogenicity study design and data analysis 2001, used in the rat study

Reviewer's findings:

Table 4: Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or the pairwise Comparisons Treated Groups and Vehicle Control Group in Mice

Sex	Organ Name	Tumor Name	0 mg Water (N=54) P - VC vs.W	0 mg Vehicle (N=54) P - Trend	10 mg Low (N=54) P - VC vs. L	30 mg Med(N=54) P - VC vs. M	100 mg High (N=54) P - VC vs. H
Male	Pituitary Gland	Adenoma	1/53 (41) 0.5190	0/54 (38) 0.0118*	0/53 (40) NC	0/54 (44) NC	3/54 (37) 0.1151
		Adenocarcinoma/ Adenoma	1/53 (41) 0.5190	0/54 (38) 0.0028*	0/53 (40) NC	0/54 (44) NC	4/54 (38) 0.0575
Female	Skin/Subcutis (Non-Protocol)	Fibrosarcoma	0/54 (39) NC	0/54 (37) 0.0224*	0/54 (36) NC	1/54 (38) 0.5067	3/54 (40) 0.1351
		Uterus	5/54 (41) 0.0355*	0/54 (37) 0.3621	3/54 (37) 0.1199	3/54 (39) 0.1300	2/54 (38) 0.2533

*: Statistically significant at 0.025 level or 0.05 level, for rare tumors for tests of dose response relationship and pairwise comparison, respectively.

Following the multiple testing adjustment method described above, this reviewer's analyses showed statistically significant increasing dose response relationships across the vehicle control and the treated groups of male mice for the incidence of adenoma, the combined adenocarcinoma and adenoma, in pituitary gland, and in female mice for the incidence of fibrosarcoma, in skin/subcutis (p-value = 0.0118, =0.0028, and = 0.0224, respectively). The pairwise comparisons showed no tumor types with a statistically significant increase in tumor incidences in A4250 treated groups, when compare to the vehicle control group in either sex of mice.

Also, this reviewer's analyses showed Statistically significant in water controls group for the incidences of polyp(s) in the uterus, when compared to the vehicle control group in female mice (P=0.0355)

Reviewer's comment on the tumor type non-protocol tissues in skin/subcutis

For the tumor dataset the number of animals examined for the tumor type non-protocol tissues in the

skin/subcutis in mice were 54 in each dose group. However, in the study report, the number of animals that were examined were 14, 13, 6, 5, and 6, in the water control, vehicle control group, low, medium, and high dose groups, in male mice, respectively, and 7, 4, 4, 4, and 6 in female mice. It has been advised by the pharm/tox reviewers and the ECAC that those discrepancies do not need to be resolved since the overall conclusions about drug-related tumorigenicity will not be impacted.

4. Summary

In this submission, the sponsor included reports of two animal carcinogenicity studies, one in regular rats and one in mice. These studies were intended to assess the carcinogenic potential of A4250, an inhibitor of the ileal bile acid transporter (IBAT), intended for the treatment of cholestatic liver disease, in rats and mice when administered orally by gavage at appropriate drug levels for about 104 weeks.

Rat Study:

In this study two separate experiments were conducted, one in male rats and one in female rats. In each of these two experiments there were three treated groups, one water control group and one vehicle control group. Two hundred and fifty Crl:WI(Han)rats of each sex were assigned to three treated groups one water control group and one vehicle control group by a stratified randomization scheme designed to achieve similar group mean body weights in equal size of 50 animals, as indicated in Table 1. The dose levels for the three treated groups were 10, 30, and 100 mg/kg/day for males and females, for up to 104 weeks. In this review, these dose groups were referred to as the low, medium, and high dose group, respectively. The water control group received distilled water, the vehicle control group received the vehicle (20% v/v propylene glycol in purified water), administered orally by gavage for about 104 weeks in the same manner as the treated groups.

This reviewer's analysis showed the numbers of rats surviving to their terminal necropsy were 33 (66%), 34 (68%), 25 (50%), 32 (64%), and 32 (64%) in the water control, vehicle control group, low, medium, and high dose groups, in male rats, respectively, and 28 (56%), 36 (72%), 32 (64%), 35 (70%), and 33 (66%) in water control, vehicle control, low, medium, and high dose groups, in female rats, respectively. This reviewer's analysis showed no statistically significant increase in mortality across the vehicle control group and the three treated groups in either sex of rats. The pairwise comparisons showed no statistically significant increase or decrease in mortality between each of the treated groups and the vehicle control group in either sex of rats.

For tumor data, following the multiple testing adjustment method described above, this reviewer's analysis showed statistically significant increasing dose response relationships across the vehicle control and the treated groups for the incidence of papilloma, sessile in non-protocol skin in of male rats (p-value =0.0229). The pairwise comparisons showed no tumor types with a statistically significant increase in tumor incidences in A4250 treated groups, when compare to the vehicle control group in either sex of rats.

Mouse Study:

Two separate experiments were conducted, one in male mice and one in female mice. In each of these two experiments there were three treated groups, one water control group and one vehicle control group. Two hundred and seventy Crl:CD-1 (ICR) mice of each sex were assigned to three treated groups one water control group and one vehicle control group by a stratified randomization scheme designed to achieve similar group mean body weights in equal size of 54 animals, as indicated in Table 3. The dose levels for the three treated groups were 10, 30, and 100 mg/kg/day for males and females, for up to 104 weeks. In this review, these dose groups were referred to as the low, medium, and high dose group, respectively. The water control group received distilled water, the vehicle control group received the vehicle (20% v/v propylene glycol in

purified water), administered orally by gavage for about 104 weeks in the same manner as the treated groups

This reviewer's analysis showed the numbers of mice surviving to their terminal necropsy were 25 (46%), 24 (44%), 28 (52%), 32 (59%), and 25 (46%), in water control, vehicle control, low, medium, and high dose groups in male mice, respectively, and 25 (46%), 22 (41%), 21 (39%), 25 (46%), and 22 (41%), in water control, vehicle control, low, medium, medium-high, and high dose groups in female mice. This reviewer's analysis showed no statistically significant increase in mortality across the vehicle control group and the three treated groups in either sex of mice. The pairwise comparisons showed no statistically significant increase or decrease in mortality between each of the treated groups and the vehicle control group in either sex of mice.

For tumor data, following the multiple testing adjustment method described above, this reviewer's analyses showed statistically significant increasing dose response relationships across the vehicle control and the treated groups of male mice for the incidence of adenoma, , the combined adenocarcinoma and adenoma in pituitary gland, and in female mice for the incidence of fibrosarcoma, in skin/subcutis (p-value = 0.0118, = 0.0028, and = 0.0224, respectively). The pairwise comparisons showed no tumor types with a statistically significant increase in tumor incidences in A4250 treated groups, when compare to the vehicle control group in either sex of mice.

Also, this reviewer's analyses showed Statistically significant in water controls group for the incidences of polyp(s) in the uterus, when compared to the vehicle control group in female mice (P=0.0355)

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cc:

Archival NDA 215498- A4250

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5. Appendix

Table 1A: Intercurrent Mortality Rate
Male Rats

Week	0 mg/kg/day Water Control		0 mg/kg/day Vehicle Control		10 mg/kg/day Low		30 mg/kg/day Medium		100 mg/kg/day High	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
0 - 52	2	4.00	1	2.00	.	.	2	4.00	2	4.00
53 - 78	7	18.00	3	8.00	6	12.00	2	8.00	4	12.00
79 - 92	3	24.00	6	20.00	7	26.00	7	22.00	7	26.00
93-106	5	34.00	6	32.00	12	50.00	7	36.00	5	36.00
Ter. Sac.	33	66.00	34	68.00	25	50.00	32	64.00	32	64.00
Total	50	100.00	50	100.00	50	100.00	50	100.00	50	100.00

Animals were assigned to the terminal sacrifice strata based on the death or sacrifice status recorded

Table 1B: Intercurrent Mortality Rate
Female Rats

Week	0 mg/kg/day Water Control		0 mg/kg/day Vehicle Control		10 mg/kg/day Low		30 mg/kg/day Medium		100 mg/kg/day High	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
0 - 52	3	6.00	.	.	1	2.00	2	4.00	1	2.00
53 - 78	5	16.00	4	8.00	1	4.00	4	12.00	8	18.00
79 - 92	4	24.00	6	20.00	8	20.00	2	16.00	2	22.00
93-104	10	44.00	4	28.00	8	36.00	7	30.00	6	34.00
Ter. Sac.	28	56.00	36	72.00	32	64.00	35	70.00	33	66.00
Total	50	100.00	50	100.00	50	100.00	50	100.00	50	100.00

Animals were assigned to the terminal sacrifice strata based on the death or sacrifice status recorded

Table 2A: Intercurrent Mortality Comparison for
Male Rats

Test Statistics	P-value for Vehicle Cont. vs Water	P-value for Vehicle Cont. Low, Med, high	P-value for Vehicle Cont. vs Low	P-value for Vehicle Cont. vs Med	P-value for Vehicle Cont. vs High
Dose-Response (Likelihood Ratio)	0.7561	0.8115	0.0976	0.6824	0.6286
Homogeneity (Log-Rank)	0.7552	0.3456	0.0958	0.6817	0.6270

Table 2B: Intercurrent Mortality Comparison for
Female Rats

Test Statistics	P-value for Vehicle Cont. vs Water	P-value for Vehicle Cont. Low, Med, high	P-value for Vehicle Cont. vs Low	P-value for Vehicle Cont. vs Med	P-value for Vehicle Cont. vs High
Dose-Response (Likelihood Ratio)	0.0993	0.6543	0.4897	0.8030	0.4755
Homogeneity (Log-Rank)	0.0971	0.8651	0.4885	0.8023	0.4740

Table 3A: Tumor Rates and P-Values for Dose Response Relationship and the pairwise comparisons

Male Rats Poly-3 Test

Organ Name	Tumor Name	0 Mg Water Cont (N=50) P - VC Vs. W	0 mg Vehicle Cont (N=50) P - Trend	10 Mg Low(N=50) P - VC Vs. L	30 Mg Med (N=50) P - VC Vs. M	100 Mg High (N=50) P - VC Vs. H
Abdominal Cavity	Haemangiosarcoma	0/50 (42) NC	0/50 (44) 0.5000	0/50 (41) NC	1/50 (43) 0.4943	0/50 (42) NC
	Leiomyosarcoma	0/50 (42) 1.0000	1/50 (44) 1.0000	0/50 (41) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
Abdominal Wall	Lipoma	0/50 (42) 1.0000	1/50 (44) 1.0000	0/50 (41) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
Adrenals	Adenocarcinoma	0/50 (42) NC	0/50 (44) 0.2471	0/50 (41) NC	0/50 (43) NC	1/50 (42) 0.4884
	Adenoma	0/50 (42) 1.0000	3/50 (44) 0.9837	3/50 (41) 0.6275	1/50 (43) 0.9390	0/50 (42) 1.0000
	Ganglioneuroma	0/50 (42) NC	0/50 (44) 0.7412	1/50 (41) 0.4824	0/50 (43) NC	0/50 (42) NC
	Phaeochromocytoma	0/50 (42) NC	0/50 (44) 0.5438	3/50 (41) 0.1079	1/50 (43) 0.4943	1/50 (42) 0.4884
Brain	Astrocytoma	1/50 (42) 0.9688	4/50 (44) 0.9991	1/50 (42) 0.9688	0/50 (43) 1.0000	0/50 (42) 1.0000
	Granular Cell	0/50 (42) 1.0000	1/50 (44) 0.9341	1/50 (41) 0.7350	0/50 (43) 1.0000	0/50 (42) 1.0000
	Medulloblastoma	0/50 (42) NC	0/50 (44) 0.7427	1/50 (42) 0.4884	0/50 (43) NC	0/50 (42) NC
	Meningeal Sarcoma	0/50 (42) NC	0/50 (44) 0.2471	0/50 (41) NC	0/50 (43) NC	1/50 (42) 0.4884
	Meningioma	0/50 (42) NC	0/50 (44) 0.5000	0/50 (41) NC	1/50 (43) 0.4943	0/50 (42) NC
	Oligodendroglioma	0/50 (42) NC	0/50 (44) 0.0621	0/50 (41) NC	0/50 (43) NC	2/50 (43) 0.2414
Caecum	Adenocarcinoma	0/50 (42) NC	0/50 (44) 0.3055	1/50 (41) 0.4824	0/50 (43) NC	1/50 (42) 0.4884
	Leiomyosarcoma	0/50 (42) NC	0/50 (44) 0.7412	1/50 (41) 0.4824	0/50 (43) NC	0/50 (42) NC
Duodenum	Adenocarcinoma	0/50 (42) NC	0/50 (44) 0.5029	0/50 (41) NC	1/50 (44) 0.5000	0/50 (42) NC
Femur and Joint (Incl. Marrow)	Osteoma	0/50 (42) 1.0000	1/50 (44) 1.0000	0/50 (41) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
Forelimbs	Papilloma	1/50 (42) 0.7412	1/50 (44) 1.0000	0/50 (41) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000

Male Rats Poly-3 Test

Organ Name	Tumor Name	0 Mg Water Cont (N=50) P - VC Vs. W	0 mg Vehicle Cont (N=50) P - Trend	10 Mg Low(N=50) P - VC Vs. L	30 Mg Med (N=50) P - VC Vs. M	100 Mg High (N=50) P - VC Vs. H
Harderian Glands	Adenocarcinoma	0/50 (42) NC	0/50 (44) 0.5000	0/50 (41) NC	1/50 (43) 0.4943	0/50 (42) NC
	Adenoma	0/50 (42) 1.0000	1/50 (44) 0.1513	0/50 (41) 1.0000	0/50 (43) 1.0000	2/50 (42) 0.4824
Heart	Endocardial Schwannoma	0/50 (42) 1.0000	1/50 (44) 1.0000	0/50 (41) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
Jejunum	Leiomyoma	0/50 (42) NC	0/50 (44) 0.2471	0/50 (41) NC	0/50 (43) NC	1/50 (42) 0.4884
	Lymphoma	0/50 (42) 1.0000	1/50 (44) 1.0000	0/50 (41) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
Caecum +Jejunum + adipose Tissue	Leiomyoma/ Leiomyosarcoma	0/50 (42) NC	0/50 (44) 0.2905	1/50 (41) 0.4824	1/50 (43) 0.4943	1/50 (42) 0.4884
Kidneys	Liposarcoma	0/50 (42) NC	0/50 (44) 0.7427	1/50 (42) 0.4884	0/50 (43) NC	0/50 (42) NC
	Mesenchymal Tumour	1/50 (42) 0.4884	0/50 (44) NC	0/50 (41) NC	0/50 (43) NC	0/50 (42) NC
	Oncocytoma	0/50 (42) NC	0/50 (44) 0.5000	0/50 (41) NC	1/50 (43) 0.4943	0/50 (42) NC
	Papilloma, Urothelium	0/50 (42) NC	0/50 (44) 0.5000	0/50 (41) NC	1/50 (43) 0.4943	0/50 (42) NC
Lip	Papilloma	0/50 (42) NC	0/50 (44) 0.7412	1/50 (41) 0.4824	0/50 (43) NC	0/50 (42) NC
	Squamous Cell Carcinoma	0/50 (42) NC	0/50 (44) 0.7412	1/50 (41) 0.4824	0/50 (43) NC	0/50 (42) NC
Liver	Adenoma	1/50 (42) 0.8706	2/50 (44) 0.3553	1/50 (41) 0.8659	0/50 (43) 1.0000	2/50 (42) 0.6741
	Cholangioma	0/50 (42) NC	0/50 (44) 0.5000	0/50 (41) NC	1/50 (43) 0.4943	0/50 (42) NC
Lungs	Adenoma	1/50 (42) 0.4884	0/50 (44) NC	0/50 (41) NC	0/50 (43) NC	0/50 (42) NC
	Carcinoma, Small Cell	0/50 (42) 1.0000	1/50 (44) 1.0000	0/50 (41) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
	Leiomyosarcoma	0/50 (42) NC	0/50 (44) 0.5000	0/50 (41) NC	1/50 (43) 0.4943	0/50 (42) NC
	Mesothelioma	0/50 (42) NC	0/50 (44) 0.7412	1/50 (41) 0.4824	0/50 (43) NC	0/50 (42) NC
Lymph Nodes	Haemangioma	1/50 (42) 0.8795	2/48 (42) 1.0000	0/50 (41) 1.0000	0/48 (41) 1.0000	0/49 (41) 1.0000

Male Rats Poly-3 Test

Organ Name	Tumor Name	0 Mg Water Cont (N=50) P - VC Vs. W	0 mg Vehicle Cont (N=50) P - Trend	10 Mg Low(N=50) P - VC Vs. L	30 Mg Med (N=50) P - VC Vs. M	100 Mg High (N=50) P - VC Vs. H
	Haemangiosarcoma	0/50 (42) NC	0/48 (42) 0.2485	0/50 (41) NC	0/48 (41) NC	1/49 (41) 0.4940
	Osteosarcoma	0/50 (42) NC	0/48 (42) 0.2485	0/50 (41) NC	0/48 (41) NC	1/49 (41) 0.4940
Mesenteric Lymph Nodes	Haemangioma	8/50 (42) 0.8768	12/50 (44) 0.7253	2/50 (41) 0.9994	5/50 (43) 0.9837	6/50 (43) 0.9650
	Haemangiosarcoma	1/50 (42) 0.4884	0/50 (44) 0.8078	2/50 (42) 0.2356	0/50 (43) NC	0/50 (42) NC
Pancreas	Adenocarcinoma	0/50 (42) 1.0000	1/50 (44) 1.0000	0/50 (41) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
	Adenoma, Exocrine Cell	3/50 (42) 0.1122	0/50 (44) 0.4329	2/50 (41) 0.2297	2/50 (43) 0.2414	1/50 (42) 0.4884
	Islet, Cell Adenoma	1/50 (42) 0.9929	6/50 (44) 0.7982	4/50 (42) 0.8236	2/50 (43) 0.9688	3/50 (42) 0.9105
	Islet, Cell Carcinoma	2/50 (42) 0.2356	0/50 (44) 0.4829	2/50 (42) 0.2356	3/50 (43) 0.1164	1/50 (42) 0.4884
	Islet, Cell Adenoma/Carcinoma	3/50 (42) 0.9105	6/50 (44) 0.7529	6/50 (43) 0.6048	5/50 (43) 0.7261	4/50 (42) 0.8236
Pituitary Gland	Adenocarcinoma	0/50 (42) NC	0/48 (42) 0.3084	1/50 (41) 0.4940	0/49 (42) NC	1/48 (41) 0.4940
	Adenoma	14/50 (44) 0.4078	12/48 (44) 0.9558	22/50 (46) 0.0361	15/49 (43) 0.2963	9/48 (42) 0.8108
Preputial Glands	Adenocarcinoma	1/49 (41) 0.4881	0/50 (44) NC	0/50 (41) NC	0/50 (43) NC	0/50 (42) NC
	Adenoma	0/49 (41) NC	0/49 (43) 0.5030	0/50 (41) NC	1/50 (43) 0.5000	0/50 (42) NC
Prostate Gland	Adenoma	0/50 (42) NC	0/50 (44) 0.7412	1/50 (41) 0.4824	0/50 (43) NC	0/50 (42) NC
Salivary Gland	Adenocarcinoma	0/50 (42) NC	0/50 (44) 0.5000	0/50 (41) NC	1/50 (43) 0.4943	0/50 (42) NC
	Fibrosarcoma	1/50 (42) 0.4884	0/50 (44) NC	0/50 (41) NC	0/50 (43) NC	0/50 (42) NC
	Malignant Schwannoma	0/50 (42) NC	0/50 (44) 0.5000	0/50 (41) NC	1/50 (43) 0.4943	0/50 (42) NC
Site of Mammary Gland	Fibroadenoma	1/50 (42) 0.4884	0/50 (44) 0.7412	1/50 (41) 0.4824	0/50 (43) NC	0/50 (42) NC
Skin	Haemangioma	0/50 (42) NC	0/50 (44) 0.2471	0/50 (41) NC	0/50 (43) NC	1/50 (42) 0.4884

Male Rats Poly-3 Test

Organ Name	Tumor Name	0 Mg Water Cont (N=50) P - VC Vs. W	0 mg Vehicle Cont (N=50) P - Trend	10 Mg Low(N=50) P - VC Vs. L	30 Mg Med (N=50) P - VC Vs. M	100 Mg High (N=50) P - VC Vs. H
Spleen	Haemangiosarcoma	0/50 (42) NC	0/50 (44) 0.5000	0/50 (41) NC	1/50 (43) 0.4943	0/50 (42) NC
Stomach	Adenoma	1/50 (42) 0.4884	0/50 (44) NC	0/50 (41) NC	0/50 (43) NC	0/50 (42) NC
	Papilloma	1/50 (42) 0.7412	1/50 (44) 1.0000	0/50 (41) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
Submandibular Lymph Nodes	Haemangioma	1/49 (41) 0.4940	0/48 (42) 0.5000	0/50 (41) NC	1/48 (41) 0.4940	0/50 (42) NC
Tail	Keratoacanthoma	0/50 (42) NC	0/50 (44) 0.2471	0/50 (41) NC	0/50 (43) NC	1/50 (42) 0.4884
	Papilloma	1/50 (42) 0.7412	1/50 (44) 0.7173	0/50 (41) 1.0000	3/50 (43) 0.2994	0/50 (42) 1.0000
Testes	Leydig Cell Tumour	1/50 (42) 0.7412	1/50 (44) 0.3517	2/50 (41) 0.4732	2/50 (43) 0.4913	2/50 (42) 0.4824
Thoracic Cavity	Thymic Lymphoma	0/50 (42) NC	0/50 (44) 0.6184	1/50 (42) 0.4884	1/50 (43) 0.4943	0/50 (42) NC
Thymus	Lymphoma	0/50 (42) NC	0/49 (43) 0.5090	0/47 (39) NC	1/50 (43) 0.5000	0/50 (42) NC
	Thymic Lymphoma	1/50 (42) 0.4941	0/49 (43) 0.5090	0/47 (39) NC	1/50 (43) 0.5000	0/50 (42) NC
	Thymoma	2/50 (42) 0.2412	0/49 (43) 0.6456	1/47 (39) 0.4756	2/50 (43) 0.2471	0/50 (42) NC
Thyroid Glands	C Cell Adenoma	0/50 (42) 1.0000	4/50 (44) 0.8961	2/50 (42) 0.8880	0/50 (43) 1.0000	1/50 (42) 0.9688
	Carcinoma, C Cell	0/50 (42) NC	0/50 (44) 0.5000	0/50 (41) NC	1/50 (43) 0.4943	0/50 (42) NC
	Adenoma/ Carcinoma C-Cell	0/50 (42) 1.0000	4/50 (44) 0.8958	2/50 (42) 0.8880	1/50 (43) 0.9706	1/50 (42) 0.9688
	Carcinoma, Follicular Cell	1/50 (42) 0.8706	2/50 (44) 0.3921	1/50 (41) 0.8659	1/50 (43) 0.8751	2/50 (42) 0.6741
	Follicular Cell Adenoma	1/50 (42) 0.7412	1/50 (44) 0.7123	3/50 (42) 0.2906	5/50 (43) 0.0959	1/50 (42) 0.7412
	Adenoma/ Carcinoma Follicular	2/50 (42) 0.8051	3/50 (44) 0.5881	4/50 (42) 0.4736	6/50 (43) 0.2304	3/50 (42) 0.6394
Adipose Tissue	Leiomyosarcoma	0/50 (42) NC	0/50 (44) 0.5000	0/50 (41) NC	1/50 (43) 0.4943	0/50 (42) NC
	Lipoma	0/50 (42) NC	0/50 (44) 0.5000	0/50 (41) NC	1/50 (43) 0.4943	0/50 (42) NC

Male Rats Poly-3 Test

Organ Name	Tumor Name	0 Mg Water Cont (N=50) P - VC Vs. W	0 mg Vehicle Cont (N=50) P - Trend	10 Mg Low(N=50) P - VC Vs. L	30 Mg Med (N=50) P - VC Vs. M	100 Mg High (N=50) P - VC Vs. H
Haemopoietic Tumour	Histiocytic Sarcoma	1/50 (42) 0.4884	0/50 (44) NC	0/50 (41) NC	0/50 (43) NC	0/50 (42) NC
	Lymphoma	2/50 (43) 0.2414	0/50 (44) 0.2161	2/50 (43) 0.2414	2/50 (44) 0.2471	2/50 (43) 0.2414
	Mononuclear Cell Leukaemia	0/50 (42) NC	0/50 (44) 0.2471	0/50 (41) NC	0/50 (43) NC	1/50 (42) 0.4884
Non-Protocol Mammary Gland	Fibroadenoma	0/50 (42) NC	0/50 (44) 0.4081	2/50 (42) 0.2356	1/50 (43) 0.4943	1/50 (42) 0.4884
Non-Protocol Nerve	Schwannoma	1/50 (42) 0.4884	0/50 (44) NC	0/50 (41) NC	0/50 (43) NC	0/50 (42) NC
Non-Protocol Skin	Basal Cell Tumour	0/50 (42) NC	0/50 (44) 0.2471	0/50 (41) NC	0/50 (43) NC	1/50 (42) 0.4884
	Carcinoma, Organ Of Zuckerkindl	0/50 (42) NC	0/50 (44) 0.5000	0/50 (41) NC	1/50 (43) 0.4943	0/50 (42) NC
	Fibroma	5/50 (42) 0.8674	8/50 (44) 0.9894	3/50 (41) 0.9676	3/50 (43) 0.9731	1/50 (42) 0.9985
	Fibrosarcoma	0/50 (42) 1.0000	1/50 (44) 0.9341	1/50 (41) 0.7350	0/50 (43) 1.0000	0/50 (42) 1.0000
	Haemangioma	0/50 (42) 1.0000	1/50 (44) 1.0000	0/50 (41) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
	Haemangiosarcoma	0/50 (42) NC	0/50 (44) 0.7412	1/50 (41) 0.4824	0/50 (43) NC	0/50 (42) NC
	Keratoacanthoma	7/50 (42) 0.6619	8/50 (45) 0.2295	6/50 (41) 0.7527	5/50 (43) 0.8674	9/50 (42) 0.4365
	Lipoma	0/50 (42) 1.0000	1/50 (44) 1.0000	0/50 (41) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
	Osteosarcoma	0/50 (42) 1.0000	1/50 (44) 1.0000	0/50 (41) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
	Papilloma	3/50 (42) 0.4777	2/50 (44) 0.5756	0/50 (41) 1.0000	0/50 (43) 1.0000	1/50 (42) 0.8706
	Papilloma, Sessile	0/50 (42) NC	0/50 (44) 0.0229*	0/50 (41) NC	2/50 (43) 0.2414	3/50 (42) 0.1122
	Schwannoma	0/50 (42) 1.0000	2/50 (44) 0.5756	0/50 (41) 1.0000	0/50 (43) 1.0000	1/50 (42) 0.8706
	Sebaceous Cell Adenoma	0/50 (42) 1.0000	1/50 (44) 0.6864	0/50 (41) 1.0000	2/50 (43) 0.4913	0/50 (42) 1.0000
	Squamous Cell Carcinoma	1/50 (42) 0.4884	0/50 (44) NC	0/50 (41) NC	0/50 (43) NC	0/50 (42) NC

Male Rats Poly-3 Test

Organ Name	Tumor Name	0 Mg Water Cont (N=50) P - VC Vs. W	0 mg Vehicle Cont (N=50) P - Trend	10 Mg Low(N=50) P - VC Vs. L	30 Mg Med (N=50) P - VC Vs. M	100 Mg High (N=50) P - VC Vs. H
	Tricho-Epithelioma	0/50 (42) NC	0/50 (44) 0.5000	0/50 (41) NC	1/50 (43) 0.4943	0/50 (42) NC
	Zymbal's Gland Tumour	0/50 (42) 1.0000	1/50 (44) 1.0000	0/50 (41) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
Whole Body	Haemangioma/ Haemangiosarcoma	9/50 (42) 0.9090	14/50 (44) 0.7375	5/50 (42) 0.9943	7/50 (43) 0.9749	8/50 (43) 0.9527
	Leiomyoma/ Leiomyosarcoma	0/50 (42) 1.0000	1/50 (44) 0.5080	1/50 (41) 0.7350	1/50 (43) 0.7471	1/50 (42) 0.7412
	Lymphoma	2/50 (43) 0.4913	1/50 (44) 0.3779	2/50 (43) 0.4913	3/50 (44) 0.3081	2/50 (43) 0.4913

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals;

ZZ=unweighted total number of animals observed;

NC = Not calculable.

*: Statistically significant at 0.005 and 0.025 level for common and rare tumor or 0.01 and 0.05 level for common and rare tumors for tests of dose response relationship and pairwise comparison, respectively.

Table 3B: Tumor Rates and P-Values for Dose Response Relationship and the pairwise comparisons

Female Rats Poly-3 Test

Organ Name	Tumor Name	0 mg Water Cont (N=50) P - VC vs. W	0 mg Vehicle Cont (N=50) P - Trend	10 mg Low(N=50) P - VC vs. L	30 mg Med (N=50) P - VC vs. M	100 mg High(N=50) P - VC vs. H
Abdominal Cavity	Malignant Schwannoma	0/50 (40) NC	0/50 (44) 0.4913	0/50 (44) NC	1/50 (43) 0.4943	0/50 (42) NC
Adrenals	Adenocarcinoma	0/50 (40) NC	0/50 (44) 0.7457	1/50 (44) 0.5000	0/50 (43) NC	0/50 (42) NC
	Adenoma	1/50 (40) 0.8610	2/50 (44) 0.3866	1/50 (44) 0.8793	2/50 (43) 0.6832	2/50 (42) 0.6741
	Phaeochromocytoma	1/50 (40) 0.4762	0/50 (44) 0.7245	2/50 (44) 0.2471	1/50 (43) 0.4943	0/50 (42) NC
Brain	Granular Cell	1/50 (40) 0.7286	1/50 (44) 1.0000	0/50 (44) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
Caecum	Leiomyosarcoma	0/50 (40) NC	0/50 (44) 0.4913	0/50 (44) NC	1/50 (43) 0.4943	0/50 (42) NC
Cervix	Adenocarcinoma	0/50 (40) NC	0/50 (44) 0.2442	0/50 (44) NC	0/49 (42) NC	1/50 (42) 0.4884
	Endometrial Sarcoma	1/50 (40) 0.7286	1/50 (44) 1.0000	0/50 (44) 1.0000	0/49 (42) 1.0000	0/50 (42) 1.0000
	Leiomyosarcoma	1/50 (40) 0.4762	0/50 (44) 0.1785	0/50 (44) NC	1/49 (42) 0.4884	1/50 (42) 0.4884
	Schwannoma	0/50 (40) NC	0/50 (44) 0.2442	0/50 (44) NC	0/49 (42) NC	1/50 (42) 0.4884
Clitoral Glands	Adenoma	0/49 (39) 1.0000	1/48 (42) 1.0000	0/48 (42) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
Colon	Adenocarcinoma	0/50 (40) NC	0/50 (44) 0.4913	0/50 (44) NC	1/50 (43) 0.4943	0/50 (42) NC
Duodenum	Adenocarcinoma	0/50 (40) NC	0/50 (44) 0.7457	1/50 (44) 0.5000	0/50 (43) NC	0/50 (42) NC
	Leiomyoma	0/50 (40) NC	0/50 (44) 0.0572	0/50 (44) NC	1/50 (43) 0.4943	2/50 (42) 0.2356
Harderian Glands	Adenoma	0/50 (40) NC	0/50 (44) 0.4913	0/50 (44) NC	1/50 (43) 0.4943	0/50 (42) NC
Ileum	Leiomyosarcoma	0/50 (40) 1.0000	1/50 (44) 1.0000	0/50 (44) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
Jejunum	Leiomyosarcoma	0/50 (40) 1.0000	1/50 (44) 1.0000	0/50 (44) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
Caecum+ Jejunum +Ileum + Duodenum + Adispose	Leiomyoma Leiomyosarcoma	1/50 (40) 0.8610	2/50 (44) 0.2867	0/50 (44) 1.0000	2/50 (43) 0.6832	2/50 (42) 0.6741

Female Rats Poly-3 Test

Organ Name	Tumor Name	0 mg Water Cont (N=50) P - VC vs. W	0 mg Vehicle Cont (N=50) P - Trend	10 mg Low(N=50) P - VC vs. L	30 mg Med (N=50) P - VC vs. M	100 mg High(N=50) P - VC vs. H
Liver	Adenocarcinoma, Hepatocellular	1/50 (41) 0.4824	0/50 (44) 0.2428	0/50 (44) NC	0/50 (43) NC	1/50 (42) 0.4884
	Adenoma	1/50 (40) 0.9296	3/50 (44) 0.7053	0/50 (44) 1.0000	1/50 (43) 0.9390	1/50 (42) 0.9361
	Adenoma /Adenocarcinoma, Hepatocellular	2/50 (41) 0.7972	3/50 (44) 0.4175	0/50 (44) 1.0000	1/50 (43) 0.9390	2/50 (42) 0.8051
	Cholangioma	0/50 (40) NC	0/50 (44) 0.6297	1/50 (44) 0.5000	2/50 (43) 0.2414	0/50 (42) NC
Lymph Nodes	Haemangioma	1/50 (40) 0.4762	0/50 (44) 0.7457	1/50 (44) 0.5000	0/50 (43) NC	0/50 (42) NC
Mesenteric Lymph Nodes	Haemangioma	1/50 (40) 0.9628	4/50 (45) 0.9899	4/50 (44) 0.6305	2/50 (43) 0.8880	0/50 (42) 1.0000
Mesentery	Mesothelioma	0/50 (40) NC	0/50 (44) 0.2428	0/50 (44) NC	0/50 (43) NC	1/50 (42) 0.4884
Ovaries	Dysgerminoma	0/49 (39) NC	0/50 (44) 0.4942	0/49 (43) NC	1/50 (43) 0.4943	0/50 (42) NC
	Tubulo-Stromal Adenoma	1/49 (39) 0.9261	3/50 (44) 0.2862	3/49 (43) 0.6508	2/50 (43) 0.8126	4/50 (42) 0.4736
Pancreas	Adenoma, Exocrine Cell	0/50 (40) NC	0/50 (44) 0.7457	1/50 (44) 0.5000	0/50 (43) NC	0/50 (42) NC
	Islet Cell Adenoma	0/50 (40) NC	0/50 (44) 0.7457	1/50 (44) 0.5000	0/50 (43) NC	0/50 (42) NC
	Islet Cell Carcinoma	0/50 (40) 1.0000	1/50 (44) 0.9364	1/50 (44) 0.7529	0/50 (43) 1.0000	0/50 (42) 1.0000
	Islet Cell Adenoma/ Carcinoma	0/50 (40) 1.0000	1/50 (44) 0.9353	2/50 (45) 0.5085	0/50 (43) 1.0000	0/50 (42) 1.0000
Pituitary Gland	Adenocarcinoma	3/50 (40) 0.2727	1/50 (44) 0.6814	3/50 (45) 0.3167	1/50 (43) 0.7471	1/50 (42) 0.7412
	Adenoma	19/50 (44) 0.8324	25/50 (49) 0.3980	19/50 (46) 0.8760	17/50 (47) 0.9530	22/50 (45) 0.6601
	Adenoma / Adenocarcinoma	22/50 (44) 0.6923	26/50 (49) 0.4476	22/50 (47) 0.7929	18/50 (47) 0.9514	23/50 (45) 0.6538
Site Of Mammary Gland	Fibroadenoma	2/50 (40) 0.2238	0/50 (44) 0.2850	1/50 (44) 0.5000	1/50 (43) 0.4943	1/50 (42) 0.4884
Spleen	Haemangioma	0/50 (40) NC	0/50 (44) 0.2428	0/50 (44) NC	0/50 (43) NC	1/50 (42) 0.4884
Tail	Papilloma	1/50 (40) 0.4762	0/50 (44) 0.7457	1/50 (44) 0.5000	0/50 (43) NC	0/50 (42) NC

Female Rats Poly-3 Test

Organ Name	Tumor Name	0 mg Water Cont (N=50) P - VC vs. W	0 mg Vehicle Cont (N=50) P - Trend	10 mg Low(N=50) P - VC vs. L	30 mg Med (N=50) P - VC vs. M	100 mg High(N=50) P - VC vs. H
Thoracic Cavity	Hibernoma	0/50 (40) NC	0/50 (44) 0.4913	0/50 (44) NC	1/50 (43) 0.4943	0/50 (42) NC
	Thymic Lymphoma	0/50 (40) NC	0/50 (44) 0.7457	1/50 (44) 0.5000	0/50 (43) NC	0/50 (42) NC
Thymus	Thymic Lymphoma	0/49 (39) 1.0000	1/50 (44) 0.5052	1/48 (42) 0.7412	1/50 (43) 0.7471	1/50 (42) 0.7412
	Thymoma	1/49 (39) 0.8559	2/50 (44) 0.3450	3/48 (42) 0.4777	1/50 (43) 0.8751	3/50 (42) 0.4777
Thyroid Glands	C Cell Adenoma	1/50 (40) 0.9648	4/50 (44) 0.9881	1/50 (44) 0.9723	1/50 (43) 0.9706	0/50 (42) 1.0000
	Carcinoma, C Cell	0/50 (40) NC	0/50 (44) 0.6155	1/50 (44) 0.5000	1/50 (43) 0.4943	0/50 (42) NC
	Adenoma / Carcinoma C-Cell	1/50 (40) 0.9648	4/50 (44) 0.9806	2/50 (44) 0.8988	2/50 (43) 0.8935	0/50 (42) 1.0000
	Follicular Cell Adenoma	1/50 (40) 0.7286	1/50 (44) 0.7807	1/50 (44) 0.7529	2/50 (43) 0.4913	0/50 (42) 1.0000
Uterus	Adenocarcinoma	0/50 (40) 1.0000	2/50 (44) 0.2867	0/50 (44) 1.0000	2/50 (43) 0.6832	2/50 (42) 0.6741
	Adenoma	0/50 (40) 1.0000	1/50 (44) 0.5948	2/50 (44) 0.5000	1/50 (43) 0.7471	1/50 (42) 0.7412
	Adenoma/ Adenocarcinoma	0/50 (40) 1.0000	3/50 (44) 0.3968	2/50 (44) 0.8198	3/50 (43) 0.6508	3/50 (42) 0.6394
	Endometrial Sarcoma	0/50 (40) NC	0/50 (44) 0.6155	1/50 (44) 0.5000	1/50 (43) 0.4943	0/50 (42) NC
	Fibrosarcoma	0/50 (40) 1.0000	1/50 (45) 1.0000	0/50 (44) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
	Haemangioma	1/50 (40) 0.4762	0/50 (44) NC	0/50 (44) NC	0/50 (43) NC	0/50 (42) NC
	Leiomyosarcoma	0/50 (40) 1.0000	1/50 (44) 1.0000	0/50 (44) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
	Malignant Schwannoma	0/50 (40) NC	0/50 (44) 0.6155	1/50 (44) 0.5000	1/50 (43) 0.4943	0/50 (42) NC
Vagina	Leiomyosarcoma	0/49 (39) NC	0/50 (44) 0.1785	0/50 (44) NC	1/48 (42) 0.4884	1/50 (42) 0.4884
Adipose Tissue	Haemangioma	0/50 (40) NC	0/50 (44) 0.2428	0/50 (44) NC	0/50 (43) NC	1/50 (42) 0.4884
	Leiomyosarcoma	1/50 (40) 0.4762	0/50 (44) NC	0/50 (44) NC	0/50 (43) NC	0/50 (42) NC

Female Rats Poly-3 Test

Organ Name	Tumor Name	0 mg Water Cont (N=50) P - VC vs. W	0 mg Vehicle Cont (N=50) P - Trend	10 mg Low(N=50) P - VC vs. L	30 mg Med (N=50) P - VC vs. M	100 mg High(N=50) P - VC vs. H
Aortic Body	Phaeochromocytoma	1/50 (40) 0.4762	0/50 (44) NC	0/50 (44) NC	0/50 (43) NC	0/50 (42) NC
Haemopoietic Tumour	Histiocytic Sarcoma	0/50 (40) NC	0/50 (44) 0.7457	1/50 (44) 0.5000	0/50 (43) NC	0/50 (42) NC
	Lymphoma	1/50 (40) 0.4762	0/50 (44) 0.4005	2/50 (45) 0.2528	1/50 (44) 0.5000	1/50 (42) 0.4884
Non-Protocol Mammary Gland	Adenocarcinoma	3/50 (40) 0.6153	3/50 (44) 0.7759	1/50 (44) 0.9418	1/50 (44) 0.9418	1/50 (42) 0.9361
	Adenoma	1/50 (40) 0.4762	0/50 (44) 0.7457	1/50 (44) 0.5000	0/50 (43) NC	0/50 (42) NC
	Fibroadenoma	10/50 (42) 0.7629	13/50 (46) 0.0142	9/50 (44) 0.8659	9/50 (43) 0.8525	19/50 (44) 0.1041
	Fibroma	0/50 (40) 1.0000	1/50 (44) 1.0000	0/50 (44) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
Non-Protocol Skeletal Muscle	Haemangiosarcoma	0/50 (40) NC	0/50 (44) 0.7457	1/50 (44) 0.5000	0/50 (43) NC	0/50 (42) NC
	Zymbal's Gland Tumour	0/50 (40) 1.0000	1/50 (44) 1.0000	0/50 (44) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
Non-Protocol Skin	Basal Cell Tumour	1/50 (40) 0.7286	1/50 (44) 1.0000	0/50 (44) 1.0000	0/50 (43) 1.0000	0/50 (42) 1.0000
	Fibroma	1/50 (40) 0.4762	0/50 (44) 0.4884	0/50 (44) NC	2/50 (43) 0.2414	0/50 (42) NC
	Haemangioma	0/50 (40) NC	0/50 (44) 0.1793	0/50 (44) NC	1/50 (43) 0.4943	1/50 (42) 0.4884
	Haemangiosarcoma	0/50 (40) NC	0/50 (44) 0.7457	1/50 (44) 0.5000	0/50 (43) NC	0/50 (42) NC
	Keratoacanthoma	0/50 (40) 1.0000	1/50 (44) 0.9109	3/50 (44) 0.3081	1/50 (43) 0.7471	0/50 (42) 1.0000
	Papilloma, Sessile	0/50 (40) NC	0/50 (44) 0.7457	1/50 (44) 0.5000	0/50 (43) NC	0/50 (42) NC
	Tricho-Epithelioma	1/50 (40) 0.4762	0/50 (44) NC	0/50 (44) NC	0/50 (43) NC	0/50 (42) NC
Whole Body	Haemangioma/ Haemangiosarcoma	3/50 (40) 0.7317	4/50 (45) 0.9035	7/50 (44) 0.2477	3/50 (43) 0.7639	2/50 (42) 0.8823
	Leiomyoma/ Leiomyosarcoma	1/50 (40) 0.9296	3/50 (44) 0.1355	0/50 (44) 1.0000	4/50 (43) 0.4870	4/50 (42) 0.4736
	Lymphoma	1/50 (40) 0.4762	0/50 (44) 0.4005	2/50 (45) 0.2528	1/50 (44) 0.5000	1/50 (42) 0.4884

Figure 1A: Kaplan-Meier Survival Curves for Male Rats

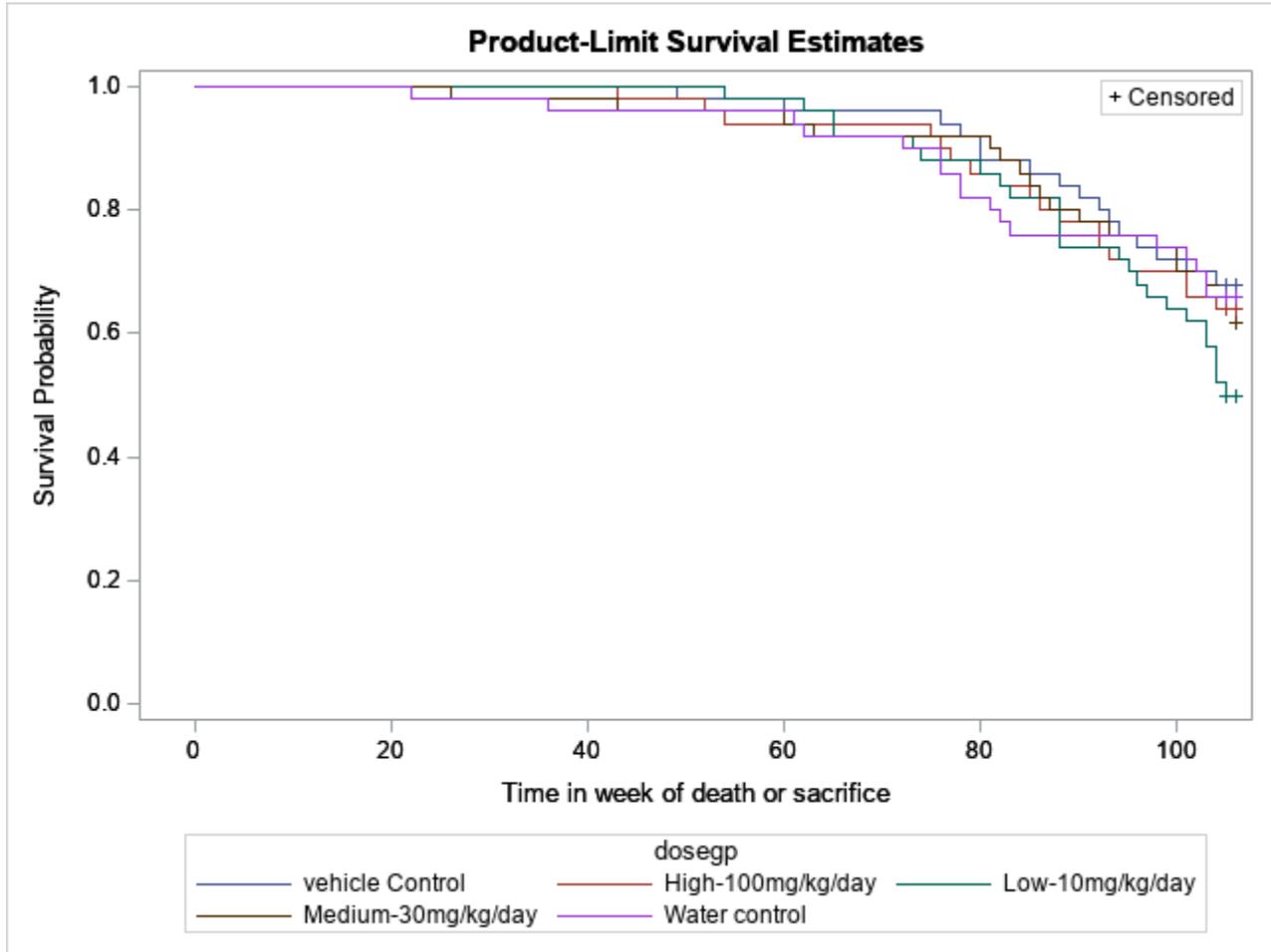


Figure 1B: Kaplan-Meier Survival Curves for Female Rats

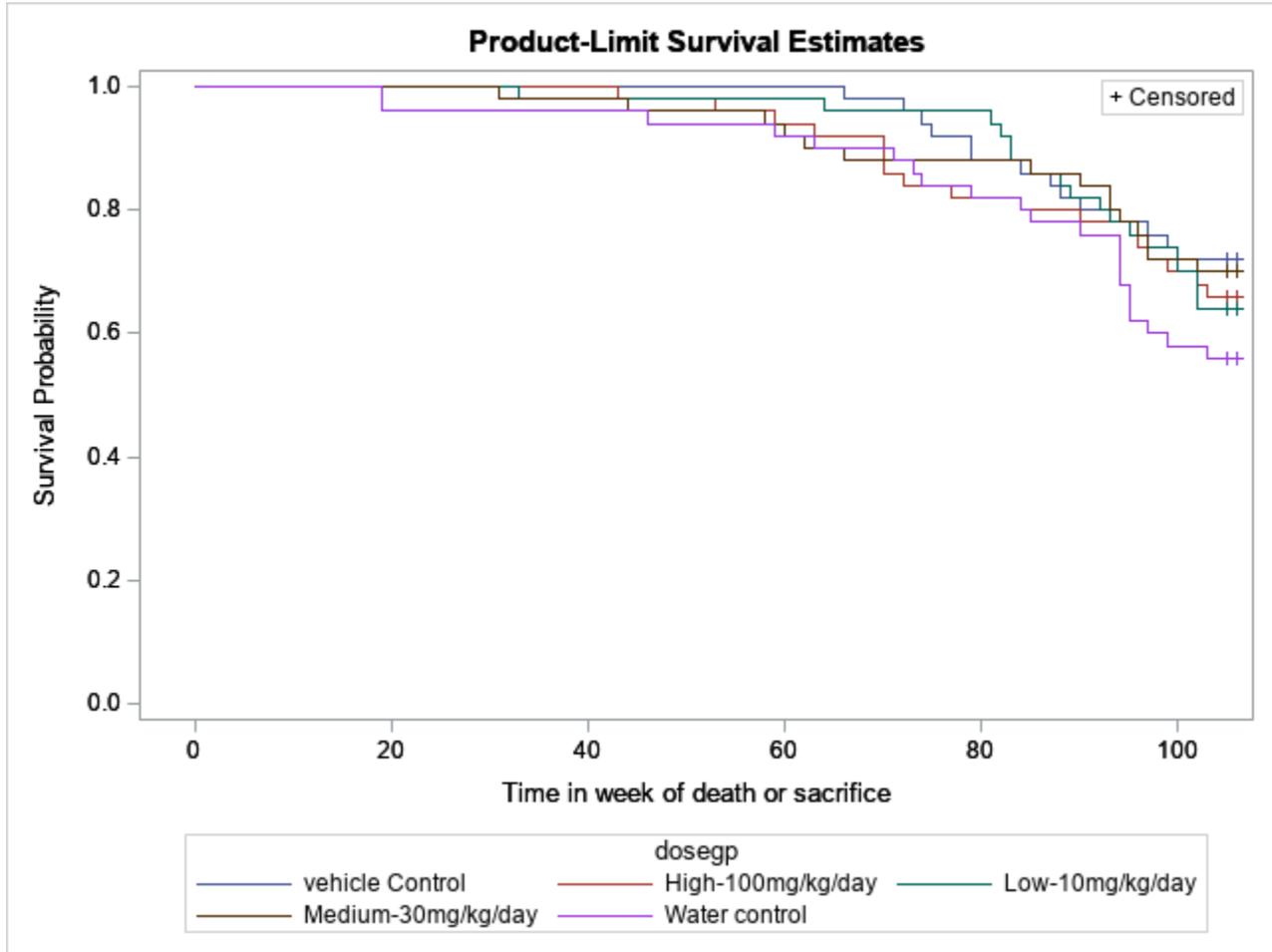


Table 4A: Intercurrent Mortality Rate
Male Mice

Week	0 mg/kg/day Water Control		0 mg/kg/day Vehicle Control		10 mg/kg/day Low		30 mg/kg/day Medium		100 mg/kg/day High	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
0 - 52	2	3.70	7	12.96	5	9.26	1	1.85	9	16.67
53 - 78	9	20.37	9	29.63	6	20.37	8	16.67	5	25.93
79 - 92	9	37.04	4	37.04	5	29.63	4	24.07	7	38.89
93 - 105	9	53.70	10	55.56	10	48.15	9	40.74	8	53.70
Ter. Sac.	25	46.30	24	44.44	28	51.85	32	59.26	25	46.30
Total	54	100.00	54	100.00	54	100.00	54	100.00	54	100.00

Animals were assigned to the terminal sacrifice strata based on the death or sacrifice status recorded

Table 4B: Intercurrent Mortality Rate
Female Mice

Week	0 mg/kg/day Water Control		0 mg/kg/day Vehicle Control		10 mg/kg/day Low		30 mg/kg/day Medium		100 mg/kg/day High	
	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %	No. of Death	Cum. %
0 - 52	4	7.41	7	12.96	4	7.41	8	14.81	1	1.85
53 - 78	9	24.07	12	35.19	13	31.48	7	27.78	14	27.78
79 - 92	4	31.48	2	38.89	7	44.44	7	40.74	5	37.04
93-104	12	53.70	11	59.26	9	61.11	7	53.70	12	59.26
Ter. Sac.	25	46.30	22	40.74	21	38.89	25	46.30	22	40.74
Total	54	100.00	54	100.00	54	100.00	54	100.00	54	100.00

Animals were assigned to the terminal sacrifice strata based on the death or sacrifice status recorded

Table 5A: Intercurrent Mortality Comparison for
Male Mice

Test Statistics	P-value for Vehicle Cont. vs Water	P-value for Vehicle Cont. Low, Med, high	P-value for Vehicle Cont. vs Low	P-value for Vehicle Cont. vs Med	P-value for Vehicle Cont. vs High
Dose-Response (Likelihood Ratio)	0.6856	0.6931	0.3551	0.0873	0.8795
Homogeneity (Log-Rank)	0.6842	0.3037	0.3523	0.0855	0.8785

Table 5B: Intercurrent Mortality Comparison for
Female Mice

Test Statistics	P-value for Vehicle Cont. vs Water	P-value for Vehicle Cont. Low, Med, high	P-value for Vehicle Cont. vs Low	P-value for Vehicle Cont. vs Med	P-value for Vehicle Cont. vs High
Dose-Response (Likelihood Ratio)	0.5731	0.8826	0.8289	0.7001	0.9432
Homogeneity (Log-Rank)	0.5710	0.9421	0.8279	0.6984	0.9427

Table 6A: Tumor Rates and P-Values for Dose Response Relationship and the Pairwise Comparisons

Male Mice Poly-3 Test

Organ Name	Tumor Name	0 mg Water Cont (N=54) P - VC vs. W	0 mg Vehicle Cont (N=54) P - Trend	10 mg Low (N=54) P - VC vs. L	30 mg Med (N=54) P - VC vs. M	100 mg High (N=54) P - VC vs. H
Adipose Tissue	Hibernoma	1/53 (40) 0.8891	2/54 (38) 0.9326	1/54 (41) 0.8933	1/54 (44) 0.9047	0/54 (37) 1.0000
Adrenals	Adenoma, Cortex	2/54 (41) 0.2661	0/54 (38) 0.1756	2/54 (41) 0.2661	1/53 (43) 0.5309	2/53 (36) 0.2332
	Adenoma, Subcapsular Cell	1/54 (41) 0.7718	1/54 (38) 0.1873	3/54 (41) 0.3370	0/53 (43) 1.0000	3/53 (36) 0.2870
	Pheochromocytoma	0/54 (40) 1.0000	1/54 (38) 1.0000	0/54 (41) 1.0000	0/53 (43) 1.0000	0/53 (36) 1.0000
Blood Vessels	Haemangioma	0/54 (40) NC	0/54 (38) 0.2360	0/54 (41) NC	0/54 (44) NC	1/54 (38) 0.5000
Colon	Adenocarcinoma	0/54 (40) NC	0/54 (38) 0.7625	1/54 (41) 0.5190	0/54 (44) NC	0/54 (37) NC
Duodenum	Adenocarcinoma	1/53 (40) 0.5128	0/54 (38) NC	0/54 (41) NC	0/54 (44) NC	0/54 (37) NC
	Adenoma	1/53 (40) 0.5128	0/54 (38) NC	0/54 (41) NC	0/54 (44) NC	0/54 (37) NC
Epididymides	Adenoma	0/54 (40) NC	0/54 (38) 0.2313	0/54 (41) NC	0/54 (44) NC	1/54 (37) 0.4933
Femur and Joint (Incl. Marrow)	Haemangiosarcoma	0/54 (40) NC	0/54 (38) 0.7625	1/54 (41) 0.5190	0/54 (44) NC	0/54 (37) NC
Haemopoietic Tissue	Lymphoma	3/54 (40) 0.8765	5/54 (39) 0.6590	3/54 (41) 0.8838	5/54 (46) 0.7316	3/54 (37) 0.8513
	Sarcoma, Histiocytic	0/54 (40) NC	0/54 (38) 0.5063	0/54 (41) NC	1/54 (44) 0.5366	0/54 (37) NC
Harderian Glands	Adenocarcinoma	0/54 (40) NC	0/54 (38) 0.4308	2/54 (41) 0.2661	0/54 (44) NC	1/54 (38) 0.5000
	Adenoma	2/54 (41) 0.9160	4/54 (38) 0.4125	8/54 (42) 0.2271	6/54 (44) 0.4667	6/54 (38) 0.3680
	Adenocarcinoma/ Adenoma	2/54 (41) 0.9160	4/54 (38) 0.3805	10/54 (42) 0.1018	6/54 (44) 0.4667	7/54 (38) 0.2580
Kidneys	Adenoma	0/54 (40) NC	0/54 (38) 0.7625	1/54 (41) 0.5190	0/54 (44) NC	0/54 (37) NC
	Oncocytoma	2/54 (41) 0.2661	0/54 (38) NC	0/54 (41) NC	0/54 (44) NC	0/54 (37) NC
Lip	Papilloma	1/54 (40) 0.5128	0/54 (38) NC	0/54 (41) NC	0/54 (44) NC	0/54 (37) NC

Male Mice Poly-3 Test

Organ Name	Tumor Name	0 mg Water Cont (N=54) P - VC vs. W	0 mg Vehicle Cont (N=54) P - Trend	10 mg Low (N=54) P - VC vs. L	30 mg Med (N=54) P - VC vs. M	100 mg High (N=54) P - VC vs. H
Liver	Adenoma, Hepatocellular	4/54 (41) 0.5432	3/54 (38) 0.8218	2/54 (42) 0.8502	0/54 (44) 1.0000	1/54 (37) 0.9393
	Carcinoma, Hepatocellular	3/54 (40) 0.6869	3/54 (38) 0.9635	2/54 (41) 0.8434	2/54 (44) 0.8626	0/54 (37) 1.0000
	Adenoma/ Carcinoma Hepatocellular	7/54 (41) 0.5397	6/54 (39) 0.9766	4/54 (42) 0.8727	2/54 (44) 0.9813	1/54 (37) 0.9930
	Cholangioma	0/54 (40) NC	0/54 (38) 0.7625	1/54 (41) 0.5190	0/54 (44) NC	0/54 (37) NC
	Haemangiosarcoma	0/54 (40) NC	0/54 (38) 0.1803	0/54 (41) NC	1/54 (44) 0.5366	1/54 (37) 0.4933
Lungs	Adenocarcinoma	7/54 (42) 0.9776	13/54 (39) 0.8760	14/54 (43) 0.6219	16/54 (46) 0.5363	9/54 (39) 0.8961
	Adenoma, Bronchiolo- Alveolar	10/54 (42) 0.6733	10/54 (39) 0.2410	6/54 (42) 0.9413	6/54 (45) 0.9568	10/54 (39) 0.6021
	Adenocarcinoma/ Adenoma, Bronchiolo-Alveolar	16/54 (43) 0.9678	22/54 (40) 0.5950	19/54 (44) 0.9035	22/54 (47) 0.8356	19/54 (40) 0.8145
	Carcinoma, Neuroendocrine	1/54 (41) 0.5190	0/54 (38) NC	0/54 (41) NC	0/54 (44) NC	0/54 (37) NC
Pancreas	Adenocarcinoma	0/54 (40) 1.0000	1/54 (38) 1.0000	0/54 (41) 1.0000	0/54 (44) 1.0000	0/54 (37) 1.0000
	Adenoma, Exocrine	0/54 (40) NC	0/54 (38) 0.5063	0/54 (41) NC	1/54 (44) 0.5366	0/54 (37) NC
	Adenoma, Islet Cell	0/54 (40) 1.0000	3/54 (38) 0.7609	1/54 (41) 0.9509	0/54 (44) 1.0000	1/54 (37) 0.9393
Pituitary Gland	Adenocarcinoma	0/53 (40) NC	0/54 (38) 0.2375	0/53 (40) NC	0/54 (44) NC	1/54 (38) 0.5000
	Adenoma	1/53 (41) 0.5190	0/54 (38) 0.0118*	0/53 (40) NC	0/54 (44) NC	3/54 (37) 0.1151
	Adenocarcinoma/ Adenoma	1/53 (41) 0.5190	0/54 (38) 0.0028*	0/53 (40) NC	0/54 (44) NC	4/54 (38) 0.0575
Preputial Glands	Adenoma	0/54 (40) 1.0000	1/52 (36) 1.0000	0/54 (41) 1.0000	0/53 (43) 1.0000	0/54 (37) 1.0000
Primary Carcinoma	Adenocarcinoma	0/54 (40) NC	0/54 (38) 0.7640	1/54 (42) 0.5250	0/54 (44) NC	0/54 (37) NC
Prostate Gland	Adenoma	1/54 (40) 0.5128	0/54 (38) NC	0/54 (41) NC	0/54 (44) NC	0/54 (37) NC
Rectum	Leiomyosarcoma	0/54 (40) 1.0000	1/54 (38) 1.0000	0/53 (40) 1.0000	0/54 (44) 1.0000	0/52 (37) 1.0000

Male Mice Poly-3 Test

Organ Name	Tumor Name	0 mg Water Cont (N=54) P - VC vs. W	0 mg Vehicle Cont (N=54) P - Trend	10 mg Low (N=54) P - VC vs. L	30 mg Med (N=54) P - VC vs. M	100 mg High (N=54) P - VC vs. H
Seminal Vesicles	Adenoma	0/54 (40) 1.0000	1/54 (38) 1.0000	0/54 (41) 1.0000	0/54 (44) 1.0000	0/54 (37) 1.0000
Site of Mammary Gland	Lipoma	0/54 (40) 1.0000	1/54 (38) 1.0000	0/54 (41) 1.0000	0/54 (44) 1.0000	0/54 (37) 1.0000
Skin/Subcutis (Non-Protocol)	Fibroma	0/54 (40) 1.0000	1/54 (38) 1.0000	0/54 (41) 1.0000	0/54 (44) 1.0000	0/54 (37) 1.0000
	Fibrosarcoma	5/54 (43) 0.1315	1/54 (38) 0.5083	1/54 (41) 0.7718	1/54 (44) 0.7883	1/54 (38) 0.7533
	Fibroma/ Fibrosarcoma	5/54 (43) 0.2705	2/54 (38) 0.6665	1/54 (41) 0.8933	1/54 (44) 0.9047	1/54 (38) 0.8800
	Papilloma	1/54 (40) 0.7659	1/54 (38) 1.0000	0/54 (41) 1.0000	0/54 (44) 1.0000	0/54 (37) 1.0000
	Sarcoma, Nos	1/54 (41) 0.5190	0/54 (38) NC	0/54 (41) NC	0/54 (44) NC	0/54 (37) NC
Spleen	Haemangioma	0/53 (40) NC	0/54 (38) 0.2313	0/54 (41) NC	0/54 (44) NC	1/54 (37) 0.4933
	Haemangiosarcoma	1/53 (41) 0.5190	0/54 (38) 0.2838	1/54 (42) 0.5250	1/54 (44) 0.5366	1/54 (37) 0.4933
Stomach	Adenocarcinoma	0/54 (40) NC	0/54 (38) 0.2360	0/54 (41) NC	0/54 (44) NC	1/54 (38) 0.5000
	Adenoma	1/54 (40) 0.5128	0/54 (38) 0.5063	0/54 (41) NC	1/54 (44) 0.5366	0/54 (37) NC
	Squamous Cell Carcinoma	0/54 (40) 1.0000	1/54 (38) 1.0000	0/54 (41) 1.0000	0/54 (44) 1.0000	0/54 (37) 1.0000
Testes	Adenoma, Leydig Cell	0/54 (40) 1.0000	2/54 (38) 0.4603	2/54 (41) 0.7207	1/54 (44) 0.9047	2/53 (37) 0.6825
	Carcinoma, Embryonal	0/54 (40) NC	0/54 (38) 0.5063	0/54 (41) NC	1/54 (45) 0.5422	0/53 (36) NC
	Haemangioma	1/54 (41) 0.5190	0/54 (38) NC	0/54 (41) NC	0/54 (44) NC	0/53 (36) NC
	Papilloma. Rete Testis	1/54 (40) 0.5128	0/54 (38) NC	0/54 (41) NC	0/54 (44) NC	0/53 (36) NC
Thyroid Glands	Adenoma, Follicular Cell	0/54 (40) NC	0/54 (38) 0.7610	1/54 (41) 0.5190	0/54 (44) NC	0/53 (36) NC
Non-Protocol Nerve	Schwannoma	0/54 (40) 1.0000	1/54 (38) 1.0000	0/54 (41) 1.0000	0/54 (44) 1.0000	0/54 (37) 1.0000
Whole Body	Haemangioma/ Haemangiosarcoma	2/54 (41) 0.2661	0/54 (38) 0.0331	2/54 (42) 0.2725	2/54 (44) 0.2849	4/54 (38) 0.0575

Table 6B: Tumor Rates and P-Values for Dose Response Relationship and the Pairwise Comparisons

Female Mice Poly-3 Test

Organ Name	Tumor Name	0 mg Water Cont (N=54) P - VC vs. L	0 mg Vehicle Cont (N=54) P - Trend	10 mg Low(N=54) P - VC vs. L	30 mg Med (N=54) P - VC vs. M	100 mg High (N=54) P - VC vs. H
Adrenals	Adenoma, Subcapsular Cell	2/54 (40) 0.2666	0/54 (37) 0.1947	0/54 (36) NC	1/54 (38) 0.5067	1/53 (38) 0.5067
Brain	Glioblastoma	0/54 (39) 1.0000	1/54 (37) 1.0000	0/54 (36) 1.0000	0/54 (37) 1.0000	0/54 (38) 1.0000
Cervix	Leiomyoma	0/53 (39) NC	0/54 (37) 0.4537	2/54 (37) 0.2466	0/54 (37) NC	1/54 (39) 0.5132
	Leiomyosarcoma	0/53 (39) NC	0/54 (37) 0.0677	0/54 (36) NC	1/54 (38) 0.5067	2/54 (39) 0.2600
	Leiomyoma/ Leiomyosarcoma	0/53 (39) NC	0/54 (37) 0.2127	2/54 (37) 0.2466	1/54 (38) 0.5067	2/54 (39) 0.2600
	Polyp	0/53 (39) NC	0/54 (37) 0.5068	0/54 (36) NC	1/54 (37) 0.5000	0/54 (38) NC
	Tumour, Epithelial With Squamous Differe	0/53 (39) NC	0/54 (37) 0.2617	0/54 (36) NC	0/54 (37) NC	1/54 (39) 0.5132
Clitoral Glands	Adenocarcinoma	0/52 (39) 1.0000	1/52 (36) 0.8323	1/50 (33) 0.7315	1/53 (36) 0.7535	0/50 (35) 1.0000
Duodenum	Adenocarcinoma	0/54 (39) NC	0/54 (37) 0.5068	0/54 (36) NC	1/54 (37) 0.5000	0/54 (38) NC
	Adenoma	0/54 (39) 1.0000	1/54 (37) 0.7584	0/54 (36) 1.0000	1/54 (37) 0.7534	0/54 (38) 1.0000
Eyelids	Papilloma	0/54 (39) NC	0/54 (37) 0.7500	1/54 (36) 0.4932	0/54 (37) NC	0/54 (38) NC
Eyes	Melanoma, Amelanotic, Cilliary Body	1/54 (40) 0.5195	0/54 (37) NC	0/54 (36) NC	0/54 (37) NC	0/54 (38) NC
Gall Bladder	Adenoma	0/54 (39) 1.0000	1/54 (37) 1.0000	0/54 (36) 1.0000	0/54 (37) 1.0000	0/52 (38) 1.0000
Haemopoietic Tissue	Lymphoma	12/54 (42) 0.8595	16/54 (43) 0.9384	13/54 (38) 0.6955	14/54 (41) 0.6984	9/54 (40) 0.9561
	Sarcoma, Histiocytic	2/54 (40) 0.2666	0/54 (37) 0.1302	1/54 (36) 0.4932	1/54 (37) 0.5000	2/54 (39) 0.2600
Harderian Glands	Adenoma	7/54 (40) 0.1887	3/54 (37) 0.1484	4/54 (36) 0.4844	2/54 (37) 0.8212	6/54 (39) 0.2673
Head	Carcinoma, Zymbal's Gland	0/54 (39) NC	0/54 (37) 0.2568	0/54 (36) NC	0/54 (37) NC	1/54 (38) 0.5067
Hindlimbs	Osteosarcoma	0/54 (39) NC	0/54 (37) 0.7517	1/54 (37) 0.5000	0/54 (37) NC	0/54 (38) NC

Female Mice Poly-3 Test

Organ Name	Tumor Name	0 mg Water Cont (N=54) P - VC vs. L	0 mg Vehicle Cont (N=54) P - Trend	10 mg Low(N=54) P - VC vs. L	30 mg Med (N=54) P - VC vs. M	100 mg High (N=54) P - VC vs. H
Ileum	Adenocarcinoma	0/54 (39) NC	0/54 (37) 0.5068	0/54 (36) NC	1/54 (37) 0.5000	0/54 (38) NC
	Lymphoma	0/54 (39) 1.0000	1/54 (38) 1.0000	0/54 (36) 1.0000	0/54 (37) 1.0000	0/54 (38) 1.0000
Liver	Adenoma, Hepatocellular	1/54 (39) 0.5132	0/54 (37) 0.5101	0/54 (36) NC	1/54 (38) 0.5067	0/54 (38) NC
	Carcinoma, Hepatocellular	0/54 (39) 1.0000	1/54 (37) 1.0000	0/54 (36) 1.0000	0/54 (37) 1.0000	0/54 (38) 1.0000
	Adenoma/ Carcinoma Hepatocellular	1/54 (39) 0.7663	1/54 (37) 0.7617	0/54 (36) 1.0000	1/54 (38) 0.7600	0/54 (38) 1.0000
	Cholangiocarcinoma	1/54 (40) 0.5195	0/54 (37) NC	0/54 (36) NC	0/54 (37) NC	0/54 (38) NC
	Haemangiosarcoma	1/54 (39) 0.5132	0/54 (37) NC	0/54 (36) NC	0/54 (37) NC	0/54 (38) NC
Lungs	Adenocarcinoma	11/54 (42) 0.5786	10/54 (39) 0.6584	7/54 (37) 0.8359	10/54 (38) 0.5759	8/54 (40) 0.8065
	Adenoma, Bronchiolo- Alveolar	7/54 (42) 0.3380	4/54 (37) 0.1776	2/54 (36) 0.8942	6/54 (37) 0.3678	6/54 (39) 0.4028
	Adenocarcinoma/ Adenoma, Bronchiolo-Alveolar	18/54 (44) 0.4048	14/54 (39) 0.4081	9/54 (37) 0.9115	15/54 (38) 0.4647	14/54 (41) 0.6549
	Haemangioma	1/54 (39) 0.5132	0/54 (37) NC	0/54 (36) NC	0/54 (37) NC	0/54 (38) NC
	Mesothelioma	0/54 (39) NC	0/54 (37) 0.7517	1/54 (37) 0.5000	0/54 (37) NC	0/54 (38) NC
Mammary Gland (Non-Protocol)	Adenocarcinoma	1/54 (40) 0.5195	0/54 (37) 0.5068	0/54 (36) NC	1/54 (37) 0.5000	0/54 (38) NC
	Carcinoma, Adenosquamous	3/54 (40) 0.3377	1/54 (37) 0.9388	1/54 (36) 0.7466	0/54 (37) 1.0000	0/54 (38) 1.0000
Mesenteric Lymph Nodes	Haemangiosarcoma	0/50 (38) NC	0/53 (36) 0.2587	0/50 (34) NC	0/53 (36) NC	1/51 (37) 0.5068
Ovaries	Adenoma, Rete Ovarii	0/54 (39) NC	0/54 (37) 0.8154	2/54 (37) 0.2466	0/54 (37) NC	0/54 (38) NC
	Adenoma, Tubulostromal	1/54 (40) 0.5195	0/54 (37) 0.5068	0/54 (36) NC	1/54 (37) 0.5000	0/54 (38) NC
	Granulosar-Thecal Cell Tumour	1/54 (39) 0.5132	0/54 (37) NC	0/54 (36) NC	0/54 (37) NC	0/54 (38) NC
	Haemangioma	0/54 (39) 1.0000	1/54 (38) 0.7310	3/54 (37) 0.2973	0/54 (37) 1.0000	1/54 (39) 0.7597

Female Mice Poly-3 Test

Organ Name	Tumor Name	0 mg Water Cont (N=54) P - VC vs. L	0 mg Vehicle Cont (N=54) P - Trend	10 mg Low(N=54) P - VC vs. L	30 mg Med (N=54) P - VC vs. M	100 mg High (N=54) P - VC vs. H
	Haemangiosarcoma	0/54 (39) NC	0/54 (37) 0.7517	1/54 (37) 0.5000	0/54 (37) NC	0/54 (38) NC
	Luteoma	0/54 (39) NC	0/54 (37) 0.6309	1/54 (37) 0.5000	1/54 (37) 0.5000	0/54 (38) NC
Oviducts	Leiomyosarcoma	0/53 (38) 1.0000	1/54 (38) 1.0000	0/53 (35) 1.0000	0/53 (36) 1.0000	0/52 (37) 1.0000
Pancreas	Adenoma, Islet Cell	0/54 (39) 1.0000	1/54 (37) 1.0000	0/54 (36) 1.0000	0/54 (37) 1.0000	0/53 (37) 1.0000
Pituitary Gland	Adenocarcinoma	0/53 (38) NC	0/53 (37) 0.7517	1/53 (37) 0.5000	0/54 (37) NC	0/54 (38) NC
	Adenoma	0/53 (38) NC	0/53 (37) 0.2155	0/53 (36) NC	2/54 (38) 0.2533	1/54 (39) 0.5132
	Adenocarcinoma/ Adenoma	0/53 (38) NC	0/53 (37) 0.3390	1/53 (37) 0.5000	2/54 (38) 0.2533	1/54 (39) 0.5132
Rectum	Leiomyosarcoma	0/54 (39) 1.0000	1/54 (37) 1.0000	0/54 (36) 1.0000	0/54 (37) 1.0000	0/54 (38) 1.0000
Salivary Gland	Adenocarcinoma	1/54 (39) 0.5132	0/54 (37) NC	0/54 (36) NC	0/54 (37) NC	0/54 (38) NC
	Myoepithelioma	0/54 (39) 1.0000	1/54 (37) 1.0000	0/54 (36) 1.0000	0/54 (37) 1.0000	0/54 (38) 1.0000
Skin	Sarcoma, Nos	0/54 (39) NC	0/54 (37) 0.7517	1/54 (37) 0.5000	0/54 (37) NC	0/54 (38) NC
Skin/Subcutis (Non-Protocol)	Fibrosarcoma	0/54 (39) NC	0/54 (37) 0.0224*	0/54 (36) NC	1/54 (38) 0.5067	3/54 (40) 0.1351
Spleen	Haemangioma	0/54 (39) NC	0/54 (37) 0.2617	0/54 (36) NC	0/54 (37) NC	1/54 (39) 0.5132
	Haemangiosarcoma	0/54 (39) 1.0000	2/54 (37) 0.9856	1/54 (37) 0.8801	0/54 (37) 1.0000	0/54 (38) 1.0000
	Histiocytoma	0/54 (39) NC	0/54 (37) 0.2568	0/54 (36) NC	0/54 (37) NC	1/54 (38) 0.5067
Sternum	Haemangioma	0/54 (39) NC	0/54 (37) 0.5101	0/54 (36) NC	1/54 (38) 0.5067	0/54 (38) NC
	Osteofibroma	0/54 (39) 1.0000	1/54 (38) 1.0000	0/54 (36) 1.0000	0/54 (37) 1.0000	0/54 (38) 1.0000
Tail	Fibrosarcoma	0/54 (39) NC	0/54 (37) 0.2568	0/54 (36) NC	0/54 (37) NC	1/54 (38) 0.5067
	Haemangiosarcoma	0/54 (39) NC	0/54 (37) 0.2617	0/54 (36) NC	0/54 (37) NC	1/54 (39) 0.5132

Female Mice Poly-3 Test

Organ Name	Tumor Name	0 mg Water Cont (N=54) P - VC vs. L	0 mg Vehicle Cont (N=54) P - Trend	10 mg Low(N=54) P - VC vs. L	30 mg Med (N=54) P - VC vs. M	100 mg High (N=54) P - VC vs. H
Thymus	Thymoma	0/52 (39) NC	0/53 (36) 0.7500	1/50 (35) 0.4930	0/52 (35) NC	0/52 (38) NC
Thyroid Glands	Adenoma, Follicular Cell	0/54 (39) NC	0/54 (37) 0.5068	0/54 (36) NC	1/54 (37) 0.5000	0/54 (38) NC
	Follicular Carcinoma	0/54 (39) NC	0/54 (37) 0.7500	1/54 (36) 0.4932	0/54 (37) NC	0/54 (38) NC
	Adenoma/ Carcinoma Follicular	0/54 (39) NC	0/54 (37) 0.6326	1/54 (36) 0.4932	1/54 (37) 0.5000	0/54 (38) NC
Urinary Bladder	Histiocytoma	0/54 (39) NC	0/54 (37) 0.2568	0/54 (36) NC	0/54 (37) NC	1/54 (38) 0.5067
	Leiomyoma	0/54 (39) NC	0/54 (37) 0.7517	1/54 (37) 0.5000	0/54 (37) NC	0/54 (38) NC
Uterus	Adenocarcinoma	0/54 (39) NC	0/54 (37) 0.8154	2/54 (37) 0.2466	0/54 (37) NC	0/54 (38) NC
	Haemangioma	0/54 (39) 1.0000	2/54 (37) 0.9643	3/54 (38) 0.5128	1/54 (37) 0.8801	0/54 (38) 1.0000
	Haemangiosarcoma	1/54 (40) 0.5195	0/54 (37) 0.5068	0/54 (36) NC	1/54 (37) 0.5000	0/54 (38) NC
	Histiocytoma	0/54 (39) NC	0/54 (37) 0.5068	0/54 (36) NC	1/54 (37) 0.5000	0/54 (38) NC
	Leiomyoma	3/54 (40) 0.3377	1/54 (37) 0.3711	2/54 (37) 0.5000	2/54 (37) 0.5000	2/54 (38) 0.5101
	Leiomyosarcoma	1/54 (40) 0.7724	1/54 (37) 0.1874	0/54 (36) 1.0000	1/54 (38) 0.7600	2/54 (40) 0.5296
	Leiomyoma/ Leiomyosarcoma	4/54 (40) 0.3756	2/54 (37) 0.1997	2/54 (37) 0.6927	3/54 (38) 0.5128	4/54 (40) 0.3756
	Polyp(S)	5/54 (41) 0.0355*	0/54 (37) 0.3621	3/54 (37) 0.1199	3/54 (39) 0.1300	2/54 (38) 0.2533
	Sarcoma, Endometrial	1/54 (40) 0.7724	1/54 (37) 0.4563	0/54 (36) 1.0000	0/54 (37) 1.0000	1/54 (39) 0.7663
Diaphragm	Adenocarcinoma	1/54 (40) 0.5195	0/54 (37) NC	0/54 (36) NC	0/54 (37) NC	0/54 (38) NC
Whole Body	Haemangioma/ Haemangiosarcoma	3/54 (40) 0.8845	5/54 (38) 0.8128	8/54 (39) 0.2896	3/54 (38) 0.8690	4/54 (40) 0.7848

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals;
ZZ=unweighted total number of animals observed;
NC = Not calculable.

*: Statistically significant at 0.005 and 0.025 level for common and rare tumor or 0.01 and 0.05 level for common and rare tumors for tests of dose response relationship and pairwise comparison, respectively

Figure 2A: Kaplan-Meier Survival Curves for Male Mice

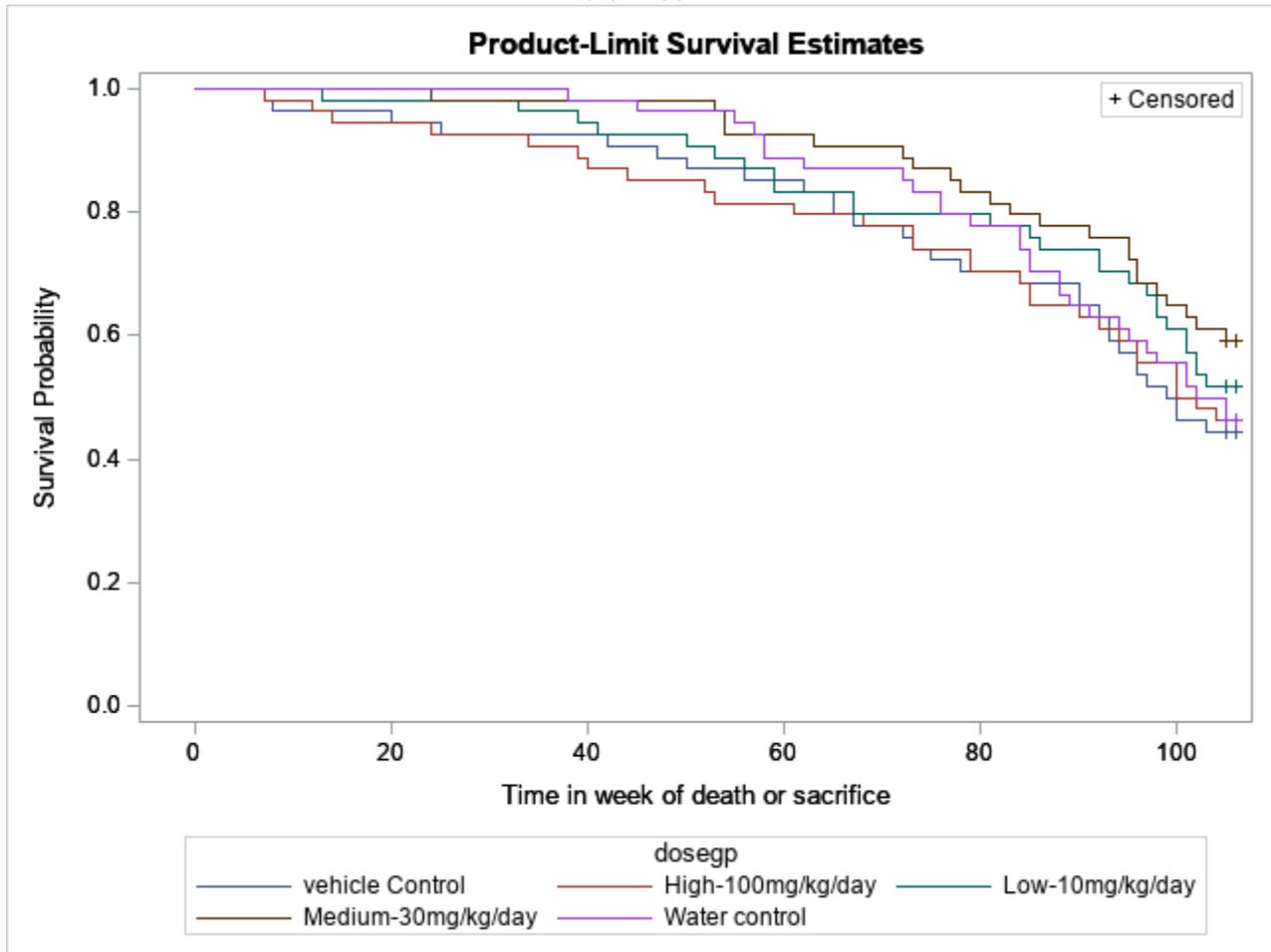
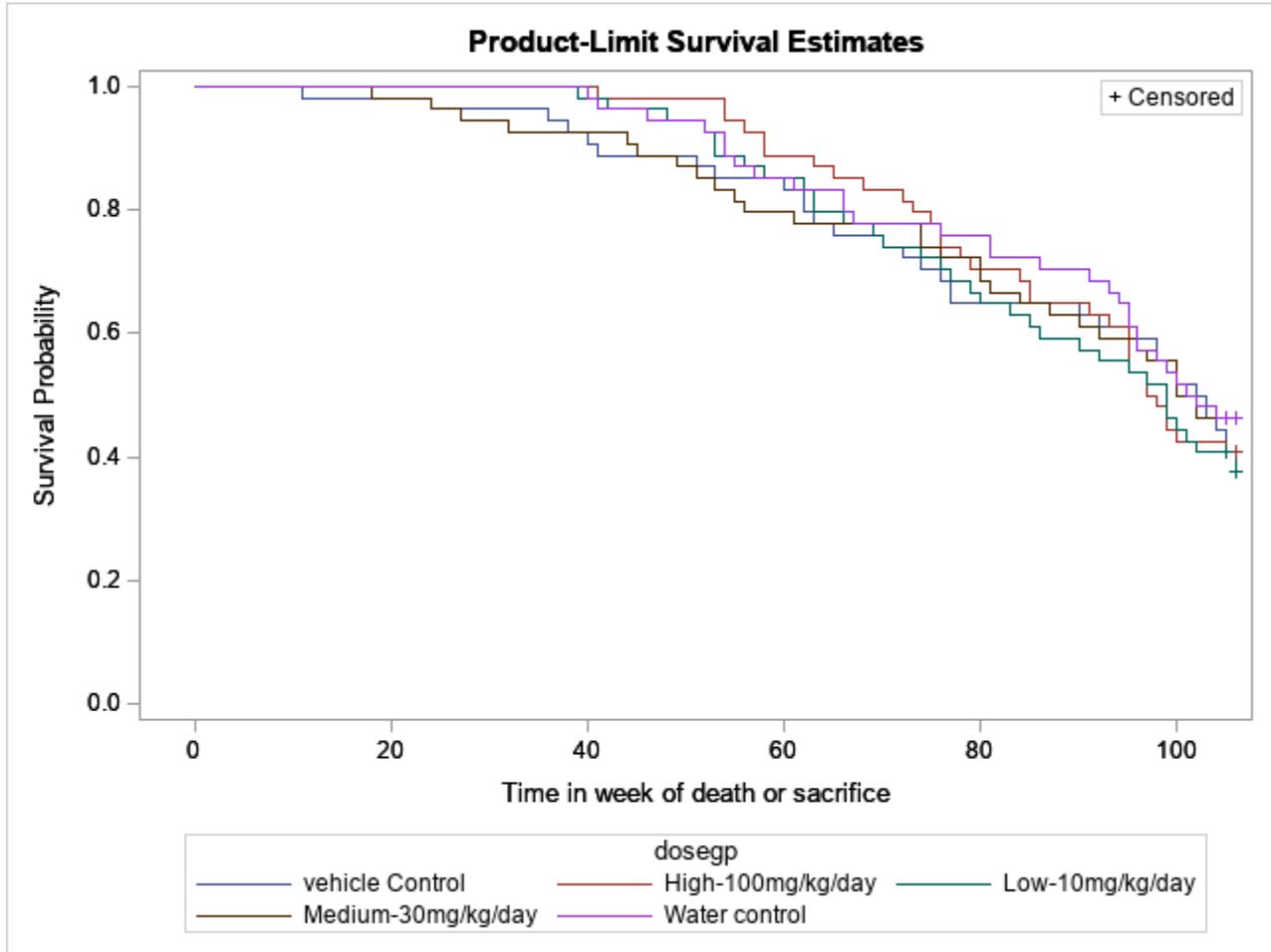


Figure 2B: Kaplan-Meier Survival Curves for Female Mice



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