

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

214018Orig1s000

INTEGRATED REVIEW

Integrated Review

Table 1. Administrative Application Information

Category	Application Information
Application type	NDA
Application number(s)	214018
Priority or standard	Priority
Submit date(s)	6/29/2020
Received date(s)	6/29/2020
PDUFA goal date	2/28/2021
Division/office	Division of Rare Diseases and Medical Genetics (DRDMG)
Review completion date	2/26/2021
Established/proper name	fosdenopterin
(Proposed) proprietary name	Nulibry
Pharmacologic class	Cyclic pyranopterin monophosphate
Code name	ALXN1101, ORGN001
Applicant	Origin Biosciences, Inc.
Dosage form(s)/formulation(s)	Lyophilized Powder for Injection
Dosing regimen	0.9 mg/kg intravenously daily
Applicant proposed indication(s)/ population(s)	Treatment of MoCD Type A, including in neonates
Proposed SNOMED indication	29692004 Combined molybdo flavoprotein enzyme deficiency (disorder)
Regulatory action	Approval
Approved dosage (if applicable)	For patients 1 year of age and older, 0.9 mg/kg administered as an intravenous infusion daily; for patients less than 12 months of age the dosage should be titrated up to 0.9 mg/kg over a period of 3 months
Approved indication(s)/ population(s) (if applicable)	Nulibry (fosdenopterin) for injection is indicated to reduce the risk of mortality in patients with molybdenum cofactor deficiency (MoCD) Type A
Approved SNOMED term for indication (if applicable)	29692004 Combined molybdo flavoprotein enzyme deficiency (disorder)

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Glossary

AE	adverse event
AET	analytical evaluation threshold
AUC	area under the concentration-time curve
BCRP	breast cancer resistance protein
CCS	container closure system
CI	confidence interval
C _{max}	maximum plasma concentration
CMC	chemistry, manufacturing, and controls
CNS	central nervous system
cPMP	cyclic pyranopterin monophosphate
CYP	cytochrome P450
E-R	exposure-response
FAS	full analysis set
FDA	Food and Drug Administration
GMAS	genotype-matched analysis set
HLM	human liver microsomes
IC ₅₀	half maximal inhibitory concentration
ICH	International Council for Harmonisation
ID	identification/identity
IIV	interindividual variation
IND	investigational new drug
ISE	integrated summary of effectiveness
IV	intravenous
HBr	monohydrobromide dihydrate
KM	Kaplan–Meier
KO	knockout
LC-MS/MS	liquid chromatography-tandem mass spectrometry
MATE	multidrug and toxin extrusion transporter
MoCD	molybdenum cofactor deficiency
MoCo	molybdenum cofactor
NDA	new drug application
NF	National Formulary
NOAEL	no observed adverse effect level
OAT	organic anion transporter
OCT	organic cation transporter
OFV	objective function value
OS	overall survival
PD	pharmacodynamic
PK	pharmacokinetic
PND	postnatal day
Q	intercompartmental clearance
RRA	remote regulatory assessment
SAE	serious adverse event

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SAP	statistical analysis plan
SD	standard deviation
SOX	sulfite oxidase
SSC	S-sulfocysteine
TEAE	treatment-emergent adverse event
TK	toxicokinetic
T _{max}	time to maximum concentration
TQT	thorough QT
UK	United Kingdom of Great Britain and Northern Ireland
V _p	peripheral volume
VPC	visual predictive check
WT	wild type

I. Executive Summary

1. Summary of Regulatory Action

Origin Biosciences, Inc. (Origin) submitted this new drug application (NDA) 214018 for Nulibry (fosdenopterin, a chemically synthesized form of cyclic pyranopterin monophosphate or cPMP), seeking approval of this first-in-class substrate replacement therapy for the rare disease molybdenum cofactor deficiency (MoCD) Type A. The estimated prevalence of MoCD Type A in the United States is 45 to 54 patients, all under 10 years of age. Patients experience intractable seizures, metabolic acidosis, failure to thrive, feeding difficulties, axial hypotonia and early death from the accumulation of toxic metabolites, primarily S-sulfocysteine (SSC), in the central nervous system. Most patients die during early childhood, most commonly from infection. The NDA was reviewed by the multidisciplinary review team. Each discipline recommends approval and the signatory authority for this application concurs with these recommendations.

Substantial evidence of effectiveness for Nulibry for the treatment of MoCD Type A was established with one adequate and well-controlled investigation plus confirmatory evidence. The adequate and well-controlled investigation demonstrated improved survival for a cohort of patients treated with synthetic or recombinant cPMP pooled from two clinical trials and a retrospective observational study compared to a matched observational cohort of untreated patients. Synthetic and recombinant cPMP are chemically equivalent, with the synthetic form being the active ingredient in Nulibry. The confirmatory evidence includes reduced urinary SSC in humans (SSC is a component of the canonical pathophysiologic pathway for this disease), an exposure-response relationship for reduced urinary SSC in humans, and animal data from a knockout mouse model of MoCD Type A showing a reduction of plasma and brain SSC and improved mortality in treated mice compared to controls. While the clinical manifestations of the disease are due to toxic metabolites in the central nervous system (CNS) and CNS SSC levels were not measured in humans, the animal data suggest that fosdenopterin can cross the blood brain barrier and that changes in peripheral SSC levels reflect reductions in brain SSC.

The available safety data show that Nulibry is safe for its intended use. The most common adverse reactions included respiratory infections and complications related to the central venous catheter, which is needed for chronic daily intravenous administration of Nulibry. The lack of a control arm for the safety assessment limits the extent to which these events can be attributed to the drug versus the disease and its comorbidities. Phototoxicity was observed in the nonclinical studies near anticipated clinical exposures, and the relevance to humans is unknown. This potential risk can be adequately mitigated through labeling and by an enhanced pharmacovigilance program.

The NDA does not include pre-approval carcinogenicity, reproductive and developmental toxicity, or QT studies. We are requiring these investigations postapproval so as not to further delay approval of Nulibry, which has shown a survival benefit for a rare, devastating condition with no other approved treatments.

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As described in Section [1.2.1](#) Benefit-Risk Framework, we conclude that Nulibry's mortality benefit outweighs the risks when Nulibry is used as recommended in the approved labeling.

2. Benefit-Risk Assessment

2.1. Benefit-Risk Framework

Table 2. Benefit-Risk Framework

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	- Molybdenum cofactor deficiency (MoCD) Type A is a rare, rapidly progressive life-threatening disease with an autosomal recessive pattern of inheritance that typically presents acutely in the neonatal period or early infancy with intractable seizures, metabolic acidosis, failure to thrive, feeding difficulties and axial hypotonia with limb hypertonia (Mechler et al. 2015; Atwal and Scaglia 2016). Mutations in the <i>MOCS1</i> gene lead to deficiency of cyclic pyranopterin monophosphate (cPMP), which is necessary for molybdenum cofactor synthesis. Without this cofactor, all molybdenum-dependent enzyme activity is deficient. Deficiency in the most important molybdenum-dependent enzyme, sulfite oxidase, results in the accumulation of neurotoxic sulfites, primarily S-sulfocysteine (SSC). Elevated levels of urinary sulfite and SSC are also characteristic findings. Elevated levels of these toxins in the central nervous system (CNS) lead to the largely irreversible neuronal injury seen in patients with this disease (Atwal and Scaglia 2016; Reiss 2016). - The estimated prevalence of MoCD Type A in the United States is 45 to 54 patients, all under 10 years of age.	MoCD Type A is a very rare, serious, and life-threatening disease with no approved treatments.
Current Treatment Options	There are no approved treatments. Current treatment is supportive to provide symptomatic relief.	There is an unmet medical need for effective and safe treatments for MoCD Type A.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Benefit	- Evidence of a survival benefit with fosdenopterin is based on an analysis of data pooled from a cohort of treated patients from two clinical trials and a retrospective observational study compared to a matched observational cohort of untreated patients; this constitutes an adequate and well-controlled clinical investigation. Thirteen patients with MoCD Type A treated with fosdenopterin (either the synthetic form, cPMP, or the recombinant form, rcPMP, which has the same active moiety as cPMP) had prolonged overall survival compared to the group of genotype-matched untreated natural history control patients. The mean survival time at 3 years was 32 months for fosdenopterin treated patients and 24 months for the untreated patients, corresponding to a hazard ratio for risk of death of 0.18 (95% confidence interval [CI] 0.04, 0.72). At 3 years, the Kaplan–Meier survival probability (95% CI) was 84% (49, 96) for fosdenopterin-treated patients and 55% (30, 74) for untreated patients. - Confirmatory evidence supporting the benefit observed in the clinical studies includes biomarker data that demonstrated a reduction of urinary SSC concentrations (normalized to creatinine) in fosdenopterin-treated patients, a reduction that was sustained with long-term treatment (up to 7 years after treatment initiation). Additionally, higher exposure to Nulibry was associated with lower levels of urinary SSC. In the mouse model of MoCD Type A, mice treated with fosdenopterin had improved survival and decreases in plasma and brain SSC levels compared to placebo-treated mice.	The conclusion that fosdenopterin provides a survival benefit for patients with MoCD Type A is established by an adequate and well-controlled investigation that demonstrated prolonged survival for fosdenopterin-treated patients compared to untreated patients. Confirmatory evidence of effectiveness of fosdenopterin treatment comes from biomarker data that demonstrated a reduction in urine SSC levels and a mouse model of MoCD showing reduced plasma SSC levels were associated with reduced SSC levels in the brain and improved survival.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Risk and Risk Management	- The safety of fodenopterin treatment for patients with MoCD Type A was demonstrated in 13 patients who received intravenous doses (administered via a central venous line) up to 1300 µg/kg daily (when received as cPMP, a hydrobromide salt form of fosdenopterin) or 1200 µg/kg daily (when received as rcPMP, a free base form of fosdenopterin). The overall exposure to fosdenopterin was 65.4 patient-years. Eight of these 13 patients initially received rcPMP prior to transitioning to cPMP (Nulibry). - The most common serious adverse events were respiratory infections and device (central line)-related complications. - Two patients died while on treatment with rcPMP. One death was due to respiratory syncytial virus pneumonia and the other death was due to necrotizing enterocolitis. - There were no treatment discontinuations or dose reductions due to treatment-emergent adverse events. - Overall, most treatment-emergent adverse events were nonserious and related to infection or device complications and unlikely drug-related. - One notable safety concern (i.e., a potential risk of phototoxicity) was identified in the nonclinical toxicology program. In vitro and in vivo animal studies demonstrated phototoxic effects of fosdenopterin. Although there was no evidence of phototoxicity in the clinical studies, potential phototoxicity was identified late in the nonclinical program, thus, the clinical studies did not include specific monitoring procedures for phototoxicity. Therefore, the clinical risk of phototoxicity is unclear.	- Overall, long-term treatment with Nulibry had an acceptable safety profile in patients with MoCD Type A. The device-related complications were expected since patients require a central venous catheter for daily administration of the drug. Risks of device-related infections and other device-related complications will be mitigated with labeling and the intended administration under the guidance of a healthcare provider. The respiratory infections observed were also expected considering the population of neurologically impaired children. In the absence of a control arm for safety, the ability to determine drug relatedness of the reported adverse events is limited. - The potential risk for phototoxicity, which was identified in the nonclinical studies and is of uncertain clinical relevance, can be adequately mitigated with labeling. Additionally, the Applicant has agreed to implement an enhanced pharmacovigilance program to monitor for potential phototoxic adverse events in the postmarket setting and further characterize the risk to patients.

2.2. Conclusions Regarding Benefit-Risk

MoCD Type A is a very rare, fatal, autosomal recessive disease with an estimated U.S. prevalence of approximately 50 patients, all under 10 years of age. It is caused by a variant in the *MOCS1* gene that leads to a deficiency of cyclic pyranopterin monophosphate (cPMP), causing deficient synthesis of molybdenum cofactor (MoCo) and the accumulation of toxic metabolites, especially

S-sulfocysteine (SSC). These toxic metabolites accumulate in the central nervous system, leading to intractable seizures, metabolic acidosis, failure to thrive, feeding difficulties, axial hypotonia and death in the first years of life mainly from infection. No drugs or biologics have been approved for the treatment of MoCD Type A.

Nulibry (fosdenopterin) is a synthetic cPMP administered intravenously once daily using weight-based dosing. The adequate and well-controlled investigation consisted of a comparison of overall survival in 13 patients with MoCD Type A who were treated with Nulibry or rcPMP (a recombinantly produced version of the drug with the same active moiety and same biologic activity) to that of an untreated natural history cohort of 18 patients with MoCD Type A who were genotype-matched to the treated patients. Patients who were treated with Nulibry (or rcPMP) had a significant improvement of overall survival compared to the untreated historical control group. The mean survival time at 3 years was 32 months for Nulibry (or rcPMP)-treated patients and 24 months for the untreated patients, corresponding to a hazard ratio for risk of death of 0.18 (95% confidence interval [CI] 0.04, 0.72). At 3 years, the Kaplan–Meier survival probability (95% CI) was 84% (49, 96) for Nulibry (or rcPMP)-treated patients and 55% (30, 74) for untreated patients.

Despite the limitations resulting from the lack of randomization, the use of an external noncontemporaneous control group, and the use of retrospective observational data in the treatment cohort, we determined that the efficacy data were adequate to support a conclusion that Nulibry provides a survival benefit in patients with MoCD Type A. The strengths of these data, including the use of a reliable and objective endpoint of mortality and the demonstration of a large treatment effect size, outweigh the limitations in the context of a very rare disease that is rapidly fatal with no other therapies known to improve survival. Additionally, the potential sources of bias were adequately addressed in the study designs and in the analyses of the efficacy data.

Safety concerns for Nulibry were identified based on nonclinical studies and from the 13 treated patients with MoCD Type A. In nonclinical studies, phototoxicity was observed in animals administered fosdenopterin at doses near human exposures. Clinical relevance could not be excluded. The adverse events (AEs) observed in more than 50% of the treated patients included catheter (central venous line)-related complications (including catheter site infections, pain and catheter dislocation) and respiratory infections. Despite the high incidence of AEs, no patients discontinued study treatment due to AEs. Although it is not possible to definitively exclude a drug effect because of the lack of a randomized control group, these AEs are likely not related to Nulibry itself, and instead reflect risks inherent to a central venous line and/or the patient population of neurologically compromised children. The safety risks of Nulibry can be adequately mitigated with labeling, including a Warning and an enhanced postmarketing pharmacovigilance program for phototoxicity.

Uncertainties about safety include the lack of pre-approval nonclinical reproductive and developmental toxicity and carcinogenicity studies, and a clinical thorough QT study. We are not requiring these investigations pre-approval so as not to further delay approval of Nulibry, which has a mortality benefit for a rare, serious, devastating disease without other treatment options. Because untreated patients with MoCD Type A do not survive into adolescence and adulthood, we agreed with the Applicant to delay the nonclinical reproductive and developmental toxicity studies until additional clinical data on patients treated with Nulibry (e.g., on longer-term survival, reproductive capability) are available to inform the necessity of reproductive toxicology studies in this particular patient

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population. Labeling will note that a dedicated thorough QT study and nonclinical carcinogenicity and reproductive toxicology studies have not been conducted.

In summary, we conclude that the benefits of Nulibry outweigh its risks when used according to the agreed-upon labeling. The availability of Nulibry for MoCD Type A will provide the first approved treatment option for this patient population.

II. Interdisciplinary Assessment

3. Introduction

Origin Biosciences, Inc. (Origin) seeks approval of fosdenopterin for the treatment of molybdenum cofactor deficiency (MoCD) Type A. Fosdenopterin is a new molecular entity. The Food and Drug Administration (FDA) granted Orphan Drug Designation on November 5, 2009, Breakthrough Therapy Designation on October 23, 2013 and Rare Pediatric Disease Designation on June 15, 2017. Refer to Section [III.12](#) for pertinent regulatory history.

MoCD Type A is a rapidly progressive, very rare, autosomal recessive neurodegenerative disease that results from mutations in the *MOCS1* gene, leading to deficient synthesis of the intermediate substrate, cPMP, which is necessary for the synthesis of MoCo (Reiss 2016). As a result of the MoCo deficiency, MoCo-dependent enzyme activity becomes undetectable. MoCo is a cofactor for four enzymes, with sulfite oxidase (SOX) the most important physiologically. The lack of effective SOX leads to elevated levels of neurotoxic sulfites and the metabolite S-sulfocysteine (SSC). Sulfites deplete cysteine and glutathione, leading to the formation of sulfur-containing metabolites including SSC. SSC levels in the plasma and urine are directly correlated with sulfite burden throughout disease progression. Elevations in these neurotoxins lead to significant and irreversible structural damage to the brain and the clinical manifestations of the disease. Elevated levels of urinary sulfite and SSC and low or absent uric acid in the urine or plasma are biochemical hallmarks of MoCD Type A (Johnson et al. 2001). Symptoms of MoCD Type A include intractable seizures, feeding difficulties, axial hypotonia with limb hypertonia, impairment of growth, and significantly delayed cognitive and motor skills. The metabolic derangements lead to rapidly progressive neurologic decline and patients usually die in the first years of life (Mechler et al. 2015).

Nulibry is a chemically synthesized form of endogenous cPMP. It treats MoCD Type A by replacing the cPMP substrate and permitting the remaining MoCo synthesis steps to proceed, enabling activation of MoCo-dependent enzymes and the elimination of sulfites.

The review team identified several issues that impacted the assessment of the benefits and risks of Nulibry. These review issues are listed and briefly described in Section [II.3.1](#). Because an understanding of the complex design of the clinical program is necessary to discuss most of these issues and how the review team addressed them, the review issues are discussed in depth later in the review (the benefit review issues in Section [II.6.3](#) and the risk review issues in Section [II.7.7](#)).

3.1. Review Issue List

3.1.1. Key Review Issues Relevant to Evaluation of Benefit

3.1.1.1. Potential for Selection Bias

Given the lack of a randomized control group for the efficacy analysis, there was a potential for selection bias. This could lead to differences between treated and untreated patients other than the use of Nulibry, yielding a biased treatment effect of Nulibry. However, the Applicant used several approaches to minimize this potential bias and the review team determined that these approaches were sufficient to overcome this potential bias. For details, refer to Section [6.3.1. Potential for Detection Bias or a Spurious Mortality Benefit](#).

Given the ability to diagnose MoCD Type A prior to symptom onset (i.e., with cascade testing, or genetic testing of relatives of known patients with MoCD including in utero prenatal testing), there was also the potential for detection bias in the evaluation of efficacy and a potential spurious mortality benefit (from diagnosing and treating patients who would have had a less severe disease course if left untreated). However, several factors led the review team to conclude that detection bias was not a contributing factor in the observed benefit. For details, refer to Section [6.3.2](#).

3.1.1.2. Regulatory Framework for Establishing Substantial Evidence of Effectiveness

Substantial evidence of effectiveness, which is the regulatory requirement for approval, generally consists of evidence from at least two adequate and well-controlled investigations. However, in certain circumstances, a single adequate and well-controlled investigation demonstrating efficacy may be sufficient to generate substantial evidence of effectiveness if appropriate confirmatory evidence is included in the application. In this NDA, biomarker data demonstrating a reduction in a metabolite with known neurotoxic effects, together with animal data demonstrating improved survival in the mouse model of the disease, provide acceptable confirmatory evidence to support the single adequate and well-controlled clinical investigation and render the lack of a second, confirmatory clinical investigation acceptable in the approval evaluation of Nulibry. For details, refer to Section [6.3.3](#).

3.1.1.3. Post Hoc Nature of the Statistical Analysis Plan

In general, statistical analysis plans (SAPs) should be submitted to the Agency in advance of knowing the outcomes to minimize the possibility of making a type I error and concluding the drug is effective when it is not. The SAP for the adequate and well-controlled investigation was formulated after mortality outcomes were already known. Although the efficacy analysis in this NDA was post hoc, we conclude that the overall data support a true effect of fosdenopterin on mortality and provide persuasive evidence that the observed mortality benefit was not due to chance. Refer to Section [6.3.4](#) for further details.

3.1.1.4. Use of Both rcPMP and cPMP in the Treatment Group

Two different drug products (rcPMP and cPMP) were used in the clinical studies. Since efficacy and safety data in this NDA are derived from both rcPMP and cPMP, it was necessary to conclude that they are chemically equivalent. Multiple analytical techniques (refer to Section [9](#) for details) demonstrated that rcPMP and cPMP have identical core structures and the same active moiety. Because rcPMP and cPMP are chemically equivalent, efficacy and safety data from rcPMP can be used to determine the safety and efficacy of cPMP (Nulibry).

3.1.2. Key Review Issues Relevant to Evaluation of Risk

3.1.2.1. Labeled Option for Administration by a Caregiver

Nulibry is primarily intended for administration by healthcare providers and requires reconstitution prior to administration. The proposed Nulibry labeling indicates an option for caregiver administration if deemed appropriate by the healthcare provider. Caregiver reconstitution and administration of an intravenous drug is not standard in clinical practice. The review team was concerned about the ability of nonhealthcare providers to safely and effectively administer Nulibry given the complex steps of reconstitution and administration. However, upon review of data comparing adverse events (AEs) (particularly those related to the central venous catheter) experienced by patients during Nulibry treatment administered by caregivers versus healthcare providers, we concluded that the Applicant had provided adequate evidence to support this administration option as described in the label. Additionally, if healthcare providers deem it appropriate for a caregiver to administer the drug, caregivers will have detailed instructions and ongoing support. See Section [7.7.1](#) for a detailed discussion.

3.1.2.2. Potential Risk of Phototoxicity

In the nonclinical studies, phototoxic potential was identified late in the drug development program and was, therefore, not specifically monitored for in the clinical studies. This potential risk can be adequately mitigated with labeling and will need to be further monitored and characterized with an enhanced pharmacovigilance monitoring program (see Section [7.7.2](#) for a detailed discussion).

3.2. Approach to the Review

[Table 3](#) provides an overview of the clinical studies conducted to support the benefit-risk assessment of fosdenopterin for the treatment of MoCD Type A. The results of Studies MCD-201, MCD-202, MCD-501, and MCD-502 provided the primary basis to establish the efficacy and safety of fosdenopterin.

Efficacy was assessed in a combined analysis of the 13 patients with genetically confirmed MoCD Type A from Studies MCD-501, MCD-201, and MCD-202 who received substrate replacement therapy with Nulibry (or rcPMP). In Study MCD-501, patients were treated with the recombinant form of the drug (rcPMP), which has the same active moiety as Nulibry (chemically synthesized cPMP). Six of these thirteen patients transitioned from Study MCD-501 to MCD-201. Efficacy was primarily assessed by comparing overall survival in these 13 patients treated with Nulibry (or rcPMP) with an untreated natural history cohort of 18 patients with

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genetically confirmed MoCD Type A (from Study MCD-502) who were genotype-matched to the treated patients.

Safety was assessed by analysis of the AE data sets for Studies MCD-201, MCD-202, and MCD-501. Safety was also assessed by analysis of laboratory data and vital sign data for these studies.

Table 3. Clinical Trials Submitted in Support of Efficacy and/or Safety Determinations¹ for Nulibry

Trial Identifier (NCT#)	Trial Population	Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	Number of Subjects (Actual Enrolled)	Number of Centers and Countries
MCD-201 (NCT02047461)	Patients with MoCD Type A	Uncontrolled, open- label Nonrandomized Unblinded Biomarkers: Blood and urine levels of SSC, xanthine, uric acid	Drug: Fosdenopterin Dosage: Up to 1200 µg/kg/day Number treated: 8	Primary: Safety Secondary: Growth, seizure frequency Feeding patterns Motor function Neurocognitive functions Neuroimaging Biomarkers, PK data Long-term safety	8	5 centers in United States, Australia, Netherlands, United Kingdom, Tunisia
MCD-202 (NCT02629393)	Neonates with confirmed or suspected MoCD Type A	Uncontrolled Nonrandomized Unblinded Biomarkers: Blood and urine levels of SSC, xanthine, uric acid Innovative design features: Open-label	Drug: fosdenopterin Dosage: 1300 µg/kg/day Number treated: 1	Primary: Safety Secondary: Growth Seizure frequency Feeding patterns Motor function Neurocognitive function Neuroimaging PK data	1	(b) (6)
MCD-501	Patients with MoCD Type A	Retrospective data collection study of patients with MoCD Type A, B, or unknown type who received rcPMP under individual patient INDs	Drug: rcPMP; 10 patients with MoCD Type A treated	Data on: Growth seizure frequency Feeding patterns Motor function Neurocognitive function Neuroimaging Safety Biomarkers	15	13 centers in Germany, United Kingdom, Netherlands, Australia, Turkey, United States

Trial Identifier (NCT#)	Trial Population	Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	Number of Subjects (Actual Enrolled)	Number of Centers and Countries
MCD-502 (NCT01735188)	Patients with MoCD Type A	Retrospective and prospective natural history study	Drug: None	N/A (not prespecified; objective was to characterize the natural history of MoCD Type A and observe clinical outcomes, including survival)	37	27 centers in Canada, Germany, Spain, United Kingdom, Israel, Italy, Japan, Malaysia, Netherlands, Poland, Saudi Arabia, Tunisia, Turkey, United States

Source: Reviewer

¹ Includes all submitted clinical trials, even if not reviewed in-depth, except for phase 1 and pharmacokinetics studies.

Abbreviations: IND, investigational new drug; MoCD, molybdenum cofactor deficiency; NCT, National Clinical Trials Register; PK, pharmacokinetics; rcPMP, recombinant cyclic pyranopterin monophosphate; SSC, S-sulfocysteine

4. Patient Experience Data

Table 4. 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		
☐	Patient-reported outcome	
☐	Observer-reported outcome	
☐	Clinician-reported outcome	
☐	Performance outcome	
Other patient experience data submitted in the application		
☐	Patient-focused drug development meeting summary	
☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel)	
☐	Observational survey studies	
☐	Natural history studies	
☐	Patient preference studies	
☐	Other: (please specify)	
☒	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
☐	Perspectives shared at patient stakeholder meeting	
☐	Patient-focused drug development meeting summary report	
☐	Other stakeholder meeting summary report	
☐	Observational survey studies	
☐	Other: (please specify)	

5. Pharmacologic Activity, Pharmacokinetics, and Clinical Pharmacology

The pharmacologic activity, pharmacokinetics, and clinical pharmacology of fosdenopterin relevant to the interpretation of benefit and risk are summarized in [Table 5](#).

Table 5. Summary of Pharmacologic Activity, Clinical Pharmacology, and Pharmacokinetics

Pharmacologic Activity																					
Established pharmacologic class	Cyclic pyranopterin monophosphate (cPMP)																				
Mechanism of action	Fosdenopterin provides an exogenous source of cPMP. cPMP is converted to molybdopterin, which is then converted to molybdenum cofactor (MoCo) and restores molybdenum cofactor biosynthesis. In MOCD Type A, mutations in MOCS1 lead to deficient MOCS1A/B-dependent synthesis of cPMP, which subsequently results in deficient molybdenum-dependent enzyme activity (e.g., sulfite oxidase).																				
Active moieties	The parent drug fosdenopterin is the active moiety.																				
QT prolongation	The Applicant has not conducted a thorough QT/QTc study to assess the effect of fosdenopterin on QT interval prolongation. We are requiring a postmarketing thorough QT/QTc study rather than a pre-approval study so as not to delay access of this drug that has shown a survival benefit for a rare, serious condition with no other approved therapies.																				
General Information																					
Bioanalysis	Two validated reverse-phase liquid chromatography methods using MS/MS detection were used to determine cPMP concentrations in human plasma and urine in Studies MCD-101, MCD-201, and MCD-202. The performance of the bioanalytical methods was acceptable.																				
Healthy subjects versus patients	The PK of fosdenopterin was characterized in healthy adult subjects in Study MCD-101 and in pediatric subjects with MoCD Type A in Studies MCD-201 and MCD-202. MCD-101 used intensive PK sampling and Studies MCD-201 and MCD-202 used sparse PK sampling. The limited PK data did not identify PK differences in patients and healthy subjects after adjustment for body weight.																				
Drug exposure at steady state following the therapeutic dosing regimen (or single dosage, if more relevant for the drug)	<table><tr><td colspan="4">The PK of parameters (mean±SD) of fosdenopterin in healthy adult subjects following single-dose intravenous (IV) infusion:</td></tr><tr><td>Parameter</td><td>0.075 mg/kg (n=6)</td><td>0.24 mg/kg (n=6)</td><td>0.68 mg/kg (n=6)</td></tr><tr><td>C_{max} (ng/mL)</td><td>285 (57)</td><td>873 (99)</td><td>2800 (567)</td></tr><tr><td>AUC_{0-last} (ng.hr/mL)</td><td>508 (74)</td><td>1760 (205)</td><td>5930 (1820)</td></tr><tr><td>AUC_{0-inf} (ng.hr/mL)</td><td>523 (75)</td><td>1790 (213)</td><td>5960 (1820)</td></tr></table>	The PK of parameters (mean±SD) of fosdenopterin in healthy adult subjects following single-dose intravenous (IV) infusion:				Parameter	0.075 mg/kg (n=6)	0.24 mg/kg (n=6)	0.68 mg/kg (n=6)	C _{max} (ng/mL)	285 (57)	873 (99)	2800 (567)	AUC _{0-last} (ng.hr/mL)	508 (74)	1760 (205)	5930 (1820)	AUC _{0-inf} (ng.hr/mL)	523 (75)	1790 (213)	5960 (1820)
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C _{max} (ng/mL)	285 (57)	873 (99)	2800 (567)																		
AUC _{0-last} (ng.hr/mL)	508 (74)	1760 (205)	5930 (1820)																		
AUC _{0-inf} (ng.hr/mL)	523 (75)	1790 (213)	5960 (1820)																		

Characteristic	Drug Information
Range of effective dosage(s) or exposure	Fosdenopterin is administered as an IV infusion once daily. For patients less than 12 months, the recommended starting dose is 0.55 mg/kg for neonates with gestational age of ≥ 37 weeks and 0.40 mg/kg for preterm neonates with gestational age of < 37 weeks. The dosage should be titrated to 0.9 mg/kg over a period of 3 months. For patients 1 year of age and older, the recommended dosage is 0.9 mg/kg. The highest dose administered in pediatric patients with MoCD Type A in clinical studies is 0.98 mg/kg/day.
Maximally tolerated dosage or exposure	A maximally tolerated dose has not been determined.
Dosage proportionality	Fosdenopterin exhibited linear pharmacokinetics. Fosdenopterin C_{max} and AUC demonstrated dose-proportional increase with increasing doses from 0.075 to 0.68 mg/kg (MCD-101).
Accumulation	No accumulation is observed at the recommended IV doses administered once daily
Time to achieve steady-state	Steady-state was achieved within 24 hr after the first dose administration.
Bridge between to-be-marketed and clinical trial formulations	The to-be-marketed formulation of fosdenopterin has been used in pivotal efficacy and safety clinical trials (Studies MCD-201 and MCD-202). The drug substance was initially developed as rcPMP, a recombinant <i>Escherichia coli</i> -derived non-salt, anhydrous form of cPMP which was used in Study MCD-501. The analytical comparability data have demonstrated that rcPMP and fosdenopterin have the same active moiety. See the OPQ review for more information. As fosdenopterin is administered via IV infusion, manufacturing changes in drug substance or drug product are not expected to affect bioavailability. A bioequivalence bridge between the to-be-marketed and clinical trial formulations is not needed.
Absorption	
Bioavailability	The bioavailability is 100%, because fosdenopterin is administered by IV infusion.
T_{max}	The T_{max} is expected to be achieved at the end of IV infusion.
Distribution	
Volume of distribution	The mean volume of distribution of fosdenopterin ranged from 341 mL/kg to 436 mL/kg following IV administration in healthy adult subjects.
Plasma protein binding	Plasma protein binding of fosdenopterin ranged from 6% to 12% at cPMP concentrations ranging from 0.1 to 10 μ M.
Drug as substrate of transporters	Fosdenopterin is not a substrate of P-gp, BCRP, OAT1, OAT3, OATP1B1, OATP1B3, OCT2, or MATE2-K. Fosdenopterin is possibly a weak MATE-1 substrate.
Elimination	
Mass balance results	The Applicant has not conducted a mass balance study for fosdenopterin. Fosdenopterin provides an exogenous source of cPMP. Patients with MoCD Type A do not produce endogenous cPMP. Because the utility of a mass balance study in healthy subjects to inform the metabolism and disposition of fosdenopterin in patients with MoCD Type A is uncertain, we are not requiring a postmarketing study to further investigate the mass balance of fosdenopterin.
Clearance	The mean clearance of fosdenopterin ranged from 167 mL/hr/kg to 180 mL/hr/kg following IV administration in healthy adult subjects. The renal clearance accounts for 40% of total clearance.
Half-life	The mean half-life of fosdenopterin ranged from 1.2 hr to 1.7 hr.
Metabolic pathway(s)	Fosdenopterin is not a substrate of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5.

Characteristic	Drug Information
Primary excretion pathways (% dosage)	Approximately 40% of the administered dose was recovered in the urine of healthy adults.
Intrinsic Factors and Specific Populations	
Body weight	The body-weight-based dosing regimen is supported by the allometric principle in pediatrics.
Age	Fosdenopterin has lower clearance in neonates as a result of immature renal function.
Renal impairment	The effect of renal impairment on the PK of fosdenopterin has not been conducted. Because renal impairment is generally not associated with MoCD Type A, further investigation of PK, efficacy, or safety of fosdenopterin to inform dosing in patients with renal impairment is not needed. Of note, the proposed initial dosage in preterm and term neonates based on their gestational age has considered the lower renal clearance of fosdenopterin in neonates.
Hepatic impairment	The effect of hepatic impairment on the PK of fosdenopterin has not been conducted. Fosdenopterin is degraded via non enzymatic degradation to its inactive oxidation product, compound Z; therefore, hepatic impairment is not expected to have a clinically significant impact on fosdenopterin PK. Furthermore, hepatic impairment is generally not associated with MoCD Type A; therefore, further investigation of the PK, efficacy, or safety of fosdenopterin to inform dosing in patients with hepatic impairment is not considered necessary.
Drug Interaction Liability (Drug as Perpetrator)	
Inhibition/induction of metabolism	Fosdenopterin does not inhibit or induce CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5.
Inhibition/induction of transporter systems	Fosdenopterin does not inhibit P-gp, BCRP, OATP1B1, OATP1B3, OCT2, OAT3, and MATE1. Fosdenopterin inhibits MATE2-K (25%) and OAT1 (33%) at 200µM.
Abbreviations: AUC, area under the plasma drug concentration over time curve; AUC _{0-inf} , AUC from time zero to infinity; AUC _{0-last} , AUC from time zero to the time of last quantifiable concentration; BCRP, breast cancer resistance protein; CYP, cytochrome P450; LC, liquid chromatography; MATE, multidrug and toxin extrusion protein; MS/MS, tandem mass spectroscopy; OAT, organic anion transporter; OCT, organic cation transporter; OPQ, Office of Pharmaceutical Quality; PK, pharmacokinetics; T _{max} , time to reach maximum concentration (C _{max})	

5.1. Nonclinical Assessment of Potential Effectiveness

5.1.1. Fosdenopterin HBr

When administered after birth, *Escherichia coli*-derived cPMP increased survival of *MOCS1* KO mice compared to untreated controls. Additionally, fosdenopterin monohydrobromide dihydrate (HBr) (synthetic cPMP) at ≥ 1.1 mg/kg/day (which $0.1\times$ the maximum recommended clinical dose of 0.9 mg/kg based on is $\geq$ body surface area) decreased plasma and brain levels of SSC, decreased urinary uric acid levels, dose-dependently increased liver SOX activity, and dose-dependently increased body weight compared to placebo and untreated *MOCS1* KO mice. *MOCS1* KO mice have been previously reported to display a severe phenotype that reflects key characteristics of human MoCD Type A (Lee et al. 2002). *MOCS1* KO mice died soon after birth and showed a significantly slower gain of body weight in comparison with their wild-type and/or heterozygous littermates.

6. Assessment of Effectiveness

6.1. Dose and Dose Responsiveness

6.1.1. The Proposed Dosage Regimen

Fosdenopterin is administered as an intravenous (IV) infusion once daily. The proposed dose of fosdenopterin in patients ≥ 1 year of age is 0.9 mg/kg. The proposed dosage regimen in patients < 12 months of age is shown in [Table 6](#), and the dose should be titrated to the target dose of 0.9 mg/kg over a period of 3 months.

Table 6. Dosage in Patients < 12 Months of Age

Titration Schedule	Gestational Age ≥ 37 Weeks (mg/kg/day)	Gestational Age < 37 Weeks (mg/kg/day)
Initial dose	0.55	0.4
Month 1	0.75	0.7
Month 3	0.9	0.9

6.1.2. Dose-Selection Rationale for Pivotal Studies

Studies MCD-501 and MCD-201 evaluated body weight-based dosing regimens that included a starting dose, a dose-escalation period, and the maximum target dose. Study MCD-202 enrolled treatment-naïve patients and tested the proposed dosing regimens. As described below, these clinical trials generally support a recommendation of fosdenopterin 0.9 mg/kg/day as the target therapeutic dose with starting doses further adjusted for infants < 12 months of age based on their gestational age.

In Study MCD-501, patients received a low initial dose (e.g., 0.06 mg/kg) which was incrementally escalated to a maximum dose of 0.18 mg/kg. Six of the eight patients in Study MCD-201 were patients who previously participated in Study MCD-501. For patients enrolled from Study MCD-501 into Study MCD-201, fosdenopterin treatment was initiated and continued

for 2 months at the same dose level as each patient's last dose of rcPMP in Study MCD-501. After 2 months on fosdenopterin treatment, fosdenopterin doses in Study MCD-201 were escalated every month by no more than 0.18 mg/kg/day. The dose-escalation regimen of fosdenopterin was guided by clinical improvement and urine levels of SSC and thiosulfate. Dose escalation continued until day 180 to the target dose of 0.9 mg/kg/day or when the plasma exposure (area under the concentration-time curve [AUC]) of fosdenopterin exceeded 5490 ng.hr/mL (derived from the 90-day interim exposure of the 5 mg/kg no observable adverse effect level (NOAEL) in a 6-month rat toxicology study). Of note, four of the eight patients enrolled in Study MCD-201 are currently receiving the target dose of 0.9 mg/kg/day; and the other four patients are receiving doses lower than 0.9 mg/kg/day, which is not due to safety concerns. In addition, the AUC_{0-3hr} at the end of the 6-month rat toxicology study was 7630 ng.hr/mL, the AUC_{0-6hr} in the juvenile rat toxicology study using an improved formulation at a NOAEL of 100 mg/kg/day was 133,000 ng.hr/mL, and the AUC_{0-6hr} in the dog toxicology study at a NOAEL of 10 mg/kg/day was 29,700 ng.hr/mL. Moreover, no maximum tolerated dose has been established in any species tested in animal studies or in human clinical trials, and no dose-dependent tolerability has been identified, indicating additional safety margins when considering the exposure of the proposed dosing regimens.

6.1.3. Dose Adjustment and Titration in Pediatric Patients <12 Months

The rationale for dose adjustment in infants <12 months of age based on gestational age takes into account lower renal maturation in neonates and the estimated 40% renal clearance of fosdenopterin observed in adults in Study MCD-101. The adjusted dosing regimens were estimated to achieve a target fosdenopterin AUC range of 4000 to 5490 ng.hr/mL. In addition, due to differences in body weight and renal maturation expected between preterm and term neonates, the dose adjustments were delineated for term and preterm neonates. The proposed starting dose in preterm neonates has been evaluated in one patient enrolled in Study MCD-202.

6.1.4. Pharmacodynamics

In MoCD Type A, the lack of active SOX leads to an elevated level of the neurotoxic sulfite S-sulfocysteine (SSC). Treatment with fosdenopterin led to a reduction of the urinary SSC level normalized to creatinine over the course of treatment. The individual pharmacodynamic data of urinary SSC level normalized to creatinine are shown in [Table 7](#).

Table 7. Urinary S-Sulfocysteine Normalized to Creatinine Level in Patients With MoCD Type A Treated With Fosdenopterin (or rcPMP) at Selected Analysis Visits (N=13)

Patient ID	Study*	Levels (μmol/mmol) at Analysis Visit										Last AVISIT
		Baseline	D7	W2	M1	M2	M3	M6	M9	M12	Last	
(b) (6)	501/ 201	80	N/A	183	51	42	19	13	14	16	8	M126
	501/ 201	94	86	28	11	12	14	9	11	25	11	M108
	501/ 201	43	22	30	15	15	13	14	13	26	9	M90
	501/ 201	78	29	18	15	11	N/A	13	4	9	7	M96
	501/ 201	12	23	23	12	5	5	N/A	N/A	23	24	M84
	501/ 201	364	38	31	27	17	11	11	11	N/A	5	M72
	501	1031	N/A	37	N/A	N/A	N/A	N/A	N/A	N/A	37	W2
	501	143	113	35	34	40	21	10	7	20	19	M15
	501	67	3	7	N/A	N/A	N/A	N/A	N/A	N/A	14	W3
	501	134	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	7	D4
	201	38	54	40	76	32	18	7	5	11	2	M36
	201	32	N/A	7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	W2
	202	90	11	6	8	6	6	5	7	9	9	M30

Source: Compiled from Table 1.7.1.2.1 Integrated Summary of Efficacy, adbio.xpt dataset, and 4-month safety update case report for Patient ID (b) (6)

* Six patients were treated with rcPMP in Study MCD-501 and continued treatment with fosdenopterin in Study MCD-201; i.e., 501/201; If multiple assessments occur within a visit window, the analysis value was defined as the median of the values across the analysis range. Baseline is defined as the measurement with the earliest date of collection for all patients.

Abbreviations: D, day; LAST AVISIT, analysis visit of last recorded measurement; M, month; MoCD, molybdenum cofactor deficiency; N/A, measurement not available; rcPMP, recombinant cyclic pyranopterin monophosphate; W, week

Urinary SSC, xanthine, and uric acid concentrations observed in patients enrolled in Study MCD-501 were measured using nonvalidated assays. The method to determine SSC, creatinine and xanthine in human urine did not meet acceptance criteria when cross-validated against the validated liquid chromatography-tandem mass spectrometry (LC/MS/MS) methods used in Studies MCD-201 and MCD-202. Consequently, it is not possible to reliably perform a quantitative comparison of values of urinary SSC normalized to creatinine determined in Study MCD-501 to the values determined in Study MCD-201. However, the bioanalytical methods used in Study MCD-501 show a comparable qualitative trend of urinary SSC normalized to creatinine before and during treatment. Based on data measured using validated bioanalytical assays, the mean $\pm$ SD levels of urinary SSC normalized to creatinine in fosdenopterin-treated patients (n=9), were 11.0 ± 8.5 μmol/mmol at Month 3, 6.3 ± 3.8 μmol/mmol at Month 6, 10.5 ± 7.6 μmol/mmol at Month 9, 13.1 ± 11.7 μmol/mmol at Month 12, and 7.0 ± 2.4 μmol/mmol at Month 48 following treatment with fosdenopterin. Only one patient had pre-treatment urinary SSC level characterized with a validated assay with a value of 89.8 μmol/mmol. (source of data: Applicant's Table 1 located in Multiple Discipline Information Amendment submitted under sequence 0047).

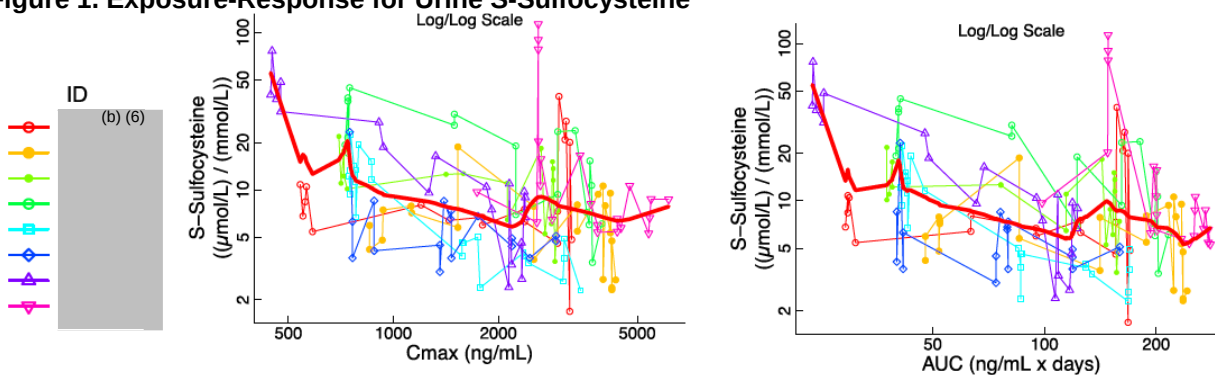
6.1.5. Exposure-Response

The dose/pharmacokinetics (PK)/pharmacodynamics (PD) relationship of fosdenopterin was evaluated in an exposure-response (E-R) analysis of PD biomarkers for individual patients in Studies MCD-201 and MCD-202. Of note, PK data were not available in the retrospective Study MCD-501; therefore, E-R analysis was not conducted for these subjects. The E-R analysis results are summarized below and generally supported the proposed dosing regimens.

- Higher plasma fosdenopterin exposures ($C_{\max}$ and AUC) were generally associated with lower creatinine-normalized urine SSC ([Figure 1](#)).
- The dose/PK/PD relationship supports a recommendation of 0.9 mg/kg/day as the target dose for maximum efficacy because the exposure-response appears to be plateaued at this dose. The single patient (n=1) who received a higher dose of 0.98 mg/kg/day in Study MCD-202 did not have any remarkable changes in biomarker responses when compared to the proposed target dose of 0.9 mg/kg/day.
- The single preterm neonate patient in Study MCD-202 who received the starting dose at 0.53 mg/kg/day demonstrated a rapid PD response and supported the proposed dosing regimen in infants <12 months of age.

Although the overall PK/PD data are limited, the available individual E-R data based on one preterm neonate, and one term neonate and seven pediatric patients aged 1 year or older, generally support the proposed dosage for fosdenopterin in patients with MoCD Type A. Refer to [Section III.14.2.3](#) for detailed E-R analysis of plasma SSC and exploratory urine and blood biomarkers.

Figure 1. Exposure-Response for Urine S-Sulfocysteine



Source: Applicant's Exposure-Response Analysis Report, Figure 13.
Color-coded thin lines connect values for each subject. Thick lines are smoothers representing average of individual data.
Abbreviations: AUC, area under the curve; $C_{\max}$, maximum concentration; ID, identification

6.2. Clinical Trials Intended to Demonstrate Efficacy

6.2.1. Studies MCD-201, MCD-202, MCD-501, and MCD-502

6.2.1.1. Design

The Applicant provided data from the four studies described below to support the efficacy of fosdenopterin.

Study MCD-201 is an ongoing prospective, open-label, single arm, phase 2 study intended primarily for the patients with MoCD Type A enrolled in Study MCD-501 (described below) to transition from treatment with rcPMP to fosdenopterin. The study took place in academic centers in five countries (the United States, Australia, the United Kingdom [UK], Tunisia, and The Netherlands). There were eight patients in this study, six of whom previously were enrolled in Study MCD-501, and two who were previously receiving rcPMP through either an expanded access investigational new drug (IND) in the United States, or a similar program if in another country. The study has two phases which include a 6-month inpatient dose escalation period followed by a 60-month extension period. The dates of recruitment were April 2, 2014 to February 24, 2020. The dates of data collection are April 2, 2014 to present. The dates of exposure are April 10, 2014 to present and follow-up is ongoing. The primary objective is to assess the safety of fosdenopterin over the first 6 months of treatment. Secondary objectives are to assess the effect of fosdenopterin on urine and blood SSC levels, on neurological function, motor and cognitive functions, and on the central nervous system (CNS) structure (e.g., cystic changes on neuroimaging). Additional objectives are to characterize the PK of increasing doses, and to assess the long-term safety of fosdenopterin in pediatric patients. At baseline, all patients had a confirmed genetic diagnosis of MoCD Type A based on mutations in the *MOCS1* gene.

Study MCD-202 is an ongoing prospective, open-label, single-arm study enrolling patients with MoCD Type A in the neonatal period who had not been previously treated with fosdenopterin. One patient was enrolled in this study (b) (6). The dates of recruitment were (b) (6) to present. The dates of data collection were (b) (6) to present. The dates of exposure were (b) (6) to present, and follow-up is ongoing. The primary objective of this study is to evaluate the safety and efficacy of fosdenopterin in neonatal patients with MoCD Type A. Secondary objectives are to evaluate the effect of fosdenopterin on the acquisition of developmental milestones, on pediatric measures of functional ability and activities of daily living, and to characterize PK. Patients were required to have either a prenatal genetic diagnosis of MoCD Type A or the onset of clinical symptoms consistent with MoCD Type A. Confirmatory genetic testing was done and if MoCD Type A was not confirmed, fosdenopterin was discontinued.

Study MCD-501 was a retrospective observational study conducted at academic centers in six countries (Australia, Germany, the Netherlands, Turkey, the UK, and the United States) to collect data in patients with MoCD Type A who received treatment with rcPMP through either an expanded access investigational new drug in the United States or a similar program if in another country. Patients in this study received rcPMP, which was in use prior to the availability of fosdenopterin. The dates of recruitment and data collection were from July 9, 2011 to October

7, 2014. The dates of exposure were from June 6, 2008 to April 10, 2014. The primary objective of this study was to collect data on MoCD disease progression in patients treated with rcPMP.

Study MCD-502 was a combined retrospective and prospective, noninterventional, natural history study to collect data on untreated patients with MoCD Type A. Study MCD-502 was conducted in academic centers in 14 countries by 27 investigators who had previously diagnosed or treated patients with MoCD. The countries were Canada, Germany, Spain, Italy, Japan, Israel, Malaysia, the Netherlands, Poland, Saudi Arabia, Tunisia, Turkey, the UK, and the United States. Additional measures for recruitment in this study were taken in the United States, including contacting state newborn screening centers for potential patients. Thirty-seven patients were enrolled in the retrospective portion and 14 in the prospective portion. The dates of recruitment were from September 24, 2013 to February 17, 2014. Data were collected from September 24, 2013 to December 11, 2015.

Additional supplemental follow-up data on survival, historic radiographic imaging and electrocardiograms in patients in Studies MCD-501 and MCD-502, were collected from November 21, 2019 to January 31, 2020.

6.2.1.2. Key Eligibility Criteria

Treatment Cohorts

Study MCD-201 Inclusion Criteria

All patients with a confirmed genetic mutation in *MOCS1*, diagnostic of MoCD Type A and on current treatment with rcPMP were eligible for inclusion.

Study MCD-201 Exclusion Criteria

Patients could not be currently on or have planned treatment with another investigational drug or device with the exception of rcPMP, from day -1 or they would be excluded.

Study MCD-202 Inclusion Criteria

Patients must have been in the first 28 days of life with a diagnosis of MoCD Type A based on prenatal genetic diagnosis or the onset of clinical symptoms consistent with MoCD Type A.

Study MCD-202 Exclusion Criteria

The exclusion criteria comprised the following:

- The patient had a diagnosis other than MoCD Type A.
- Any condition that was considered by the treating physician to be a contraindication to therapy including evidence of abnormalities on brain imaging not attributed to MoCD Type A.
- Any condition that might otherwise interfere with the patient's participation in the study or pose any additional risk to the patient or confounded patient assessments.

If there was antenatal or postnatal brain imaging prior to initiation of treatment that included cortical or subcortical cystic encephalomalacia, clinically significant intracranial hemorrhage or

other brain imaging determined by the treating physician to be clinically significant, that child would be excluded, as would a child with a Modified Glasgow Coma Scale for infants and children score of less than seven for more than 24 hr.

Study MCD-501 Inclusion Criteria

Patients of any age with genetically confirmed mutations in *MOCS1*, diagnostic of MoCD Type A, suspected Type A, or Type B, who previously received rcPMP only by the intravenous route of administration were included in the study.

Study MCD-501 Exclusion Criteria

There were no clinically relevant exclusion criteria.

Non-Treatment Cohorts

Study MCD-502 Inclusion Criteria

Documented clinical and biochemical diagnosis or genetic diagnosis of MoCD or isolated SOX deficiency was an inclusion criterion, as was a biochemical criterion of either high urine, serum, or plasma levels of SSC or a positive urine sulfite dipstick in at least two urine samples.

Study MCD-502 Exclusion Criteria

There were no clinically relevant exclusion criteria.

6.2.1.3. Statistical Analysis Plan

The adequate and well-controlled investigation that, together with confirmatory evidence, established the clinical benefit of fosdenopterin in patients with MoCD Type A compared the overall survival (OS) of treated patients from three studies (Studies MCD-501, MCD-201, and MCD-202) to those from a natural history cohort (Study MCD-502).

Study MCD-202 was originally intended to be the confirmatory, adequate and well-controlled study and planned enrollment of 5 to 10 patients with MoCD Type A. The primary endpoint was specified to be the proportion of patients alive and able to sit upright independently for at least 30 seconds at month 12. This endpoint was planned to be analyzed using a Bayesian method comparing the response rate for the primary endpoint in the treated patients to 10%, which was based on an interim analysis of the natural history study (Study MCD-502). After enrolling the first patient in June 20, 2016, further patient enrollment of Study MCD-202 was paused due to enrollment issues.

Subsequently, discussions began with the Agency regarding the combined use of data from all three studies of treated patients to compare to the untreated patients in the natural history study. The Applicant and the Agency discussed the SAP for this combined analysis, which would serve as a single adequate and well-controlled investigation for the NDA submission. Mortality, which is an objectively measured and clinically meaningful outcome, had the most reliable data capture across the studies, and was a component of the original prespecified primary endpoint in Study MCD-202. Other clinical outcomes had differences in measurement and collection frequency across the studies. Therefore, the Agency agreed that an application may be supported with OS as the primary efficacy outcome of interest, provided that there was a comparable control group.

Because all the mortality analyses performed in the SAP were post hoc (these analyses were formulated after mortality outcomes were known for each patient), the focus of the review of efficacy is to conduct various analyses of OS to ensure that the results are not driven by a single choice of statistical methodology, to assess for biases, and to address risk of type 1 error. Due to the post hoc nature of the analyses, all p-values presented in this review are nominal.

The data cutoff for the initial NDA submission was July 4, 2019, and those for the safety update were March 23, 2020 for Study MCD-201 and February 20, 2020 for Study MCD-202.

Analyses of OS incorporate the data from patients who received either rcPMP, Nulibry, or both formulations of fosdenopterin. A total of four patients participated only in Study MCD-501 (two died and two discontinued), six patients participated in both Studies MCD-501 and MCD-201, two patients participated only in Study MCD-201, and one patient participated only in Study MCD-202. Because OS is affected by all interventions prior to the time of death or censoring, the OS outcomes for those patients treated with both rcPMP and Nulibry cannot be attributed to a single formulation. Furthermore, the two formulations have the same active moiety and have been adequately bridged (refer to Section 9 for details). Due to this and the small sample sizes, the data from treated patients in Studies MCD-501, MCD-201, and MCD-202 are analyzed together.

The two main efficacy analysis populations are the full analysis set (FAS), defined as all patients (treated and untreated) with MoCD Type A from the four studies, and the genotype-matched analysis set (GMAS), defined as all treated patients with MoCD Type A in the three combined studies and the untreated patients who were a genotype match for a treated patient.

For the GMAS, treated patients were matched with one or multiple controls (untreated patients) from the natural history study based on genotype. If a treated patient did not have the same homozygous mutation as a patient in the natural history study, the SAP specified matching with an untreated patient who had mutations with a similar anticipated impact on protein function (frameshift, missense, etc.). [Table 8](#) presents the matches of the patients in the GMAS. In some matches, an untreated patient was matched to more than one treated patient; in some other matches, there was more than one untreated patient matched to a treated patient. See [Table 51](#) in Section [III.16](#) for a list of the matched patients and their resulting OS outcomes.

Table 8. Genotype-Matched Patients

Match ID	Fosdenopterin Patient(s)	Untreated Natural History Patient(s)
1	(b) (6)	(b) (6)
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		

Source: Reviewer's analysis based on the ADTTE.xpt datasets submitted in eCTD0030 on 23-Oct-2020.

Abbreviation: ID, identification

General Efficacy Analysis Methods

OS was defined as the interval in months from the date of birth, to the date of death or date last known alive. Patients who are currently enrolled in Studies MCD-201 and MCD-202 were censored at the time of data cut-off for the safety update (March 23, 2020 for Study MCD-201 and February 20, 2020 for Study MCD-202).

The SAP specified using the log-rank test to compare treated and natural history control patients and using Kaplan–Meier (KM) plots and methods to estimate survival parameters for each group, including OS rates at 6 months, 1 year, and 2 years.

Additionally, the SAP specified analyzing OS using the Cox proportional hazards model by regressing survival on an indicator variable denoting treatment status. Hazard ratios were estimated to determine the effect of treatment on the hazard of the occurrence of death, and 95% confidence intervals were estimated based on the modified score test statistic under the Cox model as described in (Lin et al. 2016). This methodology constructs confidence intervals of the hazard ratio to align with the results from the log-rank test, which is not always the case when using Wald confidence intervals.

The SAP specified repeating this analysis with additional covariates for the age at onset of the first MoCD sign/symptom subgroup (≤ 28 days, >28 days) and gender; as the age at onset of the first MoCD sign/symptom may be affected by treatment and there were no patients in the fosdenopterin group who had onset of symptoms at >28 days, analyses incorporating this variable into the modeling are not included in this review.

In addition to the Applicant's planned analyses, the Agency also presents summaries based on restricted mean survival time.

Analysis Methods for GMAS

As the GMAS is created using full matching (i.e., using all control patients who were a genotype match with a treated patient), the treatment: control allocation ratio for each match group was not 1:1 (see [Table 51](#)); therefore, several analysis methods were explored for the GMAS, some of which account for the differential match ratios:

- Unadjusted—OS analyzed using all patients in the GMAS population with no adjustment.
- Adjusted by matched identification (ID)—OS analyzed using the KM method stratified by matched ID and a stratified log-rank test. A Cox proportional hazards model with a covariate for matched ID was also used to assess the treatment effect on overall survival.
- Inversely weighted analysis—The SAP specifies estimating the average treatment effect (ATE) for the GMAS via the Cox proportional hazards model that accounts for the clustering within strata (matched IDs) by using weights as described (Austin and Stuart 2017). The weight for the j^{th} patient in the g^{th} match, w_{gj} is defined as:

$$w_{gj} = \begin{cases} \frac{qN_g}{N_{gt}}, & \text{for all treated patients in the } g^{\text{th}} \text{ matched ID} \\ \frac{(1-q)N_g}{N_{gc}}, & \text{for all control patients in the } g^{\text{th}} \text{ matched ID} \end{cases}$$

Source: page 23 of SAP

where q denotes the marginal probability of receiving treatment in the GMAS population, N_{gt} is the number of treated patients in the g^{th} matched ID, and N_{gc} is the number of natural history controls in the g^{th} matched ID, so $N_{gt} + N_{gc} = N_g$.

6.2.1.4. Results of Analyses

Efficacy analyses include only patients with MoCD Type A. A total of 13 patients with MoCD Type A were treated with fosdenopterin in Studies MCD-501, MCD-201, and MCD-202 as of the data cutoffs for the safety update of March 23, 2020 for Study MCD-201 and February 20, 2020 for Study MCD-202. Ten patients were treated with rcPMP in Study MCD-501, a retrospective, observational study. Two patients from Study MCD-501 died, two patients discontinued rcPMP, and the remaining six patients continued treatment in Study MCD-201, the open-label study in patients previously treated with rcPMP. An additional two patients were enrolled in Study MCD-201 who were not previously in Study MCD-501. As of the data-cutoff, only one patient with MoCD Type A was enrolled in Study MCD-202.

Patient disposition is presented in [Table 9](#). Study MCD-502, the natural history study, included a total of 37 patients with confirmed MoCD Type A who were included in the FAS. A total of 20 (54%) patients was already deceased and enrolled in a retrospective cohort, and 17 (46%) patients were living, of whom, 14 (38%) patients enrolled in the 12-month prospective data collection period. The GMAS untreated group included 18 patients who were genetically matched to fosdenopterin-treated patients.

Table 9. Patient Disposition

Parameter	Fosdenopterin (N=13)	Untreated FAS (N=37)	Untreated GMAS (N=18)
Cohort, n (%)			
Deceased cohort	N/A	20 (54.1%)	10 (55.6%)
Living cohort		17 (46.0%)	8 (44.4%)
Treatment status, n (%)			
Completed/ongoing/died	11 (84.6%)	N/A	N/A
Discontinued treatment	2 (15.4%)		

Source: Reviewer's analysis based on ADSL.xpt submitted in eCTD0030 on October 23, 2020.

Abbreviations: FAS, full analysis set; GMAS, genotype-matched analysis set; N, number of subjects; n, number of subjects with a given characteristic

[Table 10](#) presents baseline demographics for the combined group of patients treated with fosdenopterin and the untreated patients in the FAS and GMAS. Though there are small sample sizes, there is some imbalance between the treated and untreated cohorts in sex, birth year, and country/region. The untreated groups included more males and patients born in earlier years compared to the fosdenopterin group. Two (15.4%) patients treated with fosdenopterin were from North America, while three (8.1%) untreated patients in the FAS and zero untreated patients in the GMAS were from North America.

Table 10. Baseline Demographics

Characteristic	Fosdenopterin (N=13)	Untreated FAS (N=37)	Untreated GMAS (N=18)
Sex, n (%)			
Male	7 (53.8%)	28 (75.7%)	13 (72.2%)
Female	6 (46.2%)	9 (24.3%)	5 (27.8%)
Birth year, n (%)			
≤1999	0	7 (18.9%)	2 (11.1%)
2000 to 2004	0	1 (2.7%)	0
2005 to 2009	3 (23.1%)	17 (45.9%)	9 (50%)
2010 to 2014	8 (61.5%)	12 (32.4%)	7 (38.9%)
≥2015	2 (15.4%)	0	0
Race, n (%)			
White	10 (76.9%)	21 (56.8%)	11 (61.1%)
Asian	3 (23.1%)	10 (27%)	4 (22.2%)
Other	0	6 (16.2%)	3 (16.7%)
Ethnicity, n (%)			
Hispanic	1 (7.7%)	2 (5.4%)	0
Non-Hispanic	12 (92.3%)	31 (83.8%)	14 (77.8%)
Unknown/not reported	0	4 (10.8%)	4 (22.2%)
Region, n (%)			
North America	2 (15.4%)	3 (8.1%)	0
Europe	6 (46.2%)	14 (37.8%)	8 (44.4%)
Rest of world	5 (38.5%)	20 (54.1%)	10 (55.6%)

Characteristic	Fosdenopterin (N=13)	Untreated FAS (N=37)	Untreated GMAS (N=18)
Country of participation, n (%)			
United States	2 (15.4%)	2 (5.4%)	0
Australia	1 (7.7%)	0	0
Canada	0	1 (2.7%)	0
Germany	2 (15.4%)	3 (8.1%)	1 (5.6%)
Spain	0	1 (2.7%)	0
United Kingdom	3 (23.1%)	5 (13.5%)	2 (11.1%)
Israel	1 (7.7%)	7 (18.9%)	4 (22.2%)
Japan	0	2 (5.4%)	0
Malaysia	0	2 (5.4%)	2 (11.1%)
Netherlands	2 (15.4%)	2 (5.4%)	2 (11.1%)
Saudi Arabia	0	4 (10.8%)	2 (11.1%)
Tunisia	1 (7.7%)	3 (8.1%)	2 (11.1%)
Turkey	1 (7.7%)	5 (13.5%)	3 (16.7%)

Source: Reviewer analysis based on the ADSL.xpt submitted in eCTD0030 on October 23, 2020.

Abbreviations: FAS, full analysis set; GMAS, genotype-matched analysis set; N, number of subjects in treatment group; n, number of subjects with a given characteristic

[Table 11](#) presents the genotype-matched patients' birthdates, event time (censoring denoted in blue, death denoted in red), and the time of first treatment for those in the treated cohort. All treated patients were treated at times prior to their untreated match's time of event (e.g., patient (b) (6) was treated at day 69, which is an earlier timepoint than the death of matched patient (b) (6) at 28.8 months and the censoring of matched patient (b) (6) at 109.2 months). All but two treated patients had at least one untreated match born within 5 years. These two patients without a match born within 5 years included the only patient in Study 202 (b) (6) and a patient (b) (6) who was only enrolled in Study 201.

Table 11. Genotype-Matched Patients, Birth Year, Time of Treatment, and Event Time

Match ID	Fosdenopterin Patient(s)				Untreated Natural History Patient(s)		
	Patient ID	Birth Month/Year	Event Time (Months ¹)	Time of First Treatment (Days/Months ²)	Patient ID	Birth Month/Year	Event Time (Months ¹)
1	(b) (6)	(b) (6)	142.8	37 / 1.2	(b) (6)	(b) (6)	46.7
			123.8	9 / 0.3			
2			120.4	6 / 0.2			9
3			119.6	1 / 0.03			0.3
4			103.5	1 / 0.03			157.2
							1.2
5			64.8	1 / 0.03			49.4
							1
			44.1	1 / 0.03			50.9
6			15.9	32 / 1.1			10.7
7			0.2	1 / 0.03			88.8
							99
							51.1
							10.4
8			1.2	9 / 0.3			20.4
							28.8
9			94.4	69 / 2.3			109.2
10			21.7	12 / 0.4			12.1
11			30.4	5 / 0.2			47.8

Source: Reviewer's analysis based on the ADTTE.xpt datasets submitted in eCTD0030 on October 23, 2020.

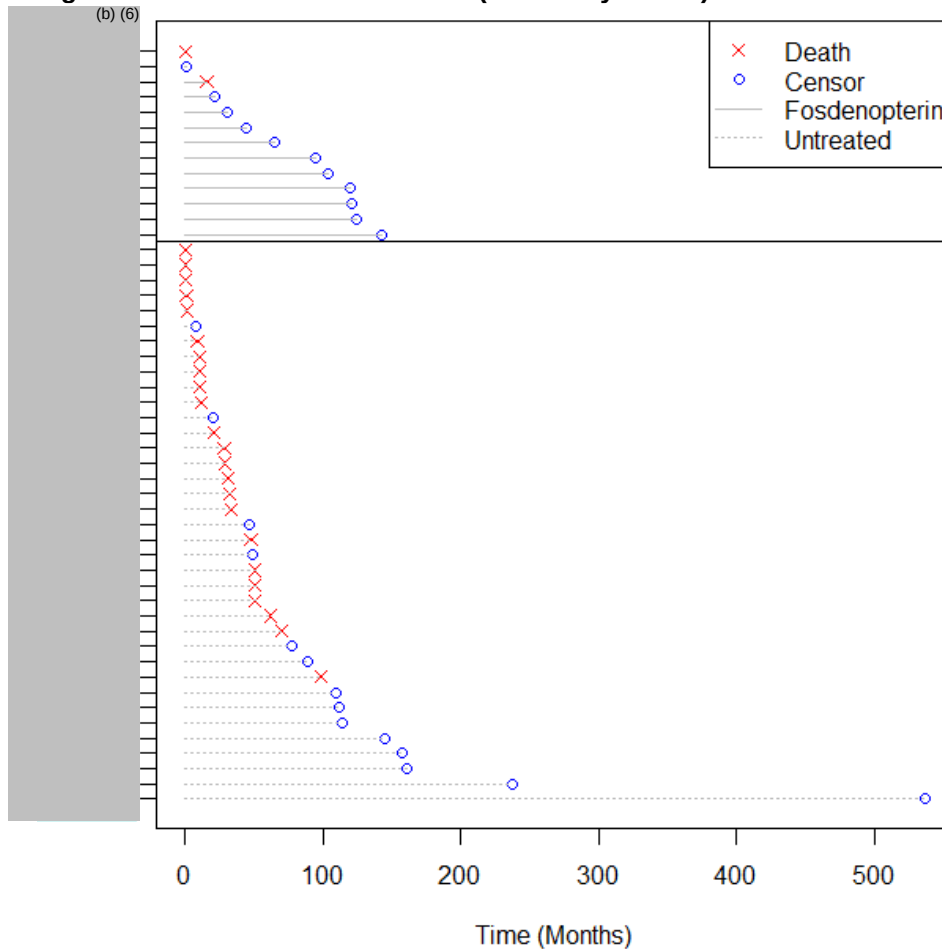
¹ Black, time of censoring; red, time of death; *, deceased cohort in Study 502 (initiated in 2013).

² Calculated as days divided by 30.4375

Abbreviation: ID, identification

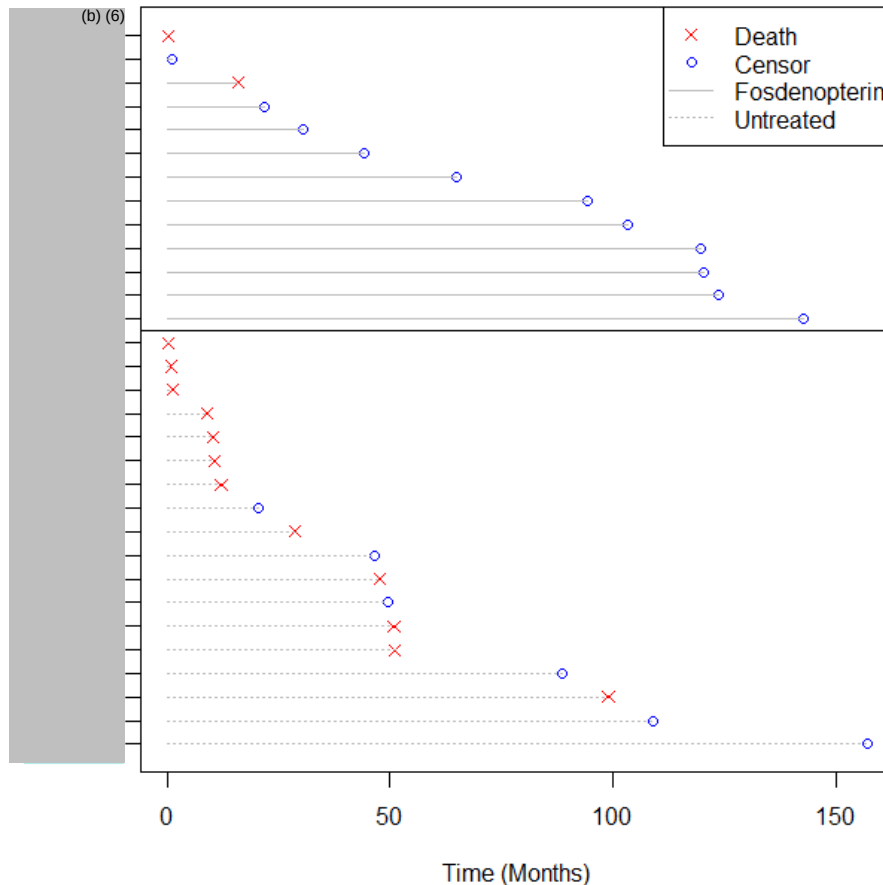
There were no missing data in the FAS or GMAS cohorts for age of onset of symptoms and genotype. [Figure 2](#) and [Figure 3](#) depict the length of follow-up time for vital status for the FAS and GMAS, respectively. Each patient is represented on the y-axis, and the x-axis depicts the follow-up time in months with a red “x” denoting the patient died at that time, and a blue “o” denoting the patient was censored at that time. The treated patients are depicted at the top of the plot, and the untreated patients are depicted below the horizontal line. Although the untreated cohort has more early deaths than the treated cohort, the follow-up time for those who were censored appear to be balanced between the two groups, except for two untreated patients with much longer follow-up times in the FAS cohort.

Figure 2. Patient-Level Event Time (Full Analysis Set)



Source: Reviewer's analysis based on the ADTTE.xpt datasets submitted in eCTD0030 on October 23, 2020.

Figure 3. Patient-Level Event Time (Genotype-Matched Analysis Set)



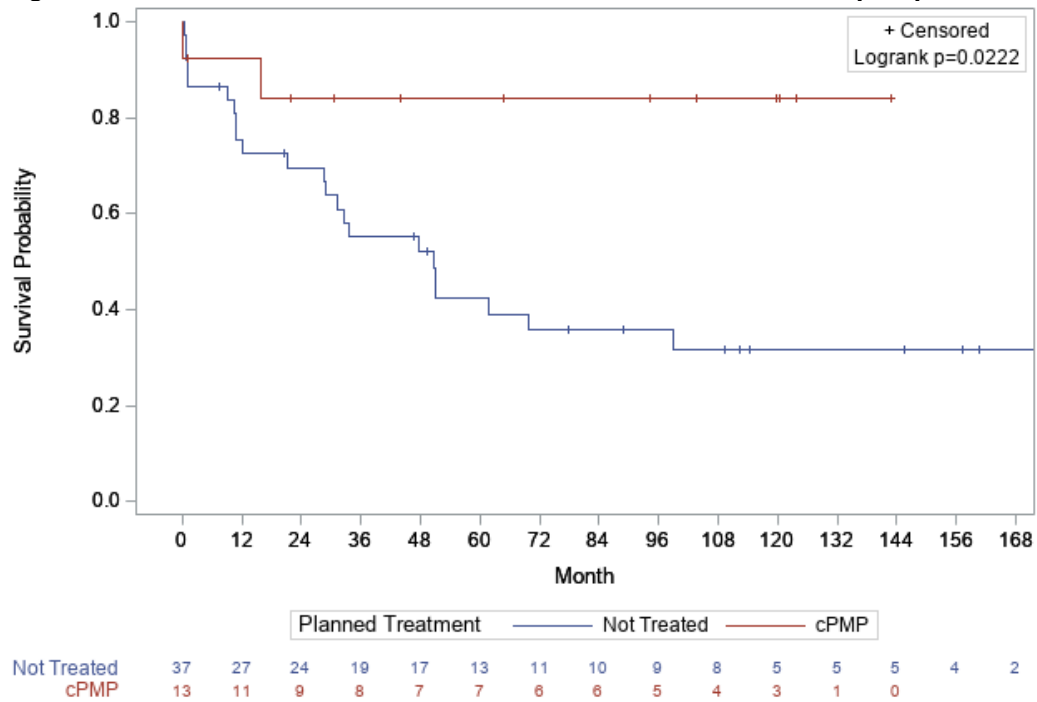
Source: Reviewer's analysis based on the ADTTE.xpt datasets submitted in eCTD0030 on October 23, 2020.

Mortality Results

Kaplan–Meier Method

[Table 52](#) in [Section III.16](#) lists the number of patients in each treatment group who were at risk, had died, or were censored by 6 months, 1 year, 2 years, 3 years, and 4 years. [Figure 4](#) and [Figure 5](#) depict the KM survival curves for the fosdenopterin group compared to the FAS and GMAS untreated groups, respectively (log-rank $p=0.022$ and 0.012 , respectively). [Figure 4](#) depicts survival probabilities up to month 200; two untreated patients are censored at 237.5 and 536.8 months and are not depicted in [Figure 4](#) in order to better visualize the patient events at earlier timepoints. The survival curves including these two patients are depicted in [Figure 28](#) in [Section III.16](#).

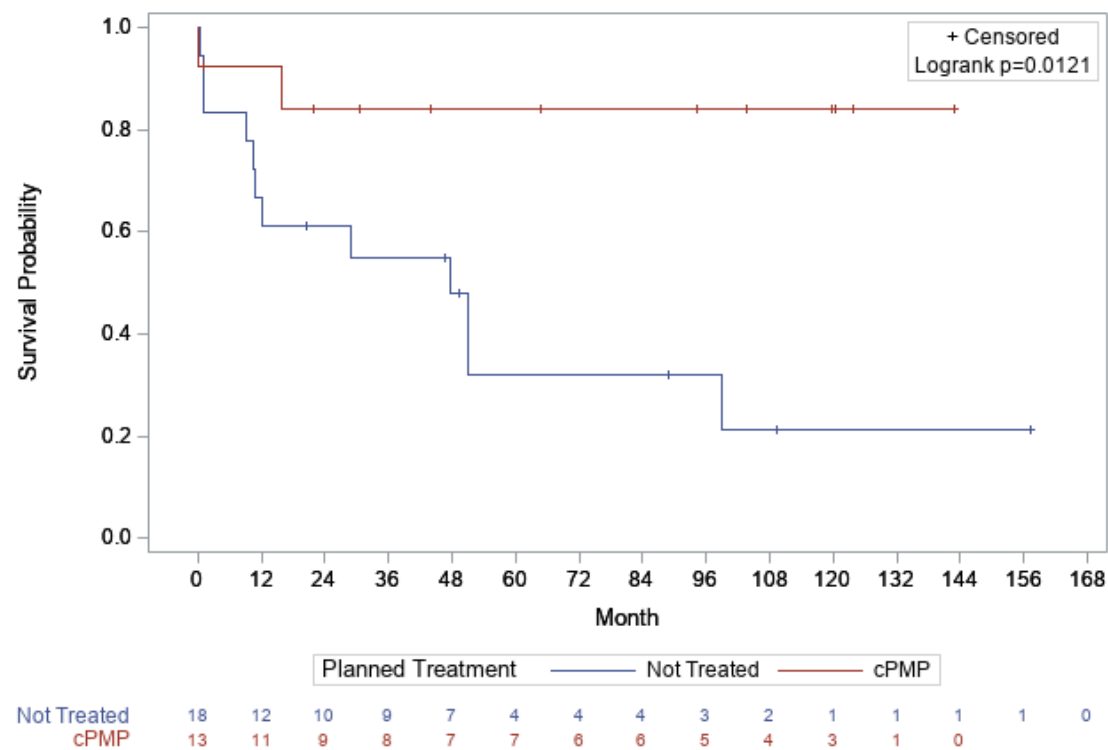
Figure 4. KM Survival Curves of the Treated Versus Untreated Groups Up to Month 200 (FAS)



Source: Reviewer's analysis based on the ADTTE.xpt dataset submitted in eCTD0030 on October 23, 2020. Two untreated patients are censored at 237.5 and 536.8 months.

Abbreviations: cPMP, cyclic pyranopterin monophosphate; FAS, full analysis set; KM, Kaplan-Meier

Figure 5. KM Survival Curves of the Treated Versus Untreated Groups (GMAS)



Source: Reviewer's analysis based on the ADTTE.xpt dataset submitted in eCTD0030 on October 23, 2020.

Abbreviations: cPMP, cyclic pyranopterin monophosphate; GMAS, genotype-matched analysis set; KM, Kaplan-Meier

Further summaries based on the KM method are presented in [Table 12](#), including survival probability estimates at 6 months, 1 year, 2 years, and 3 years. The two deaths reported in the fosdenopterin group were patients who participated solely in the retrospective Study MCD-501 and received rcPMP.

As the survival probability estimate for the fosdenopterin group does not fall below 50%, the median survival time for that group is not estimable. The median survival time for the untreated control patients was 51 months and 48 months for the FAS and GMAS untreated groups, respectively. The differences in estimated survival probabilities increase numerically over time, though the confidence intervals for the treatment groups overlap. At 2 years, the estimated survival probability is 84% (95% confidence interval [CI] 49% to 96%) in the fosdenopterin group, and 70% (95% CI 52% to 82%) and 61 (95% CI 35% to 79%) in the FAS and GMAS, respectively.

Table 12. Efficacy Results of OS—Kaplan–Meier Method

Parameter	Fosdenopterin (n=13)	Untreated FAS (n=37)	Untreated GMAS (n=18)
Number of deaths (%)	2 (15.4)	23 (62.2)	12 (66.7)
50th Percentile (median) survival time (95% CI)	N/A (16, N/A)	51 (28, 99)	48 (10, 99)
Kaplan–Meier survival probability (95% CI)			
6 Months	92 (57, 99)	86 (71, 94)	83 (57, 94)
1 Year	92 (57, 99)	75 (58, 86)	67 (40, 83)
2 Years	84 (49, 96)	70 (52, 82)	61 (35, 79)
3 Years	84 (49, 96)	55 (37, 70)	55 (30, 74)
Restricted mean survival time (95% CI)			
6 Months	5.6 (4.7, 6.4)	5.3 (4.7, 5.9)	5.1 (4.2, 6.0)
Difference ¹		0.3 (−0.8, 1.3)	0.4 (−0.8, 1.6)
1 Year	11.1 (9.4, 12.8)	10.3 (9.0, 11.5)	9.8 (7.9, 11.7)
Difference ¹		0.8 (−1.3, 2.9)	1.3 (−1.3, 3.8)
2 Years	21.5 (17.9, 25.1)	18.9 (16.1, 21.7)	17.2 (12.9, 21.3)
Difference ¹		2.6 (−1.9, 7.1)	4.3 (−1.2, 9.9)
3 Years	31.6 (25.7, 37.4)	26.5 (22.2, 30.9)	24.0 (17.3, 30.8)
Difference ¹		5.0 (−2.2, 12.3)	7.5 (−1.4, 16.4)
P-value (log-rank test)		0.0222	0.0121

Source: Reviewer's analysis based on the ADTTE.xpt dataset submitted in eCTD0030 on October 23, 2020.

¹ Treated minus untreated

Abbreviations: CI, confidence interval; FAS, full analysis set; GMAS, genotype-matched analysis set; N/A, not applicable; OS, overall survival

Although only weighted analyses utilizing the Cox proportional hazards model were specified in the SAP, [Table 13](#) presents results from the weighted KM method and the weighted log-rank test (p=0.013) using the weights described in the SAP (Section [6.2.1.3](#)). The results are similar to the unweighted results in [Table 12](#), comparing the fosdenopterin group with the untreated patients in the GMAS.

Table 13. Efficacy Results of OS Using Weighted Analysis—Kaplan–Meier Method

Parameter	Fosdenopterin (n=13)	Untreated GMAS (n=18)
50th Percentile (median) survival time (95% CI)	N/A (16, N/A)	48 (9, 99)
Kaplan–Meier survival probability (95% CI)		
6 Months	94 (52, 99)	83 (56, 94)
1 Year	94 (52, 99)	64 (38, 82)
2 Years	84 (47, 96)	58 (32, 77)
3 Years	84 (47, 96)	53 (28, 73)
P-value (log-rank test)		0.0133

Source: Reviewer's analysis based on the ADTTE.xpt dataset submitted in eCTD0030 on October 23, 2020.

Abbreviations: CI, confidence interval; GMAS, genotype-matched analysis set; N/A, not applicable; OS, overall survival

Cox Proportional Hazards Model

Analyses using the Cox proportional hazards model to compare the treated group to the untreated FAS and GMAS groups are presented in [Table 14](#), including the inversely weighted analysis to estimate the average treatment effect accounting for the different match ratios. For the inversely weighted analyses, the weights ranged from approximately 0.6 to 2.1. Most analyses resulted in a nominal p-value less than 0.05, except for the analyses of the GMAS stratified by sex based on Wald confidence intervals (p=0.055 and 0.067 for the unweighted and weighted analyses, respectively). Estimates of the hazard ratio were similar across analyses, from 0.17 to 0.24.

Table 14. Efficacy Results of OS—Cox Proportional Hazards Model

Hazard Ratio (HR) (95% CI)	Fosdenopterin (n=13) vs. Untreated FAS (n=37)	Fosdenopterin (n=13) vs. Untreated GMAS (n=18)	Weighted Analysis ¹ Fosdenopterin (n=13) vs. Untreated GMAS (n=18)
Unadjusted HR	0.22	0.18	0.18
Wald CI; p-value	(0.05, 0.92); 0.038	(0.04, 0.81) 0.025	(0.04, 0.84); 0.030
Lin et al. CI ² ; p-value	(0.06, 0.83); 0.022	(0.04, 0.72); 0.012	(0.04, 0.71); 0.011
HR stratified by sex	0.22	0.23	0.24
Wald CI; p-value	(0.05, 0.98) 0.046	(0.05, 1.03) 0.055	(0.05, 1.10) 0.067
Lin et al. CI ² ; p-value	(0.06, 0.88) 0.031	(0.06, 0.92) 0.037	(0.05, 0.90) 0.033
HR covariate for sex	0.21	0.17	0.17
Wald CI; p-value	(0.05, 0.93) 0.039	(0.04, 0.82) 0.028	(0.03, 0.95); 0.044

Source: Reviewer's analysis based on the ADTTE.xpt dataset submitted in eCTD0030 on October 23, 2020.

¹ Wald results for the weighted analysis utilize the robust sandwich variance estimator.

² CIs calculated using a published methodology and code (Lin et al. 2016). The reviewer altered the code to account for weighted analysis.

Abbreviations: CI, confidence interval; FAS, full analysis set; GMAS, genotype-matched analysis set; OS, overall survival

The SAP specified additional analyses to account for the matches, including stratifying the analysis by match ID and adjusting for match ID as a covariate (in the Cox proportional hazards model). There are few patients per treatment group per strata; however, the log-rank test stratified by match ID resulted in a nominal p<0.0001.

Sensitivity Analyses

The primary analyses censored patients at the time of their last follow-up. There were two patients (b) (6) who discontinued treatment at day 22 and day 13, respectively, but remained in the study for further follow-up to 1.2 and 21.7 months, respectively. The following two sensitivity analyses were conducted to evaluate the impact of these two patients with early treatment discontinuation:

- Sensitivity analysis 1: Censor these two patients at the time of treatment discontinuation.
- Sensitivity analysis 2: Include these two patients in the analyses as untreated patients (censored at the time of last follow-up) due to the short treatment duration and expectation that long-term treatment is needed in order to receive the benefits of Nulibry.

Both sensitivity analyses resulted in similar overall conclusions with sensitivity analysis 1, resulting in nominal p-values from the log-rank test of 0.027 for the FAS and 0.016 for the GMAS, and sensitivity analysis 2, resulting in nominal p-values by log-rank test of 0.032 for the FAS and 0.023 for the GMAS. Results for the KM survival probabilities and restricted mean survival time are similar to the primary analysis as well (see [Table 53](#) and [Table 54](#) in Section [III.16](#)).

The following additional sensitivity analyses were conducted on the GMAS cohort:

- Excluding a matched group of patients (two untreated and one treated patient) because the treated patient was from a different country and region than the genotype-matched untreated patients. The sensitivity analysis excluding this match resulted in similar overall conclusions (log-rank p=0.013; see KM curves in [Figure 29](#) in Section [III.16](#)).
- Excluding a different matched group of patients (one untreated patient and one treated patient) because the treated patient had onset of symptoms at day 1 and the genotype-matched untreated patient had onset of symptoms at day 222. The sensitivity analysis excluding this match resulted in similar overall conclusions (log-rank p=0.012; see KM curves in [Figure 30](#) in Section [III.16](#)).

Overall, the analyses of OS data, including the log rank tests and hazard ratios estimated from the Cox proportional hazards model, support the efficacy of fosdenopterin-treated patients compared to the historical control cohort. The width of the confidence intervals of other summary measures of OS, such as the estimated survival probabilities and mean survival time, is an expected consequence of the small sample size. Furthermore, the strong mechanistic evidence and other confirmatory evidence of efficacy support a conclusion that the observed treatment effect was due to a true drug effect rather than chance.

Additional Treated Patients

In addition to the 13 patients treated with fosdenopterin covered in the analyses described above, there were 4 additional patients who were treated with fosdenopterin who either had confirmed MoCD Type A or unknown type.

In the original NDA submission, the Applicant described the following two patients treated under an expanded access investigational new drug program in the United States or a similar program if in another country. Both patients had genetic results confirming a diagnosis of MoCD Type A.

- One patient was treated in (b) (6). The patient received 13 doses of fosdenopterin and was subsequently discontinued from treatment due to a complex family socioeconomic situation that prevented the ability to administer daily intravenous infusions.
- One patient was treated in the (b) (6). Treatment was discontinued after day 4 due to parental and physician decision and the apparent lack of biochemical response, the lack of clinical improvement, and poor prognosis. The patient was discharged to hospice care and died on day 77.

In the safety update dated October 23, 2020, the Applicant provided information on the following two patients treated with fosdenopterin:

- An additional treated patient (b) (6) was enrolled in Study MCD-202. The patient started treatment on (b) (6) and the patient was discontinued from the study drug on (b) (6) due to physician decision due to a poor neurologic prognosis. The patient discontinued from the study on (b) (6). This occurred after the safety update data cutoff.
- An additional patient (b) (6) received treatment with fosdenopterin under local named-patient use regulations. This patient was first dosed at (b) (6) and discontinued from treatment after day 8 due to parental decision due to a poor neurologic prognosis.

A supplemental analysis of the FAS including the data from these additional patients (including the death at day 77 of the one treated patient from the United States, and censoring the other patients at the time of discontinuation) did not change the overall conclusions (see [Figure 31](#) in Section [III.16](#)).

6.3. Key Review Issues Relevant to Evaluation of Benefit

6.3.1. Potential for Selection Bias

The lack of a randomized control group resulted in the potential for selection bias that could lead to uncertainty regarding the comparability of the treatment and control groups. To demonstrate that the survival benefit between the treated patients and the untreated historical control patients resulted from the drug and not from potential selection bias and underlying differences between the two groups, assurance of comparability between the two populations was necessary.

Background and Assessment

To conclude that the drug was responsible for the survival benefit demonstrated in the efficacy analysis, it was necessary to establish that the treated and untreated patient groups were similar in terms of the key attributes that could impact survival. These attributes were genotype, age at onset of symptoms, and the geographic location of patient care (as standard of care can vary substantially in different countries and regions of the world).

Genotype matching in the comparative analyses of treated and untreated patients is commonly used in studies evaluating inherited diseases that have variability in phenotypes. When specific genotypes are known to be predictive of clinical outcomes, this genotype matching helps to

ensure that the treated and untreated patients are expected to have similar clinical outcomes, and therefore minimizes the potential for selection bias in nonrandomized trials. Genotype matching between treated patients and untreated control patients was used in the survival analysis for Nulibry (Misko et al. 2020). Treated patients were first matched with patients in the natural history study with the same homozygous mutation if available. Treated patients who did not have an exact homozygous mutation match in the natural history control group were matched with control patients who had a mutation with a similar anticipated impact on protein function. Among the 13 treated patients, 9 patients had at least one exact genotype match. The remaining four patients were matched on mutations with a similar anticipated impact on protein function (frameshift, missense, etc.). A sensitivity analysis including only patients with an exact genotype match resulted in similar conclusions (refer to [Figure 32](#) in Section [III.16](#)).

In addition to genotype, the age at onset of common presenting symptoms (seizures, high-pitch cry, feeding difficulties, and exaggerated startle reactions) is known to be a predictive factor for progression of MoCD Type A (Hinderhofer et al. 2017). The majority of patients with MoCD Type A have an early disease onset in the neonatal period or in early infancy, which is rapidly progressive and leads to disability and early death. However, there are a few reports of patients with later onset of disease who progress less rapidly and appear to have a better survival prognosis. Evaluation of the time of onset of symptoms demonstrated that all treated patients had onset of symptoms in the first 28 days of life. In the untreated group, nearly all patients (89%) also had onset of symptoms in the first 28 days of life. There was one untreated control patient whose timing of symptom onset (day 222) was not similar to the matched treated patient (who had symptom onset at day 1). However, a sensitivity analysis that excluded these two patients determined that this match did not affect the overall conclusions of the survival analysis (refer to Section [6.2.1.4](#)). An additional sensitivity analysis including only matched patients with onset of symptoms in the first 28 days of life also did not affect the overall conclusions (refer to [Figure 33](#) in Section [III.16](#)).

Although the disease is typically fatal within the first few years of life and there are no other treatments known to alter the course of the disease, we considered whether differences in access to healthcare options among different countries and geographic regions could possibly also affect survival and therefore be a source of bias in an observed survival benefit. Overall, the treated patients and their untreated matched control patients were from the same country or geographic region. Therefore, the access to healthcare is expected to be comparable. There was only one match for which the review team was concerned that differences in medical care could bias the survivability outcome (in this match, the treated patient was from the United States and the matched control patients were from Malaysia). A sensitivity analysis conducted without this matched set demonstrated that this match had no impact on the observed survival benefit in the treated patients. Refer to Section [6.2.1.4](#) for details.

Differences in time periods of birth could also potentially affect survival. As listed in [Table 10](#), more of the natural history control patients were born before 1999 compared to the treated patients. To evaluate the impact of potential changes over time, analyses of the following two subsets of the GMAS were conducted:

- Excluding patients born before 1999

- Including only patients who were born between 2008 to 2014 (the years where both treated and untreated patients were born plus one additional year to retain sample size) with matches born in the same time period

Both analyses demonstrated a similar survival benefit for Nulibry, noting that the second analysis contains a much smaller sample size than the full GMAS cohort (refer to [Figure 34](#) and [Figure 35](#) in Section [III.16](#)).

This provides reassurance that differences in time periods of birth between the groups did not bias the observed survival benefit with Nulibry.

Conclusion

The review team concluded that the potential for selection bias was adequately overcome by the genotype matching used for the analysis and the similarity between the treated and untreated control groups in terms of the other characteristics that could have impacted survival (i.e., of age of onset of symptoms and geographic location). Furthermore, additional survival analysis using the entire untreated natural history group (without genotype matching) yielded a similar survival effect as the genotype-matched analysis (with the smaller control group). The consistency of the results from these two different analyses further supports the survival benefit.

6.3.2. Potential for Detection Bias or a Spurious Mortality Benefit

Given the ability to diagnose MoCD Type A prior to symptom onset (i.e., with cascade testing, or genetic testing of relatives of known patients with MoCD including in utero prenatal testing), there was also the potential for detection bias in the evaluation of efficacy and a potential spurious mortality benefit (from diagnosing and treating patients who would have had a less severe disease course if left untreated).

Background and Assessment

Because early-onset disease portends a worse outcome than late-onset disease, in-utero diagnosis with treatment on day 1 of life does not allow the clinician the opportunity to differentiate which form of the disease the patient has, because symptoms may not have started yet in either form of the disease. This could lead to concluding that children with late-onset disease (who would be expected to live longer) are benefiting from the treatment when in fact it may be the natural history of the disease. Genotype matching minimizes this bias. Additionally, cascade testing was not a factor that could have contributed to the observed mortality benefit of Nulibry because all of the treated patients (n=5) who were diagnosed prenatally developed symptoms in the first month of life, which is consistent with the severe phenotype of the disease characterized by early death. If the infants had not developed any signs in the neonatal period, then it would raise the possibility of detection bias because the late-onset of symptoms may indicate the presence of the less severe form of the disease, which has improved survival unrelated to the drug. Furthermore, since there are no other management options that affect survival in MoCD Type A, early diagnosis is not expected to affect the disease course. Changes in genetic testing over time can also introduce detection bias when comparing treated patients to a historical untreated control group. However, this was not a factor either, as the same genetic testing was available to the

untreated historical control patients and the treated patients. Therefore, we are reassured that detection bias was not a contributing factor and that the mortality benefit observed was not spurious.

Conclusion

We are not concerned that detection bias impacted the observed survival benefit of Nulibry.

6.3.3. Regulatory Framework for Establishing Substantial Evidence of Effectiveness

Substantial evidence of effectiveness, which is the regulatory requirement for approval, generally consists of evidence from at least two adequate and well-controlled trials. For this NDA, substantial evidence of effectiveness comes from a single adequate and well-controlled investigation (which compares treated patients from two trials and an observational study with untreated patients from a natural history control) together with confirmatory evidence.

Background and Assessment

In certain circumstances, a single adequate and well-controlled investigation demonstrating efficacy together with appropriate confirmatory evidence may be sufficient to generate substantial evidence of effectiveness. Such circumstances are described in the FDA draft guidance *Demonstrating Substantial Evidence of Effectiveness for Human Drug and Biological Products* (December 2019), which includes the persuasiveness of the single investigation; the robustness of the confirmatory evidence; the seriousness of the disease, particularly when there is an unmet medical need; the size of the patient population; and whether it is ethical and practicable to conduct more than one adequate and well-controlled investigation. These criteria and considerations listed in the aforementioned guidance are all relevant to the Nulibry development program for the very rare, serious disease of MoCD Type A with an unmet need.

The treated patients in this NDA come from two single-arm trials and from an observational study. These patients were compared to untreated patients from a matched, natural history control group. Based on our assessment of these data sources and data in the context of the disease, we conclude that this comparison of treated and untreated patients is an adequate and well-controlled investigation.

The accumulation of the toxic metabolite SSC in the CNS is the known cause of the clinical manifestations of MoCD Type A. While SSC was not measured in the CNS of treated patients, the following lines of evidence provide confirmatory evidence for Nulibry's effectiveness.

- Biomarker data demonstrated reductions in urinary SSC with Nulibry and PK/PD data demonstrated an exposure-response relationship for reduced urinary SSC normalized to creatinine. These data show that Nulibry can reduce systemic levels of the toxic metabolite.
- Elevated plasma and urinary SSC levels are biochemical hallmarks of the disease.

- Animal data using a mouse model of MoCD Type A demonstrated reduction of plasma and brain SSC levels and substantially improved survival in fosdenopterin-treated mice compared to placebo-treated mice. Tissue distribution studies in the rat following intravenous radiolabeled fosdenopterin, also demonstrated the presence of fosdenopterin in the non-circumventricular CNS. Although species differences exist for the transport of drugs to the brain, there is currently no evidence to suggest a species difference between animals and humans that would alter the permeability or transport of fosdenopterin across the blood brain barrier. These animal data also support a conclusion that Nulibry can cross the blood-brain barrier.

Although the urinary SSC data from the clinical studies have some limitations (as discussed in Section 6.1), overall these data support a conclusion that Nulibry treatment leads to a sustained reduction in this biomarker. In Study MCD-501, urinary SSC concentrations were measured using nonvalidated assays, and the method used in this study to determine SSC, creatinine and xanthine in human urine did not meet acceptance criteria when cross-validated against the validated methods used in Studies MCD-201 and MCD-202. Consequently, the quantitative comparison of urine SSC values normalized to creatinine in Study MCD-501 to those values in Studies MCD-201 and MCD-202 is limited. However, the qualitative demonstration of a similar trend of reduction in urinary SSC normalized to creatinine during treatment in Studies MCD-501 and MCD-201 and -202 was observed. Although the urinary SSC data from Studies MCD-501 and MCD-201 and -202 may not be directly comparable, the studies individually demonstrate the same qualitative trend in urinary SSC. The reproducibility of this trend in the different studies strengthens the conclusion that Nulibry treatment results in a reduction of urinary SSC. Additionally, only 4 of the 13 patients with urinary SSC biomarker data were enrolled only in Study MCD-501 (without subsequent transition to Study MCD-201). Therefore, most patients had urinary SSC results assessed utilizing the validated assays.

Conclusion

Data supporting the single adequate and well-controlled investigation in the Nulibry development program (i.e., biomarker data in humans and animals demonstrating a reduction in a metabolite with known clinical neurotoxic effects together with animal data demonstrating improved survival in the mouse model of the disease) provide acceptable confirmatory evidence for the effectiveness of Nulibry, and thereby renders the lack of a second, adequate and well-controlled clinical investigation acceptable for the approval of Nulibry.

6.3.4. Post Hoc Nature of the Analysis Plan

Issue

The SAP for the adequate and well-controlled investigation was developed after mortality outcomes were known.

Background and Assessment

The originally planned pivotal Study MCD-202 was unable to complete enrollment, and therefore, the Applicant initiated discussions with the Agency regarding the use of data across multiple studies to compare treated patients to untreated patients in the natural history study. The

mortality outcomes from patients participating in the studies were known at that time. In general, analysis plans should be submitted to the Agency in advance of knowing the outcomes to minimize the possibility of making false conclusions (e.g., that the drug is effective when it is truly not effective).

The Agency reviewed and generally agreed with the SAP for this integrated efficacy analysis prior to the NDA submission. For the NDA, the review team concluded that the post hoc analysis results were not likely due to chance nor other confounding factors based on the following considerations:

- Mortality is a clinically meaningful and objectively-measured outcome that was a component of the original prespecified primary endpoint in Study MCD-202, and the magnitude of the estimated treatment effect was large. Multiple analyses were conducted to ensure the conclusions were not driven by a single choice of statistical method.
- The review adequately explored or addressed the known potential confounders (genotype, sex, geographic region, and time of onset of symptoms) that might bias results in favor of the treated patients.
- The mortality findings were well supported by mechanistic and other confirmatory evidence.

Conclusion

The findings indicate an effect of fosdenopterin on mortality and are not likely due to chance. There is also confirmatory evidence that lends support to a drug effect (see Sections [6.1](#) and [6.3.3](#)).

6.3.5. Use of Both rcPMP and cPMP in Treated Patients

Issue

Two different drug products (rcPMP and CPMP) were used in the clinical studies.

Background and Assessment

The first patients with MoCD Type A treated with substrate replacement (in expanded access INDs and in Study MCD-501) received the recombinant form of cPMP (rcPMP). When a chemically synthetic form of the drug became available, patients receiving rcPMP switched to the synthetic form, cPMP (fosdenopterin). Because the efficacy and safety data in this NDA are derived from both rcPMP and CPMP, it was necessary to conclude that they are chemically equivalent.

The molecular structures of rcPMP and fosdenopterin (cPMP) have been compared using multiple analytical techniques (refer to Section [9](#) for details) and the results have demonstrated that rcPMP and cPMP have identical core structures and the same active moiety.

Conclusion

RcPMP and cPMP are considered chemically equivalent. Therefore, efficacy and safety data from rcPMP can be used to determine the safety and efficacy of cPMP (Nulibry).

7. Risk and Risk Management

7.1. Potential Risks or Safety Concerns Based on Nonclinical Data

7.1.1. Overall Safety Concern

A significant safety concern for phototoxicity was observed with fosdenopterin in animals. The relevance to humans cannot be excluded and, therefore, photosensitivity will be labeled in the Warnings and Precautions section of the United States Prescribing Information (as the Applicant proposed). There were no other nonclinical safety issues of significant concern as assessed by the toxicology studies conducted during the development program, and the nonclinical data support the marketing approval of fosdenopterin. Nonclinical safety was evaluated in the following studies during development:

- Safety pharmacology in the rat and dog.
- Repeat-dose toxicity studies in juvenile rats for 13 and 26 weeks and in juvenile dogs for 39 weeks ([Table 15](#)).
- Genetic toxicology studies with fosdenopterin (Ames, in vitro chromosomal aberration, and in vivo micronucleus assays); genetic toxicology studies (Ames) to assess the safety of specific impurities.
- In vitro and in vivo phototoxicity studies.

Reproductive toxicology studies and a carcinogenicity study in a single species will be conducted as postmarketing requirements.

Table 15. Safety Margins From Pivotal Toxicology Studies

Study	NOAEL (mg/kg/day)	HED (mg/kg/day)	AUC (µg.h/mL)	Safety Margin to MRHD ¹ (Based on HED)
13-Week juvenile rat	100 ²	16	532 ³	17.8×
26-Week juvenile rat	5 ²	0.8	30.5 ³	0.9×
39-Week juvenile dog	10 ²	5.6	119 ³	6.2×

Source: Reviewer generated

¹ MRHD, 0.9 mg/kg/day

² Highest dose tested

³ Applicant-supplied values of AUC from time zero to the last measurable concentration using the linear-log trapezoidal rule were multiplied by specific factors to calculate the AUC time zero to 24 hr. As a result, use of HED to determine clinical safety margins is preferred.

Abbreviations: NOAEL, no observed adverse effect level; HED, human equivalent dose (based on body surface area); AUC, area under the concentration-time curve; MRHD, maximum recommended human dose

7.1.2. Safety Pharmacology and ADME

Fosdenopterin had no effect on neurological function or behavior in a standalone 2-week neurobehavioral study in rats (up to 10 mg/kg/day) or the 6- or 9-month toxicity studies in rats (up to 5 mg/kg/day) and dogs (up to 10 mg/kg/day), respectively. Fosdenopterin had no inhibitory effect on a variety of ion channels tested in an in vitro panel, with IC₅₀ values

>300µM. In a standalone in vivo cardiovascular study in dogs, fosdenopterin had no effect on body temperature, blood pressure, heart rate, or on qualitative or quantitative electrocardiogram parameters. Fosdenopterin had no effect on respiratory function in a standalone 2-week respiratory study in rats (up to 10 mg/kg/day).

Bioavailability of fosdenopterin following subcutaneous injection was 97%. Fosdenopterin was low to moderately bound to plasma proteins in vitro, with and without ascorbic acid. (b) (4)

In the absence of ascorbic acid (e.g., in a nonacidified blood system), fosdenopterin had low affinity for the red blood cell fraction of whole blood. In the presence of blood acidified with ascorbic acid, fosdenopterin did not partition into red blood cells. In vitro metabolic profiling indicated that two cPMP-related compounds (C1 and C2) were detected in mouse, rat, dog, and human hepatocytes incubated with *E. coli*-derived cPMP in the presence of ascorbic acid. Both C1 and C2 correspond to compound Z, which is a known oxidation product of cPMP. No human-specific metabolites were identified. In vivo metabolite studies have not been conducted with fosdenopterin.

7.1.3. General Toxicology

A significant safety concern for phototoxicity with fosdenopterin was identified at doses $\geq 4.4\times$ the maximum recommended human dose of 0.9 mg/kg/day (based on the human equivalent dose [HED]) in a standalone phototoxicity study in rats. This was evidenced by cutaneous skin reactions and ophthalmic and histopathological findings indicative of phototoxicity (see Section III.13.1.8). The clinical relevance of this finding is unknown given the lack of safety monitoring in the clinical studies. There are no additional nonclinical studies that could be conducted to provide further insight into this safety concern for humans. The potential for photosensitivity is labeled in Warnings and Precautions.

There were no nonclinical safety issues of significant concern observed in the general toxicity studies initiated in juvenile rats and dogs. Fosdenopterin and impurities, and (b) (4) were all negative for genotoxic potential. Reproductive and developmental toxicity studies and carcinogenicity studies have not been conducted with fosdenopterin. These studies will be conducted as postmarketing requirements so as not to delay approval of Nulibry, which has shown a survival benefit in a rare, devastating disease without other treatment options.

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

Not Applicable. There are no other drugs in the same pharmacologic class.

7.3. Potential Safety Concerns Identified Through Postmarket Experience

Not applicable. Fosdenopterin has not been approved for marketing in any country.

7.4. FDA Approach to the Safety Review

Studies MCD-501, MCD-201, and MCD-202 were assessed individually for the safety review. The 120-day Safety Update Reports for each of these studies were also reviewed.

No major data quality or integrity issues were identified that would preclude performing a safety review for this NDA. There were no major issues identified with respect to recording, coding, and categorizing adverse events (AEs). The Applicant's translations of verbatim terms to Medical Dictionary for Regulatory Activities (MedDRA) preferred terms for the events reported in Studies MCD-501, MCD-210, and MCD-202 were reviewed and found to be acceptable. Tabulations for AEs are based on treatment-emergent AEs (TEAEs) that were protocol-defined as events that started on or after the first dose of study treatment or worsened in severity during treatment. The retrospective nature of Study MCD-501 permitted the assessment of causality for only the serious adverse events (SAEs).

7.5. Adequacy of the Clinical Safety Database

A total of 13 neonatal and pediatric patients with MoCD Type A was treated across the three Studies MCD-501, MCD-201, and MCD-202. Six of the thirteen patients initially received rcPMP while in Study MCD-501 and then transitioned to fosdenopterin when they enrolled in Study MCD-201. Four patients received only rcPMP in Study MCD-501. Two patients initially received rcPMP outside of a clinical study (through the above-described name-patient use program) and then received fosdenopterin in Study MCD-201. The one patient in Study MCD-202 received only fosdenopterin (with no prior rcPMP therapy). Patients with other types of MoCD were not included in the safety analysis because they received fosdenopterin treatment for a short duration (all <18 days) until genetic diagnostic testing confirmed that they had a different type of MoCD (i.e., not Type A). However, the safety data for these patients were reviewed and there were no notable AEs reported during their brief period of treatment with fosdenopterin.

Study MCD-101 was the first-in-human phase 1, single-dose, dose-escalation, placebo-controlled study conducted in healthy volunteers. Eighteen patients received a single dose (either 0.10 mg/kg, 0.32 mg/kg, or 0.90 mg/kg) of fosdenopterin. These patients are not included in the safety analysis and tables below, because this single-dose study is not informative for the safety analysis. The safety data were reviewed and presented in Section [III.17](#).

Overall, the 13 treated patients had 65.4 patient-years of exposure, from the first documented dose of rcPMP to the last dose of fosdenopterin. The median total time on either fosdenopterin or rcPMP was 4.6 years with a range of 6 days to 11.7 years. Among the 10 patients who received rcPMP in Study MCD-501, the median time on treatment was 1.5 years with a range of 6 days to 4.4 years. Among the eight patients who received fosdenopterin, the median time on treatment was 4.3 years and the total patient years of exposure to fosdenopterin was 37.5 years.

Patients received varying doses of fosdenopterin across the studies. Overall, four out of eight patients (50%) received a maximum initial dose of 1200 µg/kg/day (received as rcPMP, the free base form of fosdenopterin, which is equivalent to 1300 µg/kg/day of cPMP, the hydrobromide salt form of fosdenopterin)(in Study MCD-201, with all four patients continuing that dose. Four patients in MCD-201 are receiving lower doses (unrelated to safety), two because of parental request for ease of administration, one who recently enrolled and has not titrated up yet, and one

who titrated up to 960 µg/kg/day and maintained that dose due to PK results that demonstrated exposure in this patient was within 90% of the NOAEL. The one patient enrolled in Study MCD-202 is receiving 1300 µg/kg/day. The small number of patients and the lack of a consistent dose across the studies have limited the interpretation of the safety data (as a dose-response relationship for AEs was not possible). However, given the rarity of the disease and a demonstrated survival benefit, the available safety data from the three studies is adequate to inform the benefit/risk assessment of fosdenopterin. Refer to Section 6.1 for further discussion of dosing.

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

7.6.1. Safety Findings and Concerns

7.6.1.1. Overall Treatment-Emergent Adverse Event Summary, Studies MCD-501, MCD-201, and MCD-202

Table 16. Overview of Treatment-Emergent Adverse Events by Study

Event	MCD-501 N=10 n (%)	MCD-201 N=8 n (%)	MCD-202 N=1 n (%)
Any treatment-emergent AE	10 (100%)	8 (100%)	1 (100%)
Moderate or severe AEs (grades 3-5) ²	6 (60%)	8 (100%)	1 (100%)
SAEs	8 (80%)	7 (88%)	1 (100%)
SAEs with a fatal outcome	2 (20%)	0	0
AEs leading to discontinuation of study drug	0	0	0
AEs leading to dosage modification of study drug	0	0	0
AEs leading to interruption of study drug	0	0	0
AEs leading to reduction of study drug	0	0	0
AEs leading to dosage delay of study drug	0	0	0

Source: Reviewer generated

Abbreviations: AE, adverse event; SAE, serious adverse event; N, number of subjects in a group; n, number of subjects with at least one event

7.6.1.2. Deaths, Studies MCD-501, MCD-201, and MCD-202

There were two deaths reported in the three studies (both in Study MCD-501 in patients who were receiving treatment with rcPMP). A (b) (6) patient died of respiratory syncytial virus pneumonia 1.2 years after fosdenopterin treatment was initiated. This death is not likely treatment-related, because respiratory syncytial virus pneumonia is not an atypical outcome in young patients and is often more severe in patients who are neurologically compromised. The other death occurred in a (b) (6) patient who was born prematurely at a gestational age of (b) (6). This patient was diagnosed with MoCD Type A in utero and treatment with rcPMP was initiated at (b) (6). On (b) (6) the patient developed metabolic acidosis, respiratory distress and volatile blood pressure that was treated with furosemide and propranolol. She developed abdominal distension and hypotension and was managed with positive pressure ventilation, cardiac massage and vasopressors, and was then diagnosed with necrotizing enterocolitis. Her clinical status deteriorated, and she died of cardiac arrest. The determination of causality is very difficult due to the multiple confounding factors of prematurity, blood pressure

instability, and concomitant antihypertensive medicines, all of which may lead to intestinal ischemia and necrotizing enterocolitis.

7.6.1.3. Serious Adverse Events, Studies MCD-501, MCD-201, and MCD-202

The majority of patients reported at least one SAE, including 8 of the 10 patients in Study MCD-501, and 8 of the 9 patients in Studies MCD-201 and MCD-202. Device-related complications and infections were the most common SAEs observed in all three studies. All SAEs were likely unrelated to the drug, as most SAEs were infections or device related complications. Infections are common in children (especially neurologically impaired children) and device-related complications are an expected risk of a central venous catheter, which is required for the daily IV administration. See [Table 17](#) for a list of the SAEs reported in these studies. Because fosdenopterin requires a central venous catheter for daily IV administration, the device-related complications are an anticipated risk of therapy. However, the overall SAE profile is acceptable considering the benefit of prolonged survival.

Table 17. Serious Adverse Events—Safety Population, Studies MCD-501, MCD-201, and MCD-202

Serious Adverse Event ¹	MCD-501 N=10 n (%)	MCD-201 N=8 n (%)	MCD-202 N=1
Vascular device infection	0	2 (29%)	0
Catheter site infection	0	2 (29%)	0
Complication associated with device	2 (20%)	2 (29%)	1 (100%)
Pyrexia	2 (20%)	2 (29%)	0
Pneumonia	2 (20%)	2 (29%)	1 (100%)
Device dislocation	2 (20%)	2 (29%)	1 (100%)
Device-related infection	3 (30%)	1 (14%)	0
Device-related sepsis	2 (20%)	0	0
Sepsis	2 (20%)	1 (14%)	0
Respiratory tract infection	1 (10%)	1 (14%)	0
Vomiting	0	0	1 (100%)
Gastroenteritis	0	0	1 (100%)
Bacteremia	0	1 (14%)	1 (100%)
Apnea	0	0	1 (100%)
Cardiac failure	0	0	1 (100%)
Catheter site swelling	0	0	1 (100%)
Viral infection	0	1 (14%)	0
Venous thrombosis	0	1 (14%)	0
Urinary tract infection	0	1 (14%)	0
Type 1 diabetes mellitus	0	1 (14%)	0
Swelling	0	1 (14%)	0
Skin disorder	0	1 (14%)	0
Rhinovirus infection	0	1 (14%)	0
Respiratory failure	0	1 (14%)	0
Otitis media	0	1 (14%)	0
Lower respiratory infection	0	1 (14%)	0
Diabetic ketoacidosis	0	1 (14%)	0
Device-related infection	0	1 (14%)	0
Dehydration	0	1 (14%)	0
Catheter-site abscess	0	1 (14%)	0
Catheter-site discharge	0	1 (14%)	0

	MCD-501 N=10 n (%)	MCD-201 N=8 n (%)	MCD-202 N=1
Serious Adverse Event¹			
Catheter-site extravasation	0	1 (14%)	0
Catheter-site inflammation	0	1 (14%)	0
Infection	1 (10%)	0	0
Varicella	1 (10%)	0	0
Upper respiratory infection	1 (10%)	0	0
Subdural effusion	1 (10%)	0	0
Stomatitis	1 (10%)	0	0
Staphylococcal sepsis	1 (10%)	0	0
Staphylococcal infection	1 (10%)	0	0
Respiratory distress	1 (10%)	0	0
Respiratory syncytial virus pneumonia	1 (10%)	0	0
Pleural effusion	1 (10%)	0	0
Necrotizing colitis	1 (10%)	0	0
Myoclonus	1 (10%)	0	0
Irritability	1 (10%)	0	0
Febrile infection	1 (10%)	0	0
Device leakage	1 (10%)	0	0

Source: Reviewer created

¹ Coded as Medical Dictionary for Regulatory Activities preferred terms

Abbreviations: N, number of subjects in group; n, number of subjects with an adverse event

7.6.1.4. Dropouts and/or Discontinuations Due to Adverse Events, Studies MCD-501, MCD-201, and MCD-202

There were no discontinuations due to AEs in any of these studies.

7.6.1.5. Treatment-Emergent Adverse Events, Studies MCD-501, MCD-201, and MCD-202

All patients in the three studies experienced at least one TEAE. The majority of TEAEs were related either to infections or a device (i.e., central venous catheter). The device-related AEs that were reported in more than one patient in Study MCD-501 include device-related infection (three patients), device dislocation, medical device complication, device leakage, and sepsis and device-related sepsis (two patients each). Among the nine patients who were treated in Studies MCD-201 and MCD-202, eight experienced at least one device-related TEAE. The device-related events reported in more than one patient include complications associated with the device (six patients), device dislocation and catheter site infection (three patients), catheter site extravasation, catheter site pain, catheter site discharge, and vascular device infection (two patients each). See [Table 18](#) for a list of TEAEs seen in more than one patient in any study.

Device-related TEAEs are unrelated to fosdenopterin itself but are a risk related to the administration method of the drug. The benefit of prolonged survival outweighs the risks of device related complications associated with the delivery of the drug. Aside from the infections related to the device, infections overall are likely not drug-related due to the high prevalence of infections in children and in particular children with neurologic impairment.

Table 18. Treatment-Emergent Adverse Events¹ Occurring in at Least One Patient in Studies MCD-501, MCD-201, and MCD-202

Preferred Term²	MCD-501 N=10 (%)	MCD-201 N=8 (%)	MCD-202 N=1 (%)
Infection Related			
Pyrexia	3 (30%)	6 (75%)	1 (100%)
Complication associated with device	0	6 (75%)	1 (100%)
Device-related infection	3 (30%)	1 (13%)	0
Pneumonia	3 (30%)	3 (38%)	1 (100%)
Viral infection	0	4 (50%)	1 (100%)
Device-related sepsis	2 (20%)	0	0
Vascular device infection	0	2 (25%)	0
Bacteremia	0	1 (13%)	1 (100%)
Sepsis	2 (20%)	1 (13%)	0
Catheter site discharge	0	2 (25%)	0
Catheter site infection	0	2 (25%)	1 (100%)
Otitis media	2 (20%)	3 (38%)	0
Ear infection	0	2 (25%)	0
Gastroenteritis	1 (10%)	1 (13%)	1 (100%)
Upper respiratory infection	3 (30%)	2 (25%)	0
Oral candidiasis	3 (30%)	1 (13%)	0
Varicella	2 (20%)	1 (13%)	0
Viral upper respiratory infection	0	2 (25%)	0
Respiratory tract infection	1 (10%)	1 (13%)	0
Urinary tract infection	1 (10%)	1 (13%)	0
Bronchitis	1 (10%)	1 (13%)	0
Fungal skin infection	2 (20%)	0	0
Viral tonsillitis	0	1 (13%)	1 (100%)
Device Related			
Catheter site extravasation	0	2 (25%)	0
Catheter site pain	0	2 (25%)	0
Device dislocation	2 (20%)	3 (38%)	1 (100%)
Device leakage	0	2 (25%)	0
Medical device complication	2 (20%)	0	0
Catheter site inflammation	1 (10%)	1 (13%)	0
Skin			
Dermatitis	1 (10%)	0	1 (100%)
Eczema	2 (20%)	0	0
Rash maculopapular	0	2 (25%)	0
Skin disorder	0	2 (25%)	0
Other			
Vomiting	0	3 (38%)	1 (100%)
Diarrhea	0	2 (25%)	1 (100%)
Abdominal pain	0	2 (25%)	0
Constipation	1 (10%)	1 (13%)	0
Seizure	0	2 (25%)	0
Irritability	1 (10%)	1 (13%)	0
Anemia	2 (20%)	1 (13%)	1 (100%)
Eye swelling	0	2 (25%)	0
Conjunctival hemorrhage	1 (10%)	0	1 (100%)
Oropharyngeal pain	0	2 (25%)	0
Cough	1 (10%)	4 (50%)	0
Sneezing	1 (10%)	2 (25%)	0
Asthma	1 (10%)	1 (13%)	0

Preferred Term ²	MCD-501 N=10 (%)	MCD-201 N=8 (%)	MCD-202 N=1 (%)
Rhinorrhea	1 (10%)	0	1 (100%)
Strabismus	1 (10%)	1 (13%)	0

Source: Clinical reviewer

¹ Treatment-emergent adverse event defined as events that started on or after the first dose of study treatment or worsened in severity during treatment.

² Coded as Medical Dictionary for Regulatory Activities preferred terms

Abbreviations: N, number of subjects; n, number of subjects with adverse event

7.6.1.6. Laboratory Findings and Vital Signs, Studies MCD-501, MCD-201, and MCD-202

Laboratory Findings

A comprehensive review of the datasets revealed laboratory abnormalities consistent with what would be anticipated in the underlying patient population. As expected, there were isolated instances of laboratory abnormalities such as increased c-reactive protein and leukocytosis associated with infections. There were no trends of abnormally high or low values for the laboratories including hemoglobin, lymphocytes, neutrophils, platelets, bilirubin, potassium, sodium, or creatinine. Across the three studies, laboratory abnormalities were reported as TEAEs in five patients. These included anemia, increased c-reactive protein, leukocytosis, hyperbilirubinemia, hypocalcemia, and hypoglycemia. None were assessed as SAEs. There were no patients with abnormal laboratory values that met Hy's Law criteria. In summary, there were no laboratory findings in the datasets for any of the studies that were a potential safety signal.

Vital Signs

Although there were multiple minor abnormalities in vital signs throughout the three studies (as anticipated in this patient population), overall, there were no clear trends of abnormally high or low temperature, blood pressure, heart rate, or respiratory rate.

The most common vital sign abnormality that was reported as a TEAE was pyrexia, which was reported in three patients in Study MCD-501, six patients in Study MCD-201, and one patient in Study MCD-202. As noted previously, infections were commonly reported in the trials.

In Study MCD-501, TEAEs of hypertension and hypotension were reported in a critically ill neonate on day 1 of life. This patient experienced both hypertension and hypotension on day 1 of life and hypertension on day 2 and day 3 of life. There were no other TEAEs of hypertension or hypotension reported in Study MCD-501, and there were no TEAEs related to blood pressure changes reported in Studies MCD-201 or MCD-202.

In Study MCD-201, all eight patients experienced at least one outlier blood pressure value (outlier values included both increases and decreases in diastolic and systolic blood pressure). Decreases in diastolic blood pressure were the most common outliers, followed by decreases in systolic blood pressure. Review of the blood pressure data did not reveal a pattern of blood pressure changes either in individual patients or overall in the treated patients. There was no clear pattern in the timing observed for the outlier values (i.e., some patients experienced outliers soon after infusion and some experienced outliers a long duration after initiating treatment). The observance of both elevated and decreased blood pressures and the lack of a temporal relationship between drug administration and abnormal blood pressure readings support an assessment that blood pressure abnormalities were not a drug effect. None of these outliers were

reported as AEs. It is possible that the difficulties in obtaining accurate blood pressure measurements in young infants contributed to the outlier values. In summary, the data do not suggest a signal for a drug-related effect on blood pressure.

7.7. Key Review Issues Relevant to Evaluation of Risk

7.7.1. Labeled Option for Administration by a Caregiver

Issue

Nulibry is primarily intended for administration by healthcare providers and requires reconstitution prior to administration. The proposed Nulibry labeling, however, indicates an option for caregiver reconstitution and administration if deemed appropriate by the healthcare provider. Caregiver reconstitution and administration of an intravenous drug is not standard in clinical practice. Additionally, the most common AEs in the clinical studies were infections and other complications related to central venous catheters, which are required for the daily long-term administration of Nulibry. The review team was concerned about the ability of nonhealthcare providers to safely and effectively administer Nulibry given the complex steps of reconstitution and administration and that, without proper training and supervision, caregiver reconstitution and administration could increase the risk of these device-related infections and other AEs.

Background

Caregiver administration of Nulibry occurred in the clinical studies (when deemed appropriate as specified in the study protocols). However, the information included in the original NDA submission did not clarify how many patients received Nulibry administered by a caregiver, when transitions from healthcare provider to caregiver administration occurred, what training and support was required to permit this transition, and if there was a difference in AEs related to device complications with caregiver versus healthcare provider administration. In response to an information request during the review cycle, the Applicant provided additional data to make these clarifications. Briefly, all but one of the patients currently enrolled in Studies MCD-201 and MCD-202 are receiving Nulibry via caregiver administration, and the transition to caregiver administration occurred soon after treatment initiation for most of these patients. An analysis comparing AEs recorded as device complications or infections associated with devices in Studies MCD-201 and MCD-202 demonstrated similar incidences of these AEs reported with healthcare provider administration and caretaker administration. Of these 49 AEs, 22 (45%) occurred during healthcare provider administration and 27 (55%) occurred during caregiver administration. Notably, the majority of Nulibry dosing occurred during periods of caregiver administration (83% of all administered doses) rather than healthcare provider administration (17% of all administered doses). Therefore, the similar numbers of events reported during healthcare provider and caregiver administration despite the overall greater exposure with caregiver administration provide reassurance that caregiver administration does not increase the risk of these events.

Assessment and Conclusion

The Applicant provided data to support the option for caregiver administration of Nulibry. Most patients in the clinical studies have received Nulibry administered by a caregiver for a substantial period (and, consequentially, most AE data are in the context of caregiver administration). This provides adequate reassurance that caregiver administration should have an overall acceptable safety profile. Labeling will reflect that caregiver administration is only permitted if deemed appropriate by the patient's healthcare provider (which should include confirmation by the healthcare provider that caregivers have received proper training and will receive ongoing support for home administration).

7.7.2. Potential Risk of Phototoxicity

Issue

Nonclinical studies indicate a potential risk for phototoxicity with Nulibry. In the nonclinical studies, phototoxic potential was identified late in the development program and was, therefore, not specifically monitored for in the clinical studies.

Background and Assessment

The nonclinical findings of phototoxicity were identified in late 2019, after the clinical studies were initiated. The clinical studies did not include specific procedures or assessments to monitor for phototoxic AEs and characterize the clinical risk of phototoxicity. Therefore, although the AE data from the clinical studies do not suggest any phototoxic AEs, these data are not reassuring that there is no clinical risk of phototoxicity with Nulibry.

Animal studies demonstrated that pigmented rats treated with variable doses of fosdenopterin and exposed to ultraviolet radiation developed dose related corneal findings, cutaneous skin reactions, and histopathologic changes. Studies using mouse fibroblasts exposed to fosdenopterin and ultraviolet radiation also demonstrated fosdenopterin had phototoxic potential.

Using the Bradford Hill criteria for causality, it seems likely that the phototoxicity seen in nonclinical studies was causally related to fosdenopterin. Although the biological mechanism is not identified, there is experimental evidence in the animal studies that demonstrates a dose-dependent increase in eye and skin adverse findings after fosdenopterin administration, which supports the temporal sequence, and the strength of association and biologic gradient (dose-response relationship) between fosdenopterin exposure and phototoxicity. Additionally, there is consistency of evidence, with the phototoxicity observed in both the in vivo and in vitro studies. None of the other ingredients in the formulation used in these nonclinical studies are phototoxic. In humans, there is no known biologic plausibility for a phototoxic effect of fosdenopterin as a cPMP substrate replacement therapy. Clinical data specifically demonstrating phototoxic AEs would support a determination that phototoxicity is a true drug-related clinical effect of Nulibry. Analysis of AEs seen in the clinical studies did not identify AEs likely related to phototoxicity. However, the protocols for the clinical studies did not specify monitoring procedures for such events, and the amount of sun exposure in these patients was likely minimal given their degree of disability. The one patient who had phototherapy while on treatment did not have any skin findings documented on skin exam during or after phototherapy. Therefore, a conclusion cannot

be drawn regarding the drug-relatedness based on the available clinical data. In summary, although the nonclinical data provides strong evidence of phototoxic potential of Nulibry, there are no clinical data to support this association. Therefore, phototoxicity is considered a potential (rather than identified) risk of Nulibry therapy.

Conclusion

The review team concludes that phototoxicity is a serious potential risk of Nulibry therapy. Therefore, this risk will be conveyed as a labeled Warning and Precaution and labeling will include detailed instructions for patients and caregivers to mitigate this potential risk. Additionally, to monitor for AEs associated with phototoxicity and to further characterize the true clinical risk, the Applicant will implement an enhanced pharmacovigilance program as agreed upon with the Agency (refer to the review by the Division of Pharmacovigilance I dated January 7, 2021 in the Document Archiving, Reporting and Regulatory Tracking System for details regarding this program). The review team concludes that the risk of phototoxicity can be adequately mitigated and monitored with labeling and the proposed pharmacovigilance program. This potential risk is acceptable considering the anticipated survival benefit of Nulibry.

8. Therapeutic Individualization

8.1. Intrinsic Factors

The recommended dosage regimen in patients ≥ 1 year of age is based on the individual patient's body weight. The recommended dosage regimen in patients < 1 year of age is based on the individual patient's body weight and their gestational age. The currently available data do not support a need for further therapeutic individualization based on other intrinsic factors.

8.1.1. Renal Impairment or Hepatic Impairment

The effect of renal impairment or hepatic impairment on the PK of fosdenopterin has not been studied. Because renal or hepatic impairment is generally not associated with MoCD Type A, further investigation of PK, efficacy, or safety of fosdenopterin to inform dosing in patients with renal or hepatic impairment is not needed.

8.2. Drug Interactions

Fosdenopterin was not a direct time-dependent or metabolism-dependent inhibitor of cytochrome P450 (CYP)1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5 isozymes when tested in vitro in human liver microsomes. In culture human hepatocytes, fosdenopterin did not induce CYP1A2, CYP2B6, or CYP3A4. Because no CYP3A induction was observed, the potential to induce any CYP2C isozymes is inferred to be low. Fosdenopterin is not a substrate of P-glycoprotein, breast cancer resistance protein (BCRP), OAT1, OAT3, OATP1B1, OATP1B3, OCT2, or MATE2-K, and is possibly a weak substrate for MATE1. The potential for fosdenopterin to inhibit efflux or uptake transporters is low. Fosdenopterin did not inhibit P-glycoprotein, BCRP, OATP1B1, OATP1B3, OCT2, OAT3, and MATE1. Fosdenopterin slightly inhibited MATE2-K and OAT1 (25% and 33%, respectively); however, no significant drug-drug interaction is anticipated.

The potential of CYP or transporter-mediated drug-drug interactions with fosdenopterin is low. No clinical drug-drug interaction studies have been conducted with fosdenopterin.

8.3. Plans for Pediatric Drug Development

Not applicable. Nulibry is indicated for a pediatric population. Nonclinical juvenile animal study information is summarized below.

Fosdenopterin was phototoxic at doses $\geq 4.4\times$ the MRHD of 0.9 mg/kg/day (based on the human equivalent dose). The potential for photosensitivity is labeled in Warnings and Precautions. There were no other nonclinical safety issues of significant concern as assessed by the toxicology studies conducted during the development program. Nonclinical safety for pediatric patients was evaluated in repeat-dose toxicity studies initiated in juvenile rats for 13 weeks and 26 weeks, and in juvenile dogs for 39 weeks. No AEs were observed in these studies.

Table 19. Safety Margins From Pivotal Toxicology Studies

Study	NOAEL (mg/kg/day)	HED (mg/kg/day)	AUC ($\mu\text{g}\cdot\text{h}/\text{mL}$)	Safety Margin to MRHD ¹ (Based on HED)
13-Week juvenile rat	100 ²	16	532 ³	17.8×
26-Week juvenile rat	5 ²	0.8	30.5 ³	0.9×
39-Week juvenile dog	10 ²	5.6	119 ³	6.2×

Source: Reviewer generated

¹ MRHD, 0.9 mg/kg/day

² Highest dose tested

³ AUC to the last quantifiable time point values from the Applicant were multiplied by specific factors to achieve AUC up to 24 hr. As a result, use of HED to determine clinical safety margins is preferred.

Abbreviations: AUC, area under the concentration-time curve; HED, human equivalent dose (based on body surface area); MRHD, maximum recommended human dose; NOAEL, no observed adverse effect level

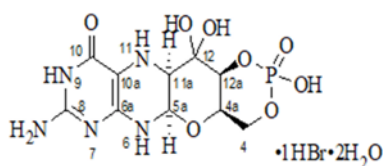
8.4. Pregnancy and Lactation

Nonclinical reproductive toxicity studies with fosdenopterin have not been conducted. These studies will be conducted as postmarketing requirements.

9. Product Quality

Nulibry, a substrate replacement therapy, is a synthetic form of cPMP ([Figure 6](#)). Nulibry (fosdenopterin) for injection is supplied as a sterile, white to pale yellow, lyophilized powder or cake in 10 mL single-dose clear glass vials. Each vial contains 9.5 mg of fosdenopterin (equivalent to 12.5 mg of fosdenopterin hydrobromide) and the following inactive ingredients: 10 mg of L-ascorbic acid United States Pharmacopeia, 187.5 mg of mannitol United States Pharmacopeia, and 62.5 mg of sucrose National Formulary (NF). Sodium hydroxide NF and hydrochloric acid NF are used to adjust the pH to 5.0 to 7.0. Ascorbic acid is used (b) (4). The vials are stoppered with rubber stoppers and capped with aluminum/ (b) (4) flip-off caps. Nulibry must be reconstituted with 5.0 mL of sterile water for injection prior to administration.

Figure 6. Structure of Fosdenopterin



Source: NDA submission

Fosdenopterin hydrobromide is the active pharmaceutical ingredient in Nulibry for injection. It is a white to pale yellow/light orange/brown/red solid. It is practically insoluble in water, methanol, ethanol and at low pH. However, it is very soluble at high pH; e.g., 0.1N KOH. It is hygroscopic. It is produced in a dihydrate crystalline form. It is a chiral molecule containing four chiral centers. The molecular weight of fosdenopterin is 480.16 Da as hydrobromide dihydrate and 363.23 Da as free base.

Fosdenopterin hydrobromide is manufactured at (b) (4)

The identity, strength, purity, and quality of fosdenopterin hydrobromide is controlled by its specification. Based on the stability studies of the multiple batches a retest period of (b) (4) was granted when stored at (b) (4) °C.

In addition, structural characterization data have shown that rcPMP and the drug substance (b) (4) have identical core structures (fosdenopterin free-base). Thus, rcPMP and cPMP are considered chemically equivalent.

Nulibry (fosdenopterin) for injection is manufactured at Alcamo Carolinas Corporation, SC. The drug product manufacturing process (b) (4)

The specification for the drug product includes the following tests: Visual appearance, Reconstitution time, pH, Identity, Fosdenopterin assay, Ascorbic acid assay, Degradation products, Content uniformity, Particulate matter, Container closure integrity, Sterility and bacterial endotoxin. The overall control strategy for the drug product's identity, strength, purity, and quality is deemed adequate based on the proposed drug product specification.

The drug product manufacturing steps, environmental monitoring program, component/equipment sterilization, depyrogenation of vials, bioburden, (b) (4), bacterial endotoxin and container closure integrity method and validation in drug product specification; stability data and postapproval stability plan were reviewed; and determined that the Applicant has met regulatory expectations for the drug product release from the microbiology perspective.

Based on the satisfactory drug product stability data provided, a 12-month expiration dating period is granted when stored at -10°C to -25°C .

The Applicant has provided sufficient chemistry, manufacturing, and controls information to assure the identity, strength, purity, and quality of the proposed Nulibry (fosdenopterin) for Injection.

The Office of Pharmaceutical Manufacturing Assessment has made an "Approval" recommendation for all manufacturing and testing facilities involved in this NDA.

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

The label/labeling issues have been satisfactorily resolved from the CMC perspective.

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

9.1. Device or Combination Product Considerations

Not applicable.

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

The results of the clinical site inspections and Applicant inspection support were hampered by the coronavirus disease-2019 restrictions placed on travel to Europe. Adjustments were made to have records copied and reviewed off site. No objectionable Good Clinical Practices violations were identified. Review of financial disclosures did not reveal any disclosures that could potentially affect the integrity of the study outcomes.

11. Advisory Committee Summary

We did not take this application to an advisory committee. We did not identify efficacy or safety issues that needed input from external experts.

III. Appendices

12. Summary of Regulatory History

On January 14, 2013, Alexion Pharmaceuticals, Inc. (Alexion) submitted a pre-investigational new drug (IND) meeting request to their pre-IND file (PIND 117502) in the Division of Gastroenterology and Inborn Errors Products (DGIEP) to discuss their development of a cyclic pyranopterin monophosphate (cPMP), fosdenopterin, also known by the company code name ALXN1101, for the treatment of patients with molybdenum cofactor deficiency (MoCD) Type A. The meeting took place on March 26, 2013, during which Alexion and the Agency discussed Alexion's proposed first in human healthy volunteers protocol. Orphan drug designation for cPMP had been previously granted on November 5, 2009.

DGIEP received Alexion's initial clinical protocol, ALXN1001-MCD-101, titled *A Phase 1, Randomized, Blinded, Placebo-Controlled, Single-Dose, Sequential-Cohort, Dose-Escalation Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of ALXN1101 in Healthy Adult Subjects*, on May 16, 2013 (IND 117502).

Breakthrough therapy designation was granted on October 23, 2013.

Alexion met with the Agency on February 4, 2014, to discuss the Agency's concerns that two noncontrolled clinical studies would not be sufficient to support a marketing application.

On June 2, 2015, Alexion and the Agency met and discussed the design of their phase 2/3 study. Alexion submitted their phase 2/3 protocol, ALXN1101-MCD-202, titled *A Phase 2/3, Multicenter, Multinational, Open-label Study to Evaluate the Efficacy and Safety of ALXN1101 in Neonates with Molybdenum Cofactor Deficiency (MoCD) Type A* on August 31, 2015.

On June 15, 2017, Alexion received rare pediatric disease designation.

Alexion met with the Agency on November 15, 2017, to discuss the clinical status of the fosdenopterin development program. The Agency provided feedback on the adequacy of clinical data to support a new drug application (NDA).

On September 26, 2018, IND 117502 was transferred from Alexion Pharmaceuticals, Inc. to Origin Biosciences, Inc. (Origin). The company code name was changed from ALXN1101 to ORGN001.

On January 21, 2019, Origin met with the Agency to receive chemistry, manufacturing, and controls (CMC) guidance, and on April 12, 2019, Origin met with the Agency and discussed the clinical and nonclinical program to support a marketing application. Additional CMC guidance was provided by written responses on May 16, 2019. On August 14, 2019, Origin and the Agency held a follow-up meeting to the April 12, 2019, meeting to continue discussion on the data needed to support an NDA, and particularly discuss the proposed safety and efficacy endpoints.

In a submission September 10, 2019, submission to the IND, Origin request a proprietary name review for the proposed tradename (b) (4)

On September 27, 2019, Origin requested to submit portions of its NDA in a rolling review, and on November 15, 2019, the Agency granted a rolling review. Written responses were provided on the content and format of the nonclinical components of Origin's NDA on November 18, 2019.

Origin submitted the first part of their NDA (NDA 214018) containing the Administrative and Nonclinical Pharmacology and Toxicology components on November 27, 2019. In that submission they requested priority review, a rare pediatric disease priority review voucher, and exclusivity.

The proposed tradename (b) (4) was found unacceptable on December 6, 2019. Origin submitted the new proposed tradename, Nulibry, for review on February 12, 2020. The proposed tradename Nulibry was found conditionally acceptable on May 6, 2020.

Two pre-NDA meetings took place: one with CMC scheduled for December 4, 2019, and one with the rest of the Agency on December 18, 2019. The CMC pre-NDA meeting scheduled for December 4, 2019, was cancelled by Origin after receiving the preliminary comments. Meeting minutes from the pre-NDA meeting on December 18, 2019, were issued on December 19, 2019.

On January 13, 2020, the Applicant submitted a request for a thorough QT (TQT) study waiver. During the review of the TQT waiver, an information request was sent to Origin on April 16, 2020, seeking additional clinical data. Origin responded to this information request on April 20, 2020. A second information request was sent to Origin on April 23, 2020, seeking clarification on how the electrocardiograms were processed. Origin responded on April 24, 2020. On June 15, 2020, the Agency informed Origin that a TQT study should be conducted prior to NDA submission and that it should submit the results with its NDA submission. The Agency and Origin had subsequent communications and on June 26, 2020, the Agency agreed that the TQT study could be completed as a postmarketing requirement considering the urgent need for an available therapy for this rare and fatal disease. On October 28, 2020, Origin submitted their TQT protocol, ORGN001-102, titled *A Blinded, Randomized, Single-Dose, Crossover Thorough QT Study to Evaluate the Effects of Fosdenopterin on Cardiac Repolarization in Healthy Subjects*.

On February 7, 2020, the Applicant submitted an update to the Nonclinical Pharmacology and Toxicology modules originally submitted on November 27, 2019.

In March 2020, the newly assembled Division of Rare Diseases and Medical Genetics assumed responsibility for the IND 117502 and NDA 214018 for fosdenopterin.

On May 5, 2020, the Applicant submitted the second section of their NDA containing a complete clinical information package.

The third section of the rolling review, CMC, and Quality Overall Summary and sections, was submitted on June 29, 2020, completing the NDA for fosdenopterin for the treatment of patients with MoCD Type A.

The Division determined that the review classification for NDA 214018 was priority because fosdenopterin is a drug for a serious disease with no approved therapy, and if approved, fosdenopterin would provide a significant advance. The application was filed on August 28, 2020. The Midcycle Communication meeting to update Origin on the status of the Agency's

review took place on October 5, 2020. The Late-Cycle Meeting between Origin and the Agency took place on December 17, 2020.

13. Pharmacology Toxicology: Additional Information and Assessment

13.1. Summary Review of Studies Submitted Under the IND

13.1.1. Pharmacology (Primary and Secondary)

In Study GTR-0483, fosdenopterin monohydrobromide dihydrate (HBr) (coded as ALXN1101) at 0.1, 1.1, 2.2, or 4.4 mg/kg/day or placebo was administered to *MOCS1* knockout (KO) mice via intraperitoneal injection once daily from postnatal day (PND) 1 to 28. At PND 9, fosdenopterin at ≥ 1.1 mg/kg decreased plasma and brain levels of S-sulfocysteine (SSC), decreased urinary uric acid levels, increased liver sulfite oxidase (SOX) activity, and increased body weight compared to placebo and untreated *MOCS1* KO mice. Beyond PND 9, body weight gains in animals administered < 4.4 mg/kg/day were decreased compared to wild-type (WT) or heterozygous littermates. A comparison to untreated *MOCS1* KO controls beyond PND 9 cannot be made because these mice died or were euthanized by PND 8. Body weights and body weight gains at 4.4 mg/kg/day were maintained similarly to WT and heterozygous littermates until PND 22. From PND 22 to 28, body weights and body weight gains at 4.4 mg/kg/day were reduced compared to WT and heterozygous littermates, though no body weight loss was reported. At PND 28, dose-dependent reductions in brain SSC, decreased plasma SSC levels and urinary uric acid levels, and dose-dependent increases in liver SOX activity were observed in fosdenopterin HBr-treated animals. Further, brain SSC levels and liver SOX activity at ≥ 2.2 mg/kg/day were comparable to WT and heterozygous littermates. Notably, on PND 9 and PND 28 plasma SSC levels were decreased and urinary uric acid levels were increased at ≥ 1.1 mg/kg/day compared to WT and heterozygous littermates, with no obvious dose response. As a result, the Applicant concluded that plasma SSC and urinary uric acid may not be adequate biomarkers to guide therapeutic dosing in patients with MoCD Type A.

In Study GTR-0466, fosdenopterin HBr (coded as ALXN1101), *Escherichia coli*-derived cPMP or placebo was administered to *MOCS1* KO mice from PND 1 to 107. From PND 1 to 21, fosdenopterin HBr and *E. coli*-derived cPMP were administered at 2 μ g/mouse three times per week via intrahepatic injection. From PND 22 to 58, fosdenopterin HBr and *E. coli*-derived cPMP were administered at 4 μ g/mouse three times per week via intrahepatic injection. From PND 59 to 107, fosdenopterin HBr and *E. coli*-derived cPMP were administered at 4 μ g/mouse three times per week via intraperitoneal injection. Placebo-treated *MOCS1* KO mice were observed with severe abnormalities including reduced body size, dehydration, slow movement, and limb paralysis, with death occurring between PND 8 and PND 16. Treatment of *MOCS1* KO mice with either fosdenopterin or *E. coli*-derived cPMP substantially improved animal survival (70 to 80% survival), body weight, physical function, and production of plasma SSC levels.

Fosdenopterin was evaluated in an in vitro panel of enzyme and radioligand binding assays, and no significant interactions were observed.

13.1.2. Safety Pharmacology

Table 20. Safety Pharmacology Findings

Safety Pharmacology Data	Findings
2087-002: CNS and respiratory	
Rats; Sprague–Dawley	
Eight males/group for pulmonary function	
Ten females/group for CNS test	
Doses: 1, 3, and 10 mg/kg	
Intravenous infusion/ 14 days	
Frequency: Once daily	
2087-001: Cardiovascular (in vivo)	
Dogs; Beagle	
Four males; Latin-square design	
Doses: 1, 3, and 10 mg/kg	
Intravenous infusion	
Frequency: Single dose, 7-day washout between dosage levels	
109129: Cardiovascular (in vitro)	
Automatic parallel patch-clamp system	
Concentrations: 10, 30, 100, and 300µM	
Source: Reviewer generated	
Abbreviations: CNS, central nervous system; ECG, electrocardiogram; IC ₅₀ half-maximal inhibitory concentration	

- No effects on pulmonary function (respiratory rate, tidal volumes, or minute volume)
- No effect on neurobehavioral function (autonomic, sensorimotor, neuromuscular and activity/arousal)
- No qualitative or quantitative changes in ECG parameters
- No inhibitory effect (IC₅₀ >300µM) on any ion channels tested, including hCav1.2 L-type, hCav1.2 T-type, hHCN2, hHCN4, hERG, hKir2.1, hKir3.1/3.4, Kir6.2/SUR2A, hKv1.5, hKv4.3/kChIP2.2, hKvLQT1/hminK, hNav1.5

13.1.3. ADME / PK / TK

Pharmacokinetic analysis following a single dose of fosdenopterin HBr (5 mg/kg) administered to juvenile dogs (lactational day 8 or day 9) either via intravenous or subcutaneous injections, revealed similar systemic exposure to fosdenopterin between the two routes of administration. Bioavailability following subcutaneous injection was 97%. Notably, the maximum concentration (C_{max}) of fosdenopterin was 36% higher when administered via intravenous injection compared to subcutaneous injection. No sex differences in exposure were observed with either route of administration. Fosdenopterin was low to moderately bound to plasma proteins in vitro, with and without ascorbic acid. (b) (4)

E. coli-derived cPMP was relatively stable when cultured in vitro in mouse, rat, dog, and human hepatocytes without ascorbic acid. In the presence of ascorbic acid, in vitro metabolic profiling indicated that two cPMP-related compounds (C1 and C2) were detected in mouse, rat, dog, and human hepatocytes incubated with 10µM *E. coli*-derived cPMP. No human-specific metabolites were identified in vitro, and these compounds were detected in all species tested. Comparison to the analysis of a compound Z-spiked human hepatocyte sample suggests that both C1 and C2 correspond to compound Z, a known oxidation product of cPMP. Notably, loss of fosdenopterin was observed after 240 min of incubation in mouse (25%), rat (21%), dog (20%), and human (34%) hepatocytes. Extensive loss of fosdenopterin was observed in boiled/denatured hepatocytes from mouse (71%), rat (65%), dog (65%), and human (55%), suggesting that the metabolism of fosdenopterin /cPMP in hepatocytes occurs predominantly through nonenzymatic degradation processes. In vivo metabolism studies have not been conducted with fosdenopterin. Treatment of cultured human hepatocytes with *E. coli*-derived cPMP (up to 100µM) produced little or no

increase in cytochrome P450 (CYP) 1A2, CYP2B6, or CYP3A4 mRNA and enzyme activity levels. Similarly, treatment of human liver microsomes with *E. coli*-derived cPMP produced little or no evidence ($IC_{50} > 500 \mu M$) of direct, time-dependent, or metabolism-dependent inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5.

E. coli-derived cPMP at $20 \mu M$ did not inhibit P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), organic anion transporter (OAT) P1B1, OATP1B3, organic cation transporter (OCT) 2, OAT3, multidrug and toxin extrusion transporter (MATE) 1, and MATE2-K (half-maximal inhibitory concentration [IC_{50}] $> 200 \mu M$). However, *E. coli*-derived cPMP at $200 \mu M$ inhibited MATE2-K (25%) and OAT1 (33%). *E. coli*-derived cPMP was a substrate for MATE1 but was not a substrate of P-gp, BCRP, OAT1, OAT3, OATP1B1, OATP1B3, OCT2, or MATE2-K, evidenced by either an efflux ratio below 2 or < 2 -fold accumulation of cPMP in cells. Excretion studies with fosdenopterin HBr have not been conducted.

Table 21. Toxicokinetics Findings

Toxicokinetic Data	Findings
9000563: 13-Week Study in Juvenile Rats	
Sample collection times: 0, 10 min, 30 min, and 1, 2.5, 4, and 6 hr postdose	Week-13 TK Parameters
Accumulation: Yes, all doses	C_{max} 181 $\mu g/mL$
Dose proportionality: Linear	AUC_{0-6h} 133 $\mu g \cdot hr/mL$
NOAEL: 100 mg/kg/day (highest dose)	AUC_{0-24h}^* 532 $\mu g \cdot hr/mL$
Safety margin: 17.8 $\times$ (based on HED)	* AUC_{0-6h} multiplied by 4 to calculate AUC_{0-24h}
902668: 26-Week Study in Juvenile Rats	
Sample Collection Times: 0, 5 min, 15 min, 30 min, and 1, 2, 3, and 4 hr postdose	Week-26 TK Parameters
Accumulation: No	C_{max} 16.9 $\mu g/mL$
Dose proportionality: Linear	AUC_{0-3h} 7.63 $\mu g \cdot hr/mL$
NOAEL: 5 mg/kg/day (highest dose)	AUC_{0-24h}^* 61.0 $\mu g \cdot hr/mL$
Safety margin: 0.89 $\times$ (based on HED)	* AUC_{0-3h} multiplied by 8 to calculate AUC_{0-24h}
2087-006: 39-Week Study in Juvenile Dogs	
Sample collection times: 0.083, 0.25, 0.5, 1, 2, 3, 4, and 6 hr postdose	Week-39 TK Parameters
Accumulation: Yes, all doses	C_{max} 15.6 $\mu g/mL$
Dose proportionality: Linear	AUC_{0-6h} 29.7 $\mu g \cdot hr/mL$
NOAEL: 10 mg/kg/day (highest dose)	AUC_{0-24h}^* 119 $\mu g \cdot hr/mL$
Safety margin: 6.2 $\times$ (based on HED)	* AUC_{0-6h} multiplied by 4 to calculate AUC_{0-24h}

Source: Reviewer generated

Abbreviations: AUC_{0-3h} , area under the curve up to 3 hr; AUC_{0-6h} , area under the curve to 6 hr; AUC_{0-24h} , area under the curve up to 24 hr; C_{max} , maximum concentration; HED, human equivalent dose; NOAEL, no observable adverse effect level; TK, toxicokinetics

13.1.4. General Toxicology

To support the chronic use of fosdenopterin, 13- and 26-week toxicity studies in juvenile rats and a 39-week toxicity study in juvenile dogs were conducted. No adverse effects (AEs) were observed in rats or dogs at doses up to 17.8 $\times$ and 6.2 $\times$, respectively, the maximum recommended human dose, when compared to the human equivalent dose (mg/kg/day), based on body surface area.

13.1.4.1. 9000563 / A 13-Week Study of the Potential Toxicity of Daily Parenteral Injection of ALXN1101 in Juvenile Rats With a 28-Day Recovery Period

Key Study Findings

- No AEs were observed
- No observable adverse effect level (NOAEL) is 100 mg/kg/day

Methods

Table 22. Study 9000563 Methods

Dose and frequency of dosing:	25, 50, 100 mg/kg/day; once daily for 13 weeks
Route of administration:	Subcutaneous injection from PND 7-20 Intravenous injection from PND 21 onwards
Formulation/vehicle:	L-Ascorbic Acid, USP (b) (4) Mannitol USP (b) (4) (b) (4) in Sterile Water for Injection, USP, pH (b) (4)
Species/strain:	Rat, Sprague–Dawley
Number/sex/group:	Main study: 10/sex/group Recovery: 5/sex/group Toxicokinetic: 3/sex/vehicle; 12/sex/fosdenopterin HBr groups
Age:	PND 7 at initiation
Satellite groups/unique design:	Satellite groups were utilized to collect plasma samples for toxicokinetic analyses
Deviation from study protocol affecting interpretation of results:	No

Source: Reviewer generated

Abbreviations: PND, postnatal day; USP, United States Pharmacopeia

Observations and Results: Changes from the Control

Table 23. Study 9000563 Results

Parameter	Major Findings
NOAEL	100 mg/kg; based on lack of adverse treatment-related findings
Mortality	No treatment-related mortalities
Clinical signs	No treatment-related clinical signs
Body weight	No biologically relevant effects
Ophthalmoscopy	None
Hematology	Neutrophil counts increased 37% in 100 mg/kg/day males compared to controls at the end of the main study, and elevated (28%) following the recovery period. Neutrophil counts unaffected in 100 mg/kg/day females at the end of the main study but increased 97% compared to controls after the recovery period.
Clinical chemistry	None
Urinalysis	None
Gross pathology	Dark focal areas of the adrenal glands were observed in two 100 mg/kg/day females but lacked histopathological correlates.
Organ weights	None
Histopathology	None
Adequate battery: Yes	
Sexual maturation	No effect

NDA 214018
Nulibry (fosdenopterin)

Parameter	Major Findings
Functional observation battery	No effect
Motor activity	No effect
Bone length measurements	No effect

Source: Reviewer generated

Abbreviation: NOAEL, no observable adverse effect level

13.1.4.2. 902668 / A 26-Week Study of the Potential Toxicity of Daily Parenteral Injection of ALXN1101 in Juvenile Rats With a 28-Day Recovery Period

Key Study Findings

- No AEs were observed
- NOAEL is 5 mg/kg/day

Methods

Table 24. Study 902668 Methods

Dose and frequency of dosing:	1, 3, 5 mg/kg/day; once daily up to 26 weeks
Route of administration:	Subcutaneous injection from PND 7-20 Intravenous injection from PND 21 onwards
Formulation/vehicle:	L-Ascorbic Acid, USP (2 mg/mL); Mannitol USP (b) (4) and (b) (4); in Sterile Water for Injection, USP, pH (b) (4)
Species/strain:	Rat, Sprague–Dawley
Number/sex/group:	3-month interim sacrifice: Main study: 10/sex/group Recovery: 5/sex/group Full 26-week duration: Main study: 10/sex/group Recovery: 5/sex/group Toxicokinetics: 3/sex/vehicle, 9/sex/fosdenopterin HBr groups
Age:	PND 7 at initiation
Satellite groups/unique design:	Toxicokinetic animals were administered a single dose of vehicle or fosdenopterin HBr on day 1 of dosing (PND 7): 3/sex/vehicle, 18/sex/treatment groups A 3-month interim sacrifice was conducted in this study
Deviation from study protocol affecting interpretation of results:	No

Source: Reviewer generated

Abbreviations: HBr, monohydrobromide dihydrate; PND, postnatal day; USP, United States Pharmacopeia

Observations and Results: Changes From Control

Table 25. Study 902668 Results

Parameter	Major Findings
NOAEL	5 mg/kg; based on lack of adverse treatment-related findings
Mortality	None
Clinical signs	None
Body weights	No effect
Ophthalmoscopy	None
Hematology	None
Clinical chemistry	None
Urinalysis	None
Gross pathology	None
Organ weights	None
Histopathology	None
Adequate battery: Yes	
Sexual maturation	No effect
Functional observation battery	No effect
Motor activity	No effect
Bone length measurements	No effect

Source: Reviewer generated

Abbreviation: NOAEL, no observable adverse effect level

13.1.4.3. 2087-006 / A 9-Month Intravenous Toxicity Study in Juvenile Beagle Dogs With a 3-Month Recovery Period

Key Study Findings

- No AEs observed
- NOAEL is 10 mg/kg/day

Methods

Table 26. Study 2087-006 Methods

Dose and frequency of dosing:	1, 3, 5, 10 mg/kg/day; once daily for 39 weeks
Route of administration:	PND 5-25: Subcutaneous injection PND 25 onwards: Either intravenous injection alone or both intravenous and subcutaneous injections
Formulation/vehicle:	L-Ascorbic Acid, USP (2 mg/mL), Mannitol USP (b) (4) and Sterile Water for Injection, USP; pH (b) (4)
Species/strain:	Dog, beagle
Number/sex/group:	Main study: 5-6/sex/group Recovery: 3/sex/group
Age:	PND 5-6 at dosing initiation
Satellite groups/unique design:	The maximum feasible intravenous dose was determined to be 5 mg/kg (10 mL/kg). To achieve a dose of 10 mg/kg/day, animals in Group 6 were coadministered an additional 5 mg/kg (10 mL/kg) subcutaneous dose of fosdenopterin HBr (total of 20 mL fosdenopterin HBr/kg/animal). A second placebo group (Group 5) was included to act as a control for coadministration of a subcutaneous dose with an intravenous dose (total dose of 20 mL/kg), as administered to Group 6.

Dose and frequency of dosing:	1, 3, 5, 10 mg/kg/day; once daily for 39 weeks
Deviation from study protocol affecting interpretation of results:	No
Source: Reviewer generated	
Abbreviations: HBr, monohydrobromide dihydrate; PND, postnatal day; USP, United States Pharmacopeia	

Observations and Results: Changes From Control

Table 27. Study 2087-006 Results

Parameter	Major Findings
NOAEL	10 mg/kg/day (intravenous + subcutaneous dosing); based on lack of adverse treatment-related findings
Mortality	No treatment-related mortalities
Clinical signs	No treatment-related clinical signs
Body weights	No effect
Ophthalmoscopy	None
Electrocardiogram	No effect
Hematology	Neutrophil counts increased at 10 mg/kg/day but decreased at 1, 3, and 5 mg/kg/day. Red cell mass decreased 10% and absolute reticulocytes decreased 49% at 5 mg/kg/day. APTT was prolonged 22%. No other indices observed to suggest these findings negatively affected the health of the animals.
Clinical chemistry	Increased ALP levels at all doses, increased lipase levels at 10 mg/kg/day. No histopathological correlates.
Urinalysis	None
Gross pathology	None
Organ weights	Increased spleen weight in 10 mg/kg/day during main study and recovery periods. No histopathological correlates.
Histopathology	No definitive treatment-related histopathological findings
Adequate battery: Yes	
Neurological examination	No effect
Bone-length measurements	No effect

Source: Reviewer generated
Abbreviations: ALP, alanine aminotransferase; APTT, activated partial thromboplastin time; NOAEL, no observable adverse effect level

13.1.5. Genetic Toxicology

Fosdenopterin was negative in the standard battery of genotoxicity studies. Process intermediate (b) (4), and impurity (b) (4) were negative in Ames test.

13.1.6. Carcinogenicity

Carcinogenicity studies with fosdenopterin have not been conducted. A carcinogenicity study in a single species will be conducted as a postmarketing requirement.

13.1.7. Reproductive Toxicology

Nonclinical reproductive and developmental toxicity studies with fosdenopterin have not been conducted. These studies will be conducted as a postmarketing requirement.

13.1.8. Other Toxicology / Specialized Studies

13.1.8.1. 20202895 / A Repeat-Dose Phototoxicity Study to Determine the Effects of Intravenous Administration of ORGN001 on Eyes and Skin in Pigmented Rats

Key Study Findings

- Dose-dependent cutaneous skin reactions (25, 50, and 100 mg/kg/day; erythema, edema, flaking, and eschar), ophthalmic findings (50 and 100 mg/kg/day; corneal lesions), and histopathologic changes in ocular anterior chamber, ciliary body, cornea, and lens indicative of phototoxicity (50 and 100 mg/kg/day) were observed in this study.
- Effects observed at doses $\geq 4.4\times$ the maximum recommended human dose (based on the human equivalent dose).

13.1.9. 2087-004 / An In Vitro Hemolysis Assay With Human Whole Blood

Key Study Findings

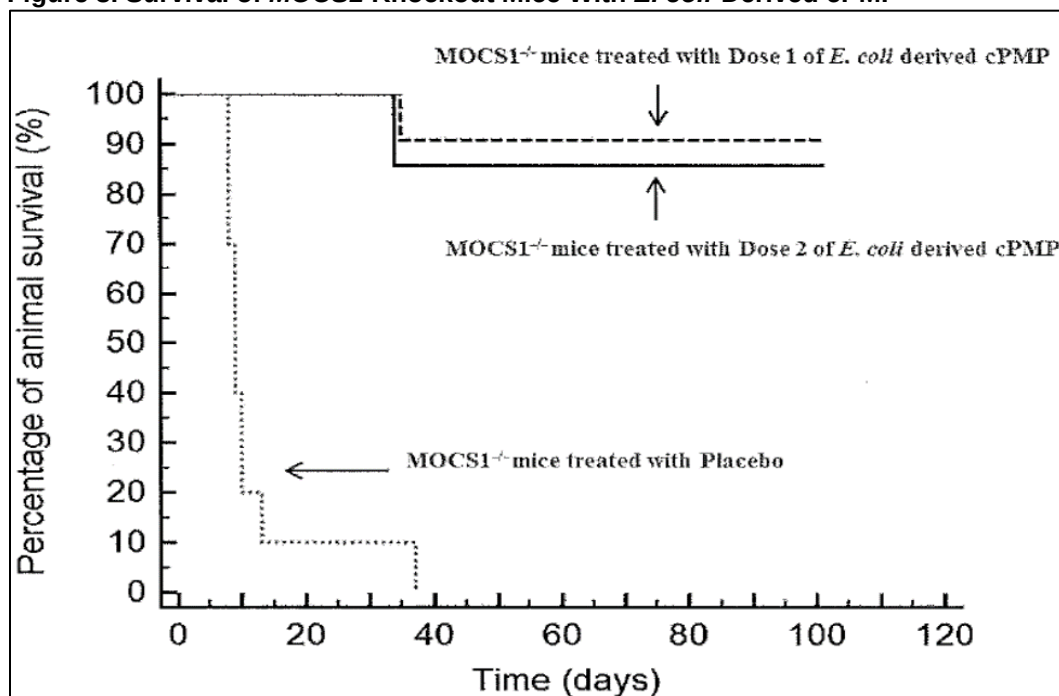
- Fosdenopterin, at concentrations 0.045 to 0.25 mg/mL, did not produce any precipitation, turbidity, or hemolysis when incubated with human blood.

13.2. Individual Reviews of Studies Submitted to the NDA

13.2.1. Pharmacology (Primary and Secondary)

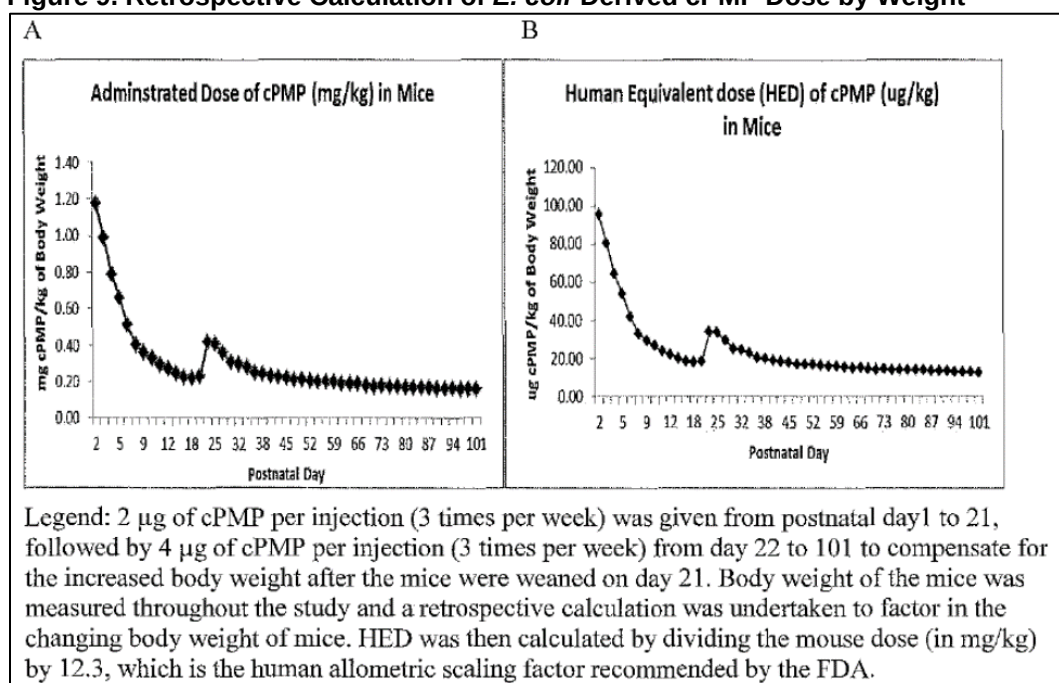
In Study GTR-0465, *E. coli*-derived cPMP at 1, 2, or 4 $\mu\text{g}/\text{mouse}$ or placebo was administered to transgenic mice with the molybdenum cofactor deficiency (*MOCS1* KO mice) three times per week via intrahepatic injection from PND 1 to 67, and intraperitoneal injection from PND 68 to 101. *MOCS1* KO mice intrahepatically administered the placebo were observed with small body size, dehydration, slow movement, and paralysis of the limbs. Mortalities in this placebo group were observed between PND 8 and PND 37, with a mean lifespan of 12.1 days ([Figure 8](#)). Intrahepatic administration of *E. coli*-derived cPMP increased survival of *MOCS1* KO mice. By the end of the study at PND 101, 10 of 11 *MOCS1* KO mice administered 1 to 2 μg *E. coli*-derived cPMP/mouse and 7 of 8 *MOCS1* KO mice administered 2 to 4 μg *E. coli*-derived cPMP/mouse were alive and were not observed with abnormal behavior. Though body weight and growth were similar between *E. coli*-derived cPMP treated *MOCS1* KO mice and their WT and heterozygous littermates from PND 1 to 18, from PND 18 to 101, body weight and growth were reduced. This is likely because the animals were given a flat dose of *E. coli*-derived cPMP over time and did not account for increases in body weight observed from PND 1 to 18 ([Figure 9](#)). *E. coli*-derived cPMP also decreased serum SSC levels 65% in *MOCS1* KO mice (mean 79.8 $\mu\text{mol}/\text{L}$) compared to the placebo controls (mean 226 $\mu\text{mol}/\text{L}$), though not to the levels in WT or heterozygous littermates (0.00 to 4.97 $\mu\text{mol}/\text{L}$).

Figure 8. Survival of *MOCS1* Knockout Mice With *E. coli*-Derived cPMP



Source: Applicant-provided table within Study GTR-0465
Abbreviation: cPMP, cyclic pyranopterin monophosphate

Figure 9. Retrospective Calculation of *E. coli*-Derived cPMP Dose by Weight



Source: Applicant-provided table within Study GTR-0465
Abbreviations: cPMP, cyclic pyranopterin monophosphate; FDA, Food and Drug Administration

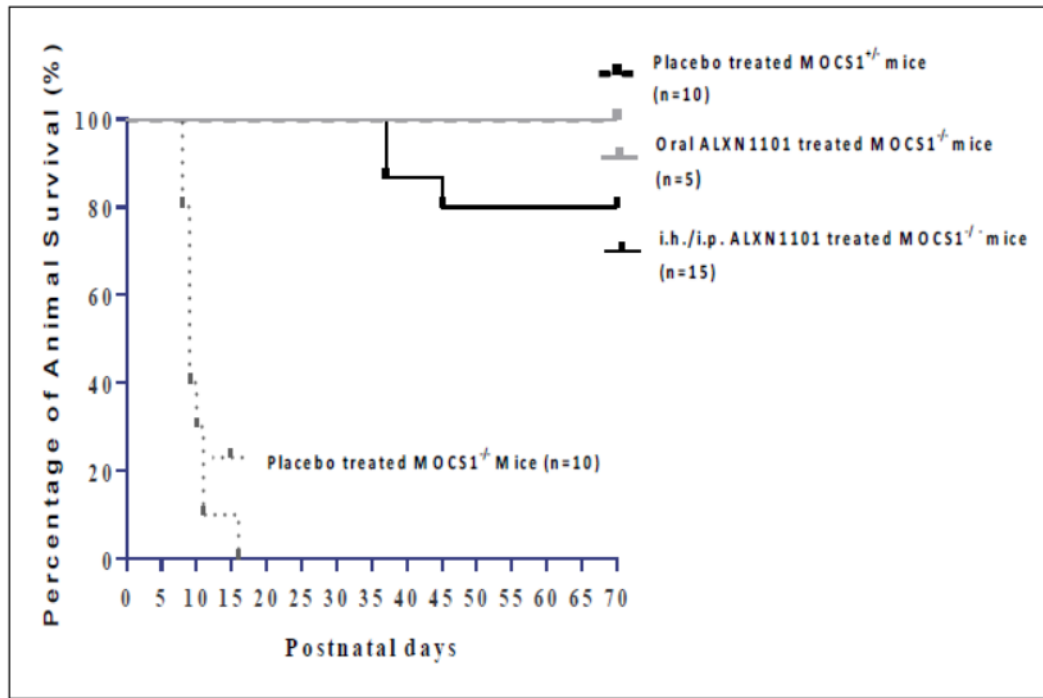
In Study GTR-0478, serum profiles of three metabolites associated with the lethal MoCD, SSC, taurine, and urea, were compared to identify a sensitive and quantitative biomarker that facilitates accurate assessment of both pathophysiological changes and cPMP dose response in the *MOCS1*

KO mouse model. This study was designed to observe changes of biomarkers SSC, taurine, and urea from baseline to 24 hr post single-dose treatment, or 120 hr post multidose treatment. *MOCS1* KO mice treated with either a single dose or multiple doses of either *E. coli*-derived cPMP or fosdenopterin HBr (coded ALXN1101), a synthetic formulation of cPMP, were observed with reductions in serum SSC, taurine, and urea at multiple time points post-treatment. However, only serum SSC levels correlated with markers of disease progression, including survival. The most robust reductions in serum SSC were achieved in response to increased dose frequency of either *E. coli*-derived cPMP or fosdenopterin, indicating a cumulative pharmacodynamic effect after repeated administration. However, reductions in serum SSC did not correlate with increased liver SOX activity. Because severe neurological damage observed in patients with MoCD is primarily attributable to the accumulation of sulfite as a result of the loss of SOX activity, the Applicant concluded that SSC, therefore, may not be an adequate biomarker to guide therapeutic dosing in patients with MOCD Type A.

In Study RTR-0002, plasma and brain SSC levels and liver SOX activity in age-matched WT and heterozygous littermates of the *MOCS1* mouse model were compared. Fosdenopterin HBr (coded ALXN1101) at 2 to 4 µg/mouse or placebo was administered to *MOCS1* mice three times per week via intrahepatic injection from PND 1 to 58 and intraperitoneal injection from PND 59 to 145. Plasma and brain SSC levels and liver SOX activity were similar between age-matched WT and heterozygous mice, suggesting that a single copy of the *MOCS1* gene in heterozygous mice produces no obvious deleterious phenotype. As a result of these data, the Applicant stated they do not need to conduct a clinical trial in MoCD heterozygous subjects.

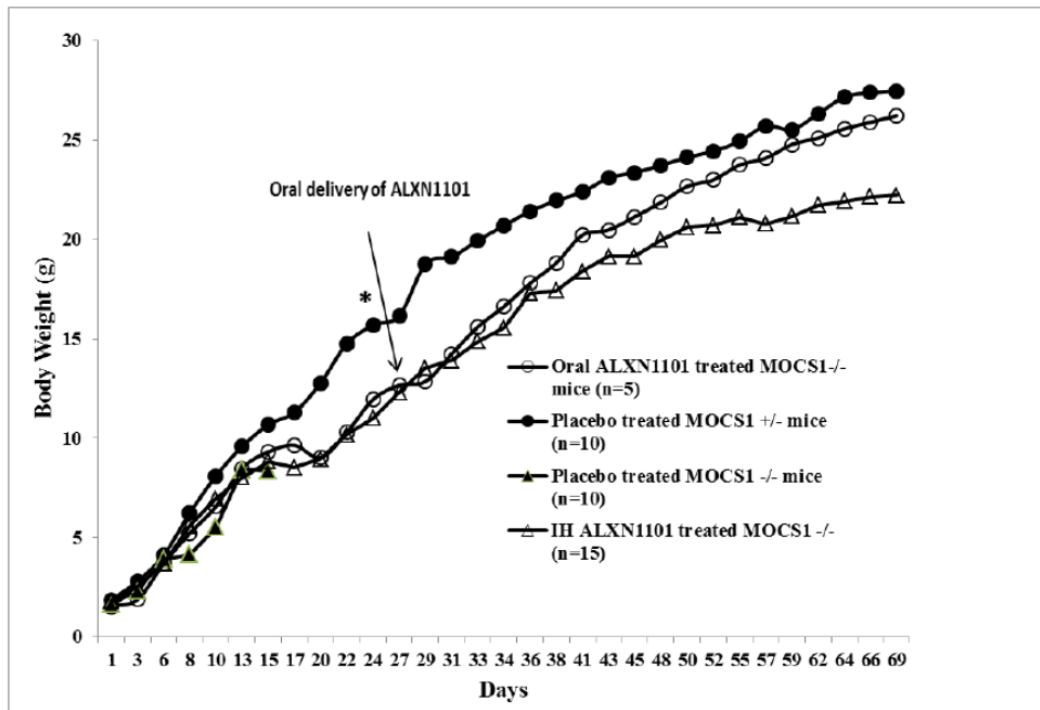
In Study RTR-0010, the feasibility of oral administration of fosdenopterin HBr (coded ALXN1101) in *MOCS1* KO mice was investigated. Fosdenopterin HBr at 2 µg/mouse was administered to *MOCS1* KO mice three times per week by intrahepatic injection from PND 1 to 24, followed by oral administration at 500 µg/mouse from PND 27 to 69. Oral administration of fosdenopterin HBr improved survival of adult *MOCS1* KO mice (Figure 10) and increased body weights (Figure 11) compared to mice administered fosdenopterin HBr by intrahepatic and intraperitoneal injections but did not normalize plasma levels of SSC (Figure 12). However, *MOCS1* KO mice orally administered fosdenopterin HBr appeared alert and agile, similar to their heterozygous littermates. Notably, this study was designed and conducted without consideration of efficacious dose, pharmacokinetics, or bioavailability of fosdenopterin, but may support the therapeutic potential of orally administered fosdenopterin HBr.

Figure 10. Survival of *MOCS1* Knockout Mice With Oral Fosdenopterin HBr



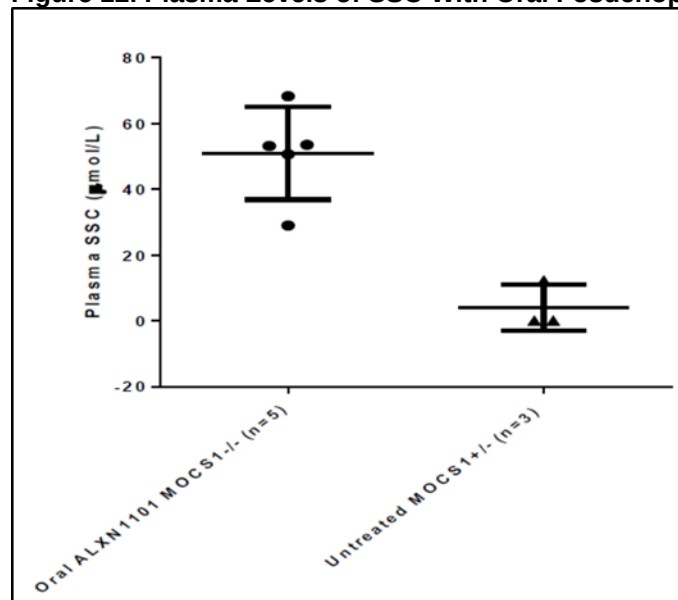
Source: Applicant-provided table within Study RTR-0010
Abbreviation: ALXN1101, fosdenopterin; HBr, monohydrobromide dihydrate

Figure 11. Body Weights of *MOCS1* Knockout Mice With Oral Fosdenopterin HBr; PND 1 to 69



Source: Applicant-provided table within Study RTR-0010
Abbreviations: ALXN1101, fosdenopterin; HBr, monohydrobromide dihydrate; PND, postnatal day

Figure 12. Plasma Levels of SSC With Oral Fosdenopterin HBr; PND 70



Source: Applicant-provided table within Study RTR-0010

Abbreviations: ALXN1101, fosdenopterin; HBr, monohydrobromide dihydrate; PND, postnatal day; SSC, S-sulfocysteine

13.2.2. ADME / PK / TK

In the absence of ascorbic acid (nonacidified blood system), fosdenopterin had a low affinity for the red blood cell fraction of whole blood (Study XS-0484). Fosdenopterin partitioned to red blood cells highest in mouse (32.1 to 27.8%), followed by rat (25.9 to 26.3%), human (23.0 to 15.1%), and dog (6.4 to 5.3%). In the presence of acidified blood with ascorbic acid, fosdenopterin partitioning to red blood cells was null (0%) in samples from all species tested.

Tissue distribution of radiolabeled fosdenopterin after intravenous administration to pigmented and nonpigmented rats was extensive, including to non-circumventricular central nervous system tissues at 0.5 hr postdose (cerebellum, medulla oblongata, and spinal cord) (Study 8311531). In both the pigmented and nonpigmented rats, the highest concentrations of fosdenopterin were observed in the kidney, bone epiphyseal line, skin, liver, and esophagus. Peak distribution was observed at 0.5 hr postdose for 98% of the evaluated tissues. Elimination was completed by 24 hr postdose in 75 to 80% of tissues, with measurable amounts of fosdenopterin remaining in the bone epiphyseal line, kidneys, cecum (pigmented rats only), and liver. Fosdenopterin did not appear to exhibit an affinity for ocular tissues or skin containing melanin in the pigmented male rats.

Fosdenopterin is unlikely to be a substrate of CYP enzymes, and CYP enzymes are unlikely to catalyze the conversion of fosdenopterin HBr (cPMP) to its oxidation product. Though extensive substrate loss was observed after incubation of *E. coli*-derived cPMP (0.1 and 1 μ M) with a panel of recombinant human CYP enzymes (rCYP1A2, rCYP286, rCYP2C8, rCYP2C9, rCYP2C19, rCYP2D6, and rCYP3A4) in human liver microsomes, the loss occurred at the same rate in the presence or absence of a nicotinamide adenine dinucleotide phosphate (NADPH)-generating system. Thus, cPMP degradation is not caused by CYP enzymes and the loss of cPMP substrate is likely due to cPMP instability in aqueous solutions (Study XT134084).

13.2.3. Genetic Toxicology

Impurities (b) (4) were negative in the Ames test and in vitro chromosomal aberration assay, with and without metabolic activation by S9.

Impurities (b) (4) µg/plate) and (b) (4) µg/plate), were negative in the Ames test, with and without metabolic activation (Study 9600210). Notably, an invalid positive control result ((b) (4)-fold increase compared to the concurrent vehicle control instead of 2-fold) was obtained with WP2 *uvrA* treated with (b) (4) in the presence of S9. The assay was still considered to be valid as the sensitivity of the WP2 *uvrA* was confirmed in the absence of S9 using (b) (4), and the effectiveness of the S9 mix was confirmed by the positive results for the other strains in presence of S9. When compared to the untreated plates, the revertants increased 2-fold for WP2 *uvrA* treated with (b) (4), and the revertant colony count was within the historical positive control range.

Impurity (b) (4) (up to (b) (4) µg/mL) and impurity (b) (4) (up to (b) (4) µg/mL) did not induce chromosomal damage (chromatid or chromosome gaps, or polyploidy) when cultured with human peripheral blood lymphocytes, with and without metabolic activation (Study 9600211).

13.2.4. Impurities / Degradants / Excipients / Extractables / Leachables

All impurities, identified leachables, and elemental impurities are controlled or qualified adequately for fosdenopterin HBr. There are no novel excipients, or excipients of human or animal origin used in the manufacturing of fosdenopterin HBr drug product. Levels of inactive ingredients used in this drug product are similar to or lower than those used in other Food and Drug Administration (FDA)-approved products.

13.2.4.1. Impurities / Degradation Products

Fosdenopterin HBr impurities (b) (4) and degradation product (b) (4) have each been qualified for in vivo safety in the rat and toxicology studies in accordance with the International Council for Harmonisation (ICH) Q3A/B guidelines (Table 28). Degradation product (b) (4) has not been specifically qualified in in vivo studies and, therefore, is controlled as an unspecified degradation product in the drug product at no more than (b) (4)%, per the ICH Q3B identification threshold for a maximum daily dose of >10 mg to 2 g.

Table 28. Summary of Impurities/Degradation Product Specifications

(b) (4)

Abbreviations: ICH, International Council on Harmonisation; N/A, not applicable; NMT, no more than

Table 29. Qualification of Fosdenopterin HBr Impurities in Toxicology Studies

(b) (4)

Source: Applicant-provided table
Abbreviation: HBr, monohydrobromide dihydrate

13.2.4.2. Extractables/Leachables

Fosdenopterin HBr is administered daily via intravenous infusion at a recommended dose of 0.9 mg/kg/day. Fosdenopterin HBr is lyophilized powder providing 9.5 mg of fosdenopterin per vial. The vial contents should be reconstituted in 5 mL of sterile water. (b) (4)

(b) (4) All identified leachables are controlled or qualified adequately.

The analytical evaluation threshold (AET) was calculated based on ICH M7(R1). Leachables of concern were identified at the toxicological threshold of concern for chronic exposure to a genotoxic or carcinogenic compound of 1.5 µg/day, used as the safety concern threshold. This was used to derive an AET of (b) (4) µg/mL or (b) (4) µg/vial.

(b) (4)

The container closure system (CCS) for fosdenopterin HBr consists of a glass vial (b) (4) closed with a rubber stopper (b) (4). Fosdenopterin HBr in the proposed primary container closure system is sensitive to light and a cardboard carton is proposed as the secondary package to protect the product from light.

The Applicant conducted extraction and simulated leachables studies with the vial and stopper of the primary CCS to inform the leachable assessment. Solvents chosen for the extraction studies

were water, ethanol, hexanes, and 6% acid (5% nitric acid/1% hydrochloric acid). Fosdenopterin HBr (coded as ORGN001 Drug Product) was used for the simulated leachables study. Each sample was analyzed by direct injection gas chromatography-mass spectrometry to screen for semivolatile organic compounds and by headspace gas chromatography-mass spectrometry to screen for volatile organic compounds. Liquid chromatography with ultraviolet and mass spectrometry detection was used to screen for nonvolatile organic compounds. An elemental analysis was performed using inductively coupled plasma-mass spectrometry.

A simulated leachables study is acceptable for this drug product, considering the drug product is a lyophilized powder. During the simulated leachables study, a set of CCS each with lyophilized drug product were incubated in an inverted position at 25°C for 1 month (32 days). This duration and temperature simulate a 24-month storage period. After the incubation period, the samples were reconstituted with 5 mL of water and held inverted at room temperature for 4 hr, which is the maximum time allowed for reconstituted drug product. During the final 30 min of inverted incubation at room temperature, a portion of plastic tubing was cut and submerged in the reconstituted drug product solution in each vial. A control solution of the liquid drug product that was not exposed to the vial/stopper or tubing was prepared in the same manner as the sample preparation (i.e., it was stored at 25°C for 32 days and then stored at room temperature for 4 hr) to ensure that there was no contamination from the labware or reagents used that could be misinterpreted as a leachable compound.

The results of the extractables study are presented in [Table 30](#).

Table 30. Extractables Study Results

CCS Component	Extraction Technique	Solvents	Reportable Compounds	
			Primary Analysis	Extracted Ion Analysis ¹
Stopper	Direct-injection GC/MS	Water		(b) (4)
		Ethanol		
		Hexanes		
	Headspace GC/MS	Dry		
		Water		
		Ethanol		
		Hexanes		
Vial	ICP/MS	6% Acid ³		
		6% Acid		

¹ Extracted ion analysis was performed to determine if compounds reported in the simulated leachables study were present, but below the reporting limit, in the extractables study.

² If no compounds were reportable in the simulated leachables study, the extraction analysis was not performed.

³ 6% Acid: 5% nitric acid/1% hydrochloric acid

⁴ RL for (b) (4)

Source: Reviewer generated

Abbreviations: CCS, container closure system; GC, gas chromatography; ICP, inductively coupled plasma; LC, liquid chromatography; MS, mass spectrometry; RL, reporting limit; UV, ultraviolet

The results of the simulated leachables study are presented in [Table 31](#). Six potential (b) (4) leachables and (b) (4) were identified and evaluated for safety. (b) (4) and two of the reportable compounds (b) (4)

(b) (4) were detected at concentrations calculated to be greater than the AET of (b) (4) µg/mL, at levels of (b) (4) µg/mL, (b) (4) µg/mL, and (b) (4) µg/mL, respectively. Notably, the levels of (b) (4) are below the qualification threshold of (b) (4) µg/day with respect to systemic toxicity for nongenotoxic leachables in parenteral products (see Document Archiving, Reporting, and Regulatory Tracking System December 10, 2020). Still, a safety assessment was conducted for the identified leachables (b) (4)

With a maximum daily dose volume of (b) (4) mL, patients would be exposed to (b) (4) at (b) (4) µg/day (b) (4) µg/mL) and (b) (4) at (b) (4) µg/day ((b) (4) µg/mL). These levels are (b) (4) × and (b) (4) × below the permitted daily exposure calculated for (b) (4) (based on (b) (4) of (b) (4) µg/day ([Table 32](#)), and thus, there are no safety concerns for the levels of leachables (b) (4)

Table 31. Identified Leachables

Analysis	Tentative Identification	CAS#	Estimated Concentration (µg/mL) (b) (4)
Direct injection GC/MS (semivolatile compounds)	(b) (4)	(b) (4)	(b) (4)
Headspace GC/MS (volatile compounds)			
LC/UV/MS (Nonvolatile compounds)			
ICP/MS			

Source: Reviewer generated

Abbreviations: CAS, Chemical Abstracts Service; GC/MS, gas chromatography/mass spectrometry; ICP/MS, inductively coupled plasma/mass spectrometry; LC/UV/MS, liquid chromatography/ultraviolet/mass spectrometry

(b) (4)

The Applicant identified (b) (4) µg/mL, for a maximum daily exposure of (b) (4) (b) (4). The FDA has established a reference daily intake of (b) (4) for adults and children 4 years of age and older (21 CFR 101.9). Considering that the potential daily exposure to (b) (4) is far lower than the reference daily intake set by the FDA, the presence of (b) (4) µg/day is of no safety concern.

(b) (4)

Adequate repeated dose toxicity studies for the leachable chemicals (b) (4) are either limited or not available. The Applicant selected (b) (4) and (b) (4) as suitable surrogates for the risk assessment of (b) (4)

The Extractables and Leachables PTCC Subcommittee was consulted to assist with this safety assessment (see Document Archiving, Reporting, and Regulatory Tracking System December 10, 2020). The CDER/OTS/OCP/DARS Computational Toxicology Consultation Service was consulted under NDA (b) (4) for a similar leachable assessment and

determined that (b) (4) was an acceptable surrogate for the risk assessment of (b) (4) due to the structural similarity. Likewise, the Extractables and Leachables PTCC Subcommittee determined that information about (b) (4) could be extrapolated to cover (b) (4)

Table 32. PDE Calculation for Leachables (b) (4)

Modifying Factor	Factor	Rationale
F1 (Extrapolation between species)	5	Rat to human
F2 (Variability between individuals)	10	Uniform
F3 (Toxicology studies of short duration)	5	3-Month inhalation toxicology study in rats (FDA)
F4 (Severe toxicity)	1	No severe toxicity concerns
F5 (NOAEL established?)	1	NOAEL: 36 mg/kg
F6 (Change in route of administration)	10	Bioavailability following inhalation exposure, 3%
PDE		(b) (4) µg/day

Source: Reviewer generated

Abbreviations: FDA, Food and Drug Administration; NOAEL, no observable adverse effect level; PDE, permitted daily exposure

13.2.4.3. Elemental Impurities

The Applicant conducted a risk assessment for the (b) (4). The levels of each element were below the control threshold defined in ICH Q3D ((b) (4) % of the established permitted daily exposure in the drug product). Thus, there are no safety concerns for the levels of elemental impurities in fosdenopterin HBr drug product.

13.2.4.4. Excipients

There are no novel excipients, or excipients of human or animal origin used in the manufacturing of fosdenopterin HBr drug product. Levels of excipients used in this drug product are similar to or lower than those used in other FDA-approved products (Table 33).

Table 33. Excipient Levels in Fosdenopterin HBr Drug Product

Excipient	% (w/v) in Reconstituted Drug Product	% (w/v) in IIG [1]	
		Lowest	Highest
Mannitol	3.75	2	75
Sucrose	1.25	1	90
Ascorbic acid	0.20	0.088	62.50

[1] Source: <https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm>

Source: Applicant-provided table

Abbreviation: IIG, inactive ingredient

14. Clinical Pharmacology: Additional Information and Assessment

14.1. In Vitro Studies

14.1.1. Metabolism

14.1.1.1. XT135111: In Vitro Evaluation of cPMP as an Inhibitor of Cytochrome P450 Enzymes in Human Liver Microsomes

The ability of fosdenopterin to inhibit major cytochrome P450 (CYP) enzymes, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 was determined in human liver microsomes (HLM). HLM from a mixed-gender pool of 16 individuals were incubated with marker substrates in the presence or absence of cPMP. cPMP concentrations ranging from 0.5 to 500µM were used to evaluate the potential for direct inhibition. The nature of inhibition, metabolism-dependent or time-dependent, was determined by preincubating cPMP with HLM for 30 min with and without an NADPH-generating system, respectively, prior to the incubation with the marker substrates. There was little or no evidence of potential direct, time-dependent or metabolism-dependent inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5 by cPMP. IC₅₀ values were reported as >500µM.

14.1.1.2. XT133120: In Vitro Evaluation of cPMP as an Inducer of CYP Expression in Cultured Human Hepatocytes

The ability of fosdenopterin to induce CYP1A2, CYP2B6, and CYP3A4 was evaluated in human hepatocytes. Three preparations of cultured human hepatocytes (HC10-1, HC5-30, and HC7-4) from three separate livers were treated once daily for three consecutive days with dimethyl sulfoxide (DMSO; 0.1% v/v, vehicle control), ascorbic acid (11mM, vehicle control), flumazenil (25µM, negative control), one of six concentrations of cPMP (0.3, 1, 3, 10, 30, or 100µM), or one of three known human CYP inducers, 50µM omeprazole (CYP1A2), 750µM phenobarbital (CYP2B6), and 20µM rifampin (CYP3A4). LC-MS/MS methods were used to analyze metabolites to determine enzyme activity and quantitative RT-PCR were used to determine enzyme mRNA levels.

In HC10-1, the 10 and 30µM cPMP treatment groups showed increases of 2.12-fold and 2.01-fold in HC5-30, and the 100µM cPMP treatment group showed an increase of 2.02-fold. However, these increases were <5% as effective as the positive control inducer, omeprazole, and did not appear to be concentration dependent. Overall, treatment of hepatocytes with up to 100µM cPMP had little or no effect in CYP1A2 mRNA levels. Treatment of cultured human hepatocytes with up to 100µM cPMP also did not increase in CYP2B6 or CYP3A4 mRNA levels.

Treatment of cultured human hepatocytes with up to 100µM cPMP resulted in little or no increase in CYP1A2, CYP2B6, or CYP3A4 enzyme activity levels.

14.1.1.3. XT138074: In Vitro Evaluation of cPMP as an Inhibitor of Human P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, and MATE2-K Transporters and as a Substrate of Human P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, and MATE2-K Transporters

Evaluation of cPMP as an Inhibitor of Transporters

The potential of cPMP to inhibit efflux transporters P-gp and BCRP was evaluated in the bidirectional permeability of probe substrate (digoxin [10µM] or prazosin [1µM], respectively) across a monolayer of Caco-2 and MDCKII-BCRP cells. The results showed that cPMP (20 to 200µM) did not inhibit the efflux of digoxin across Caco-2 cells or of prazosin across MDCKII-BCRP cells.

The potential of cPMP to inhibit uptake transporters OATP1B1, OATP1B3, OCT1, OCT2, OAT1 and OAT3 was evaluated by measuring the accumulation of probe substrates (estradiol-17β-glucuronide [OATP1B1 and OATP1B3], tetraethylammonium bromide [OCT1], metformin [OCT2], p-aminohippurate [OAT1] or estrone-3-sulfate [OAT3]) in transporter-expressing and control HEK293 cells in the presence of cPMP (20 to 200µM). The results showed that cPMP (20 to 200µM) did not inhibit the uptake of the probe substrate for each transporter.

The potential of cPMP to inhibit MATE1 and MATE2-K efflux transporters was evaluated by measuring the accumulation of probe substrates (metformin) into transporter-expressing and control cells in the presence of cPMP (20 to 200µM). In the presence of cPMP, the cleared volume of metformin in MATE1-expressing cells was not reduced. In the presence of cPMP, the cleared volume of metformin in MATE2-K expressing cells demonstrated up to 25% inhibition.

Evaluation of cPMP as a Substrate of Transporters

In the evaluation of bidirectional permeability of cPMP across MDCKII-MDR1 and control cells, and MDCKII-BCRP and control cells to determine if cPMP (1 and 10µM) is a substrate of P-gp and BCRP, the efflux ratios of cPMP (1µM) in MDCKII-MDR1 and MDCKII-BCRP were less than 0.5 indicating the cPMP was not a substrate of P-gp and BCRP.

The accumulation cPMP was evaluated in transporter-expressing and control cells to determine if cPMP (1, 5, and 25µM) was a substrate of OATP1B1, OATP1B3, OCT2, OAT1, and OAT3. Uptake of probe substrates (estradiol-17β-glucuronide [OATP1B1 and OATP1B3], tetraethylammonium bromide [OCT1], metformin [OCT2], p-aminohippurate [OAT1] or estrone-3-sulfate [OAT3]) in the presence and absence of inhibitors (10µM rifampin [OATP1B1 and OATP1B3], 100µM quinidine [OCT1], 300µM quinidine [OCT2], 100µM probenecid [OAT1 and OAT3]) served as positive controls for transporter function.

The uptake ratios of cPMP in OATP1B1, OATP1B3, OCT2, OAT1, and OAT3 cells were generally <2 across different timepoints and were not affected by the positive control inhibitors, supporting that cPMP is not a substrate of these transporters.

14.1.1.4. XT134084: In Vitro Cytochrome P450 Reaction Phenotyping of cPMP in Human Liver Microsomes and Recombinant Human CYP Enzymes

Three concentrations of cPMP (0.1, 1, and 10 μ M) were incubated with different concentrations (0.5, 1, and 2 mg protein/mL) of HLM pooled from 200 individuals for 60 min to establish appropriate incubation conditions and evaluate the suitability of an LC-MS/MS method developed to analyze cPMP concentrations of 0.01 to 12.5 μ M. Additional incubations of cPMP were conducted with HLM (1 mg protein/mL) for multiple incubation durations (0, 30, 60, 120, and 240 min). At up to 240 min, cPMP disappearance ranged from 0 to 32% in the HLM and the NADPH-generating system. In the absence of protein, cPMP loss ranged from 8 to 20% in the 60 min incubations.

To determine the role of CYP enzymes involved in the metabolism of cPMP, cPMP (0.1 and 1 μ M) was incubated with a panel of recombinant human CYP enzymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) with and without an NADPH-generating system. At 0.1 μ M, disappearance of cPMP in the presence of an NADPH-generating system ranged from 68 to 100%. At 1 μ M, loss of cPMP ranged from 63 to 88%. In the absence of NADPH, loss of cPMP (0.1 μ M) ranged from 77 to 100%. At 1 μ M, disappearance was 75 to 96%. The disappearance of cPMP was not dependent on CYP metabolism because cPMP was consumed regardless of the presence or absence of protein and NADPH.

14.1.1.5. XT134092: Metabolic Stability of cPMP in Cryopreserved Mouse, Rat, Dog, and Human Hepatocytes

The metabolic stability of cPMP (1 μ M) was evaluated in cryopreserved hepatocytes from mouse (pool of nine, male), rat (pool of four, male), dog (pool of three, male), and human (pool of 100, mixed gender) to establish substrate loss and estimate in vitro half-life and in vitro intrinsic clearance. Loss of cPMP ranged from 0 to 22% after 240 min across the four species evaluated. Substrate loss observed in incubations with boiled hepatocytes (denatured cells) was 4 to 39%.

cPMP was metabolically stable and the substrate loss observed in incubations of live hepatocytes and boiled hepatocytes of the species evaluated were comparable. In vitro half-life and in vitro intrinsic clearance could not be estimated because cPMP largely remained in incubation over the 240 min incubation period. Loss of cPMP over 240 min was not attributable to metabolism.

14.1.1.6. XT134093: Metabolite Characterization of cPMP in Mouse, Rat, Dog, and Human Hepatocytes

The in vitro metabolism of cPMP (10 μ M) was characterized following incubation with pooled and cryopreserved CD-1 mouse, Sprague–Dawley rat, Beagle dog, and human hepatocytes. Supernatants of 0, 60, 120, and 240 min incubations were analyzed by LC-MS/MS. Zero- and 240-min incubations of cPMP were performed in denatured hepatocytes to distinguish true metabolites from chemical degradation products. After 240 min of incubation of cPMP with mouse, rat, dog and human hepatocytes, loss of substrate was approximately 25, 21, 20, and 34%, respectively; and 71, 65, 65, and 55% was observed in denatured mouse, rat, dog and human hepatocytes, respectively. Two cPMP-related components (C1 and C2) were detected at all time points in hepatocytes of all species evaluated. No human-specific metabolites were identified, and all components were detected in all species. The product ion spectral data and the

analysis of compound Z-spiked human hepatocyte sample suggested that both C1 and C2 correspond to compound Z, which is a known oxidation product of cPMP.

14.2. In Vivo Studies

14.2.1. ALXN1101-MCD-101 (MCD-101)

MCD-101 was a first-in-human study that evaluated the pharmacokinetics of fosdenopterin in healthy subjects. A total of 24 subjects was randomized into three sequential cohorts (6 active: 2 placebo) and received a single IV infusion of fosdenopterin at doses of 0.075, 0.25, and 0.68 mg/kg. Of note, a fourth dose, 1.14 mg/kg, cohort was planned but was not enrolled after the safety review committee determined that the geometric mean area under the concentration-time curve from time zero to infinity for the 0.68 mg/kg cohort exceeded the AUC from time zero to the last quantifiable time point (4270 ng.hr/mL) at the NOAEL (the nonclinical toxicology thresholds identified at the time). Intensive pharmacokinetics (PK) sampling up to approximately 96 hr after infusion were evaluated. The PK results are summarized in [Table 34](#).

Table 34. PK Parameters (Mean±SD) of Fosdenopterin in Healthy Subjects Following Single-Dose Intravenous Administration (Study MCD-101)

Parameter	0.075mg/kg (n=6)	0.25 mg/kg (n=6)	0.68 mg/kg (n=6)
C _{max} (ng/mL)	285 (56.5)	873 (98.7)	2800 (567)
T _{max} (hr) [#]	0.500 (0.500-0.900)	0.510 (0.500-0.920)	0.875 (0.500-1.08)
Half-life (hr)	1.22 (0.162)	1.67 (0.433)	1.64 (0.300)
AUC _{0-last} (ng.hr/mL)	508 (73.7)	1760 (205)	5930 (1820)
AUC _{0-inf} (ng.hr/mL)	523 (74.8)	1790 (213)	5960 (1820)
CL (mL/h/kg)	195 (29.5)	181 (19.5)	167 (66.0)
CL _r (mL/h/kg)	84.0 (10.7)	81.7 (13.5)	79.1 (30.5)
V _d (mL/kg)	341 (73.1)	436 (118)	375 (75.5)
AUC ₀₋₆ (ng.hr/mL)	498 (69.9)	1680 (192)	5640 (1650)
Ae ₀₋₆ (mg)	3.33 (0.78)	9.89 (2.3)	29.7 (4.2)

Source: Applicant's noncompartmental analysis

[#]T_{max} reported as median (range)

Abbreviations: Ae₀₋₆, amount of drug excreted unchanged in urine during the 0 to 6.75 hr interval post-beginning of infusion (BOI); AUC, area under the concentration-time curve; AUC₀₋₆, AUC during the 0 to 6.75 hr interval post-BOI; AUC_{0-inf}, AUC from time zero to infinity; CL, clearance; CL_r, renal clearance; C_{max}, maximum concentration; SD, standard deviation; T_{max}, time to C_{max}; V_d, volume of distribution

14.2.2. Population PK Analysis

The Applicant performed the population PK analysis for fosdenopterin using plasma PK data from a total of seven infants and children previously treated with rcPMP in Study MCD-201 and one neonate in Study MCD-202. Descriptive statistics of baseline demographics for categorical demographic data are presented in [Table 35](#) and descriptive statistics of continuous demographic data are presented in [Table 36](#).

Table 35. Summary of Categorical Covariates

Covariate	Value	Descriptor	Count	Percentage
Race	1	White	5	62.5
	3	Asian	3	37.5
Sex	1	male	3	37.5
	2	female	5	62.5

Source: Applicant's Population PK Analysis Report, Table 8.

Table 36. Summary of Continuous Covariates

Descriptor	Mean	Standard Deviation	Median	Minimum	Maximum
Baseline Age (years)	3.29376	2.25322	3.91665	0.0001	6
Baseline Weight (kg)	14.6638	8.71309	14.4	2.88	27.8
Baseline Height/Length (cm)	100.808	19.182	105.6	68.5	124.46
Alkaline Phosphatase (SIU)	180.375	89.8522	172.5	22	305
ALT (SIU)	20	10.2539	20.5	6	34
AST (SIU)	29.875	7.23952	30	17	39
Albumin (gm/dL)	42.4	4.80862	41.5	37	50
Serum Creatinine (mg/dL)	0.367762	0.1511	0.33375	0.2	0.6
Total Bilirubin (mg/dL)	0.58698	0.897063	0.2924	0.08772	2.78
GFR (Schwartz; ml/min/1.73 m ²)	165.338	69.8256	173.9	77.1	273.8

Source: Applicant's Population PK Analysis Report, Table 9.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GFR, glomerular filtration rate; SIU, standard international unit

The Applicant found a two-compartment model yielded consistently better fits (a smaller objective function and improved goodness-of-fit graphics) as compared to the one-compartment structural model. The modeling strategy was then to examine the following two sets of issues concurrently to evaluate whether other covariates should be incorporated into the model:

- Scaling of systemic parameters for body size with an estimated or fixed value of (b) (4) for clearance and (b) (4) for volume of distribution.
- Incorporating an age-related maturation factor (Rhodin et al. 2009), (b) (4).

The Applicant found that fosdenopterin PK was best described by a two-compartment disposition model with estimated power term for scaling of systemic parameters and maturation factor to (b) (4) % of clearance. Interindividual variabilities (IIV) were added to clearance, central compartment volume, distribution clearance (Q) and peripheral volume (V_P) (Table 37). The random residual error was described using a combined proportional and additive error structure.

The parameter estimates for the final PK model are presented in Table 38. The median and 90% confidence interval (CI) of the parameter estimates were generally similar to the derived estimates based on 500 bootstrap (Table 39). The Applicant stated that the goodness-of-fit plots in Figure 13, the visual predictive check (VPC) and prediction-corrected VPC in Figure 14, demonstrated the adequacy of the final PK model to describe the plasma concentration-time profile of fosdenopterin.

NDA 214018
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Table 37. Results of the Bootstrap Analysis for the Final Model With Estimated Power for Scaling

THETA	Estimated Value	Mean	Standard Deviation	Percentile		
				5	50	95
<i>All (N = 499)</i>						
CL						
V ₁						
CLdist						
V ₂						
Power term						

Source: Applicant's Population Pharmacokinetics Analysis Report, Table 23.

Abbreviations: CL, clearance; CL_{dist}, distribution clearance; V₁, apparent volume of the central compartment; V₂, apparent volume of the peripheral compartment

Table 38. Parameter Estimates for the Final Fosdenopterin Population PK Model

Parameter	Estimate	Inter-Individual Variability*
SCALE		(b) (4)
RHODIN		
Clearance (L/day)		
Central Compartment Volume (L)		
Distribution Clearance (L/day)		
Peripheral Volume (L)		

OMEGA #	Applies to THETA	Variance	Correlation
(b) (4)			

Description	Variance	Square Root Variance
Proportional component of error		(b) (4)
Additive component of error		

Source: Applicant's Population PK Analysis Report; Tables 2, 17, and 18.

Abbreviations: PK, pharmacokinetics; V_1 , apparent volume of the central compartment; V_2 , apparent volume of the peripheral compartment

Table 39. Results of the Bootstrap Analysis for the Final Model With Estimated Power for Scaling

THETA	Estimated	Mean	Standard	Percentile		
	Value		Deviation	5	50	95
All (N = 499)						
CL						
V ₁						
CLdist						
V ₂						
Power term						

(b) (4)

(b) (4)

Source: Applicant's Population Pharmacokinetics Analysis Report, Table 23.

Abbreviations: CL, clearance; CL_{dist}, distribution clearance; V₁, apparent volume of the central compartment; V₂, apparent volume of the peripheral compartment

Figure 13. Goodness-of-Fit Plots for the Final Pharmacokinetics Model

(b) (4)

(b) (4)

Source: Applicant's Population Pharmacokinetics Analysis Report; Figures 7, 8, and 9.

Figure 14. VPC Based on Time-After-Dose for the Model With Estimated Power for Scaling (Left, VPC; Right, Prediction-Corrected VPC)

(b) (4)

Source: Applicant's Population Pharmacokinetics Analysis Report, Figure 11.

Abbreviation: VPC, visual predictive check

The post hoc estimated parameters are displayed in [Table 40](#).

Table 40. Post Hoc Estimated Parameters for the Model With Estimated Power for Scaling

	Standard				
Description	Mean	Deviation	Median	Minimum	Maximum
Clearance/F (L/day)	(b) (4)				
V ₁ /F (L)					
Distributional clearance/F (L/day)					
V ₂ /F (L)					

Source: Applicant's Population Pharmacokinetics Analysis Report, Table 20.

Abbreviations: F, bioavailability; V₁, apparent volume of the central compartment; V₂, apparent volume of the peripheral compartment

The Applicant also highlighted the following limitations of the analysis:

- The number of subjects was extremely small. Of the eight subjects, only one subject started treatment in the first months of life (as a neonate). This limits the ability to evaluate the effects of maturational change during the period when it is likely to change most rapidly. The target patient population for further administration of fosdenopterin is likely to be newly-diagnosed subjects in the first days-to-weeks of life. Data from only one subject inform the PK model during this period.
- The sampling regimen included a few predose samples. However, the marked decrease in concentration between the end-of-infusion and samples approximately 4 hr later, coupled with the PK data in adults, suggested that residual predose concentrations are likely to be extremely low.
- The sampling regimen typically included a sample at the end-of-infusion and one sample approximately 4 hr later. The marked decrease in the second sample suggested that samples 1 to 2 hr postinfusion might have provided more insight into the distribution phase of the PK profile (and, thereby, better estimation of area-under-the-curve or AUC).

Reviewer's Comments:

Per the FDA guidance regarding population PK analysis, covariate-parameter relationships should be formulated based on the current knowledge of biology, physiology, or allometric principles. The Applicant's final population PK model is reasonable to include allometric scaling for clearance and volume of distribution, and an age-related maturation factor to adjust clearance in pediatrics. However, it seems not acceptable to apply the renal maturation factor to (b) (4)% of clearance in pediatrics, because a PK study suggested that approximately 40% of maturation was via the kidney in adults. In addition, the Applicant's final PK model did not converge because 1) the limited PK data did not support such an over-parameterized model incorporating IIV for all PK parameters with a full variance-covariance matrix; and 2) the estimated power of (b) (4) for allometric scaling of clearance and volume of distribution reached the predefined lower limit of initial value (b) (4).

The reviewer made the following modifications of the Applicant's final model:

- 1) assuming the same fraction of 40% renal elimination in pediatrics and adults;
- 2) remove IIV for Q and Vp

The reviewer's modified PK model converged successfully with similar objective function value (OFV) (1443.8 versus 1438.6) and slightly different PK parameter estimates ([Table 41](#)) compared to the Applicant's final model. A different allometric scaling exponent was also tested for clearance (CL, Q) and volume of distribution (Vc, Vp). However, the model did not achieve a significant decrease in OFV ($p > 0.05$) but resulted in higher uncertainty of the PK parameter estimates due to the limited data.

Table 41. Parameter Estimates of the Reviewer's Modified PK Model

Theta		Estimate*	SE	RSE	95% CI**
1	Typical CL(L/day) for a BW of 75kg	368	42.7	11.6%	284.308 - 451.692
2	Typical Vc(L) for a BW of 75kg	13.5	1.21	9%	11.128 - 15.872
3	Typical Q (L/day) for a BW of 75kg	198	47.2	23.8%	105.488 - 290.512
4	Typical Vp(L) for a BW of 75kg	10.4	2.14	20.6%	6.206 - 14.594
5	Exponent of power function for BW on CL and V	0.825	0.0907	11%	0.647 - 1.003
Omega		1	2	Etabar (SE)	p val
1	IIV for CL	0.0474		-0 (0.076)	0.9994
2	IIV for Vc	0.0386	0.0315	-0 (0.062)	0.9992
Omega (on SD scale) *		1	2		
1	IIV for CL	21.8%			
2	IIV for Vc	99.9%	17.7%		
Sigma		1	2		
1	Proportional RUV	0.0546 (28.2%)			
2	Additive RUV	0	54.9 (110.7%)		

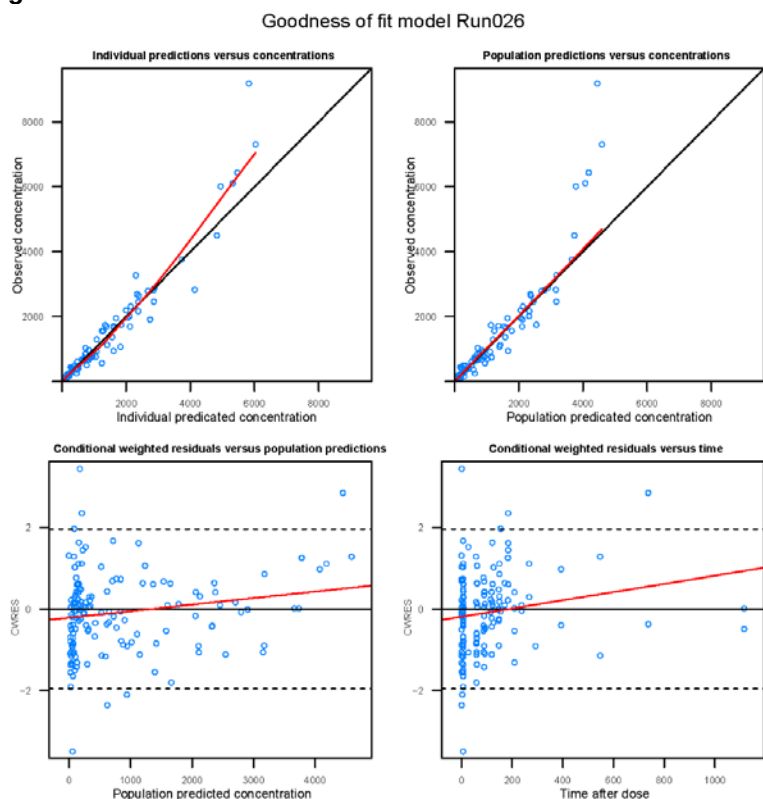
* Correlations in omega are shown as the off-diagonal elements. SAME blocks are not shown.

Source: Reviewer's analysis.

Abbreviations: CL, clearance; PK, pharmacokinetics; Q, blood flow; RUV, residual unexplained variability; Vc, central volume of distribution; Vp, peripheral volume of distribution

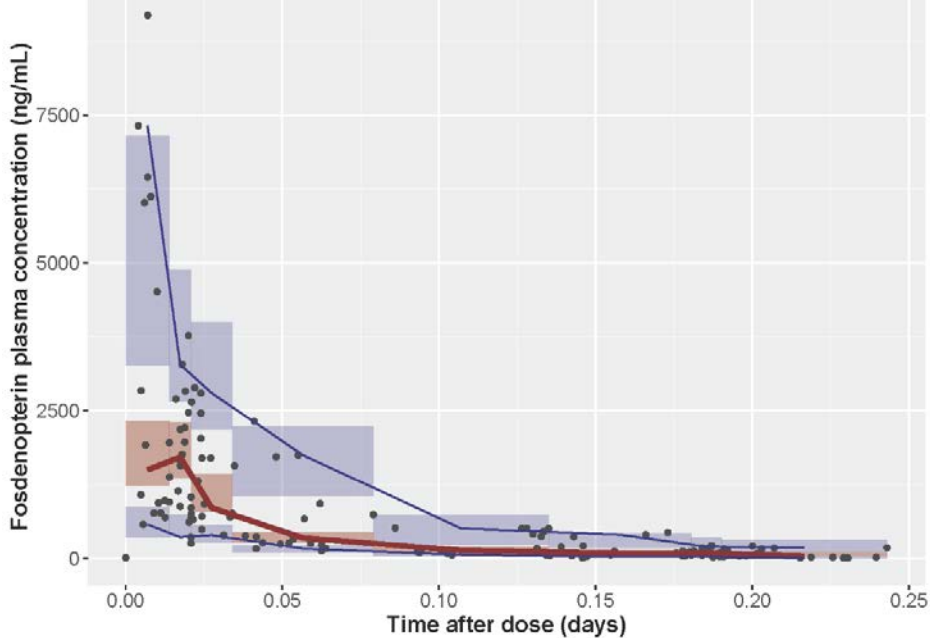
The goodness-of-fit plots ([Figure 15](#)) and VPC ([Figure 16](#)) showed that the modified PK model described the time profile of fosdenopterin plasma concentration reasonably well.

Figure 15. Goodness-of-Fit Plots for the Reviewer's Modified Pharmacokinetics Model



Source: Reviewer's analysis.

Figure 16. Visual Predictive Check for the Reviewer’s Modified Pharmacokinetics Model



Source: Reviewer’s analysis.

The post hoc PK parameter estimates of the reviewer’s modified model are displayed in [Table 42](#).

Table 42. Post Hoc PK Parameter Estimates of the Reviewer’s Modified Model

Parameter	Mean	Standard Deviation	Median	Minimum	Maximum
CL/F (L/day)	93.4	49.2	97.2	12.6	159.3
Vc/F (L)	3.48	1.75	3.57	0.69	5.89
Q/F (L/day)	50.2	25.8	50.6	13.5	87.4
Vp/F (L)	2.64	1.35	2.65	0.71	4.58

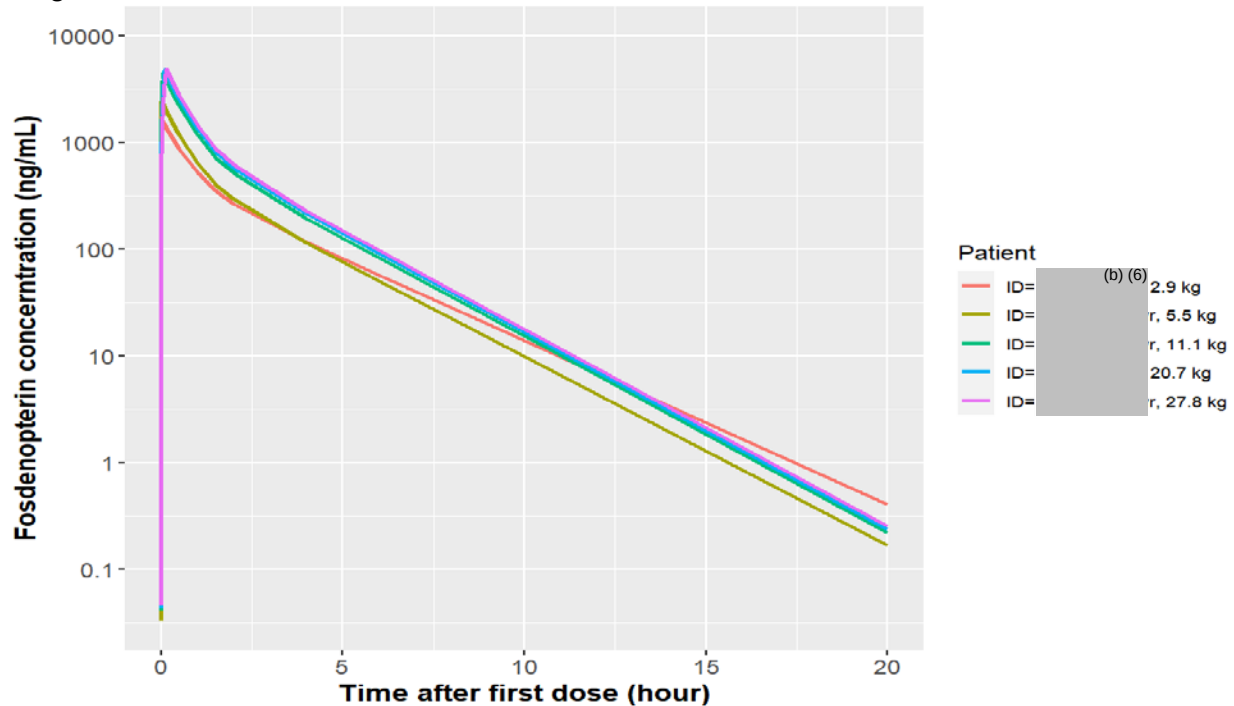
Source: Reviewer’s analysis.

Abbreviations: CL, clearance, F, bioavailability; PK, pharmacokinetics; Q, blood flow; Vc, central volume of distribution; Vp, peripheral volume of distribution

Overall, population PK data were too limited to conduct conclusive covariate analysis to guide dose adjustment based on patients’ demographics.

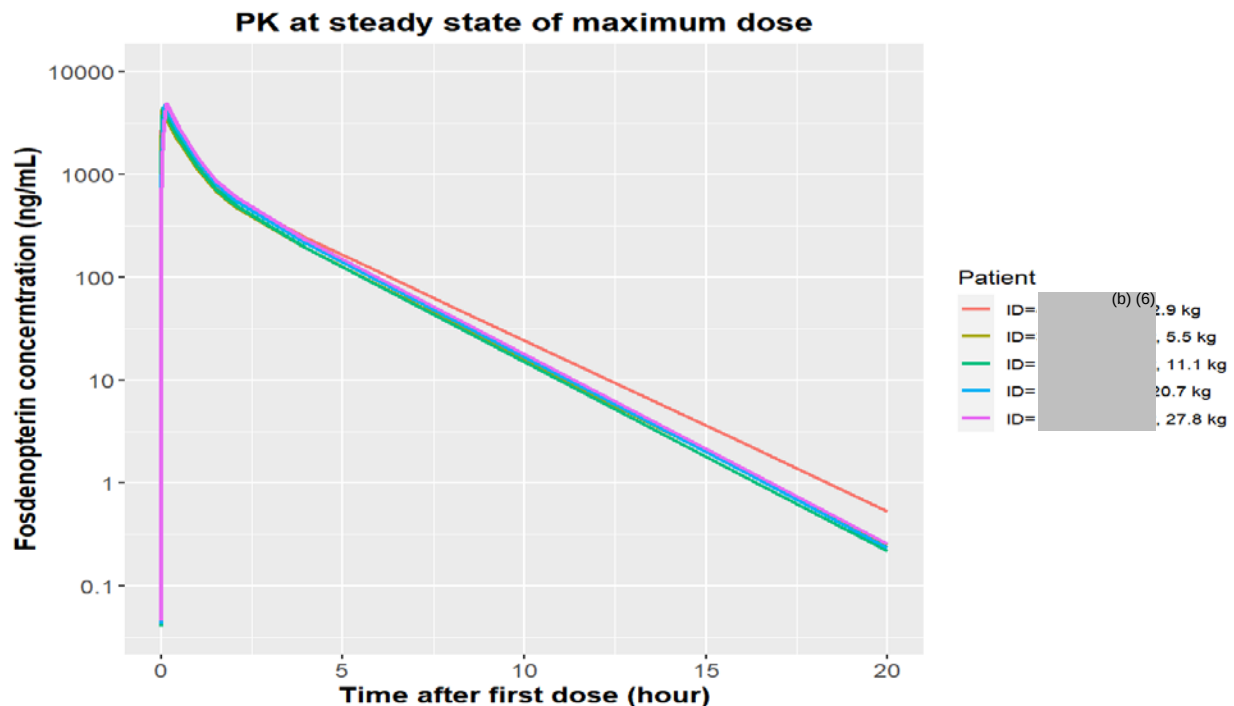
To illustrate the PK profiles across different age range (0 to 5 years old) and body weight range (2.5 to 30 kg), simulations were conducted using the Reviewer’s refined final model without IIV and residual errors in five typical patients at steady state of the proposed initial dose ([Figure 17](#)) and maximum dose ([Figure 18](#)) with a proposed infusion rate of 1.5 mL/min. The proposed initial dose was 0.4 mg/kg for patients <1 year old with a gestational age of <37 weeks, 0.55 mg/kg for patients <1 year old with a gestational age of ≥37 weeks, and 0.9 mg/kg for patients with ≥1 year old, while the proposed maximum dose was 0.9 mg/kg for all patients. As expected, due to lower initial dose level, simulation results showed that patients <1 year old have lower steady-state C_{max} at the proposed initial dose, as compared to patients ≥1 year old ([Table 43](#)). Simulation results also showed that fosdenopterin steady-state C_{max} at the proposed maximum dose was generally comparable across different ages (0 to 5 years old) and body weights (2.5 to 30 kg), which justified the same maximum dose level of 0.9 mg/kg for all patients regardless of age.

Figure 17. Fosdenopterin Pharmacokinetic Profiles at Steady State With the Proposed Initial Dose and Proposed Infusion Rate (1.5 mL/min) in Five Typical Patients of Different Ages and Body Weights



Source: Reviewer's analysis.

Figure 18. Fosdenopterin Pharmacokinetic Profiles at Steady State of the Proposed Maximum Dose and Proposed Infusion Rate (1.5 mL/min) in Five Typical Patients of Different Ages and Body Weights



Source: Reviewer's analysis.

Table 43. Fosdenopterin C_{max} at Steady State at the Proposed Initial Dose and Maximum Dose and the Proposed Infusion Rate of 1.5 mL/min in Five Typical Patients of Different Ages and Body Weights

Patient	Baseline Age (Years)	Baseline Weight (kg)	C _{max} at Steady State of Initial Dose (ng/mL)	C _{max} at Steady State of Maximum Dose (ng/mL)
	(b) (6)	2.88	1686.6	4208.6
		5.53	2478.5	4277.3
		11.1	4538.2	4527.3
		20.7	4834.4	4846.2
		27.8	4952.0	4964.9

Source: Reviewer's analysis.

Abbreviation: C_{max}, maximum concentration

Per initial protocols of Studies 201 and 202, fosdenopterin doses were administered by IV infusion with a dosing solution concentration of 0.38 mg/mL over approximately 10 to 15 min and the infusion duration increased proportionally with increasing dose. In 2016, both protocols were amended to specify an infusion rate of 1.5 mL/min with a dosing solution concentration of 1.9 mg/mL. In the current NDA, the proposed intravenous infusion rate is 1.5 mL/min with a final concentration of 1.9 mg/mL in reconstituted solution, which is consistent with the current study protocols.

Based on the Applicant's summary of available infusion durations in Study 201, the median infusion time ranged from 20 min to 30 min across the doses studied when the 0.38 mg/mL dosing solution concentration was used (n=7 patients), while the median infusion duration was 2.0 min with the 1.9 mg/mL concentration (n=1 patient). In Study 202, the median infusion time ranged from 2 min to 14 min (n=2 patients) across the doses administered including treatment initiation. Thus, the proposed infusion duration could be dramatically shorter than that in Studies 201 and 202, especially in neonates <1 year of age with a low body weight. Therefore, simulations were conducted to compare fosdenopterin C_{max} at steady state of the proposed initial dose and maximum dose between the proposed infusion rate of 1.5 mL/min and assuming an infusion duration of 10, 20, or 30 min in two neonates (ID (b) (6) and (b) (6)). Simulation results (Table 44) showed that steady-state C_{max} in two neonates at the proposed infusion rate of 1.5 mL/min was ~11%, ~24%, and ~39% higher than that at an infusion duration of 10, 20, and 30 min, respectively; these variations are not considered clinically meaningful. Note that infusion rate or infusion time is not expected to affect systemic exposure of fosdenopterin based on AUC, which was used to guide dose escalation in early fosdenopterin clinical trials. Also note that all patients in Studies 201 and 202 are currently receiving treatment with fosdenopterin infusion with 1.9 mg/mL concentration. Overall, the PK simulations, the available clinical experiences, and the protocol for the ongoing clinical studies support the proposed infusion rate of 1.5 mL/min with the to-be-marketed formulation.

Table 44. Fosdenopterin C_{max} at Steady State of the Proposed Initial Dose and Maximum Dose at the Proposed Infusion Rate (1.5 mL/min) and Assuming an Infusion Duration of 10, 20, or 30 min in Two Neonates (ID (b) (6))

Patient	Baseline Age (Years)	Baseline Weight (kg)	Infusion Rate/Duration	C_{max} at Steady State of Initial Dose (ng/mL)	C_{max} at Steady State of Maximum Dose (ng/mL)
(b) (6)	2.88		Proposed infusion rate (1.5 mL/min)	1686.6	4208.6
			Infusion duration of 10 min	1510.0	3810.9
			Infusion duration of 20 min	1355.4	3394.8
			Infusion duration of 30 min	1226.8	3051.5
(b) (6)	5.53		Proposed infusion rate (1.5 mL/min)	2478.5	4277.3
			Infusion duration of 10 min	2205.5	3866.2
			Infusion duration of 20 min	1949.7	3410.7
			Infusion duration of 30 min	1740.5	3039.2

Source: Reviewer's analysis.

Abbreviation: C_{max} , maximum concentration

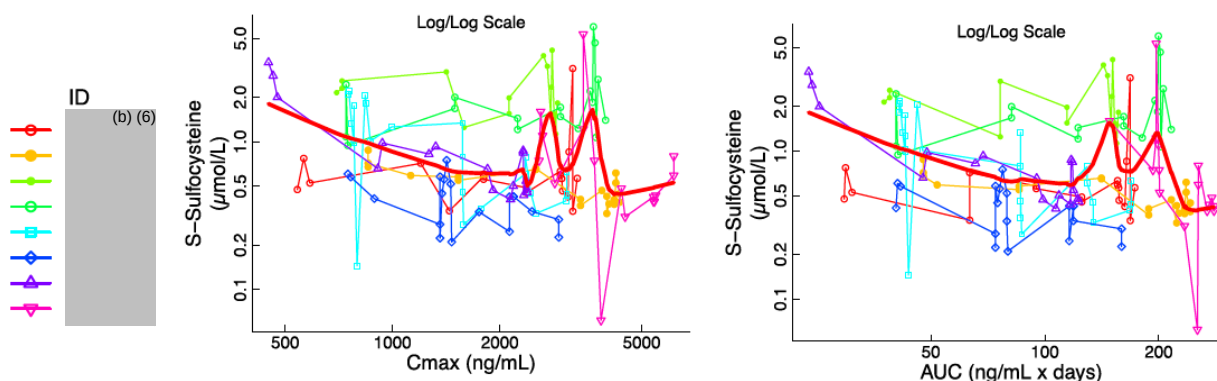
14.2.3. Exposure-Response Analysis

The Applicant conducted the descriptive exposure-response analyses for plasma biomarkers, including s-sulfocysteine, uric acid, xanthine, and urothione, as well as urine biomarkers, including SSC, uric acid, and xanthine based on data from seven infants and children previously treated with rcPMP in Study MCD-201 and one newly diagnosed neonate in Study MCD-202.

14.2.3.1. Plasma Biomarkers

There was a trend that higher exposure (C_{max} and AUC) of fosdenopterin was associated with a lower value of plasma S-sulfocysteine; however, a definitive conclusion of E-R relationship could not be made due to the small number of subjects and large inter-subject variability (Figure 19).

Figure 19. Exposure-Response for Plasma S-Sulfocysteine

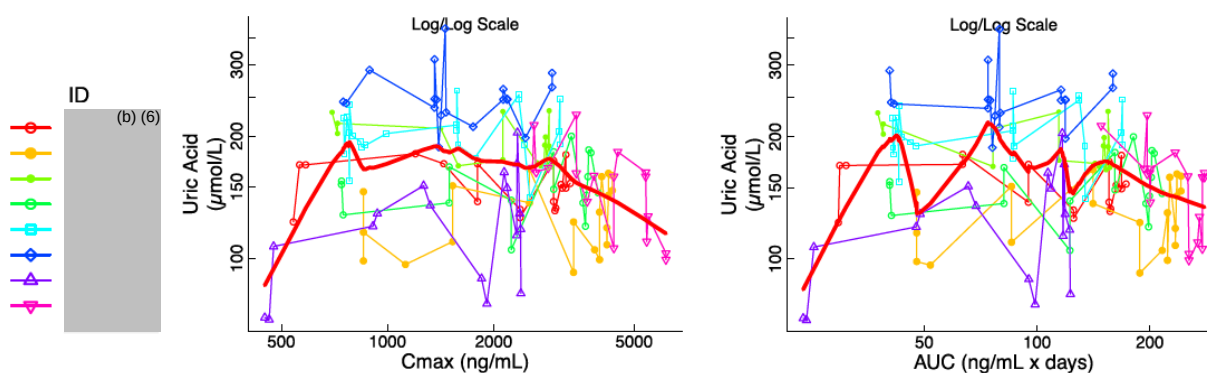


Source: Applicant's Population Pharmacokinetics Analysis Report, Figure 7.

Abbreviations: AUC, area under the curve; C_{max} , maximum concentration; ID, identification

There was no relationship between plasma uric acid and fosdenopterin C_{max} or AUC (Figure 20).

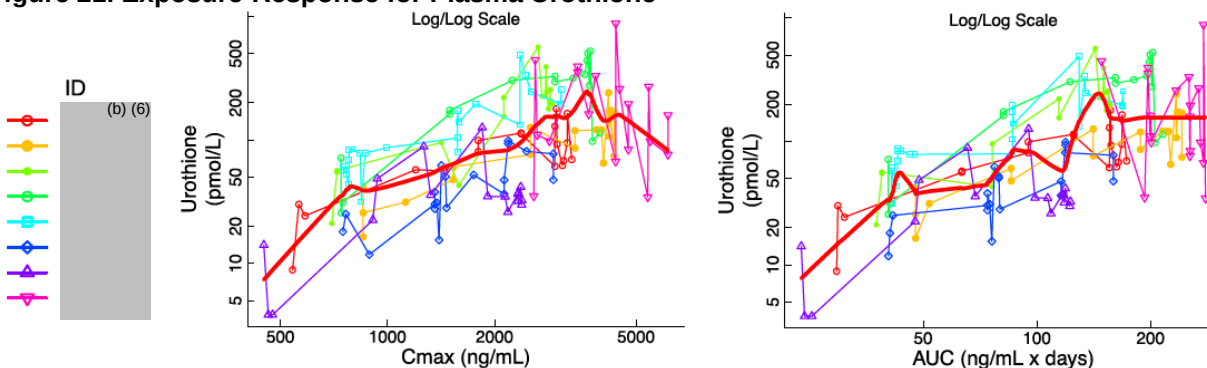
Figure 20. Exposure-Response for Plasma Uric Acid



Source: Applicant's Population Pharmacokinetics Analysis Report, Figure 8.
Abbreviations: AUC, area under the curve; C_{max}, maximum concentration; ID, identification

There was no relationship between plasma xanthine and fosdenopterin C_{max} or AUC; however, there was a strong positive E-R relationship for plasma urothione ([Figure 21](#)), a metabolic degradation product of MoCo that is undetectable in untreated patients with MoCD Type A. The Applicant stated that this finding may be viewed as indirect evidence of the restoration of MoCo synthesis with fosdenopterin administration.

Figure 21. Exposure-Response for Plasma Urothione

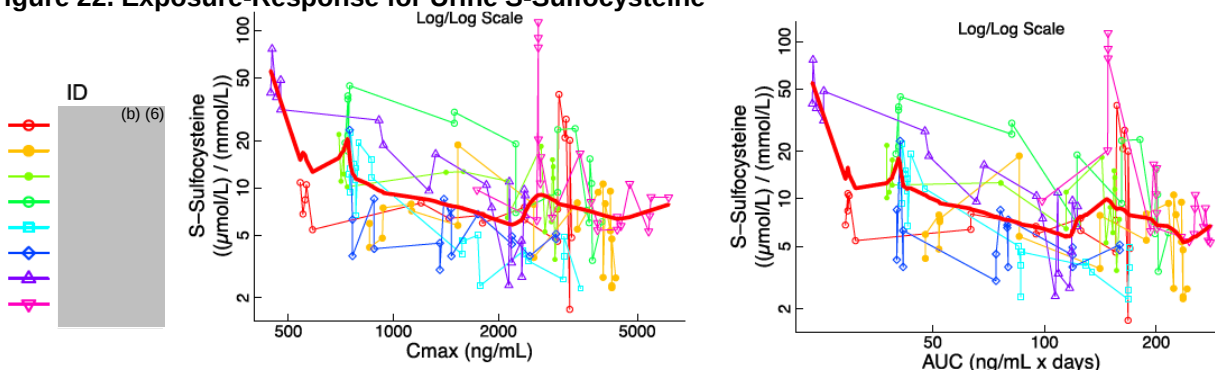


Source: Applicant's Population Pharmacokinetics Analysis Report, Figure 10.
Abbreviations: AUC, area under the curve; C_{max}, maximum concentration; ID, identification

14.2.3.2. Urine Biomarkers

Higher exposure (C_{max} and AUC) of fosdenopterin was associated with a lower value of creatinine-normalized urine SSC ([Figure 22](#)).

Figure 22. Exposure-Response for Urine S-Sulfocysteine

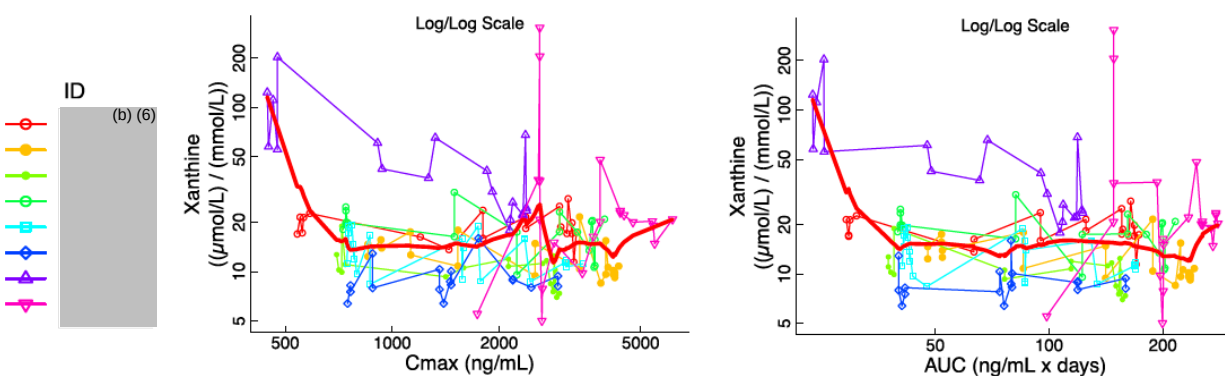


Source: Applicant's Exposure-Response Analysis Report Figure 13.
Abbreviations: AUC, area under the curve; C_{max}, maximum concentration; ID, identification

There was no relationship between creatinine-normalized urine uric acid and fosdenopterin C_{max} or AUC.

Creatinine-normalized urine urothione decreases with increasing exposure at the lowest exposures, but no relationship above a C_{max} of ~800 ng/mL or an AUC of 40 ng/mL (Figure 23).

Figure 23. Exposure-Response for Plasma Urothione

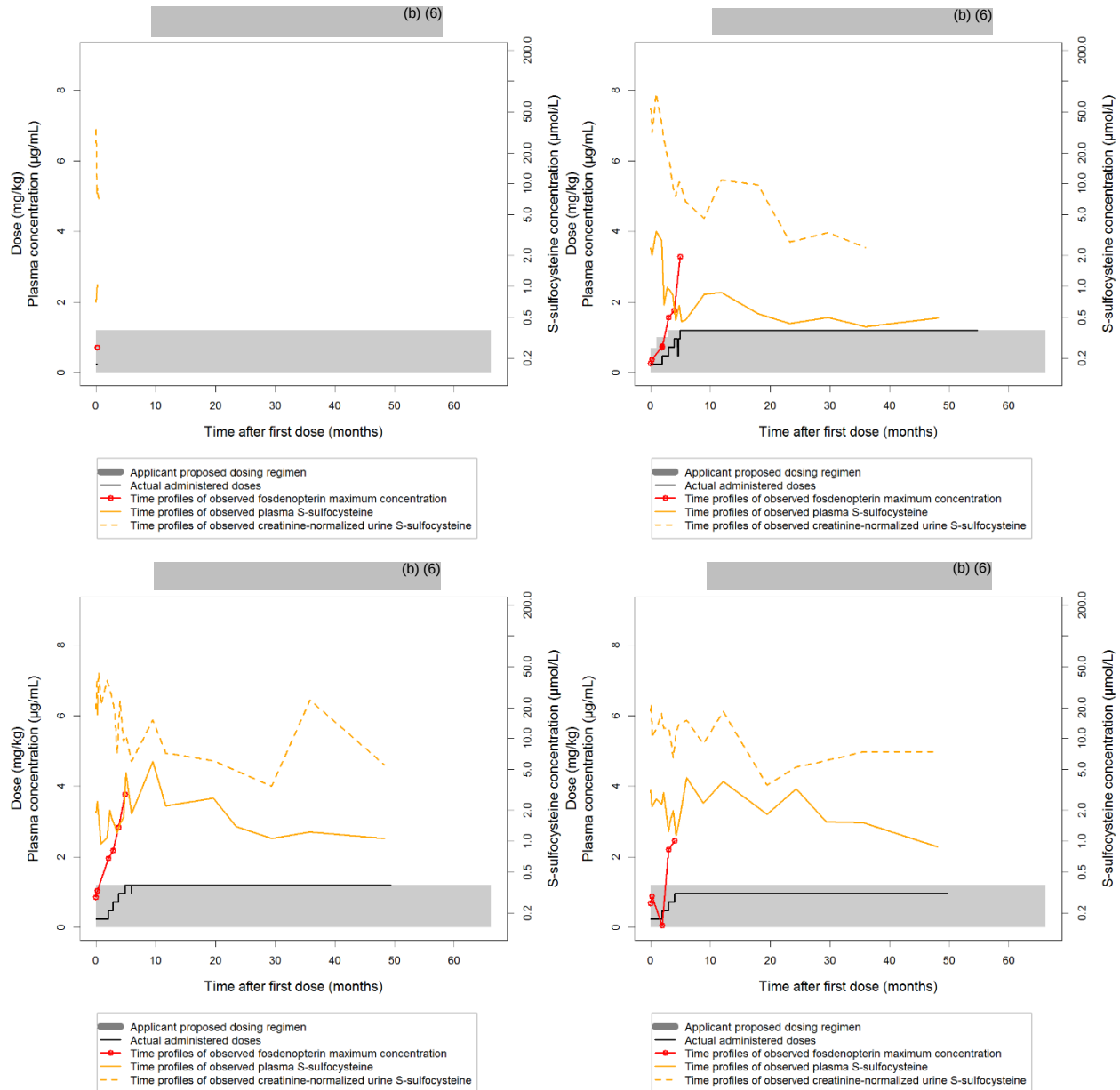


Source: Applicant's Exposure-Response Analysis Report Figure 15.
Abbreviations: AUC, area under the curve; C_{max}, maximum concentration; ID, identification

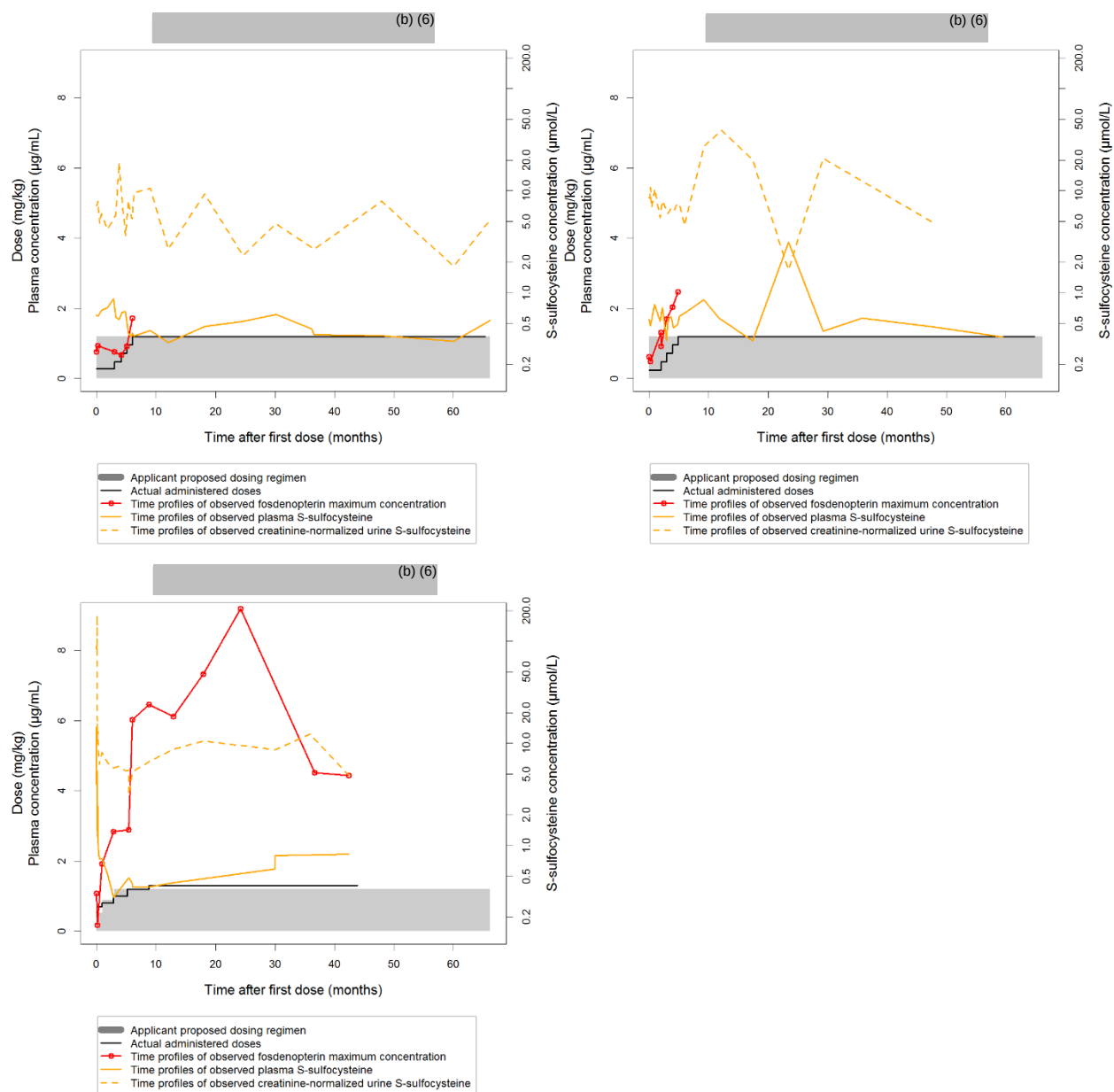
Reviewer's Comments:

In addition to limited sample size, the Applicant's descriptive exposure-response analysis for biomarkers may be confounded by prior treatment and patients' demographics. The reviewer conducted independent analyses to illustrate the relationships among actual administered doses, fosdenopterin maximum concentrations, and plasma and creatinine-normalized urine biomarkers at the individual patient level (Figure 24 to Figure 27). Despite some fluctuations of biomarkers over time, changes in plasma SSC, creatinine-normalized urine SSC and plasma urothione generally followed a similar or reverse pattern of fosdenopterin C_{max} after dose titration or modification. The observed dose-dependent changes of biomarkers from individual patient further support the proposed dosing regimen.

Figure 24. Individual Time Profiles of Observed Fosdenopterin Maximum Concentration, Plasma S-Sulfocysteine, and Creatinine-Normalized Urine S-Sulfocysteine

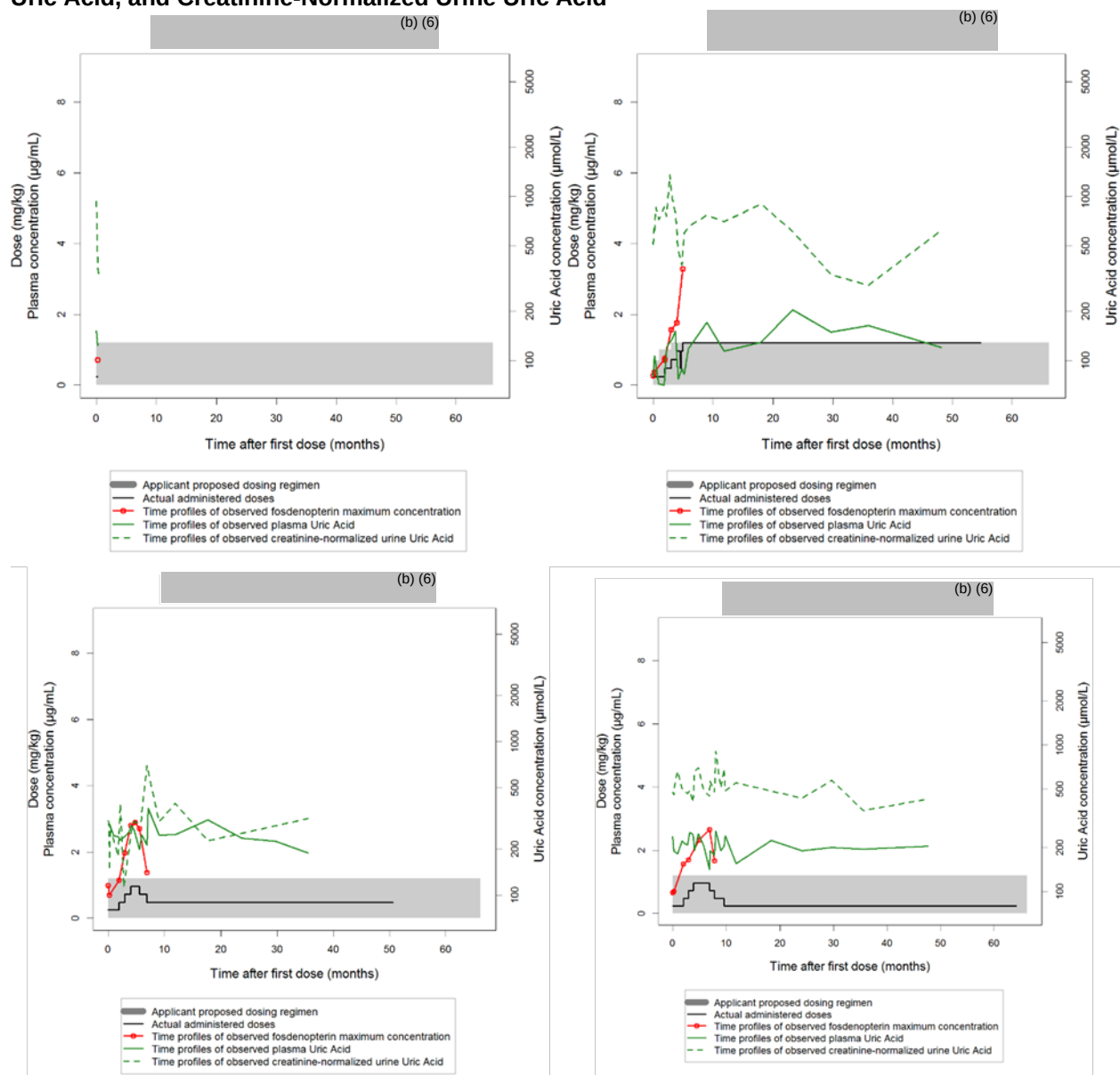


NDA 214018
Nulibry (fosdenopterin)

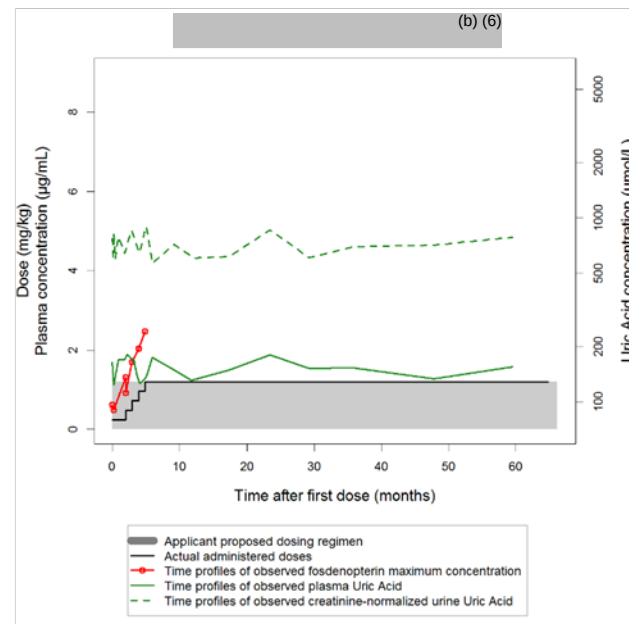
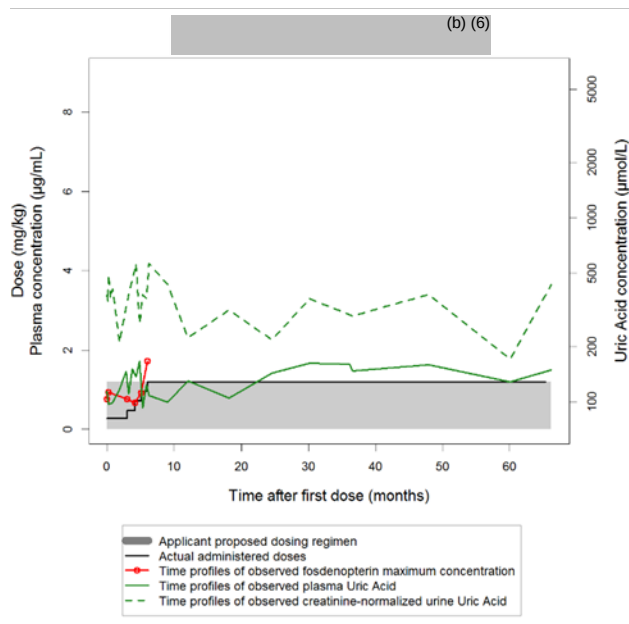
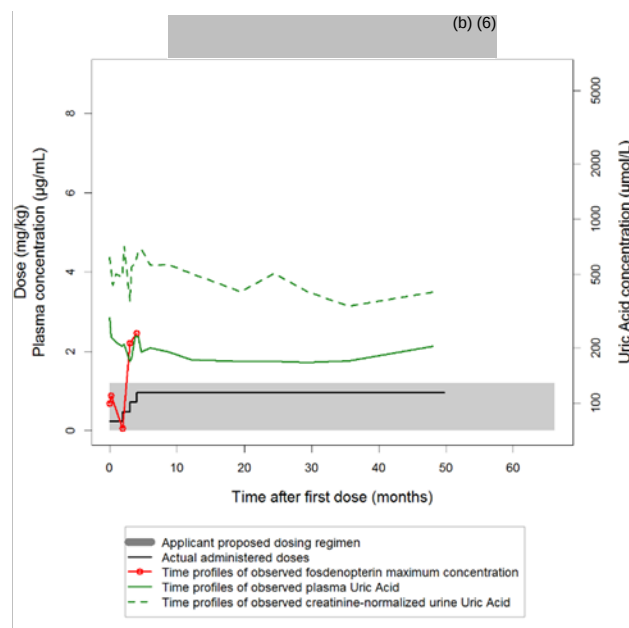
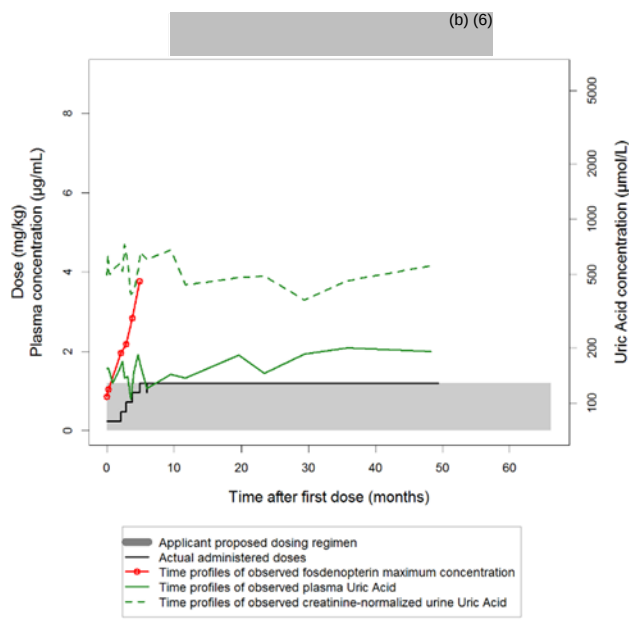


Source: FDA reviewer's analysis results.

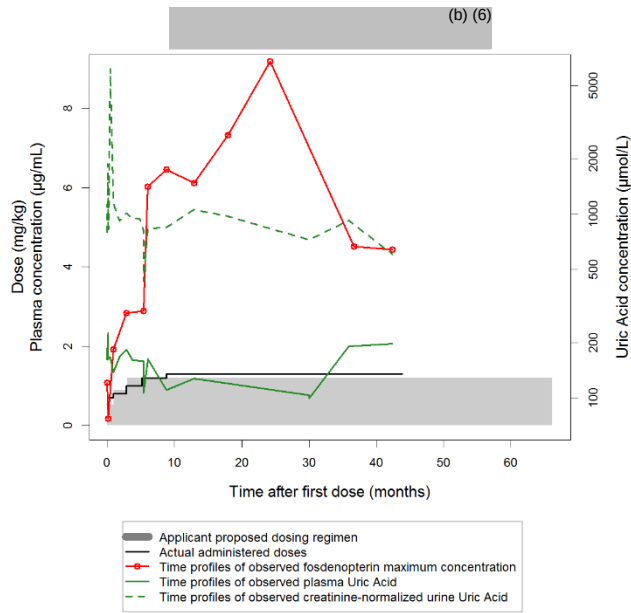
Figure 25. Individual Time Profiles of Observed Fosdenopterin Maximum Concentration, Plasma Uric Acid, and Creatinine-Normalized Urine Uric Acid



NDA 214018
Nulibry (fosdenopterin)

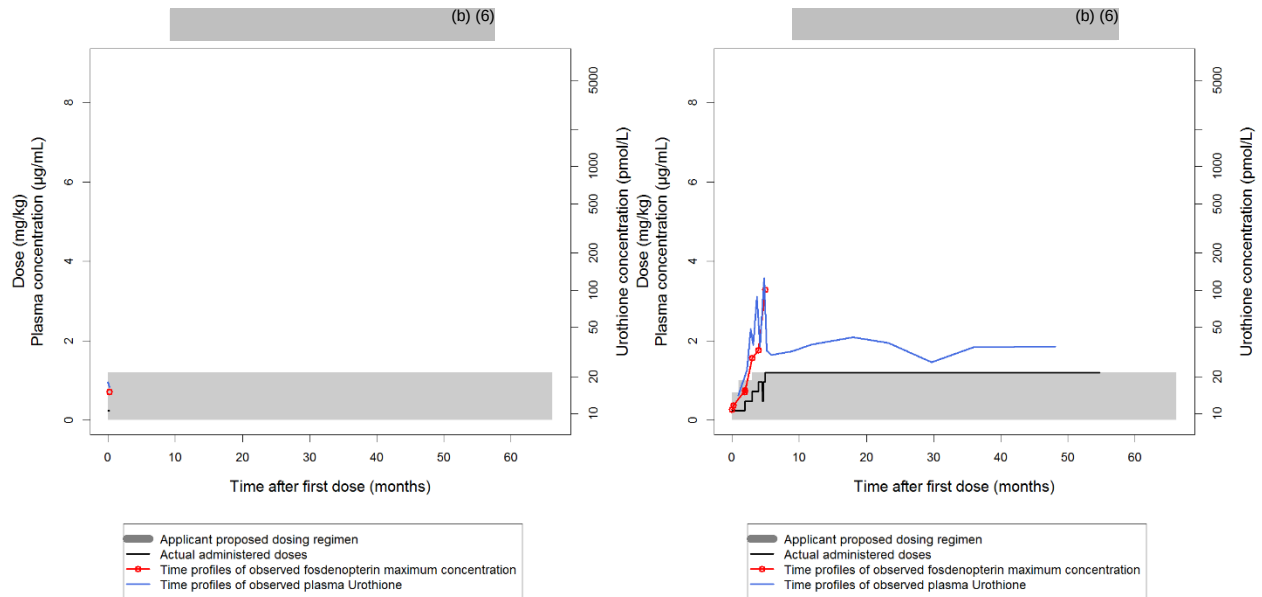


NDA 214018
Nulibry (fosdenopterin)

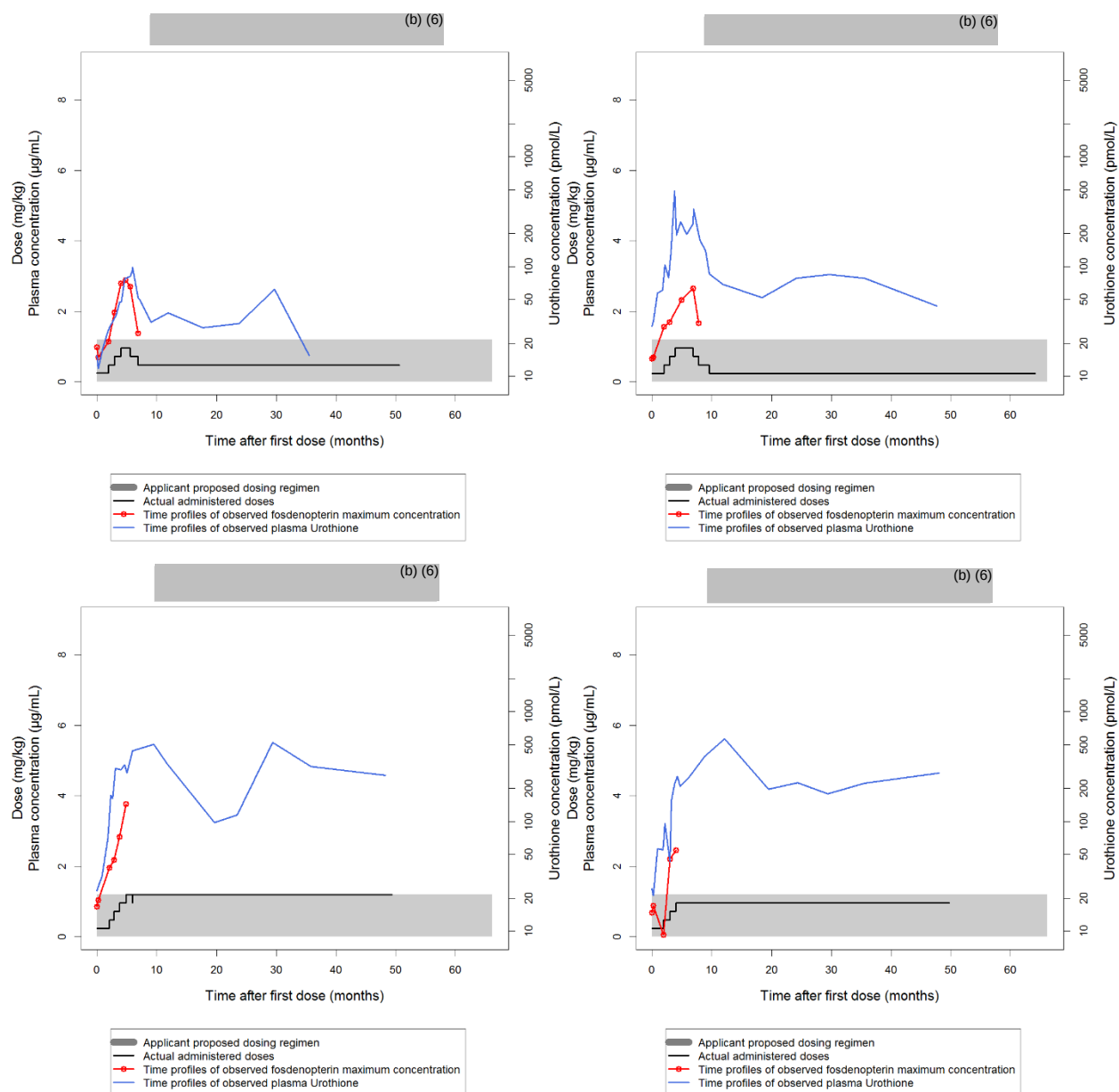


Source: Reviewer's analysis.

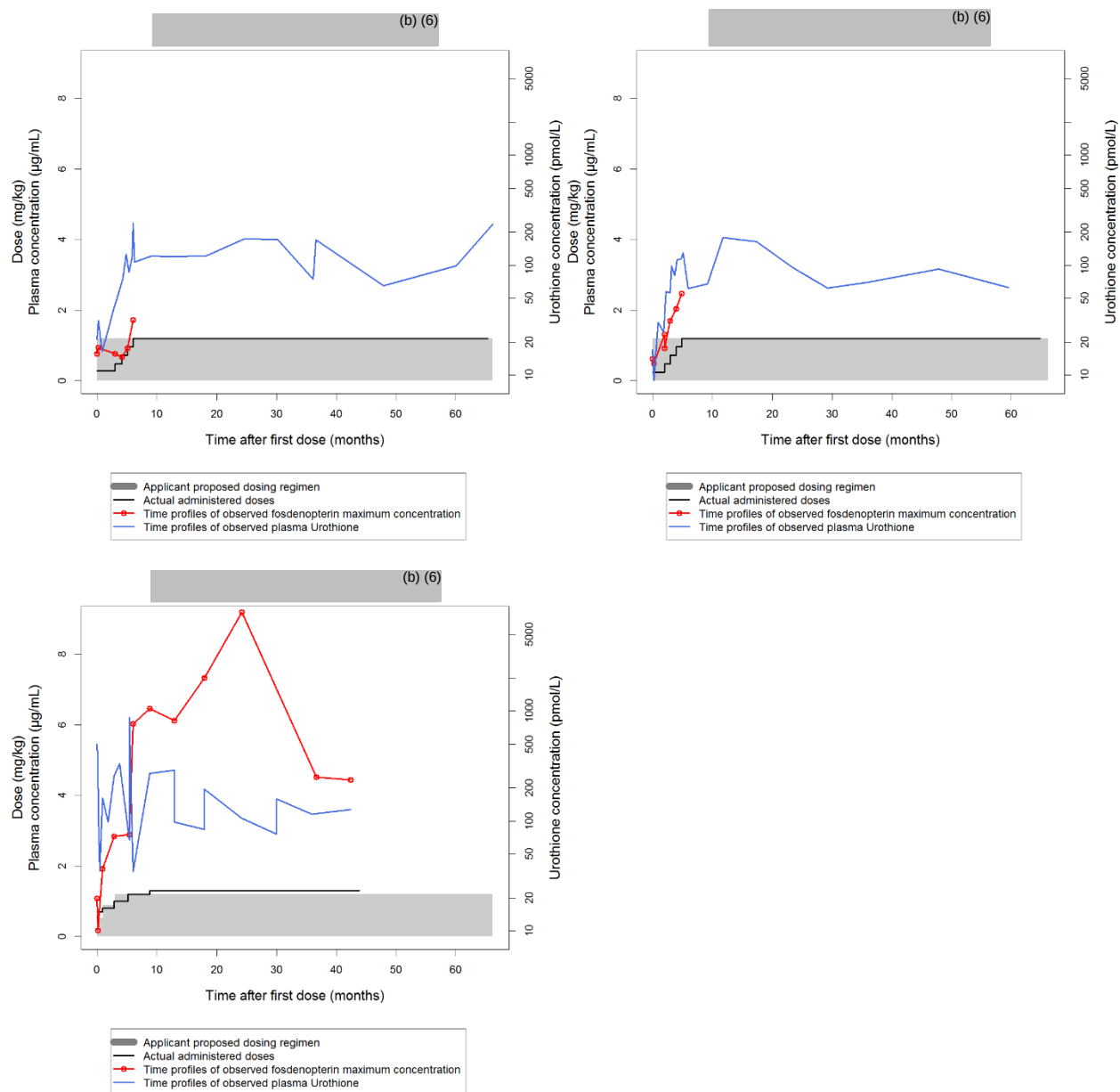
Figure 26. Individual Time Profiles of Observed Fosdenopterin Maximum Concentration and Plasma Urothione



NDA 214018
Nulibry (fosdenopterin)

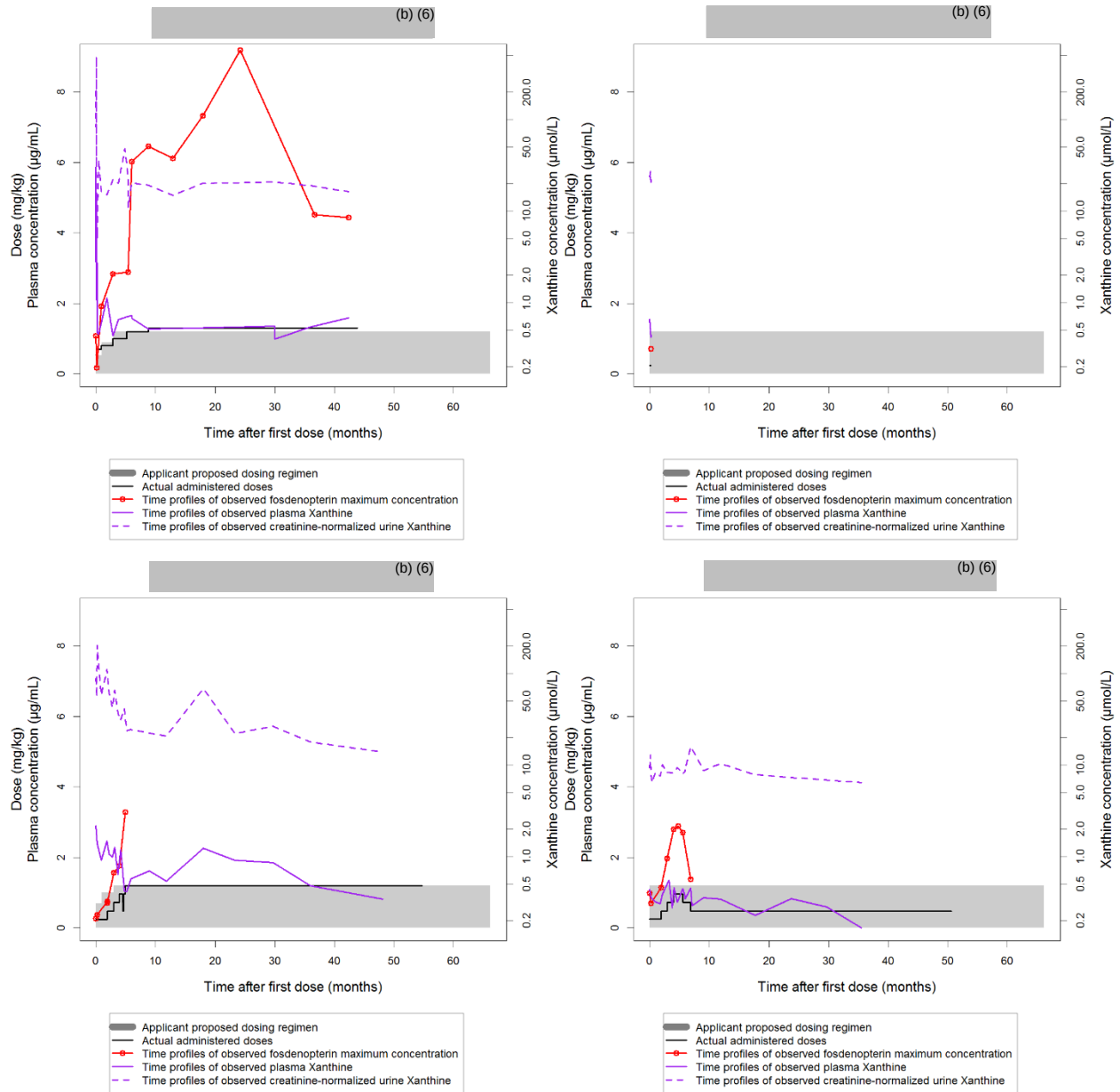


NDA 214018
Nulibry (fosdenopterin)

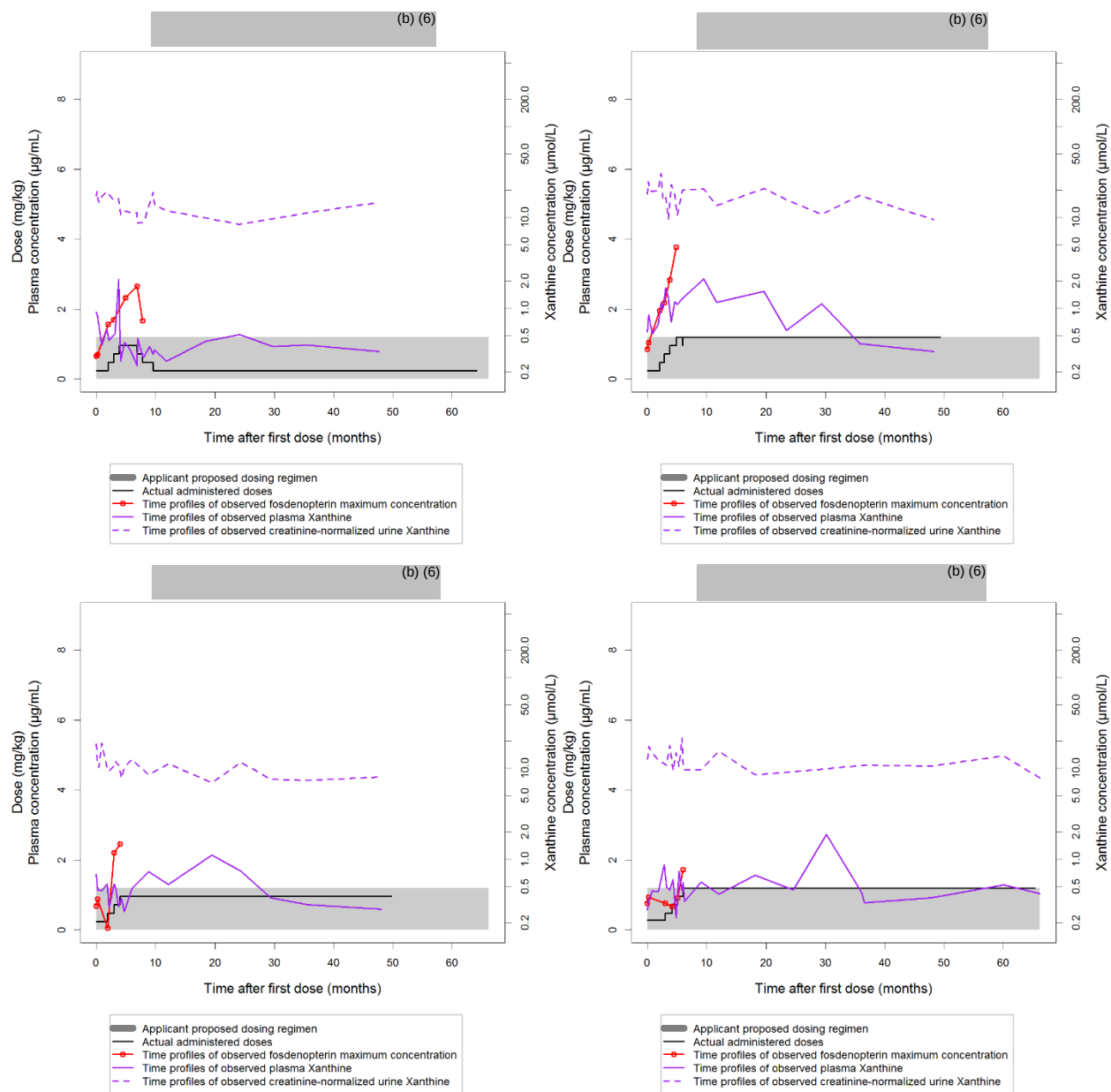


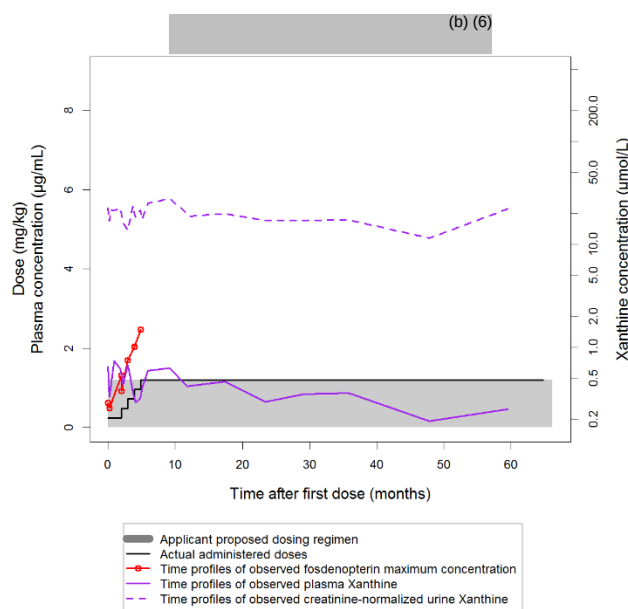
Source: Reviewer's analysis.

Figure 27. Individual Time Profiles of Observed Fosdenopterin Maximum Concentration, Plasma Xanthine, and Creatinine-Normalized Urine Xanthine



NDA 214018
Nulibry (fosdenopterin)





Source: Reviewer's analysis.

14.3. Summary of Bioanalytical Method Validation and Performance

The bioanalytical methods for concentration determination and sample analysis of plasma fosdenopterin, urine biomarkers (SSC, uric acid, xanthine, creatinine, urothione) and blood biomarkers (SSC, uric acid and xanthine) used for Studies MCD-201 and MCD-202, were validated at (b) (6). The Office of Study Integrity and Surveillance determined that a site inspection of (b) (4) was not necessary because the Office of Study Integrity and Surveillance conducted a remote regulatory review for the site in (b) (4) and found the data for the reviewed studies reliable. The method validations were acceptable.

The bioanalytical method for the determination of fosdenopterin concentrations in human plasma was validated at (b) (6) and was used for Study MCD-101. The performance of the method and the assay validation parameters are summarized in [Table 45](#).

Table 45. Bioanalytical Method Validation for Fosdenopterin Determination in Human Plasma (Method MET129)

Bioanalytical method validation report name, amendments, and hyperlinks	• (b) (4) MET129 vI–Analytical Method and Validation Report for Determination of ALXN1101 in Human Plasma by LC/MS/MS • (b) (4) /MET129 vI Addendum-1–Analytical Stability Report for ALXN1101 in Treated Human Plasma and in Solution		
Method description	Reverse-phase liquid chromatography separation and detection by tandem mass spectrometry (LC-MS/MS).		
Materials used for calibration curve and concentration	Cyclic pyranopterin monophosphate monohydrobromide dihydrate (cPMP HBr.2H ₂ O); ALXN1101: (b) (4) Lot 856_GMP/cPMP/002 Human plasma treated with 0.25M L-Ascorbic Acid (10:1 plasma:ascorbic acid)		
	Standard	Concentration	
	Std 1	5.02 ng/mL	
	Std 2	10.03 ng/mL	
	Std 3	21.93 ng/mL	
	Std 4	56.48 ng/mL	
	Std 5	160.54 ng/mL	
	Std 6	248.22 ng/mL	
	Std 7	509.27 ng/mL	
	Std 8	1016.73 ng/mL	
Validated assay range	5.02 ng/mL to 1016.73 ng/mL		
Materials used for QC and concentrations	Cyclic pyranopterin monophosphate monohydrobromide dihydrate (cPMP HBr 2H ₂ O); ALXN1101: (b) (4) Lot 856_GMP/cPMP/002 Human Plasma treated with 0.25M L-ascorbic acid (10:1 plasma:ascorbic acid)		
	Control	Concentration	
	High QC	728.83 ng/mL	
	Mid QC	60.74 ng/mL	
	Low QC	13.12 ng/mL	
	LLOQ	5.02 ng/mL	
Minimum required dilutions	1:4 with Internal Standard (b) (4) -IS) 1:2 with sample dilution buffer 0.25M L-ascorbic acid		
Source and lot of reagents	• Cyclic pyranopterin monophosphate monohydrobromide dihydrate (cPMP HBr 2H₂O); ALXN1101: (b) (4) Lot 856_GMP/cPMP/002 • [¹³C3] cPMP HBr 2H₂O; ALXN1101: (b) (4) Batch 7119SJR031-2		
Regression model and weighting	Linear, weighted 1/x ²		
Validation parameters	Method validation summary		
Standard calibration curve performance	Number of standard calibrators from LLOQ to ULOQ		8
during accuracy and precision	Cumulative accuracy (%bias) from LLOQ to ULOQ		87.2 to 129.2%
	Cumulative precision (%CV) from LLOQ to ULOQ		≤8.2%
QC performance during accuracy and precision	Cumulative accuracy (%bias) in three QCs		
	QCs: ALXN1101		
	Low		84.3 to 112.4%
	Mid		90.8 to 103.1%
	High		88.9 to 105.9%
	Interbatch %CV		
	QCs: ALXN1101		
	Low		≤8.1%
	Mid		≤4.5%
	High		≤3.7%

Bioanalytical method validation report name, amendments, and hyperlinks	(b) (4) MET129 vI–Analytical Method and Validation Report for Determination of ALXN1101 in Human Plasma by LC/MS/MS (b) (4) MET129 vI Addendum-1–Analytical Stability Report for ALXN1101 in Treated Human Plasma and in Solution																										
Selectivity and matrix effect	Six lots of blank matrix (human plasma) spiked with ALXN1101 at LLOQ (5.02 ng/mL). Accuracy 86.1 to 107.9% %CV 8.7%																										
Interference and specificity	Six lots of blank matrix (human plasma) evaluated. No interference at the retention time of ALXN1101 observed.																										
Hemolysis effect	Analytic recovery in hemolytic plasma was evaluated in triplicate at two levels (145 mg/dL and 400 mg/dL) after spiking to QC Low, Mid, and High levels with ALXN1101. Assay unaffected by hemolyzed plasma within the tested ranges. <table><tr><th colspan="2">Hemolysis for:</th><th>Recovery</th><th>CV</th></tr><tr><td rowspan="3">145 mg/dL</td><td>QC Low</td><td>85.2 to 112.7%</td><td>14.2%</td></tr><tr><td>QC Mid</td><td>83.0 to 96.7%</td><td>7.9%</td></tr><tr><td>QC High</td><td>90.6 to 96.8%</td><td>3.3%</td></tr><tr><td rowspan="3">400 mg/dL</td><td>QC Low</td><td>85.5 to 86.9%</td><td>1.3%</td></tr><tr><td>QC Mid</td><td>85.2 to 92.7%</td><td>4.3%</td></tr><tr><td>QC High</td><td>90.4 to 93.2%</td><td>1.6%</td></tr></table>			Hemolysis for:		Recovery	CV	145 mg/dL	QC Low	85.2 to 112.7%	14.2%	QC Mid	83.0 to 96.7%	7.9%	QC High	90.6 to 96.8%	3.3%	400 mg/dL	QC Low	85.5 to 86.9%	1.3%	QC Mid	85.2 to 92.7%	4.3%	QC High	90.4 to 93.2%	1.6%
Hemolysis for:		Recovery	CV																								
145 mg/dL	QC Low	85.2 to 112.7%	14.2%																								
	QC Mid	83.0 to 96.7%	7.9%																								
	QC High	90.6 to 96.8%	3.3%																								
400 mg/dL	QC Low	85.5 to 86.9%	1.3%																								
	QC Mid	85.2 to 92.7%	4.3%																								
	QC High	90.4 to 93.2%	1.6%																								
Lipemic effect	Analytic recovery in one lot of lipemic plasma (medium level by visual inspection) was evaluated after spiking to QC Low, Mid, and High levels with ALXN1101. Assay was not affected by lipemic plasma within the tested ranges. <table><tr><th colspan="2">Medium Lipemia for:</th><th>Recovery</th><th>CV</th></tr><tr><td colspan="2">QC Low</td><td>95.0 to 101.7%</td><td>3.6%</td></tr><tr><td colspan="2">QC Mid</td><td>86.0 to 100.2%</td><td>8.0%</td></tr><tr><td colspan="2">QC High</td><td>94.0 to 99.6%</td><td>3.0%</td></tr></table>			Medium Lipemia for:		Recovery	CV	QC Low		95.0 to 101.7%	3.6%	QC Mid		86.0 to 100.2%	8.0%	QC High		94.0 to 99.6%	3.0%								
Medium Lipemia for:		Recovery	CV																								
QC Low		95.0 to 101.7%	3.6%																								
QC Mid		86.0 to 100.2%	8.0%																								
QC High		94.0 to 99.6%	3.0%																								
Dilution linearity and hook effect	Dilution integrity: 5101.79 ng/mL ALXN1101 spiked into plasma and diluted 10-fold. No bias was introduced by the dilution procedure. <table><tr><th colspan="2">Dilution integrity (n=6)</th><th>Recovery</th><th>CV</th></tr><tr><td colspan="2"></td><td>85.7 to 107.7%</td><td>8.0%</td></tr></table> Hook effect: Not applicable for chromatographic assays			Dilution integrity (n=6)		Recovery	CV			85.7 to 107.7%	8.0%																
Dilution integrity (n=6)		Recovery	CV																								
		85.7 to 107.7%	8.0%																								
Benchtop/process stability	Benchtop stability of six replicates for the QC Low and High was evaluated after storage for 4 hr at room temperature. <table><tr><th colspan="2">Benchtop Stability</th><th>Recovery</th><th>CV</th></tr><tr><td colspan="2">QC Low</td><td>90.8 to 103.4%</td><td>5.0%</td></tr><tr><td colspan="2">QC High</td><td>90.4 to 102.7%</td><td>5.0%</td></tr></table> Process stability(extract/autosampler) of six replicates of the QC Low and High extracts evaluated after storage in the autosampler tray for 48 hr at 4°C <table><tr><th colspan="2">Extract Stability</th><th>Recovery</th><th>CV</th></tr><tr><td colspan="2">QC Low</td><td>93.3 to 105.1%</td><td>4.0%</td></tr><tr><td colspan="2">QC High</td><td>88.9 to 99.3%</td><td>3.7%</td></tr></table>			Benchtop Stability		Recovery	CV	QC Low		90.8 to 103.4%	5.0%	QC High		90.4 to 102.7%	5.0%	Extract Stability		Recovery	CV	QC Low		93.3 to 105.1%	4.0%	QC High		88.9 to 99.3%	3.7%
Benchtop Stability		Recovery	CV																								
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QC Low		93.3 to 105.1%	4.0%																								
QC High		88.9 to 99.3%	3.7%																								
Freeze-thaw stability	Freeze-thaw stability of six replicates of the QC Low and High. Controls stored at -70°C were evaluated after three freeze-thaw cycles. <table><tr><th colspan="2">Freeze-Thaw Stability</th><th>Recovery</th><th>CV</th></tr><tr><td colspan="2">QC Low</td><td>93.6 to 102.8%</td><td>3.7%</td></tr><tr><td colspan="2">QC High</td><td>93.5 to 106.8%</td><td>4.8%</td></tr></table>			Freeze-Thaw Stability		Recovery	CV	QC Low		93.6 to 102.8%	3.7%	QC High		93.5 to 106.8%	4.8%												
Freeze-Thaw Stability		Recovery	CV																								
QC Low		93.6 to 102.8%	3.7%																								
QC High		93.5 to 106.8%	4.8%																								

Bioanalytical method validation report name, amendments, and hyperlinks	(b) (4) MET129 vI–Analytical Method and Validation Report for Determination of ALXN1101 in Human Plasma by LC/MS/MS (b) (4) MET129 vI Addendum-1–Analytical Stability Report for ALXN1101 in Treated Human Plasma and in Solution																												
Long-term storage	Long-term storage of treated plasma was evaluated by testing six replicates of the QC Low and High controls made using treated plasma stored for 34, 68, and 117 days at –70°C. <table><tr><th>Storage Duration</th><th>Sample</th><th>Recovery</th><th>CV</th></tr><tr><td rowspan="2">34 Days</td><td>QC Low</td><td>89.6 to 113.1%</td><td>9.2%</td></tr><tr><td>QC High</td><td>86.9 to 104.0%</td><td>6.0%</td></tr><tr><td rowspan="2">68 Days</td><td>QC Low</td><td>96.2 to 117.7%</td><td>7.3%</td></tr><tr><td>QC High</td><td>99.2 to 107.5%</td><td>3.0%</td></tr><tr><td rowspan="2">117 Days</td><td>QC Low</td><td>86.2 to 104.0%</td><td>6.7%</td></tr><tr><td>QC High</td><td>87.0 to 108.1%</td><td>9.2%</td></tr></table> Long-term storage of ALXN1101 stock solution was evaluated by testing six replicates of stock solution stored for 35, 66, and 118 days at –20°C compared to freshly prepared stock solution.				Storage Duration	Sample	Recovery	CV	34 Days	QC Low	89.6 to 113.1%	9.2%	QC High	86.9 to 104.0%	6.0%	68 Days	QC Low	96.2 to 117.7%	7.3%	QC High	99.2 to 107.5%	3.0%	117 Days	QC Low	86.2 to 104.0%	6.7%	QC High	87.0 to 108.1%	9.2%
Storage Duration	Sample	Recovery	CV																										
34 Days	QC Low	89.6 to 113.1%	9.2%																										
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	QC High	99.2 to 107.5%	3.0%																										
117 Days	QC Low	86.2 to 104.0%	6.7%																										
	QC High	87.0 to 108.1%	9.2%																										
Parallelism	Not applicable																												
Carry over	Sample carryover was evaluated by running a blank or rinse after the highest standard of each analyte and assessing the relative peak height of this blank versus the signal at LLOQ. Carryover signal was below detection limit.																												
Method Performance in Study Number ALX1101-MCD-101																													
Assay passing rate	All six (6) assays performed on subject plasma samples passed the assay acceptance criteria (including all re-analyses).																												
Standard curve performance	Cumulative bias range: 95.1 to 104.0% Cumulative precision: ≤9.9% CV																												
QC performance	Cumulative bias range: 96.8 to 104.4% Cumulative precision: ≤11.1% CV																												
Method reproducibility	Incurred sample re-analysis was performed in 9.8% (46/469) of study samples, and 87.0% (40/46) of the samples met the prespecified criteria.																												
Study sample analysis/stability	Storage stability for standards/QC samples was 118 days at –70°C. Storage stability for study samples was 26 days at –70°C.																												
Standard calibration curve performance during accuracy and precision runs	Standard	Concentration	Standard	Concentration																									
	Std 1 (LLOQ)	5.01 ng/mL	Std 5	167.14 ng/mL																									
	Std 2	9.59 ng/mL	Std 6	265.43 ng/mL																									
	Std 3	22.23 ng/mL	Std 7	530.34 ng/mL																									
	Std 4	56.37 ng/mL	Std 8 (ULOQ)	1058.57 ng/mL																									

Abbreviations: ALXN1101, fosdenopterin; CV, coefficient of variation; LLOQ, lower limit of quantification; QC, quality control; ULOQ, upper limit of quantification

Method (b) (4) MET129 LC/MS/MS for ALXN1101 was subsequently revalidated at (b) (4) and used to determine fosdenopterin concentrations in Studies MCD-201 and MCD-202. The assay validation parameters are summarized in [Table 46](#).

Table 46. Bioanalytical Method Validation for Fosdenopterin Determination in Human Plasma (Method LCMSC 669)

Bioanalytical method validation report name, amendments, and hyperlinks	• Method Validation Report: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; April 2014 • Method Validation Report Addendum 1: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; April 2016 • Method Validation Report Addendum 2: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; February 2020
Method description	Reverse-phase liquid chromatography separation and detection by tandem mass spectrometry (LC-MS/MS) using positive ion electrospray.

Bioanalytical method validation report name, amendments, and hyperlinks	• Method Validation Report: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; April 2014 • Method Validation Report Addendum 1: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; April 2016 • Method Validation Report Addendum 2: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; February 2020		
Materials used for calibration curve and concentration	Cyclic pyranopterin monophosphate monohydrobromide dihydrate (cPMP HBr 2H ₂ O); ALXNI101 human plasma K ₂ EDTA with 4 mg/mL ascorbic acid		
	Standard	Concentration	
	CAL 1 LLOQ	5.0 ng/mL	
	CAL 2	9.00 ng/mL	
	CAL 3	15.0 ng/mL	
	CAL 4	40.0 ng/mL	
	CAL 5	125 ng/mL	
	CAL 6	350 ng/mL	
	CAL 7	800 ng/mL	
	CAL 8 ULOQ	1000 ng/mL	
Validated assay range	5.00 ng/mL to 1000 ng/mL		
Materials used for QC and concentrations	Cyclic pyranopterin monophosphate monohydrobromide dihydrate (cPMP HBr 2H ₂ O); ALXNI101 human plasma K ₂ EDTA with 4 mg/mL ascorbic acid		
	Control	Concentration	
	QC 0	5.00 ng/mL	
	QC 1	12.0 ng/mL	
	QC 2	25.0 ng/mL	
	QC 3	75.0 ng/mL	
	QC 4	200 ng/mL	
	QC 5	750 ng/mL	
Minimum required dilutions	~1:8		
Source and lot of reagents (LBA)	Material	Source	Lot
	cPMP.HBr.2H ₂ O	(b) (4)	856 GMP/cPMP/002
	ALXN-1101- ¹³ C3		7119SJR031-2
	Acetonitrile, LC-MS CHROMASOLV		Product 34967
	Ammonium acetate		Product 431311-250G
	Formic acid (98%)		Product 94318
	Dulbecco's PBS		Product D8537
	Glacial acetic acid (99.7% min)		Product MK250414
	L-ascorbic acid		Product A5960-250G
	Methanol, LC-MS CHROMASOLV		Product 34966
	Human urine		Not provided
Regression model and weighting	Linear, weighted 1/x ²		
Validation parameters	Method validation summary		
Standard	Number of standard calibrators from LLOQ to ULOQ		8
calibration curve	Cumulative accuracy (%bias) from LLOQ to ULOQ		97.93 to 101.69%
performance in accuracy/ precision	Cumulative precision (%CV) from LLOQ to ULOQ		≤6.02%

Bioanalytical method validation report name, amendments, and hyperlinks	• Method Validation Report: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; April 2014 • Method Validation Report Addendum 1: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; April 2016 • Method Validation Report Addendum 2: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; February 2020																								
QC performance during accuracy and precision	<table> <tr> <td colspan="2">Cumulative accuracy (%bias) in three QCs:</td></tr> <tr> <td>QC 0</td><td>88.0 to 108.4%</td></tr> <tr> <td>QC 1</td><td>96.0 to 106.7%</td></tr> <tr> <td>QC 2</td><td>97.1 to 107.7%</td></tr> <tr> <td colspan="2">Interbatch %CV:</td></tr> <tr> <td>QC 0</td><td>≤6.44%</td></tr> <tr> <td>QC 1</td><td>≤4.65%</td></tr> <tr> <td>QC 2</td><td>≤5.52%</td></tr> <tr> <td>QC 3</td><td>≤3.43%</td></tr> <tr> <td>QC 4</td><td>≤3.22%</td></tr> <tr> <td>QC 5</td><td>≤2.19%</td></tr> <tr> <td>Total error</td><td>Not applicable</td></tr> </table>	Cumulative accuracy (%bias) in three QCs:		QC 0	88.0 to 108.4%	QC 1	96.0 to 106.7%	QC 2	97.1 to 107.7%	Interbatch %CV:		QC 0	≤6.44%	QC 1	≤4.65%	QC 2	≤5.52%	QC 3	≤3.43%	QC 4	≤3.22%	QC 5	≤2.19%	Total error	Not applicable
Cumulative accuracy (%bias) in three QCs:																									
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QC 3	≤3.43%																								
QC 4	≤3.22%																								
QC 5	≤2.19%																								
Total error	Not applicable																								
Selectivity and matrix effect	Matrix Factor The matrix factor experiment was conducted to evaluate the suppression or enhancement of ionization of analytes by the presence of matrix components in the sample extracts. Matrix factor samples (MF) from eight different individual matrix lots were fortified post extraction to approximate low- and high-concentration QC levels. The matrix lots included four normal human plasma lots, two hemolyzed lots (5% hemolysis), and two lipemic lots (>300 mg/dL triglyceride) and were analyzed (n=1) along with one injection of a comparable external standard at each concentration level. The analyte responses of the fortified matrix samples were divided by the response of the external standard prepared at the same analyte concentrations in neat solution (free from matrix components). The matrix factor results were also calculated using peak response ratios (IS-normalized). While the coefficient of variation calculated using individual component responses was >15%, the matrix factor results were deemed acceptable since the coefficient of variation met the criteria of ≤15% at each level for the IS-normalized evaluation indicating that matrix effects were consistent across the lots tested. Recovery The general extraction recovery of the analyte and internal standard from human plasma was evaluated by comparing the analyte responses of pre-extraction fortified samples to those of post extraction fortified samples representing 100% recovery. The overall cPMP recovery was calculated to be 36.2% and that of the internal standard 34.4%.																								

Bioanalytical method validation report name, amendments, and hyperlinks	• Method Validation Report: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; April 2014 • Method Validation Report Addendum 1: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; April 2016 • Method Validation Report Addendum 2: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; February 2020												
Interference and specificity	Specificity Human plasma samples from six individuals were extracted and analyzed for cPMP and the internal standard. There were no significant chromatographic peaks detected at the mass transitions and expected retention times of the internal standard that would interfere with quantitation. There were peaks detected at the mass transitions and expected retention time of the analyte in three out of the six specificity samples fortified with internal standard that was >20% of the mean peak area response ratio of the LLOQ. A review of these data indicates that there is an approximate 10% contribution to cPMP from the labeled internal standard. Since the specificity double blanks do not contain a similar peak and the fortified specificity experiment (see below) was found to quantitate acceptably (and was evaluated using the same specificity lots), selectivity is not deemed to be compromised. Additional specificity samples, fortified with cPMP at 5.00 ng/mL, were prepared from six individuals and analyzed in triplicate to evaluate potential matrix suppression effects. A seventh lot (SPF 7), prepared from the matrix pool used to prepare the calibration standards and quality controls, was analyzed as a control. There were no significant matrix suppression effects indicated that could compromise the sensitivity or accuracy of the assay.												
Hemolysis effect	The effect of hemolysis on the quantitation of cPMP was evaluated by analyzing blanks, with and without internal standard, and low- and high-level QCs (HEMQC), fortified with hemolyzed human whole blood according to the validation plan to represent 5% hemolysis. There were no significant chromatographic peaks detected at the mass transitions and expected retention times of the analyte or the internal standard that would interfere with quantitation. There was no effect from hemolysis on the quantitation of cPMP. <table><tr><th>Hemolysis Evaluation</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td>QC 1</td><td>12.0 ng/mL</td><td>106.40%</td><td>5.09%</td></tr><tr><td>QC 5</td><td>750 ng/mL</td><td>96.41%</td><td>1.92%</td></tr></table>	Hemolysis Evaluation	Concentration	Recovery	CV	QC 1	12.0 ng/mL	106.40%	5.09%	QC 5	750 ng/mL	96.41%	1.92%
Hemolysis Evaluation	Concentration	Recovery	CV										
QC 1	12.0 ng/mL	106.40%	5.09%										
QC 5	750 ng/mL	96.41%	1.92%										
Lipemic effect	The effect of lipemia on the quantitation of cPMP was evaluated by analyzing blanks, with and without internal standard, and low- and high-level QCs (LIPQC), prepared in lipemic human plasma with a triglyceride concentration of >300 mg/dL. A peak was detected at the mass transitions and expected retention time of the analyte in the lipemic sample fortified with internal standard (LP/IS 1-1) that was >20% (20.1%) of the mean peak area response ratio of the LLOQ. A review of the analyte interference data indicates that there is an approximate 10% contribution to cPMP from the labeled internal standard. Since the lipemic double blank sample (LP 1-1) does not contain a similar peak and the fortified lipemic sample experiments (LIPQC 1 and LIPQC 5) were found to quantitate acceptably (and were evaluated using the same lipemic plasma pool), selectivity of lipemic samples is deemed not to be compromised. There was no effect of lipemia on the quantitation of cPMP. <table><tr><th>Lipemia Evaluation</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td>QC 1</td><td>12.0 ng/mL</td><td>102.18%</td><td>4.92%</td></tr><tr><td>QC 5</td><td>750 ng/mL</td><td>99.77%</td><td>2.43%</td></tr></table>	Lipemia Evaluation	Concentration	Recovery	CV	QC 1	12.0 ng/mL	102.18%	4.92%	QC 5	750 ng/mL	99.77%	2.43%
Lipemia Evaluation	Concentration	Recovery	CV										
QC 1	12.0 ng/mL	102.18%	4.92%										
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Dilution linearity and hook effect	The ability to analyze samples with insufficient volume for a full aliquot was validated by analyzing six replicate QCs, containing 75.0 ng/mL cPMP, as two-and-a-half-fold dilutions (using blank human plasma as the diluent). The ability to dilute samples originally above the upper limit of the calibration range was validated by analyzing six replicate QCs, containing 2000 or 4000 ng/mL cPMP, as four-fold dilutions (using blank human plasma as the diluent). The intra-assay quality control data for the diluted QC pools met the performance criteria. <table><tr><th>Dilution Integrity</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td>DF=2.5 QC 3</td><td>75.0 ng/mL</td><td>90.82%</td><td>6.96%</td></tr><tr><td>DF=4 QC 6</td><td>2000 ng/mL</td><td>96.75%</td><td>1.75%</td></tr><tr><td>DF=4 QC 7</td><td>4000 ng/mL</td><td>95.86%</td><td>4.27%</td></tr></table> Hook effect: Not applicable for chromatographic assays.	Dilution Integrity	Concentration	Recovery	CV	DF=2.5 QC 3	75.0 ng/mL	90.82%	6.96%	DF=4 QC 6	2000 ng/mL	96.75%	1.75%	DF=4 QC 7	4000 ng/mL	95.86%	4.27%																																
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DF=4 QC 6	2000 ng/mL	96.75%	1.75%																																														
DF=4 QC 7	4000 ng/mL	95.86%	4.27%																																														
Benchtop/process stability	Thawed Matrix Stability This was evaluated by allowing a set of low- and high-level quality controls to thaw and remain at room temperature for 24 hr prior to extraction and analysis. The thawed matrix stability data met the acceptance criteria. <table><tr><th>Thawed Matrix Stability</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td>QC 1</td><td>12.0 ng/mL</td><td>97.02%</td><td>5.30%</td></tr><tr><td>QC 5</td><td>750 ng/mL</td><td>92.04%</td><td>2.94%</td></tr></table> Post-preparative Extract Stability Post-preparative extract stability was evaluated by analyzing quality controls that were extracted and injected and stored at 2-8°C for approximately 127 hr prior to reanalysis versus freshly prepared calibrators. The post-preparative extract stability data met the acceptance criteria. <table><tr><th>Post-preparative Extract</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td>QC 1</td><td>12.0 ng/mL</td><td>100.46%</td><td>5.06%</td></tr><tr><td>QC 5</td><td>750 ng/mL</td><td>98.54%</td><td>2.98%</td></tr></table> Reinjection Reproducibility Reinjection reproducibility (RR) was evaluated by analyzing calibration standards and quality controls extracted and injected and stored at 2-8°C prior to and during reanalysis. The reinjection reproducibility data met the acceptance criteria. <table><tr><th>Sample</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td>RR 1</td><td>12.0 ng/mL</td><td>96.30%</td><td>2.95%</td></tr><tr><td>RR 2</td><td>25.0 ng/mL</td><td>97.42%</td><td>5.04%</td></tr><tr><td>RR 3</td><td>75.0 ng/mL</td><td>96.76%</td><td>3.71%</td></tr><tr><td>RR 4</td><td>200 ng/mL</td><td>96.05%</td><td>1.71%</td></tr><tr><td>RR 5</td><td>750 ng/mL</td><td>97.83%</td><td>2.05%</td></tr></table>	Thawed Matrix Stability	Concentration	Recovery	CV	QC 1	12.0 ng/mL	97.02%	5.30%	QC 5	750 ng/mL	92.04%	2.94%	Post-preparative Extract	Concentration	Recovery	CV	QC 1	12.0 ng/mL	100.46%	5.06%	QC 5	750 ng/mL	98.54%	2.98%	Sample	Concentration	Recovery	CV	RR 1	12.0 ng/mL	96.30%	2.95%	RR 2	25.0 ng/mL	97.42%	5.04%	RR 3	75.0 ng/mL	96.76%	3.71%	RR 4	200 ng/mL	96.05%	1.71%	RR 5	750 ng/mL	97.83%	2.05%
Thawed Matrix Stability	Concentration	Recovery	CV																																														
QC 1	12.0 ng/mL	97.02%	5.30%																																														
QC 5	750 ng/mL	92.04%	2.94%																																														
Post-preparative Extract	Concentration	Recovery	CV																																														
QC 1	12.0 ng/mL	100.46%	5.06%																																														
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RR 1	12.0 ng/mL	96.30%	2.95%																																														
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RR 4	200 ng/mL	96.05%	1.71%																																														
RR 5	750 ng/mL	97.83%	2.05%																																														
Freeze-thaw stability	Freeze-thaw stability was evaluated by analyzing two sets of low- and high-level quality controls subjected to five freeze-thaw cycles. Samples were thawed at room temperature. One set was frozen at -20°C (FTF) and the other at -70°C (FTC) for each freeze cycle. The freeze/thaw stability data met the acceptance criteria <table><tr><th colspan="2">Freeze-Thaw Stability</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td rowspan="2">-20°C</td><td>QC 1</td><td>12.0 ng/mL</td><td>94.42%</td><td>3.40%</td></tr><tr><td>QC 5</td><td>750 ng/mL</td><td>90.36%</td><td>1.65%</td></tr><tr><td rowspan="2">-70°C</td><td>QC 1</td><td>12.0 ng/mL</td><td>99.62%</td><td>5.50%</td></tr><tr><td>QC 5</td><td>750 ng/mL</td><td>100.96%</td><td>1.63%</td></tr></table>	Freeze-Thaw Stability		Concentration	Recovery	CV	-20°C	QC 1	12.0 ng/mL	94.42%	3.40%	QC 5	750 ng/mL	90.36%	1.65%	-70°C	QC 1	12.0 ng/mL	99.62%	5.50%	QC 5	750 ng/mL	100.96%	1.63%																									
Freeze-Thaw Stability		Concentration	Recovery	CV																																													
-20°C	QC 1	12.0 ng/mL	94.42%	3.40%																																													
	QC 5	750 ng/mL	90.36%	1.65%																																													
-70°C	QC 1	12.0 ng/mL	99.62%	5.50%																																													
	QC 5	750 ng/mL	100.96%	1.63%																																													

Bioanalytical method validation report name, amendments, and hyperlinks	• Method Validation Report: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; April 2014 • Method Validation Report Addendum 1: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; April 2016 • Method Validation Report Addendum 2: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; February 2020																																																
Long-term storage	Analyte stability in frozen matrix containing 4 mg/mL ascorbic acid or 16 mg/mL ascorbic acid was evaluated by analyzing samples which had been stored at -20°C (STABF) and -70°C (STABC) versus freshly prepared calibration standards. The analyte in frozen matrix stability data met the acceptance criteria. <table><tr><th>With 4 mg/mL Ascorbic Acid</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td rowspan="2">190 Days At -70°C</td><td>QC 1</td><td>12.0 ng/mL</td><td>106.77%</td><td>7.96%</td></tr><tr><td>QC 5</td><td>750 ng/mL</td><td>100.29%</td><td>4.44%</td></tr><tr><td rowspan="2">56 Days At -20°C</td><td>QC 1</td><td>12.0 ng/mL</td><td>91.12%</td><td>2.97%</td></tr><tr><td>QC 5</td><td>750 ng/mL</td><td>93.79%</td><td>1.63%</td></tr><tr><th colspan="5">With 16 mg/mL Ascorbic Acid</th></tr><tr><td rowspan="2">54 Days At -20°C</td><td>QC 1</td><td>12.0 ng/mL</td><td>99.71%</td><td>8.52%</td></tr><tr><td>QC 5</td><td>750 ng/mL</td><td>103.39%</td><td>2.21%</td></tr><tr><td rowspan="2">142 Days At -70°C</td><td>QC 1</td><td>12.0 ng/mL</td><td>94.36%</td><td>7.19%</td></tr><tr><td>QC 5</td><td>750 ng/mL</td><td>97.18%</td><td>3.20%</td></tr></table>				With 4 mg/mL Ascorbic Acid	Concentration	Recovery	CV	190 Days At -70°C	QC 1	12.0 ng/mL	106.77%	7.96%	QC 5	750 ng/mL	100.29%	4.44%	56 Days At -20°C	QC 1	12.0 ng/mL	91.12%	2.97%	QC 5	750 ng/mL	93.79%	1.63%	With 16 mg/mL Ascorbic Acid					54 Days At -20°C	QC 1	12.0 ng/mL	99.71%	8.52%	QC 5	750 ng/mL	103.39%	2.21%	142 Days At -70°C	QC 1	12.0 ng/mL	94.36%	7.19%	QC 5	750 ng/mL	97.18%	3.20%
With 4 mg/mL Ascorbic Acid	Concentration	Recovery	CV																																														
190 Days At -70°C	QC 1	12.0 ng/mL	106.77%	7.96%																																													
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Parallelism	Not applicable for chromatographic assays																																																
Carry over	The potential for carryover from a sample containing a high concentration of analyte to the following sample in an injection sequence was evaluated by injecting duplicate extracted matrix blanks immediately after the ULOQ calibration standards in each validation run. There were no contributions from chromatographic peaks, at the expected retention time of the analyte in the blank samples, greater than 20% of the mean analyte response for the LLOQ calibration standards and quality controls in the validation run																																																
Method Performance in Study Number ALX1101-MCD-201																																																	
Assay passing rate	43/50 runs (86.00%) acceptable																																																
Standard curve performance	• Cumulative bias range: 98.43 to 101.71% • Cumulative precision: ≤6.58% CV																																																
QC performance	• Cumulative bias range: 96.70 to 98.15% • Cumulative precision: ≤9.31% CV																																																
Method reproducibility	Incurred sample reanalysis was performed in 9.01% of study samples and 90.0% of samples met the prespecified criteria.																																																
Study sample analysis/stability	A maximum of 337 days passed between sample collection and analysis. The currently demonstrated matrix stability timeframe of 200 days is insufficient to cover the time from sample collection to sample analysis for the study samples.																																																
Standard calibration curve performance in accuracy and precision runs	Standard	Concentration	Standard	Concentration																																													
	Std 1 (LLOQ)	5.00 ng/mL	Std 5	125 ng/mL																																													
	Std 2	9.00 ng/mL	Std 6	350 ng/mL																																													
	Std 3	15.0 ng/mL	Std 7	800 ng/mL																																													
	Std 4	40.0 ng/mL	Std 8 (ULOQ)	1000 ng/mL																																													
Method Performance in Study Number ALX1101-MCD-202																																																	
Assay passing rate	18/24 runs (75.00%) acceptable																																																
Standard curve performance	• Cumulative bias range: 98.86 to 101.93% • Cumulative precision: ≤6.99% CV																																																
QC performance	• Cumulative bias range: 96.55 to 99.37% • Cumulative precision: ≤8.28% CV																																																
Method reproducibility	Incurred sample reanalysis was performed in 9.09% of study samples and 100% of samples met the prespecified criteria.																																																

Bioanalytical method validation report name, amendments, and hyperlinks	• Method Validation Report: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; April 2014 • Method Validation Report Addendum 1: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; April 2016 • Method Validation Report Addendum 2: Quantitation of cPMP in Human Plasma via HPLC with MS/MS Detection; February 2020			
Study sample analysis/stability	A maximum of 198 days passed between sample collection and analysis. All samples were analyzed within the 200 days demonstrated long-term storage stability in human plasma containing dipotassium EDTA at -80°C.			
Standard calibration curve performance in accuracy and precision runs	Standard	Concentration	Standard	Concentration
	Std 1 (LLOQ)	5.00 ng/mL	Std 5	125 ng/mL
	Std 2	9.00 ng/mL	Std 6	350 ng/mL
	Std 3	15.0 ng/mL	Std 7	800 ng/mL
	Std 4	40.0 ng/mL	Std 8 (ULOQ)	1000 ng/mL

Abbreviations: cPMP, cyclic pyranopterin monophosphate; CV, coefficient of variation; EDTA, ethylenediaminetetraacetic acid; HPLC, high-performance liquid chromatography; IS, internal standard; LLOQ, lower limit of quantification; MS, mass spectrometry; PBS, phosphate-buffered saline; QC, quality control; ULOQ, upper limit of quantification

Methods MET129 and LCMSC 669 were also cross-validated at (b) (4) and the methods were deemed comparable.

Clinical plasma samples for the PD biomarkers of Study MCD-201 were partly analyzed using the original validated method (LCMS 636.1; [Table 47](#)). The method was changed during the study, and as of May 12, 2015, the remaining samples were analyzed by the revalidated method (LCMSC 636.1 Project), which was subsequently used in Study MCD-202. The summary of validation parameters is presented in [Table 48](#).

Table 47. Method LCMS 636.1-Bioanalytical Method Validation for S-Sulfocysteine Determination in Human Plasma

Bioanalytical method validation report name, amendments, and hyperlinks	• Method Validation Report LCMS 636.1-Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection; Sep 2013; Project BTZ2 • Method Validation Report Addendum 1 LCMS 636.1-Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection Feb 2014; Project BTZ3	
Method description	High-performance liquid chromatography (HPLC) with tandem mass spectrometry (LC-MS/MS).	
Materials used for calibration curve and concentration	S-Sulphocysteine; Dulbecco's PBS	
	Standard	Concentration (Theoretical)
	CAL 1 LLOQ	0.0250 µg/mL
	CAL 2	0.0400 µg/mL
	CAL 3	0.0750 µg/mL
	CAL 4	0.200 µg/mL
	CAL 5	0.625 µg/mL
	CAL 6	1.75 µg/mL
	CAL 7	4.00 µg/mL
	CAL 8 ULOQ	5.00 µg/mL
Validated assay range	0.0250 to 5.00 µg/mL	

Bioanalytical method validation report name, amendments, and hyperlinks	• Method Validation Report LCMS 636.1–Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection; Sep 2013; Project BTZ2		
	• Method Validation Report Addendum 1 LCMS 636.1–Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection Feb 2014; Project BTZ3		
	Material used for QC and concentration		
	Control	Concentration (Theoretical)	Matrix
	QC 0	0.0250 µg/mL	Surrogate
	QC 1	0.0600 µg/mL	Surrogate
	QC 2	0.125 µg/mL	Surrogate
	QC 3	0.375 µg/mL	Surrogate
	QC 4	1.00 µg/mL	Surrogate
	QC 5	3.75 µg/mL	Surrogate
Minimum required dilutions	1:25.5		
Source and lot of reagents (LBA)	Analyte	Source	Lot
	S-Sulphocysteine	(b) (4)	9-DSD-60-2
	S-Sulphocysteine-d3		E537P18
	Dulbecco's PBS (10×)		Product 20-031-CV
	Dulbecco's PBS		Product D8537
	Human plasma		N/A
Regression model and weighting	Linear, weighted 1/x ²		
Validation parameters			
Standard calibration curve performance during accuracy and precision	Number of standard calibrators from LLOQ to ULOQ		8
	Cumulative accuracy (%bias) from LLOQ to ULOQ		98.6 to 101.3%
	Cumulative precision (%CV) from LLOQ to ULOQ		≤6.9%
QC performance during accuracy and precision	Cumulative accuracy (%bias) in four QCs:		
	QC 0		95.66 to 101.99%
	QC 1		100.81 to 101.79%
	QC 2		102.21 to 102.34%
	QC 3		100.39 to 102.99%
	QC 4		98.32 to 103.38%
	QC 5		97.64 to 98.66%
	QC 11		93.89 to 99.85%
	QC 15		91.22 to 96.33%
	Interbatch %CV		
	QC 0		≤5.47%
	QC 1		≤4.57%
	QC 2		≤2.56%
	QC 3		≤2.19%
QC 4		≤2.46%	
QC 5		≤1.46%	
QC 11		≤3.11%	
QC 15		≤2.80%	
Selectivity and matrix effect	S-Sulphocysteine is an endogenous compound; therefore, measurable levels are expected to be present in human plasma. The validation was conducted using samples prepared in two types of matrix: human plasma containing dipotassium EDTA and an analyte-free surrogate matrix of Dulbecco's PBS.		

Bioanalytical method validation report name, amendments, and hyperlinks	• Method Validation Report LCMS 636.1–Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection; Sep 2013; Project BTZ2 • Method Validation Report Addendum 1 LCMS 636.1–Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection Feb 2014; Project BTZ3																
	Six lots of human plasma spiked with S-sulfocysteine at 3.00 µg/mL. • Accuracy 96.7 to 99.6% • CV ≤1.06% General extraction recovery of S-sulfocysteine from human plasma was evaluated by comparing the analyte responses of pre-extraction fortified samples to those of post-extraction fortified samples. Overall recovery of S-sulfocysteine was 82.3%.																
Interference and specificity	Chromatographic Considerations Because S-sulfocysteine is an endogenous compound, chromatographic peaks were detected at the expected mass transitions and retention times for S-sulfocysteine, xanthine, and uric acid. There were no significant chromatographic peaks detected at the mass transitions and expected retention times of the internal standards that would interfere with quantitation. Matrix-Related Ion Suppression Interference The potential for variable matrix-related ion suppression interference was evaluated by fortifying human plasma samples from six different individuals. Endogenous levels of S-sulfocysteine and xanthine were measured by analyzing samples fortified with only internal standard in triplicate. The same sample lots were then fortified with approximately 0.500 µg/mL S-sulfocysteine and internal standard at the level of use. The results indicated that matrix suppression effects do not compromise the accuracy of the assay. Cross-Analyte Interference No significant cross-analyte interference was noted with the quantitation of S-sulfocysteine in human plasma. Concomitant Medication Interference An interference check was performed to determine if samples fortified with acetaminophen, caffeine, acetylsalicylic acid, salicylic acid, ibuprofen, naproxen, chlorpheniramine, ethinyl estradiol, norgestrel or norethindrone interfered with the quantitation of S-sulfocysteine in human plasma. Results indicated there was no effect on the quantitation of S-sulfocysteine in human plasma fortified with these compounds to the stated concentrations.																
Hemolysis effect	The effect of hemolysis on the quantitation of S-sulfocysteine was evaluated by analyzing surrogate matrix and human plasma blanks, prepared in hemolyzed human plasma containing 5% fully lysed whole blood. The endogenous levels of S-sulfocysteine (0.220 µg/mL) and xanthine (0.153 µg/mL) were determined by analyzing the blank hemolyzed human plasma pool in triplicate. The sum of the fortified amount plus the endogenous level of each analyte was used as the “theoretical concentration.” There were no significant chromatographic peaks detected at the mass transitions and expected retention times of the analyte or the internal standard that would interfere with quantitation. There was no effect from hemolysis on the quantitation of S-sulfocysteine. <table><tr><th>Hemolysis Effect (n=3)</th><th>Sample</th><th>Recovery</th><th>CV</th></tr><tr><td>Plasma</td><td>0.220 µg/mL</td><td>99.84%</td><td>1.07%</td></tr><tr><td>QC 11</td><td>0.420 µg/mL</td><td>98.68%</td><td>2.21%</td></tr><tr><td>QC 15</td><td>3.22 µg/mL</td><td>98.35%</td><td>2.54%</td></tr></table>	Hemolysis Effect (n=3)	Sample	Recovery	CV	Plasma	0.220 µg/mL	99.84%	1.07%	QC 11	0.420 µg/mL	98.68%	2.21%	QC 15	3.22 µg/mL	98.35%	2.54%
Hemolysis Effect (n=3)	Sample	Recovery	CV														
Plasma	0.220 µg/mL	99.84%	1.07%														
QC 11	0.420 µg/mL	98.68%	2.21%														
QC 15	3.22 µg/mL	98.35%	2.54%														
Lipemic effect	The effect of lipemia on the quantification of S-sulfocysteine was not evaluated.																

Bioanalytical method validation report name, amendments, and hyperlinks	- Method Validation Report LCMS 636.1–Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection; Sep 2013; Project BTZ2 - Method Validation Report Addendum 1 LCMS 636.1–Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection Feb 2014; Project BTZ3				
Dilution linearity and hook effect	The ability to analyze samples with insufficient volume for a full aliquot was validated by analyzing six replicate human plasma QCs, containing 3.25 µg/mL S-sulfocysteine, as 2-fold dilution. The ability to dilute samples originally above the upper limit of the calibration range was validated by analyzing six replicate human plasma QCs, containing 10.2 µg/mL S-sulfocysteine, as ten-fold dilutions.				
		Dilution	Recovery	CV	
	Dilution linearity (n=6)	1:2	98.76%	1.39%	
		1:10	92.99%	3.48%	
	Hook effect: Not applicable for chromatographic assays.				
Bench-top/process stability	Thawed Analyte Stability Thawed analyte stability was evaluated by evaluating QC controls [six replicates of two surrogate buffer (QC 1 and 5) and two plasma (QC 11 and 15)] that had been thawed and kept on wet ice for 4 or 25 hr prior to extraction. Thawed analytes were stable for up to 25 hr on wet ice prior to extraction.				
	Time on Wet Ice	Sample	Concentration	Recovery	CV
	4 hr	QC 1	0.0600 µg/mL	98.28%	3.22%
		QC 5	3.75 µg/mL	96.71%	2.91%
		QC 11	0.446 µg/mL	99.66%	3.26%
		QC 15	3.25 µg/mL	93.92%	3.81%
	25 hr	QC 1	0.0600 µg/mL	102.00%	5.19%
		QC 5	3.75 µg/mL	97.72%	1.92%
		QC 11	0.446 µg/mL	114.30%	3.15%
		QC 15	3.25 µg/mL	90.78%	3.83%
	Reinjection Reproducibility Reinjection reproducibility was demonstrated by analyzing surrogate matrix calibration and human plasma quality controls that were extracted and injected then stored at 15°C prior to and during reanalysis. Reanalysis was demonstrated to be reproducible.				
	Reinjection Reproducibility		Concentration	Recovery	CV
	QC 1		0.0600 µg/mL	107.95%	2.43%
	QC 2		0.125 µg/mL	104.69%	3.33%
	QC 3		0.375 µg/mL	102.68%	2.78%
	QC 4		1.00 µg/mL	102.32%	2.54%
	QC 5		3.75 µg/mL	98.46%	1.66%
	QC 11		0.446 µg/mL	94.76%	6.13%
	QC 15		3.25 µg/mL	96.62%	2.44%

Bioanalytical method validation report name, amendments, and hyperlinks	- Method Validation Report LCMS 636.1–Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection; Sep 2013; Project BT22 - Method Validation Report Addendum 1 LCMS 636.1–Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection Feb 2014; Project BTZ3				
	Post-preparative Extract Stability				
	Post-preparative extract stability was evaluated by analyzing QC controls that were extracted and tested then stored at 15°C for approximately 89 or 138 hr prior to reanalysis versus freshly prepared calibrators.				
	Post-preparative extract was demonstrated to be stable for 138 hr at 15°C.				
	Duration	Sample	Concentration	Recovery	CV
	89 hr	QC 1	0.0600 µg/mL	102.74%	5.16%
		QC 5	3.75 µg/mL	97.24%	2.10%
		QC 11	0.446 µg/mL	95.80%	1.66%
		QC 15	3.25 µg/mL	94.56%	0.885%
	38 hr	QC 1	0.0600 µg/mL	104.83%	3.85%
QC 5		3.75 µg/mL	100.72%	2.00%	
QC 11		0.446 µg/mL	97.39%	1.78%	
QC 15		3.25 µg/mL	97.25%	2.43%	
Whole Blood Stability					
Whole blood was fortified at approximately 3.00 µg/mL of S-sulfocysteine processed after 1 to 2 hr on ice. A control sample was prepared in whole blood and immediately processed to plasma using a 2-8°C centrifuge or a room temperature centrifuge.					
S-sulfocysteine in whole blood was demonstrated not to be stable for 2 hr on ice regardless of whether the centrifuge was refrigerated or not.					

Bioanalytical method validation report name, amendments, and hyperlinks	- Method Validation Report LCMS 636.1–Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection; Sep 2013; Project BTZ2 - Method Validation Report Addendum 1 LCMS 636.1–Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection Feb 2014; Project BTZ3																																																										
Long-term storage	Analyte Stability in Surrogate Solution S-Sulfocysteine stability in frozen surrogate matrix (QC 1 and 5) were evaluated by analyzing samples which had been stored for 32 days at –20°C or –70°C. S-Sulfocysteine in frozen surrogate matrix was demonstrated to be stable for 32 days when stored at –20°C or –70°C. <table><tr><th>Temperature</th><th>Sample</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td rowspan="2">–20°C</td><td>QC 1</td><td>0.0600 µg/mL</td><td>103.96%</td><td>3.4%</td></tr><tr><td>QC 5</td><td>3.75 µg/mL</td><td>100.42%</td><td>1.88%</td></tr><tr><td rowspan="2">–70°C</td><td>QC 1</td><td>0.0600 µg/mL</td><td>103.91%</td><td>5.17%</td></tr><tr><td>QC 5</td><td>3.75 µg/mL</td><td>100.05%</td><td>1.40%</td></tr></table> Long-term storage of S-sulfocysteine in surrogate matrix was evaluated after storage for 201 days at –70°C by testing versus freshly prepared calibration standards (six replicates). <table><tr><th>S-Sulphocysteine Sample</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td>STABC1</td><td>0.0600 µg/mL</td><td>108.52%</td><td>5.21%</td></tr><tr><td>STABC5</td><td>3.75 µg/mL</td><td>96.51%</td><td>3.08%</td></tr></table> Analyte Stability in Plasma S-Sulphocysteine stability in plasma (QC 11 and 15) were evaluated by analyzing samples which had been stored for 30 days at –20°C or –70°C. S-Sulphocysteine in frozen plasma was demonstrated not to be stable for 30 days when stored at –20°C but was stable for 30 days when stored at –70°C. <table><tr><th>Temperature</th><th>Sample</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td rowspan="2">–20°C</td><td>QC 11</td><td>0.446 µg/mL</td><td>39.80%</td><td>4.00%</td></tr><tr><td>QC 15</td><td>3.25 µg/mL</td><td>21.7%</td><td>3.16%</td></tr><tr><td rowspan="2">–70°C</td><td>QC 11</td><td>0.446 µg/mL</td><td>96.57%</td><td>1.90%</td></tr><tr><td>QC 15</td><td>3.25 µg/mL</td><td>96.40%</td><td>2.12%</td></tr></table>	Temperature	Sample	Concentration	Recovery	CV	–20°C	QC 1	0.0600 µg/mL	103.96%	3.4%	QC 5	3.75 µg/mL	100.42%	1.88%	–70°C	QC 1	0.0600 µg/mL	103.91%	5.17%	QC 5	3.75 µg/mL	100.05%	1.40%	S-Sulphocysteine Sample	Concentration	Recovery	CV	STABC1	0.0600 µg/mL	108.52%	5.21%	STABC5	3.75 µg/mL	96.51%	3.08%	Temperature	Sample	Concentration	Recovery	CV	–20°C	QC 11	0.446 µg/mL	39.80%	4.00%	QC 15	3.25 µg/mL	21.7%	3.16%	–70°C	QC 11	0.446 µg/mL	96.57%	1.90%	QC 15	3.25 µg/mL	96.40%	2.12%
Temperature	Sample	Concentration	Recovery	CV																																																							
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Parallelism	Not applicable for chromatographic assays																																																										
Carry over	The potential for carryover from a sample containing a high concentration of analyte to the following sample in an injection sequence was evaluated by injecting duplicate extracted matrix surrogate matrix blanks immediately after the ULOQ calibration standards in each validation run. There were contributions from chromatographic peaks, at the expected retention time of the xanthine in the blank samples, greater than 20% of the mean analyte response for the LLOQ calibration standards and quality controls in the validation runs ranging from 21.1 to 35.1%. To minimize the impact of potential carryover, study samples were analyzed in pharmacokinetic order and two matrix blanks were injected after high-concentration samples that preceded low-concentration samples.																																																										

Abbreviations: CV, coefficient of variation; EDTA, ethylene diaminetetraacetic acid; LLOQ, lower limit of quantification; PBS, phosphate-buffered saline; QC, quality control; RT, room temperature; ULOQ, upper limit of quantification

Abbreviations: CV, coefficient of variation; EDTA, ethylene diaminetetraacetic acid; LLOQ, lower limit of quantification; PBS, phosphate-buffered saline; QC, quality control; RT, room temperature; ULOQ, upper limit of quantification

Table 48. Method LCMS 636.1—Bioanalytical Method Validation for S-Sulfocysteine Determination in Human Plasma

Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Report LCMS 636.1–Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection; May 2016
Method description	High-performance liquid chromatography (HPLC) with tandem mass spectrometry (LC-MS/MS).

Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Report LCMSC 636.1–Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection; May 2016		
Materials used for calibration curve and concentration	S-Sulphocysteine, Dulbecco's PBS		
	Standard	Concentration (Theoretical)	
	CAL 1 LLOQ	0.124µM	
	CAL 2	0.199µM	
	CAL 3	0.373µM	
	CAL 4	0.994µM	
	CAL 5	3.11µM	
	CAL 6	8.70µM	
	CAL 7	19.9µM	
CAL 8 ULOQ	24.9µM		
Validated assay range	0.0250 to 5.00 µg/mL		
Material used for QCs and concentration	S-Sulphocysteine, Dulbecco's PBS, human plasma		
	Control	Concentration (Theoretical)	Matrix
	QC 0	0.124µM	Surrogate
	QC 1	0.298µM	Surrogate
	QC 3	1.86µM	Surrogate
	QC 5	18.6µM	Surrogate
	QC 11	0.586µM	Plasma
	QC 15	18.7µM	Plasma
Minimum required dilutions	1:14.5		
Source and lot of reagents (LBA)	Analyte	Source	Lot
	S-Sulphocysteine	(b) (4)	9-DSD-60-2
	S-Sulphocysteine-d3		E537P18
	Dulbecco's PBS (10×)		Product 20-031-CV
	Dulbecco's PBS		Product D8537
Human plasma	N/A		
Regression model and weighting	Linear, weighted 1/x²		
Validation parameters			
Standard calibration curve performance during accuracy and precision	Number of standard calibrators from LLOQ to ULOQ		8
	Cumulative accuracy (%bias) from LLOQ to ULOQ		97.94 to 101.38%
	Cumulative precision (%CV) from LLOQ to ULOQ		≤6.22%
QC performance during accuracy and precision	Cumulative accuracy (%bias) in four QCs:		
	QC 0	97.92 to 104.11%	
	QC 1	99.70 to 101.53%	
	QC 3	98.45 to 104.10%	
	QC 5	97.30 to 103.33%	
	QC 11	97.54 to 102.27%	
	QC 15	98.63 to 102.61%	
	Interbatch %CV		
	QC 0	≤9.42%	
	QC 1	≤4.81%	
	QC 3	≤4.02%	
	QC 5	≤3.68%	
QC 11	≤5.55%		
QC 15	≤4.57%		
Total error: Not applicable			

Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Report LCMSC 636.1–Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection; May 2016																	
Selectivity and matrix effect	S-Sulphocysteine is an endogenous compound; therefore, measurable levels are expected to be present in human plasma. The validation was conducted using samples prepared in two types of matrix: human plasma containing dipotassium EDTA and an analyte-free surrogate matrix of Dulbecco's PBS. Recovery of the analyte and internal standard was carried out at low and high levels in surrogate matrix by comparing responses of pre-extraction fortified samples to those of post-extraction fortified samples representing 100% recovery. Due to the endogenous nature of the analyte, recovery in primary matrix was evaluated using the labeled internal standard only; high concentration primary matrix QCs were fortified pre- and post-extraction with internal standard and responses compared. Overall recovery of S-sulfocysteine was 101%.																	
Interference and specificity	Chromatographic Considerations Because S-sulfocysteine is an endogenous compound, chromatographic peaks were detected at the expected mass transitions and retention times for S-sulfocysteine. There were no significant chromatographic peaks detected at the mass transitions and expected retention times of the internal standards that would interfere with quantitation. Matrix-Related Ion Suppression Interference The potential for variable matrix-related ion suppression interference was evaluated by fortifying human plasma samples from six different individuals. Endogenous levels of S-sulfocysteine were measured by analyzing samples fortified with only internal standard in triplicate. The same sample lots were then fortified with approximately 0.497µM SSC in addition to endogenous levels for lot 1, 2, 3, and 4, and 0.248µM SSC for lots 5 and 6. The theoretical concentration for each SPF lot was the sum of the endogenous level plus the amount fortified. The results indicated that matrix suppression effects do not compromise the accuracy of the assay.																	
Hemolysis effect	The effect of hemolysis on the quantitation of SSC was evaluated by analyzing hemolyzed surrogate matrix with (HSIMS) and without (HSM) internal standard and low- and high-level QCs (HEMQC) prepared from human plasma fortified with 5% hemolyzed human blood. Aliquots of each low- and high human plasma quality control sample were fortified with 2.5 µL of hemolyzed whole blood (equivalent to 5% of volume) and extracted per the method. Due to the endogenous nature of the analytes, the whole blood used for hemolysis testing was expected to contain measurable baseline levels. In order to correct for this, aliquots of surrogate matrix were prepared containing 2.5 µL of hemolyzed whole blood (equivalent to 5% of volume); these samples were fortified with internal standard (HSMIS) and were analyzed (n=6) to determine baseline levels. The nominal concentration for each level was the sum of the baseline concentration plus the established theoretical QC concentration. Because the analytes are endogenous compounds, chromatographic peaks were detected at the expected mass transition and expected retention time xanthine and uric acid in the blank (HSM) sample. There were no significant chromatographic peaks detected at the mass transition and expected retention time of the internal standard that would interfere with quantitation.																	
	<table><tr><th></th><th>Sample</th><th>Recovery</th><th>CV</th></tr><tr><td rowspan="4">Hemolysis effect (n=6)</td><td>QC 11 (0.586µM)</td><td>104.84%</td><td>5.95%</td></tr><tr><td>QC 15 (18.7µM)</td><td>84.8%</td><td>3.56%</td></tr><tr><td>QC 21 (0.596µM)</td><td>94.62%</td><td>1.44%</td></tr><tr><td>QC 25 (18.7µM)</td><td>91.76%</td><td>6.03%</td></tr></table>		Sample	Recovery	CV	Hemolysis effect (n=6)	QC 11 (0.586µM)	104.84%	5.95%	QC 15 (18.7µM)	84.8%	3.56%	QC 21 (0.596µM)	94.62%	1.44%	QC 25 (18.7µM)	91.76%	6.03%
	Sample	Recovery	CV															
Hemolysis effect (n=6)	QC 11 (0.586µM)	104.84%	5.95%															
	QC 15 (18.7µM)	84.8%	3.56%															
	QC 21 (0.596µM)	94.62%	1.44%															
	QC 25 (18.7µM)	91.76%	6.03%															

Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Report LCMSC 636.1–Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection; May 2016			
Lipemic effect	N/A			
Dilution linearity and hook effect	The ability to analyze samples with insufficient volume for a full aliquot was validated by analyzing six replicate human plasma QCs, containing 18.7µM as 2-fold dilution.			
	Dilution linearity (n=6)	Dilution	Recovery	CV
		1:2	100.91%	2.37%
	Hook effect: Not applicable for chromatographic assays			
Bench-top/process stability	Thawed Analyte Stability Thawed analyte stability was evaluated by evaluating QC controls [six replicates of two surrogate buffer (QC 1 and 5) and two plasma (QC 11 and 15)] that had been thawed and kept on wet ice for 4 or 25 hr prior to extraction. Two sets of low- and high-level surrogate matrix and human plasma quality controls were allowed to thaw and remain at room temperature (RT) for 24 hr prior to extraction. Thawed analytes were stable for up to 25 hr on wet ice prior to extraction.			
	Time on Wet Ice	Sample	Concentration	Recovery
				CV
	4 hr	QC 1	0.0600 µg/mL	98.28%
		QC 5	3.75 µg/mL	96.71%
		QC 11	0.446 µg/mL	99.66%
		QC 15	3.25 µg/mL	93.92%
	25 hr	QC 1	0.0600 µg/mL	102.00%
		QC 5	3.75 µg/mL	97.72%
		QC 11	0.446 µg/mL	114.30%
		QC 15	3.25 µg/mL	90.78%
	Time at RT	Sample	Concentration	Recovery
				CV
	24 hr	QC 1	0.298µM	99.52%
		QC 5	18.6µM	97.89%
		QC 11	0.568µM	92.47%
		QC 15	18.7µM	94.96%
	Reinjection Reproducibility Reinjection reproducibility (RR) was evaluated by analyzing calibration standards and quality controls that were extracted and injected then stored at 30°C prior to and during reanalysis. RR met the acceptance criteria.			
	Sample	Concentration	Recovery	CV
	QC 1	0.298µM	100.49%	5.01%
	QC 3	1.86µM	93.96%	4.90%
	QC 5	18.6µM	97.96%	3.66%
	QC 11	0.586µM	96.66%	7.69%
	QC 15	18.7µM	96.83%	6.15%
	Post-preparative Extract Stability Post-preparative extract stability was evaluated by analyzing quality controls that were extracted and injected and stored at 30°C for approximately 160 hr. Post-preparative extract stability met the acceptance criteria.			
	Sample	Concentration	Recovery	CV
	QC 1	0.298µM	100.36%	5.02%
	QC 5	18.6µM	98.12%	0.498%
	QC 11	0.586µM	98.88%	2.33%
	QC 15	18.7µM	97.92%	1.49%

Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Report LCMSC 636.1–Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection; May 2016																																											
	Whole Blood Stability Whole blood was fortified at approximately 3.00 µg/mL S-sulfocysteine processed after 1 to 2 hr on ice. A control sample was prepared in whole blood and immediately processed to plasma using a 2-8°C centrifuge or a room temperature centrifuge. S-sulfocysteine was stable in human whole blood for up to 2 hr on ice prior to processing to plasma in either a 2-8°C or room temperature centrifuge. <table><tr><th>Analyte Storage in Whole Blood</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td>1 hr/ref centrifuge WBIB 1</td><td>3.00 µg/mL</td><td>96.49%</td><td>2.38%</td></tr><tr><td>2 hr/ref centrifuge WBIB 2</td><td>3.00 µg/mL</td><td>94.79%</td><td>1.07%</td></tr><tr><td>1 hr/RT centrifuge WBIB 1</td><td>3.00 µg/mL</td><td>99.87%</td><td>4.57%</td></tr><tr><td>2 hr/RT centrifuge WBIB 2</td><td>3.00 µg/mL</td><td>93.84%</td><td>3.64%</td></tr></table>					Analyte Storage in Whole Blood	Concentration	Recovery	CV	1 hr/ref centrifuge WBIB 1	3.00 µg/mL	96.49%	2.38%	2 hr/ref centrifuge WBIB 2	3.00 µg/mL	94.79%	1.07%	1 hr/RT centrifuge WBIB 1	3.00 µg/mL	99.87%	4.57%	2 hr/RT centrifuge WBIB 2	3.00 µg/mL	93.84%	3.64%																			
Analyte Storage in Whole Blood	Concentration	Recovery	CV																																									
1 hr/ref centrifuge WBIB 1	3.00 µg/mL	96.49%	2.38%																																									
2 hr/ref centrifuge WBIB 2	3.00 µg/mL	94.79%	1.07%																																									
1 hr/RT centrifuge WBIB 1	3.00 µg/mL	99.87%	4.57%																																									
2 hr/RT centrifuge WBIB 2	3.00 µg/mL	93.84%	3.64%																																									
Freeze-thaw stability	Freeze-thaw stability was evaluated by analyzing two sets of low- and high-level surrogate matrix and human plasma quality controls that were subjected to five freeze/thaw cycles. Samples were thawed on wet ice. One set was frozen at –20°C (FTF) and the other set was frozen at –70°C (FTC) for each freeze cycle. The freeze/thaw stability data met the acceptance criteria with the exception of the S-sulfocysteine human plasma samples frozen at –20°C, which had negative biases (LCMS 636.1). Freeze/thaw stability was also evaluated by analyzing two sets of low- and high-level surrogate matrix and human plasma quality controls that were subjected to three freeze/thaw cycles. Samples were thawed on wet ice. One set was frozen at –20°C (FTF) and the other set was frozen at –70°C (FTC) for each freeze cycle. The freeze/thaw stability data met the acceptance criteria. <table><tr><th>Conditions</th><th>Sample</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td rowspan="4">–20°C (FTF; 3FT)</td><td>QC 1</td><td>0.298µM</td><td>101.29%</td><td>3.96%</td></tr><tr><td>QC 5</td><td>18.6µM</td><td>98.81%</td><td>4.88%</td></tr><tr><td>QC 11</td><td>0.586µM</td><td>93.49%</td><td>4.58%</td></tr><tr><td>QC 15</td><td>18.7µM</td><td>94.60%</td><td>1.46%</td></tr><tr><td rowspan="4">–70°C (FTFC; 3FT)</td><td>QC 1</td><td>0.298µM</td><td>101.34%</td><td>3.97%</td></tr><tr><td>QC 5</td><td>18.6µM</td><td>99.33%</td><td>2.48%</td></tr><tr><td>QC 11</td><td>0.586µM</td><td>97.82%</td><td>2.12%</td></tr><tr><td>QC 15</td><td>18.7µM</td><td>93.59%</td><td>1.98%</td></tr></table>					Conditions	Sample	Concentration	Recovery	CV	–20°C (FTF; 3FT)	QC 1	0.298µM	101.29%	3.96%	QC 5	18.6µM	98.81%	4.88%	QC 11	0.586µM	93.49%	4.58%	QC 15	18.7µM	94.60%	1.46%	–70°C (FTFC; 3FT)	QC 1	0.298µM	101.34%	3.97%	QC 5	18.6µM	99.33%	2.48%	QC 11	0.586µM	97.82%	2.12%	QC 15	18.7µM	93.59%	1.98%
Conditions	Sample	Concentration	Recovery	CV																																								
–20°C (FTF; 3FT)	QC 1	0.298µM	101.29%	3.96%																																								
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	QC 11	0.586µM	93.49%	4.58%																																								
	QC 15	18.7µM	94.60%	1.46%																																								
–70°C (FTFC; 3FT)	QC 1	0.298µM	101.34%	3.97%																																								
	QC 5	18.6µM	99.33%	2.48%																																								
	QC 11	0.586µM	97.82%	2.12%																																								
	QC 15	18.7µM	93.59%	1.98%																																								
Long-term storage	Analyte Stability in Surrogate Matrix Analyte stability in frozen surrogate matrix was evaluated by analyzing samples which had been stored for 32 days at –20°C (STABF) versus freshly prepared calibration standards and those stored for 435 days at –70°C (STABC). Analyte in frozen surrogate matrix was demonstrated to be stable for 32 days when stored at –20°C and for 435 days when stored at –70°C. <table><tr><th>Conditions</th><th>Sample</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td rowspan="2">–20°C; 32 days</td><td>STABF1</td><td>0.0600 µg/mL</td><td>103.96%</td><td>3.4%</td></tr><tr><td>STABF5</td><td>3.75 µg/mL</td><td>100.42%</td><td>1.88%</td></tr><tr><td rowspan="2">–70°C; 435 days</td><td>STABF1</td><td>0.298µM</td><td>92.74%</td><td>13.7%</td></tr><tr><td>STABF5</td><td>18.6µM</td><td>99.89%</td><td>10.6%</td></tr></table>					Conditions	Sample	Concentration	Recovery	CV	–20°C; 32 days	STABF1	0.0600 µg/mL	103.96%	3.4%	STABF5	3.75 µg/mL	100.42%	1.88%	–70°C; 435 days	STABF1	0.298µM	92.74%	13.7%	STABF5	18.6µM	99.89%	10.6%																
Conditions	Sample	Concentration	Recovery	CV																																								
–20°C; 32 days	STABF1	0.0600 µg/mL	103.96%	3.4%																																								
	STABF5	3.75 µg/mL	100.42%	1.88%																																								
–70°C; 435 days	STABF1	0.298µM	92.74%	13.7%																																								
	STABF5	18.6µM	99.89%	10.6%																																								

Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Report LCMSC 636.1–Quantitation of S-Sulphocysteine, Xanthine, and Uric Acid in Human Plasma via HPLC with MS/MS Detection; May 2016											
	Analyte Stability in Frozen Plasma											
	Analyte stability in frozen plasma at –70°C (STABC) for S-sulfocysteine was evaluated by analyzing samples which had been stored for 433 and 441 days respectively versus freshly prepared calibration standards.											
	Analyte in frozen plasma was demonstrated to be stable up to 441 days when stored at –70°C.											
	<table><tr><th>Time Stored at –70°C</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td>441 days; STABC11</td><td>2.22µM</td><td>110.1%</td><td>6.00%</td></tr><tr><td>433 days; STABC15</td><td>16.1µM</td><td>92.56%</td><td>9.87%</td></tr></table>	Time Stored at –70°C	Concentration	Recovery	CV	441 days; STABC11	2.22µM	110.1%	6.00%	433 days; STABC15	16.1µM	92.56%
Time Stored at –70°C	Concentration	Recovery	CV									
441 days; STABC11	2.22µM	110.1%	6.00%									
433 days; STABC15	16.1µM	92.56%	9.87%									
Parallelism	Not applicable for chromatographic assays											
Carry over	The potential for carryover from a sample containing a high concentration of analyte to the following sample in an injection sequence was evaluated by injecting duplicate extracted matrix blanks immediately after the ULOQ calibration standards in each validation run. There were no contributions from chromatographic peaks, at the expected retention time of the analyte in the blank samples, greater than 20% of the mean analyte response for the LLOQ calibration standards in the validation run.											

Abbreviations: CV, coefficient of variation; EDTA, ethylenediaminetetraacetic acid; LLOQ, lower limit of quantification; N/A, not applicable; PBS, phosphate-buffered saline; RT, room temperature; QC, quality control; SSC, S-sulfocysteine; ULOQ, upper limit of quantification

The clinical urine samples for the PD biomarkers of Study MCD-201 were partly analyzed using the original validated method (LCMS 636; [Table 49](#)). The method was changed during the study, and as of May 12, 2015, the remaining samples were analyzed by the revalidated method (LCMSC 636), which was subsequently used in Study MCD-202. The summary of validation parameters is presented in [Table 50](#).

Table 49. Method LCMS 636-Bioanalytical Method Validation for S-Sulfocysteine Determination in Human Urine

Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Report LCMS 636–Quantitation of S-sulfocysteine, Xanthine, Uric Acid, and Creatinine in Human Urine via HPLC with MS/MS Detection; Sep 2013; Project ATZ2																		
Method description	High-performance liquid chromatography (HPLC) with tandem mass spectrometry (LC-MS/MS) using negative ion electrospray.																		
Materials used for calibration curve and concentration	S-Sulphocysteine; Dulbecco's PBS <table> <tr> <th>Standard</th><th>Concentration (Theoretical)</th></tr> <tr> <td>CAL 1 LLOQ</td><td>0.100 µg/mL</td></tr> <tr> <td>CAL 2</td><td>0.160 µg/mL</td></tr> <tr> <td>CAL 3</td><td>0.300 µg/mL</td></tr> <tr> <td>CAL 4</td><td>0.800 µg/mL</td></tr> <tr> <td>CAL 5</td><td>2.50 µg/mL</td></tr> <tr> <td>CAL 6</td><td>7.00 µg/mL</td></tr> <tr> <td>CAL 7</td><td>16.0 µg/mL</td></tr> <tr> <td>CAL 8 ULOQ</td><td>20.0 µg/mL</td></tr> </table>	Standard	Concentration (Theoretical)	CAL 1 LLOQ	0.100 µg/mL	CAL 2	0.160 µg/mL	CAL 3	0.300 µg/mL	CAL 4	0.800 µg/mL	CAL 5	2.50 µg/mL	CAL 6	7.00 µg/mL	CAL 7	16.0 µg/mL	CAL 8 ULOQ	20.0 µg/mL
Standard	Concentration (Theoretical)																		
CAL 1 LLOQ	0.100 µg/mL																		
CAL 2	0.160 µg/mL																		
CAL 3	0.300 µg/mL																		
CAL 4	0.800 µg/mL																		
CAL 5	2.50 µg/mL																		
CAL 6	7.00 µg/mL																		
CAL 7	16.0 µg/mL																		
CAL 8 ULOQ	20.0 µg/mL																		
Validated assay range	0.100 to 20.0 µg/mL																		

Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Report LCMS 636–Quantitation of S-sulfocysteine, Xanthine, Uric Acid, and Creatinine in Human Urine via HPLC with MS/MS Detection; Sep 2013; Project ATZ2		
Material used for QCs and concentration	S-Sulphocysteine, Dulbecco's PBS, human plasma		
	Control	Concentration (Theoretical)	Matrix
	QC 0	0.100 µg/mL	Surrogate
	QC 1	0.225 µg/mL	Surrogate
	QC 2	0.500 µg/mL	Surrogate
	QC 3	1.50 µg/mL	Surrogate
	QC 4	4.00 µg/mL	Surrogate
	QC 5	15.0 µg/mL	Surrogate
	QC 11	0.759 µg/mL (interassay mean)	Urine
	QC 15	15.7 µg/mL	Urine
Minimum required dilutions	1:24.5		
Source and lot of reagents (LBA)	Analyte	Source	Lot
	S-Sulphocysteine	(b) (4)	9-DSD-60-2
	S-Sulphocysteine-d3		E537P18
	Dulbecco's PBS (10×)		Product 20-031-CV
	Dulbecco's PBS		Product D8537
Human urine	N/A		
Regression model and weighting	Linear, weighted 1/x ²		
Validation parameters			
Standard calibration curve performance during accuracy and precision	Number of standard calibrators from LLOQ to ULOQ		8
	Cumulative accuracy (%bias) from LLOQ to ULOQ		99.15 to 100.94%
	Cumulative precision (%CV) from LLOQ to ULOQ		≤2.66%
QCs performance during accuracy and precision	Cumulative accuracy (%bias) in four QCs:		
	QC 0	98.47 to 105.39%	
	QC 1	102.08 to 103.72%	
	QC 2	100.43 to 102.18%	
	QC 3	100.01 to 100.90%	
	QC 4	99.76 to 101.79%	
	QC 5	99.26 to 102.35%	
	QC 11	99.50 to 100.98%	
	QC 15	99.61 to 103.41%	
	Interbatch %CV:		
	QC 0	≤4.51%	
	QC 1	≤2.39%	
	QC 2	≤2.04%	
	QC 3	≤1.36%	
	QC 4	≤1.67%	
	QC 5	≤2.74%	
	QC 11	≤1.53%	
	QC 15	≤2.55%	
	Total error		
QC 1, 2, 3, 4, 5, 11, and 15		Not applicable	

Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Report LCMS 636–Quantitation of S-sulfocysteine, Xanthine, Uric Acid, and Creatinine in Human Urine via HPLC with MS/MS Detection; Sep 2013; Project ATZ2											
Selectivity and matrix effect	S-Sulphocysteine is an endogenous compound; therefore, measurable levels are expected to be present in human urine. The validation was conducted using samples prepared in two types of matrix: human urine and an analyte-free surrogate matrix of Dulbecco's PBS Six lots of human urine spiked with S-sulfocysteine at 13.5 µg/mL. - Accuracy 97.1 to 99.9% - CV ≤1.53% General extraction recovery of S-sulfocysteine from human urine was evaluated by comparing the analyte responses of pre-extraction fortified samples to those of post extraction fortified samples. Overall recovery of S-sulfocysteine was 103%.											
Interference and specificity	Chromatographic Considerations Because S-sulfocysteine is an endogenous compound, chromatographic peaks were detected at the expected mass transitions and retention times for S-sulfocysteine. There were no significant chromatographic peaks detected at the mass transitions and expected retention times of the internal standards that would interfere with quantitation. Matrix-Related Ion Suppression Interference The potential for variable matrix-related ion suppression interference was evaluated by fortifying human urine samples from six different adults and six pediatric individuals. Endogenous levels of S-sulfocysteine were measured by analyzing samples fortified with only internal standard in triplicate. The same sample lots were then fortified with approximately 2.00 µg/mL S-sulfocysteine and internal standard at the level of use. The results indicated that matrix suppression effects do not compromise the accuracy of the assay. Cross-Analyte Interference No cross-analyte interference was noted with the quantitation of S-sulfocysteine in human urine. Concomitant Medication Interference An interference check was performed to determine if samples fortified with acetaminophen, caffeine, acetylsalicylic acid, salicylic acid, ibuprofen, naproxen, chlorpheniramine, ethinyl estradiol, norgestrel or norethindrone interfered with the quantitation of S-sulfocysteine in human urine. Results indicated there was no effect on the quantitation of S-sulfocysteine in human urine fortified with these compounds to the stated concentrations.											
Hemolysis effect	The effect of hemolysis on the quantification of SSC was not evaluated.											
Lipemic effect	The effect of lipemia on the quantification of SSC was not evaluated.											
Dilution linearity and hook effect	The ability to analyze samples with insufficient volume for a full aliquot was validated by analyzing six replicate human urine QCs, containing 15.7 µg/mL S-sulfocysteine, as a 2-fold dilution. The ability to dilute samples originally above the upper limit of the calibration range was validated by analyzing six replicate human urine QCs, containing 98.7 µg/mL S-sulfocysteine, as 10-fold dilutions. <table><tr><td></td><td>Dilution</td><td>Recovery</td><td>CV</td></tr><tr><td rowspan="2">Dilution linearity (n=6)</td><td>1:2</td><td>106.44%</td><td>3.19%</td></tr><tr><td>1:10</td><td>101.07%</td><td>2.58%</td></tr></table> Hook effect: Not applicable for chromatographic assays		Dilution	Recovery	CV	Dilution linearity (n=6)	1:2	106.44%	3.19%	1:10	101.07%	2.58%
	Dilution	Recovery	CV									
Dilution linearity (n=6)	1:2	106.44%	3.19%									
	1:10	101.07%	2.58%									

Bioanalytical method validation report name, amendments, and hyperlinks

- Method Validation Report LCMS 636–Quantitation of S-sulfocysteine, Xanthine, Uric Acid, and Creatinine in Human Urine via HPLC with MS/MS Detection; Sep 2013; Project ATZ2

Bench-top/process stability

Thawed Analyte Stability

Thawed S-sulfocysteine stability was evaluated by evaluating QC controls (six replicates of two surrogate buffer (QC 1 and 5) and two urine [QC 11 and 15]) that had been thawed and kept at room temperature (RT) for 24 hr or at 2-8°C for 168 hr prior to extraction and analysis.
Thawed S-sulfocysteine was stable for up to 24 hr at room temperature and or 168 hr at 2-8°C prior to extraction and analysis.

Storage Prior to Extraction	Concentration	Recovery	CV
24 hr at RT			
QC 1	0.225 µg/mL	101.2%	1.04%
QC 5	15.0 µg/mL	99.24%	1.38%
QC 11	0.759 µg/mL	107.99%	1.73%
QC 15	15.7 µg/mL	101.14%	1.18%
168 hr at 2-8°C			
QC 1	0.225 µg/mL	101.29%	0.83%
QC 5	15.0 µg/mL	99.40%	1.06%
QC 11	0.759 µg/mL	107.33%	1.72%
QC 15	15.7 µg/mL	100.54%	0.77%

Reinjection Reproducibility

Reinjection reproducibility was demonstrated by analyzing surrogate matrix calibration and human urine quality controls that were extracted and injected then stored at 15°C prior to and during reanalysis. Reanalysis demonstrated reproducibility.

Reinjection Reproducibility	Concentration	Recovery	CV
QC 1	0.225 µg/mL	101.71%	1.28%
QC 2	0.500 µg/mL	100.36%	1.84%
QC 3	1.50 µg/mL	101.56%	1.78%
QC 4	4.00 µg/mL	102.41%	1.58%
QC 5	15.0 µg/mL	101.25%	1.46%
QC 11	0.759 µg/mL	100.96%	0.79%
QC 15	15.7 µg/mL	99.56%	0.99%

Post-preparative Extract Stability

Post-preparative extract stability was evaluated by analyzing QC controls that were extracted and tested then stored at 15°C for approximately 83 hr prior to reanalysis versus freshly prepared calibrators.
Post-preparative extract demonstrated stability for 83 hr at 15°C.

Sample	Concentration	Recovery	CV
QC 1	0.225 µg/mL	98.99%	0.80%
QC 5	15.0 µg/mL	100.17%	1.92%
QC 11	0.759 µg/mL	98.46%	1.75%
QC 15	15.7 µg/mL	98.89%	0.89%

Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Report LCMS 636–Quantitation of S-sulfocysteine, Xanthine, Uric Acid, and Creatinine in Human Urine via HPLC with MS/MS Detection; Sep 2013; Project ATZ2																																														
Freeze-thaw stability	Freeze-thaw stability was evaluated by analyzing QC controls [six replicates of two surrogate buffer (QC 1 and 5) and two urine (QC 11 and 15)] that had been subjected to five freeze-thaw cycles at –20°C or –70°C. Urine samples were stable after repeated freeze-thaws at –20°C or –70°C <table><tr><th>Temperature</th><th>Sample</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td rowspan="4">–20°C</td><td>QC 1</td><td>0.225 µg/mL</td><td>102.38%</td><td>0.9%</td></tr><tr><td>QC 5</td><td>15.0 µg/mL</td><td>99.60%</td><td>0.63%</td></tr><tr><td>QC 11</td><td>0.759 µg/mL</td><td>101.61%</td><td>1.64%</td></tr><tr><td>QC 15</td><td>15.7 µg/mL</td><td>100.83%</td><td>1.58%</td></tr><tr><td rowspan="4">–70°C</td><td>QC 1</td><td>0.225 µg/mL</td><td>103.23%</td><td>0.94%</td></tr><tr><td>QC 5</td><td>15.0 µg/mL</td><td>100.40%</td><td>0.91%</td></tr><tr><td>QC 11</td><td>0.759 µg/mL</td><td>102.33%</td><td>2.15%</td></tr><tr><td>QC 15</td><td>15.7 µg/mL</td><td>100.42%</td><td>0.96%</td></tr></table>	Temperature	Sample	Concentration	Recovery	CV	–20°C	QC 1	0.225 µg/mL	102.38%	0.9%	QC 5	15.0 µg/mL	99.60%	0.63%	QC 11	0.759 µg/mL	101.61%	1.64%	QC 15	15.7 µg/mL	100.83%	1.58%	–70°C	QC 1	0.225 µg/mL	103.23%	0.94%	QC 5	15.0 µg/mL	100.40%	0.91%	QC 11	0.759 µg/mL	102.33%	2.15%	QC 15	15.7 µg/mL	100.42%	0.96%							
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	QC 15	15.7 µg/mL	100.42%	0.96%																																											
Long-term storage	Analyte Stability in Surrogate Solution S-Sulphocysteine stability in frozen surrogate matrix (QC 1 and 5) was evaluated by analyzing samples stored for 60 days at –20°C or –70°C. S-Sulphocysteine in frozen surrogate matrix demonstrated stability for 60 days when stored at –20°C or –70°C. <table><tr><th>Temperature</th><th>Sample</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td rowspan="2">–20°C</td><td>QC 1</td><td>0.225 µg/mL</td><td>101.18%</td><td>1.26%</td></tr><tr><td>QC 5</td><td>15.0 µg/mL</td><td>98.99%</td><td>1.04%</td></tr><tr><td rowspan="2">–70°C</td><td>QC 1</td><td>0.225 µg/mL</td><td>100.76%</td><td>2.07%</td></tr><tr><td>QC 5</td><td>15.0 µg/mL</td><td>98.98%</td><td>1.86%</td></tr></table> Analyte Stability in Urine S-Sulphocysteine stability in urine (QC 11 and 15) were evaluated by analyzing samples which had been stored for 60 days at –20°C or –70°C. S-Sulphocysteine in frozen urine was demonstrated to be stable for 60 days when stored at –20°C or –70°C. <table><tr><th>Temperature</th><th>Sample</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td rowspan="2">–20°C</td><td>QC 11</td><td>0.759 µg/mL</td><td>101.09%</td><td>1.38%</td></tr><tr><td>QC 15</td><td>15.7 µg/mL</td><td>98.35%</td><td>2.04%</td></tr><tr><td rowspan="2">–70°C</td><td>QC 11</td><td>0.759 µg/mL</td><td>101.93%</td><td>1.11%</td></tr><tr><td>QC 15</td><td>15.7 µg/mL</td><td>100.12%</td><td>1.10%</td></tr></table>	Temperature	Sample	Concentration	Recovery	CV	–20°C	QC 1	0.225 µg/mL	101.18%	1.26%	QC 5	15.0 µg/mL	98.99%	1.04%	–70°C	QC 1	0.225 µg/mL	100.76%	2.07%	QC 5	15.0 µg/mL	98.98%	1.86%	Temperature	Sample	Concentration	Recovery	CV	–20°C	QC 11	0.759 µg/mL	101.09%	1.38%	QC 15	15.7 µg/mL	98.35%	2.04%	–70°C	QC 11	0.759 µg/mL	101.93%	1.11%	QC 15	15.7 µg/mL	100.12%	1.10%
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	QC 15	15.7 µg/mL	100.12%	1.10%																																											
Parallelism	Not applicable for chromatographic assays																																														
Carry over	The potential for carryover from a sample containing a high concentration of analyte to the following sample in an injection sequence was evaluated by injecting duplicate extracted matrix surrogate matrix blanks immediately after the ULOQ calibration standards in each validation run. There were no contributions from chromatographic peaks, at the expected retention time of the analyte in the blank samples, greater than 20% of the mean analyte response for the LLOQ calibration standards and quality controls in the validation run. To minimize the impact of potential carryover, study samples were analyzed in pharmacokinetic order and two matrix blanks were injected after high-concentration samples that proceeded low-concentration samples.																																														

Abbreviations: CV, coefficient of variation; LLOQ, lower limit of quantification; N/A, not applicable; PBS, phosphate-buffered saline; RT, room temperature; QC, quality control; SSC, S-sulfocysteine; ULOQ, upper limit of quantification

Table 50. Method LCMSC 636—Bioanalytical Method Validation for S-Sulfocysteine Determination in Human Urine

Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Report LCMSC 636–Quantitation of S-Sulphocysteine, Xanthine, Uric Acid and Creatinine in Human Urine via HPLC with MS/MS Detection; May 2016		
Method description	High-performance liquid chromatography (HPLC) with tandem mass spectrometry (LC-MS/MS).		
Materials used for calibration curve and concentration	S-Sulphocysteine, Dulbecco's PBS		
	Standard	Concentration (Theoretical)	
	CAL 1 LLOQ	0.497µM	
	CAL 2	0.795µM	
	CAL 3	1.49µM	
	CAL 4	3.98µM	
	CAL 5	12.4µM	
	CAL 6	34.8µM	
	CAL 7	79.5µM	
CAL 8 ULOQ	99.4µM		
Validated assay range	0.497 to 99.4µM		
Material used for QCs and concentration	S-Sulphocysteine, Dulbecco's PBS, human plasma		
	Control	Concentration (Theoretical)	Matrix
	QC 0	0.497µM	Surrogate
	QC 1	1.12µM	Surrogate
	QC 3	7.45µM	Surrogate
	QC 5	74.5µM	Surrogate
	QC 11	0.870µM	Urine
	QC 15	29.2µM	Urine
	Minimum required dilutions	1:2.25	
Source and lot of reagents (LBA)	Analyte	Source	Lot
	S-Sulphocysteine	(b) (4)	9-DSD-60-2
	S-Sulphocysteine-d3		E537P18
	Dulbecco's PBS (10×)		Product 20-031-CV
	Dulbecco's PBS		Product D8537
	Human Plasma		N/A
Regression model & weighting	Linear, weighted 1/x²		
Validation parameters			
Standard calibration curve performance during accuracy and precision	Number of standard calibrators from LLOQ to ULOQ		8
	Cumulative accuracy (%bias) from LLOQ to ULOQ		97.58 to 101.45%
	Cumulative precision (%CV) from LLOQ to ULOQ		≤5.79%
QCs performance during accuracy and precision	Cumulative accuracy (%bias) in four QCs		
	QC 0		95.85 to 100.81%
	QC 1		97.34 to 104.07%
	QC 3		98.0 to 99.06%
	QC 5		96.49 to 101.56%
	QC 11		99.31 to 100.67%
	QC 15		94.67 to 103.42%

Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Report LCMSC 636–Quantitation of S-Sulphocysteine, Xanthine, Uric Acid and Creatinine in Human Urine via HPLC with MS/MS Detection; May 2016		
	Interbatch %CV		
	QC 0		≤7.95%
	QC 1		≤5.73%
	QC 3		≤3.96%
	QC 5		≤4.09%
	QC 11		≤5.38%
	QC 15		≤4.89%
	Total error		
	Not applicable		
Selectivity and matrix effect	Because S-sulfocysteine is endogenous compound in human urine, PBS was used as surrogate matrix for quantitation. The calibration standards and quality controls were prepared in PBS, with additional quality controls prepared in human urine		
Interference and specificity	Chromatographic Considerations Human urine samples from six individuals were extracted and analyzed. Because S-sulfocysteine is an endogenous compound, chromatographic peaks were detected at the expected mass transitions and retention times for S-sulfocysteine. There were no significant chromatographic peaks detected at the mass transitions and expected retention times of the internal standards that would interfere with quantitation. Matrix-Related Ion Suppression Interference The potential for variable matrix-related ion suppression interference was evaluated by fortifying human urine samples from six different individuals. Endogenous levels of S-sulfocysteine were measured by analyzing samples fortified with only internal standard in triplicate. The same sample lots were then fortified with approximately 6.46µM S-sulfocysteine and internal standard at the level of use. The results indicated that matrix suppression effects do not compromise assay accuracy.		
Hemolysis effect	N/A		
Lipemic effect	N/A		
Dilution linearity and hook effect	The ability to analyze samples with insufficient volume for a full aliquot was validated by analyzing six replicate human urine QCs, containing 29.2µM S-sulfocysteine, as two-fold dilution using surrogate matrix.		
	Dilution linearity (n=6)	Dilution 1:2	Recovery 103.65% CV 3.59%
	Hook effect: Not applicable for chromatographic assays		
Bench-top/process stability	Thawed Analyte Stability Thawed S-sulfocysteine stability was evaluated by evaluating QC controls [six replicates of two surrogate buffer (QC 1 and 5) and two urine (QC 11 and 15)] that had been thawed and kept at room temperature (RT) for 24 hr or at 2-8°C for 168 hr prior to extraction and analysis. Thawed S-sulfocysteine was stable for up to 24 hr at RT or 168 hr at 2-8°C prior to extraction and analysis (LCMS 636). A set of low and high human urine QCs were thawed at RT and placed in a 37°C water bath for 1 hr prior to extraction. Data met the acceptance criteria.		
	Sample	Concentration	Recovery CV
	QC 11	0.870µM	96.54% 6.56%
	QC 15	29.2µM	99.04% 3.10%

Bioanalytical method validation report name, amendments, and hyperlinks	Method Validation Report LCMSC 636–Quantitation of S-Sulphocysteine, Xanthine, Uric Acid and Creatinine in Human Urine via HPLC with MS/MS Detection; May 2016																																				
	Reinjection Reproducibility Reinjection reproducibility was demonstrated by analyzing surrogate matrix calibration and human urine quality controls that were extracted and injected then stored at 30°C prior to and during reanalysis. Reanalysis demonstrated reproducibility. <table><tr><th>Reinjection Reproducibility</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td>QC 1</td><td>1.12µM</td><td>98.81%</td><td>2.96%</td></tr><tr><td>QC 3</td><td>7.45µM</td><td>98.48%</td><td>2.63%</td></tr><tr><td>QC 5</td><td>74.5µM</td><td>100.55%</td><td>3.47%</td></tr><tr><td>QC 15</td><td>29.2µM</td><td>97.29%</td><td>1.97%</td></tr></table> Post-preparative Extract Stability Post-preparative extract stability was evaluated by analyzing surrogate matrix and human urine QC controls that were extracted and tested then stored at 30°C for approximately 107 hr prior to reanalysis versus freshly prepared calibrators. Post-preparative extract demonstrated stability for 107 hr at 30°C. <table><tr><th>Sample</th><th>Concentration</th><th>Recovery</th><th>CV</th></tr><tr><td>QC 1</td><td>1.12µM</td><td>96.27%</td><td>6.30%</td></tr><tr><td>QC 5</td><td>74.5µM</td><td>103.55%</td><td>3.03%</td></tr><tr><td>QC 15</td><td>29.2µM</td><td>103.96%</td><td>3.56%</td></tr></table>	Reinjection Reproducibility	Concentration	Recovery	CV	QC 1	1.12µM	98.81%	2.96%	QC 3	7.45µM	98.48%	2.63%	QC 5	74.5µM	100.55%	3.47%	QC 15	29.2µM	97.29%	1.97%	Sample	Concentration	Recovery	CV	QC 1	1.12µM	96.27%	6.30%	QC 5	74.5µM	103.55%	3.03%	QC 15	29.2µM	103.96%	3.56%
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QC 15	29.2µM	103.96%	3.56%																																		
Freeze-thaw stability	Freeze-thaw stability was evaluated by analyzing QC controls [six replicates of two surrogate buffer (QC 1 and 5) and two urine (QC 11 and 15)] that had been subjected to five freeze-thaw cycles at –20°C or –70°C. Urine samples were stable after repeated freeze-thaws at –20°C or –70°C.																																				
Long-term storage	Analyte Stability in Surrogate Solution S-Sulphocysteine stability in frozen surrogate matrix (QC 1 and 5) was evaluated by analyzing samples stored for 60 days at –20°C or –70°C. S-Sulphocysteine in frozen surrogate matrix demonstrated stability for 60 days when stored at –20°C or –70°C (LCMS 636). Analyte Stability in Urine S-Sulphocysteine stability in urine (QC 11 and 15) were evaluated by analyzing samples which had been stored for 60 days at –20°C or –70°C. S-Sulphocysteine in frozen urine was demonstrated to be stable for 60 days when stored at –20°C or –70°C (LCMS 636).																																				
Parallelism	Not applicable for chromatographic assays.																																				
Carry over	The potential for carryover from a sample containing a high concentration of analyte to the following sample in an injection sequence was evaluated by injecting duplicate extracted matrix surrogate matrix blanks immediately after the ULOQ calibration standards in each validation run. There were no contributions from chromatographic peaks, at the expected retention time of the analyte in the blank samples, greater than 20% of the mean analyte response for the LLOQ calibration standards and quality controls in the validation run.																																				

Abbreviations: CV, coefficient of variation; LLOQ, lower limit of quantification; N/A, not applicable; PBS, phosphate-buffered saline; RT, room temperature; QC, quality control; ULOQ, upper limit of quantification

15. Trial Design: Additional Information and Assessment

Not applicable.

16. Efficacy: Additional Information and Assessment

Table 51. Genotype-Matched Patients and Outcomes

Match Identity	Fosdenopterin Patient(s)	Event Time (Months)	Natural History Patient(s)	Event Time (Months)
Data cutoff 23 March 2020 (Study MCD-201) and 20 February 2020 (Study MCD-202)				
1	(b) (6)	142.8	(b) (6)	46.7
		123.8		
2		120.4		9
3		119.6		0.3
4		103.5		157.2
				1.2
5		64.8		49.4
				1
		44.1		50.9
6		15.9		10.7
7		0.2		88.8
				99
				51.1
				10.4
8		1.2		20.4
				28.8
9		94.4		109.2
10		21.7		12.1
11		30.4		47.8

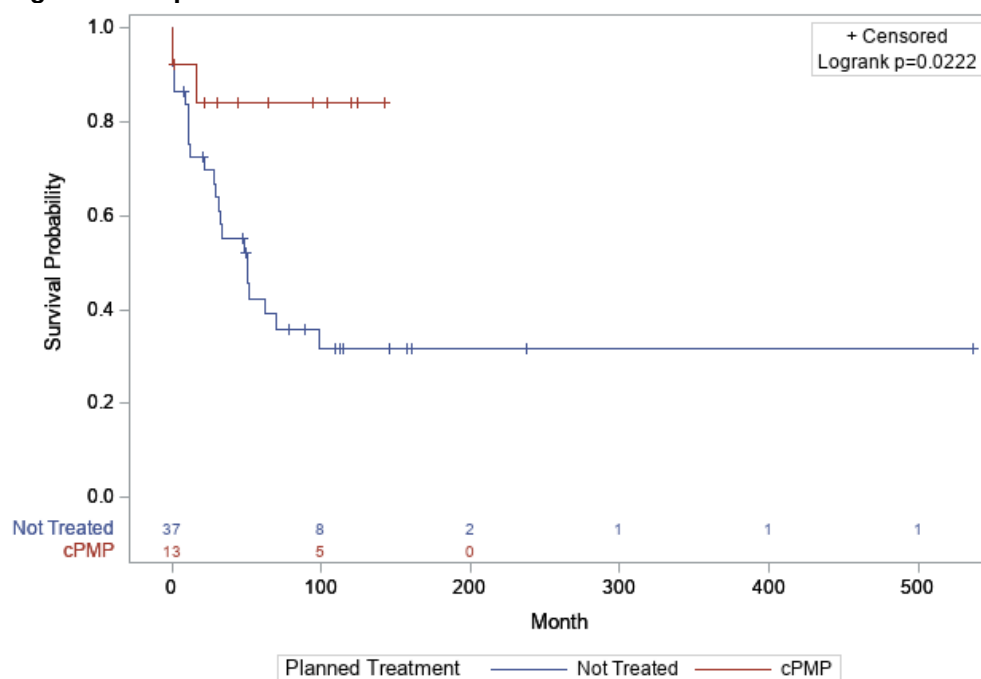
Source: Reviewer's analysis based on the ADSL.xpt and ADTTE.xpt datasets submitted in eCTD0030 on October 23, 2020. Blue indicates a censoring event and red indicates a death event.

Table 52. Follow-up Summary

	Fosdenopterin (n=13)	FAS Untreated (n=37)	GMAS Untreated (n=18)
6 Months			
At risk	11	32	15
Died	1	5	3
Censored	1	0	0
1 Year			
At risk	11	27	12
Died	1	9	6
Censored	1	1	0
2 Years			
At risk	9	24	10
Died	2	11	7
Censored	2	2	1
3 Years			
At risk	8	19	9
Died	2	16	8
Censored	3	2	1
4 Years			
At risk	7	17	7
Died	2	17	9
Censored	4	3	2

Source: Reviewer's analysis based on the ADTTE.xpt dataset submitted in eCTD0030 on October 23, 2020. Abbreviations: FAS, full analysis set; GMAS, genotype-matched analysis set

Figure 28. Kaplan–Meier Survival Curves of the Treated Versus Untreated Groups (FAS)



Source: Reviewer's analysis based on the ADTTE.xpt dataset submitted in eCTD0030 on October 23, 2020.
Abbreviations: cPMP, cyclic pyranopterin monophosphate; FAS, full analysis set

Table 53. Efficacy Results of Overall Survival—Kaplan–Meier Method (Sensitivity Analysis 1)

Parameter	Fosdenopterin (n=13)	Untreated FAS (n=37)	Untreated GMAS (n=18)
Number of deaths (%)	2 (15.4)	23 (62.2)	12 (66.7)
50th Percentile (median) survival time (95% CI)	N/A (16, N/A)	51 (28, 99)	48 (10, 99)
Kaplan–Meier survival probability (95% CI)			
6 Months	92 (57, 99)	86 (71, 94)	83 (57, 94)
1 Year	92 (57, 99)	75 (58, 86)	67 (40, 83)
2 Years	83 (47, 96)	70 (52, 82)	61 (35, 79)
3 Years	83 (47, 96)	55 (37, 70)	55 (30, 74)
Restricted mean survival time (95% CI)			
6 Months	5.6 (4.7, 6.4)	5.3 (4.7, 5.9)	5.1 (4.2, 6.0)
Difference ¹		0.3 (−0.8, 1.3)	0.4 (−0.8, 1.6)
1 Year	11.1 (9.4, 12.8)	10.3 (9.0, 11.5)	9.8 (7.9, 11.7)
Difference ¹		0.8 (−1.3, 2.9)	1.3 (−1.3, 3.8)
2 Years	21.4 (17.8, 25.0)	18.9 (16.1, 21.7)	17.2 (12.9, 21.4)
Difference ¹		2.5 (−2.0, 7.1)	4.3 (−1.3, 9.8)
3 Years	31.4 (25.4, 37.4)	26.5 (22.2, 30.9)	24.0 (17.3, 30.8)
Difference ¹		4.9 (−2.5, 12.3)	7.3 (−1.6, 16.3)
P-value (log-rank test)		0.0273	0.0160

Source: Reviewer's analysis based on the ADTTE.xpt dataset submitted in eCTD0030 on October 23, 2020.

¹Treated minus untreated

Abbreviations: CI, confidence interval; FAS, full analysis set; GMAS, genotype-matched analysis set

Table 54. Efficacy Results of Overall Survival—Kaplan–Meier Method (Sensitivity Analysis 2)

Parameter	Fosdenopterin (n=11)	Untreated FAS (n=39)	Untreated GMAS (n=13 ²)
Number of deaths (%)	2 (18.2)	23 (59.0)	8 (61.5)
50th Percentile (median) survival time (95% CI)	N/A (16, N/A)	51 (29, 99)	48 (1.2, N/A)
Kaplan–Meier survival probability (95% CI)			
6 Months	91 (51, 99)	87 (72, 94)	77 (44, 92)
1 Year	91 (51, 99)	76 (59, 87)	62 (31, 82)
2 Years	82 (45, 95)	71 (53, 83)	62 (31, 82)
3 Years	82 (45, 95)	56 (38, 70)	54 (25, 76)
Restricted mean survival time (95% CI)			
6 Months	5.5 (4.5, 6.5)	5.3 (4.8, 5.9)	4.8 (3.6, 6.0)
Difference ¹		0.1 (–1.0, 1.3)	0.7 (–0.9, 2.2)
1 Year	10.9 (8.9, 12.9)	10.4 (9.2, 11.5)	9.1 (6.6, 11.6)
Difference ¹		0.6 (–1.8, 2.9)	1.8 (–1.4, 5.0)
2 Years	21.1 (17.0, 25.2)	19.1 (16.5, 21.8)	16.5 (11.1, 21.9)
Difference ¹		2.0 (–2.9, 6.9)	4.6 (–2.2, 11.4)
3 Years	30.9 (24.2, 37.6)	26.8 (22.6, 31.1)	23.3 (15.0, 31.6)
Difference ¹		4.1 (–3.8, 11.9)	7.6 (–3.0, 18.3)
P-value (log-rank test)		0.0323	0.0477

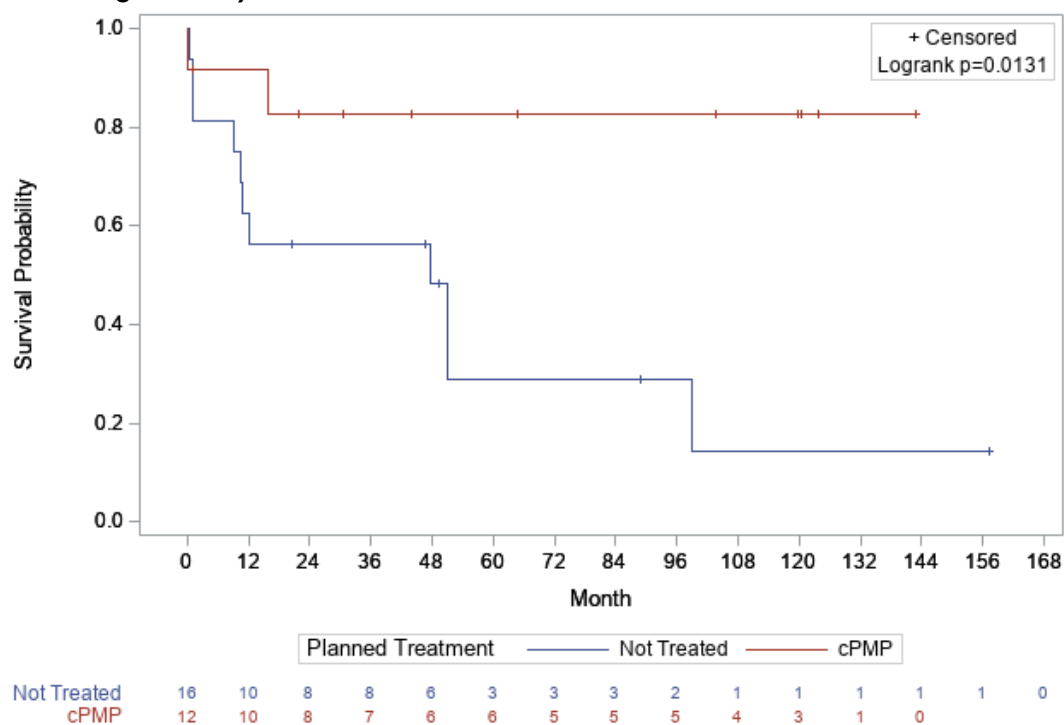
Source: Reviewer's analysis based on the ADTTE.xpt dataset submitted in eCTD0030 on October 23, 2020.

¹ Treated minus untreated

² Includes only untreated patients who are genotype-matched to those in the fosdenopterin group excluding the two patients who discontinued treatment early.

Abbreviations: CI, confidence interval; FAS, full analysis set; GMAS, genotype-matched analysis set; N/A, not applicable

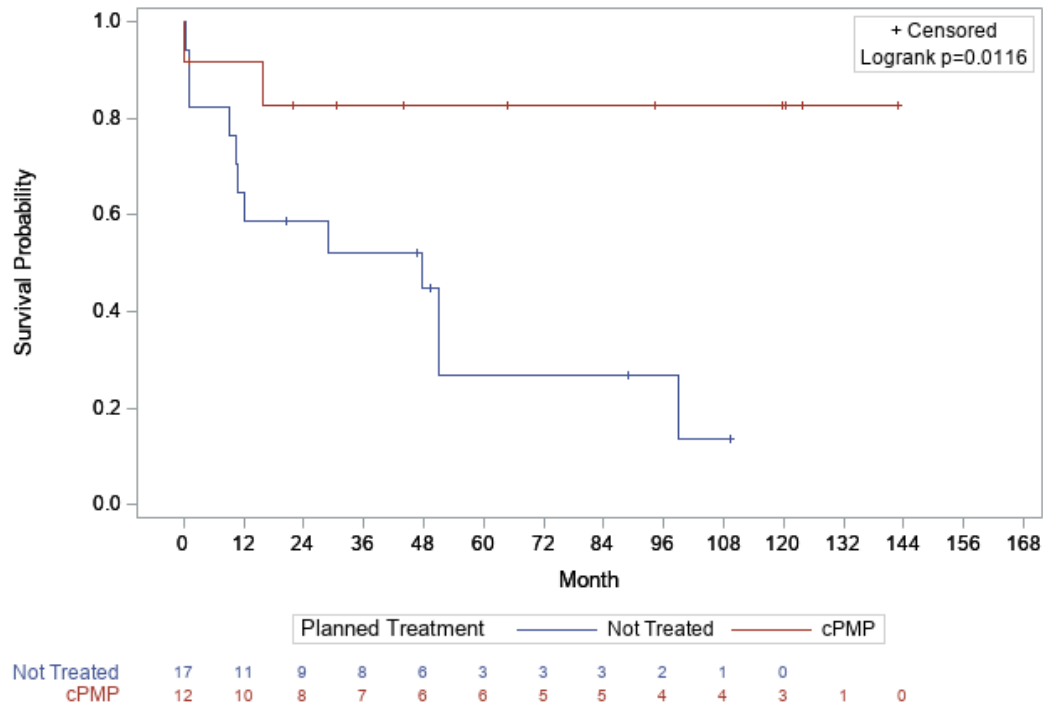
Figure 29. Kaplan–Meier Survival Curves of the Treated Versus Untreated Groups (GMAS, Excluding Match 9)



Source: Reviewer's analysis based on the ADTTE.xpt dataset submitted in eCTD0030 on October 23, 2020.

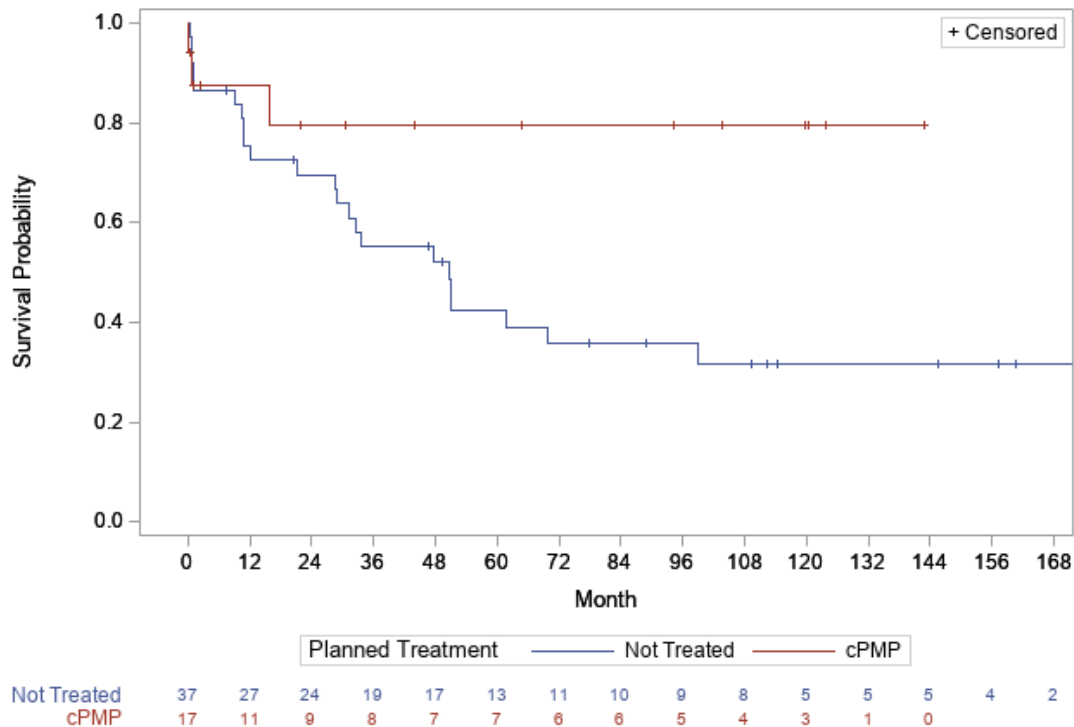
Abbreviations: cPMP, cyclic pyranopterin monophosphate; GMAS, genotype-matched analysis set

Figure 30. Kaplan–Meier Survival Curves of the Treated Versus Untreated Groups (GMAS, Excluding Match 4)



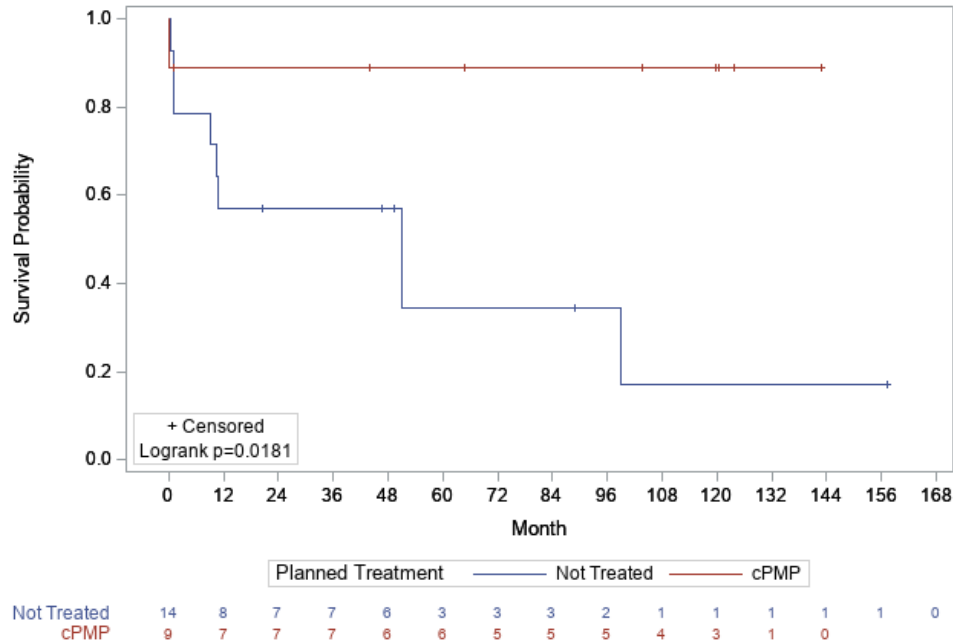
Source: Reviewer analysis based on the ADTTE.xpt dataset submitted in eCTD0030 on October 23, 2020.
Abbreviations: cPMP, cyclic pyranopterin monophosphate; GMAS, genotype-matched analysis set

Figure 31. Kaplan–Meier Survival Curves of the Treated Versus Untreated Groups (FAS and Additional Treated Patients)



Source: Reviewer's analysis based on the ADTTE.xpt dataset submitted in eCTD0030 on October 23, 2020, and information provided in the Safety Update. Two untreated patients are censored at 237.5 and 536.8 months.
Abbreviations: cPMP, cyclic pyranopterin monophosphate; FAS, full analysis set

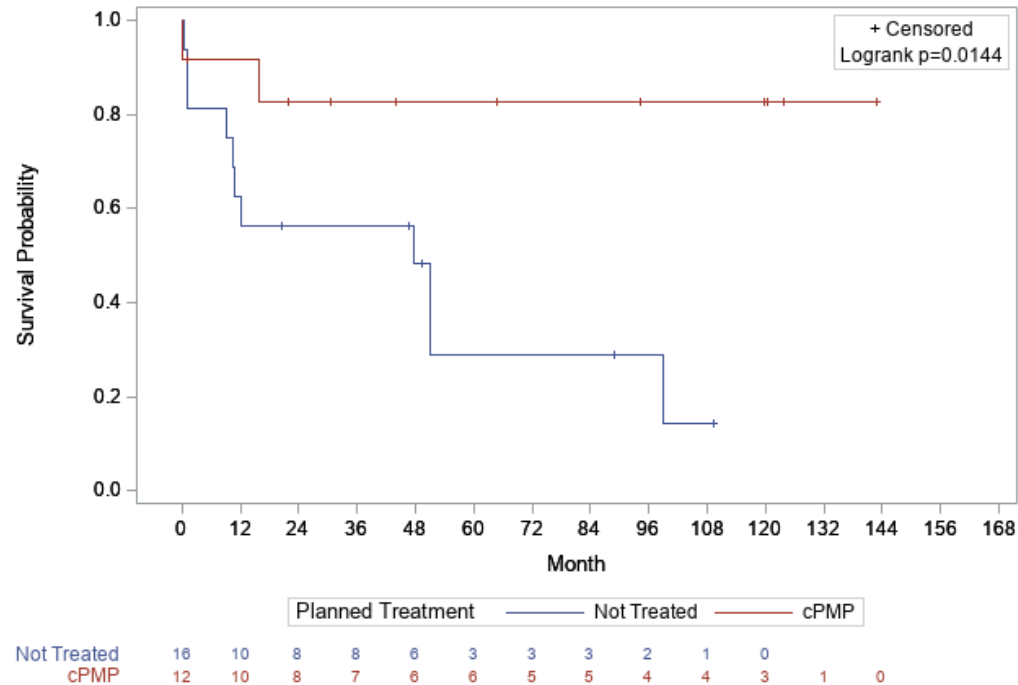
Figure 32. Kaplan–Meier Survival Curves of the Treated Versus Untreated Groups (GMAS Excluding Patients Without Exact Genotype Match)



Source: Reviewer's analysis based on the ADTTE.xpt dataset submitted in eCTD0030 on October 23, 2020, and information provided in the Safety Update.

Abbreviations: cPMP, cyclic pyranopterin monophosphate; GMAS, genotype-matched analysis set

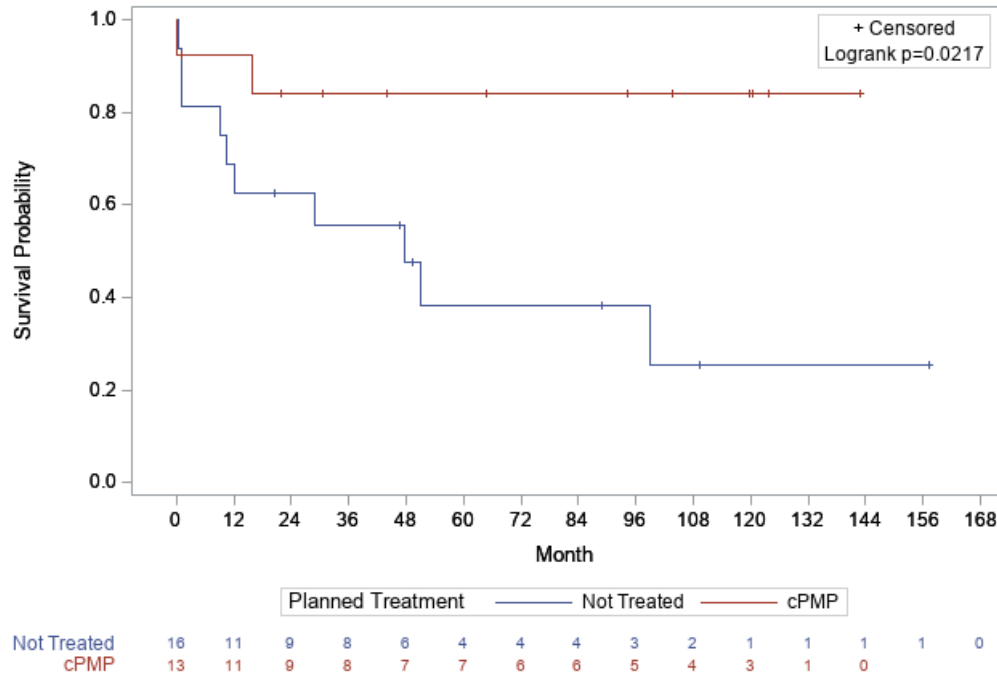
Figure 33. Kaplan–Meier Survival Curves of the Treated Versus Untreated Groups (GMAS Including Only Matched Patients With Onset of Symptoms Before Day 28 of Life)



Source: Reviewer's analysis based on the ADTTE.xpt dataset submitted in eCTD0030 on October 23, 2020, and information provided in the Safety Update.

Abbreviations: cPMP, cyclic pyranopterin monophosphate; GMAS, genotype-matched analysis set

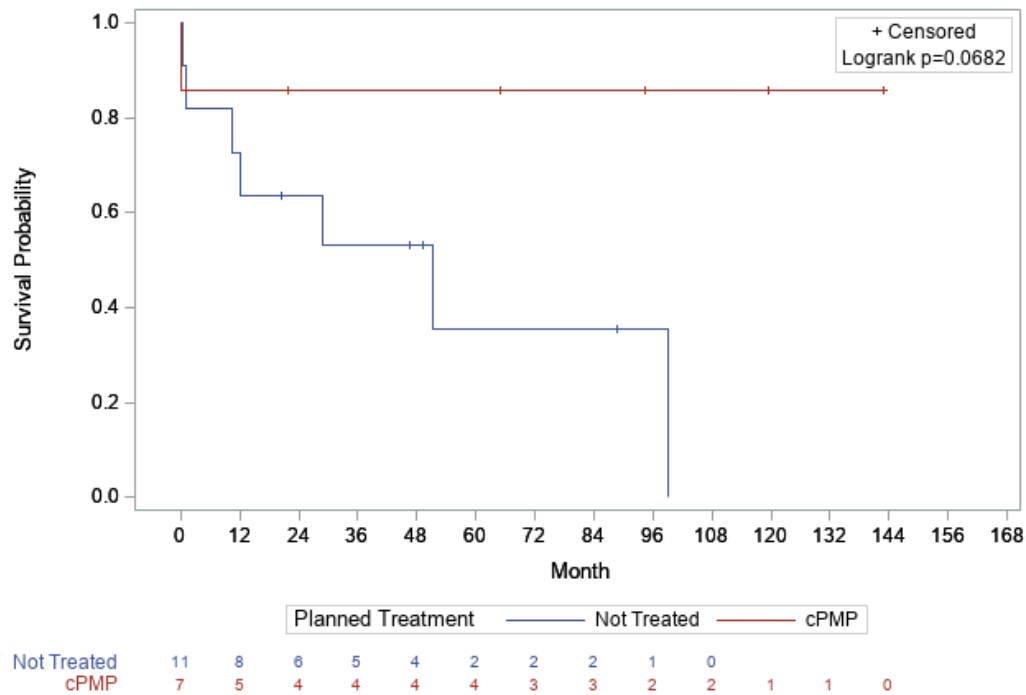
Figure 34. Kaplan–Meier Survival Curves of the Treated Versus Untreated Groups (GMAS Excluding Two Patients Born Before 1999)



Source: Reviewer's analysis based on the ADTTE.xpt dataset submitted in eCTD0030 on October 23, 2020, and information provided in the Safety Update.

Abbreviations: cPMP, cyclic pyranopterin monophosphate; GMAS, genotype-matched analysis set

Figure 35. Kaplan–Meier Survival Curves of the Treated Versus Untreated Groups (GMAS Including Matched Patients Born 2008 to 2014)



Source: Reviewer's analysis based on the ADTTE.xpt dataset submitted in eCTD0030 on October 23, 2020, and information provided in the Safety Update.

Abbreviations: cPMP, cyclic pyranopterin monophosphate; GMAS, genotype-matched analysis set

17. Clinical Safety: Additional Information and Assessment

While the first in human trial is not included in the primary safety analyses of treated patients, here we briefly describe the trial design and safety results. Trial MCD-101 was the first in human, phase 1, single dose dose-escalation, placebo-controlled study conducted in healthy volunteers. Three cohorts with six patients each, received a single dose (either 0.10, 0.32, or 0.90 mg/kg) of the drug. Overall, four patients who received fosdenopterin experienced six treatment-emergent adverse events (TEAEs), and three patients who received placebo experienced five TEAEs. All TEAEs were mild-to-moderate in intensity and all were resolved by the end of the study. No TEAE led to discontinuation or death. There was one serious adverse event reported in a placebo-treated patient. TEAEs were reported at similar rates in subjects treated with fosdenopterin or placebo. No TEAE was reported more than once by any subject treated with fosdenopterin and none appeared to be treatment-related.

18. Mechanism of Action / Drug Resistance: Additional Information and Assessment

Nothing further to cover in this section.

19. Other Drug Development Considerations: Additional Information and Assessment

None.

20. Data Integrity-Related Consults (Office of Scientific Investigations, Other Inspections)

Two studies were audited remotely in support of this NDA review. In auditing the two studies, two clinical investigator sites that participated in the interventional study (ALXN-MCD-201) and the contract research organization that coordinated the natural history study (ALX-MCD-502) were selected for Good Clinical Practices inspection. Remote regulatory assessments (RRAs) were performed in lieu of on-site inspections since on-site inspections were not achievable due to the ongoing COVID-19 pandemic-related travel restrictions. Overall, no objectionable Good Clinical Practices violations were identified during the RRAs of all three entities. Based on the results of the RRAs, the clinical data generated by the two clinical investigators and the contract research organization the investigators concluded that the audit appears to be supportive of this NDA. Refer to the complete report by the Office of Scientific Investigations entered into DARRTS on January 29, 2021 for full details.

21. Labeling Summary of Considerations and Key Additional Information

Major changes to the initial proposed prescribing information (PI) include the following:

Section 1: The indication was revised from (b) (4) to “to reduce the risk of mortality in patients with molybdenum cofactor deficiency (MoCD) Type A.” This change reflects the findings for which we have substantial evidence of effectiveness. The originally proposed (b) (4) indication is broader and could imply improvement on other signs or symptoms of the disease, for which we do not have substantial evidence of effectiveness.

Section 2.1: The team decided it is necessary to inform the prescriber to institute treatment in patients at the time MoCD Type A is suspected pending genetic confirmation and to discontinue use if the diagnosis is not confirmed by genetic testing. This is important so as to not delay treatment while awaiting genetic testing.

Section 2.3: The Applicant was asked to revise the preparation instructions in step 1 to include more detailed directions for calculating the number of vials needed to provide a dose, including an example. This was advised to help caregivers with administration.

Section 5.1: The team decided to add language to advise patients to seek care if they develop phototoxicity.

Section 6.1: In the initial PI, the Applicant (b) (4) The review team requested the Applicant include all AEs that occurred while patients were receiving rcPMP. This section was updated to reflect that request.

Section 8.6: This section was added to support use of Nulibry in adult patients with MoCD Type A. There are currently no patients with MoCD Type A who survive into adulthood. However, if Nulibry is effective at extending survival into adulthood, we concluded that there would be substantial evidence of effectiveness for adults based on our findings of effectiveness in children. This conclusion is based on adults having the same genetic and pathophysiologic basis of disease and the fact that the pharmacokinetics of fosdenopterin are similar in adults and children.

Section 14: The review team determined that Table 4 (overall survival data) (b) (4) The table was revised to include the survival data at 1 and 3 years. Figure 1 (the Kaplan–Meier curve for overall survival) was truncated to reflect only the amount of time treated patients have been followed. (b) (4) was removed after the team determined that the data were not sufficient to support this labeling claim.

22. Postmarketing Requirements and Commitments

The following postmarketing requirements were deemed necessary and were agreed upon with the Applicant:

- Fertility and embryofetal development (FEED/EFD) study in mice.

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- Embryofetal development (EFD) study in rabbits.
- Pre- and postnatal development (PPND) study in mice.
- 104-Week carcinogenicity study in mice.
- Thorough QT/QTc Study.

We are not requiring these studies pre-approval so as not to further delay approval of Nulibry, a drug with a mortality benefit for a rare, serious, devastating disease without other treatment options.

Because untreated patients with MoCD Type A do not survive into adolescence and adulthood, we agreed upon a delay of the nonclinical reproductive and developmental toxicity studies until additional clinical data on patients treated with Nulibry (e.g., on longer-term survival, reproductive capability) are available to inform the necessity of reproductive toxicology studies in this particular patient population. Specifically, we agreed to prioritize the conduct of the FEED/EFD studies in mice and rabbits, followed by the conduct of the PPND study in mice. The reason for prioritizing the FEED/EFD studies is that the oldest patient currently treated with Nulibry is a 12-year old female who is or could become reproductively capable soon, and the EFD studies are the most useful studies to determine the effects of a drug on reproductive capacity and characterize any teratogenic potential. The manufacturing and availability of the drug was also taken into account in the development of the postmarketing requirement milestones.

23. Financial Disclosure

Table 55. Covered Clinical Studies: MCD-201, MCD-202, MCD-501, and MCD-502

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 118		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): None disclosed		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 00		
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)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: Enter text here. Significant payments of other sorts: Enter text here. Proprietary interest in the product tested held by investigator: Enter text here. Significant equity interest held by investigator: Enter text here. Sponsor of covered study: Enter text here.		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☐	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☐	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3): 00		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

24. References

- Atwal, PS and F Scaglia, 2016, Molybdenum cofactor deficiency, *Mol Genet Metab*, 117(1):1-4.
- Austin, PC and EA Stuart, 2017, The performance of inverse probability of treatment weighting and full matching on the propensity score in the presence of model misspecification when estimating the effect of treatment on survival outcomes, *Stat Methods Med Res*, 26(4):1654-1670.
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Reiss, J, 2016, Molybdenum cofactor and sulfite oxidase deficiency, *Journal of Postgenomics Drug and Biomarker Development*, 3(6).

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25. Review Team

Table 56. Reviewers of Integrated Assessment

Role	Name(s)
Regulatory Project Manager	Nicolas Kong
Nonclinical Reviewer	Jackye Peretz
Nonclinical Team Leader	Mukesh Summan
Office of Clinical Pharmacology Reviewer(s)	Oluseyi Adeniyi; Liang Li
Office of Clinical Pharmacology Team Leader(s)	Jie (Jack) Wang; Lian Ma
Clinical Reviewer	Sheila Farrell
Clinical Team Leader	Jacqueline Karp
Statistical Reviewer	Rebecca Hager
Statistical Team Leader	Yan Wang
Cross-Disciplinary Team Leader	Jacqueline Karp
Division Director (Pharm/Tox)	Mukesh Summan
Division Director (OCP)	Michael Pacanowski
Division Director (OB)	Dionne Price
Division Director (Clinical)	Kathleen Donohue
Associate Director for Labeling	Jane Filie
Office Director (or Designated Signatory Authority)	Hylton Joffe

OCP, Office of Clinical Pharmacology
OB, Office of Biostatistics

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Table 57. Additional Reviewers of Application

Office or Discipline	Name(s): Reviewer/Team Leader
DMPP	Sharon Mills/Barbara Fuller
OPQ	Hitesh Shroff
OPQ/Drug Product	Larry Perez/Donna Christner
OPQ/Drug Substance	Jane Chang/Wendy Wilson
OPQ/Environmental Assessment	Yan Xu/Joanne Wang
OPQ/Microbiology	Shannon Heine/Daniel Schu
OPDP	Adewale Adeleye/Katie Klemm
OSI	John Lee/Phillip Kronstein
OSE/DEPI	Catherine Lerro/Catherine Callahan
OSE/DMEPA	Sherly Abraham/Idalia Rychlik
OSE/DRISK	Mona Patel/Laura Zendel
OSE/DPV	Ivon Kim/Carmen Cheng
Other	

OPQ, Office of Pharmaceutical Quality

OPDP, Office of Prescription Drug Promotion

OSI, Office of Scientific Investigations

OSE, Office of Surveillance and Epidemiology

DEPI, Division of Epidemiology

DMEPA, Division of Medication Error Prevention and Analysis

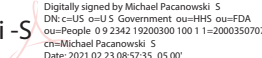
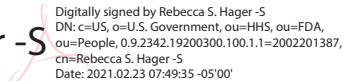
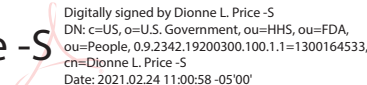
DPV, Division of Pharmacovigilance

DRISK, Division of Risk Management

Table 58. Signatures of Reviewers

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved
Clinical	Kathleen Donohue	OND/DRDMG	IA & ES ☐ Authored ☒ Approved
Division Director	Signature: Kathleen Donohue, M.D.  Digitally signed by Kathleen Donohue, M.D. DN: cn=Kathleen Donohue, M.D., o=FDA, ou=DGIEP OND CDER, email=Kathleen.Donohue@fda.hhs.gov, c=US Date: 2021.02.23 10:13:23 -05'00'		
Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved
Clinical	Jacqueline Karp	OND/DRDMG	ES & IA 3,4,6,7 ☒ Authored Entire doc. ☒ Approved
Cross-Disciplinary Team Lead	Signature: Jacqueline E. Karp -S  Digitally signed by Jacqueline E. Karp -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2002101371, cn=Jacqueline E. Karp -S Date: 2021.02.23 09:34:43 -05'00'		
Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved
Clinical	Sheila Farrell	OND/DRDMG	3,4,6,7 ☒ Authored ☐ Approved
Reviewer	Signature: Sheila M. Farrell -S  Digitally signed by Sheila M. Farrell -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2002968569, cn=Sheila M. Farrell -S Date: 2021.02.23 07:34:34 -05'00'		
Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved
Regulatory	Michael G. White	OND/DRO- RPURM	12 ☒ Authored ☐ Approved
Chief, Project Management Staff	Signature: Michael White -S  Digitally signed by Michael White -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Michael White -S, 0.9.2342.19200300.100.1.1=2001600058 Date: 2021.02.22 20:19:01 -05'00'		
Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved
Regulatory	Pamela Lucarelli	OND/DRO- RPURM	12 ☐ Authored ☒ Approved
Director, Project Management Staff	Signature: Pamela K. Lucarelli -S  Digitally signed by Pamela K. Lucarelli -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000426455, cn=Pamela K. Lucarelli -S Date: 2021.02.23 18:21:19 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved
Pharmacology/Toxicology	Jackye Peretz	OND/DPT- RPURM	5.1, 7.1, 8.4, 13, 22 ☒ Authored ☐ Approved
Reviewer	Signature: Jackye R. Peretz -S Digitally signed by Jackye R. Peretz -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2001856434, cn=Jackye R. Peretz -S Date: 2021.02.23 08:27:38 -05'00'		
Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved
Pharmacology/Toxicology	Mukesh Summan	OND/DPT- RPURM	5.1, 7.1, 8.4, 13, 22 ☐ Authored ☒ Approved
Division Director	Signature: Mukesh Summan -S Digitally signed by Mukesh Summan -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000337340 cn=Mukesh Summan -S Date: 2021.02.24 10:40:50 -05'00'		
Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved
Clinical Pharmacology	Oluseyi Adeniyi	OCP/DTPM	5, 6.1, 8.1, 8.2, 14, 22, 24 ☒ Authored ☐ Approved
Reviewer	Signature: Oluseyi Adeniyi -S Digitally signed by Oluseyi Adeniyi -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2001901490, cn=Oluseyi Adeniyi -S Date: 2021.02.22 20:27:47 -05'00'		
Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved
Clinical Pharmacology	Jie Wang	OCP/DTPM	5, 6.1, 8.1, 8.2, 14, 22, 24 ☐ Authored ☒ Approved
Team Leader	Signature: Jie Wang -S Digitally signed by Jie Wang -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Jie Wang -S 0.9.2342.19200300.100.1.1=2000739081 Date: 2021.02.22 21:38:48 -05'00'		
Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved
Clinical Pharmacology/Pharmacometrics	Liang Li	OCP/DPM	5, 6.1, 8.1, 8.2, 14, 22, 24 ☒ Authored ☐ Approved
Reviewer	Signature: Liang Li -S Digitally signed by Liang Li -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Liang Li -S, 0.9.2342.19200300.100.1.1=2001459144 Date: 2021.02.23 00:23:26 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved
Clinical Pharmacology/Pharmacometrics	Lian Ma	OCP/DPM	5, 6.1, 8.1, 8.2, 14, 22, 24 ☐ Authored ☒ Approved
Team Leader	Signature: Lian Ma -S		
Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved
Clinical Pharmacology	Michael Pacanowski	OCP/DTPM	5, 6.1, 8.1, 8.2, 14, 22, 24 ☐ Authored ☒ Approved
Division Director	Signature: Michael Pacanowski -S		
Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved
Statistical	Rebecca Hager	OB/DBIII	6.2, 6.3, 16 ☒ Authored ☐ Approved
Reviewer	Signature: Rebecca S. Hager -S		
Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved
Statistical	Yan Wang	OB/DBIV	6.2, 6.3, 16 ☐ Authored ☒ Approved
Team Leader	Signature: Yan Wang -S		
Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved
Statistical	Dionne Price	OB/DBIV	6.2, 6.3, 16 ☐ Authored ☒ Approved
Division Director	Signature: Dionne L. Price -S		

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Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved
Product Quality	Hitesh Shroff	OPQ/ONDP	9 ☒ Authored ☒ Approved
Team Leader	Signature: Hitesh N. Shroff -S 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: 2021.02.23 12:56:29 -05'00'		

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/

MICHAEL G WHITE
02/26/2021 02:44:50 PM

HYLTON V JOFFE
02/26/2021 02:50:39 PM

**Response to Consult Request - PTCC Extractables and Leachables
Subcommittee**

DATE: 11/10/2020
TO: Jackye Peretz, Ph.D.
FROM: Timothy W. Robison, Ph.D., D.A.B.T.
SUBJECT: Toxicological Assessment of Leachables, (b) (4)

COMMITTEE MEMBERS: Dan Mellon (DAAP), Timothy McGovern (OND IO), David Joseph, Jane Sohn (OPND), Luqi Pei (DPACC), Eleni Salicru (DRTM), Xiaochun Chen (DRTM), Huiqing Hao (OCHEN), Irene Surh (DAAP), Sree Rayavarapu (OGD), Carlic Huynh (DAAP), Katie Sokolowski (DAAP), Misol Ahn (DAAP), Jaime D'Agostino (DAAP), Newton Woo (DAAP), Jay Chang (DAAP), Rama Dwivedi (OCHEN), Narendranath Reddy Chintagari (OGD), Melanie Mueller (OGD), Naomi Kruhlak (DARS), Lidiya Stavitskaya (DARS), Robert Dorsam (OGD), Taro Akiyama (OPND), Imran Khan (DAAP), Marlene Kim (DARS), Jenny Li (OID), Cindy Li (OII/DPTII), Jonathan Cohen (ORDPURM/DPTRDPURM), Armaghan Emami (DAAP), Silvia De Paoli (CBER), Amy Skinner (OOD/DHOT), and Anne Pilaro (CBER)

Questions to the Committee:

In the simulated leachables study, the Applicant identified two (b) (4) (b) (4) (b) (4) at concentrations calculated to be greater than the AET of (b) (4) µg/mL; (b) (4) µg/mL, and (b) (4) µg/mL respectively. Thus, the Applicant conducted a toxicological assessment to further understand the potential risks associated with these impurities and qualify them at the measured levels. However, the Applicant did not evaluate the reported leachables due to lack of toxicological information for them and, instead, they assessed (b) (4) in the toxicological assessment). Further, the derivation of the PDE for (b) (4) is based on occupational exposure in human workers.

1. Is the Applicant's toxicological assessment of these leachables necessary to determine the safety of two compounds identified in the leachables study, (b) (4)
2. Is the Applicant's toxicological assessment acceptable to support the levels of these two compounds?

Background and Relevant Data:

The container closure system (CCS) for fosdenopterin HBr consists of a glass vial (b) (4) closed with a rubber stopper (b) (4) and (b) (4) tubing (b) (4). Fosdenopterin HBr in the proposed primary container closure system is sensitive to light and a cardboard carton is proposed as the secondary package to protect the product from light.

The Applicant conducted extraction and simulated leachables studies with the vial and stopper of the primary CCS to inform the leachable assessment. Solvents chosen for the extraction studies were water, ethanol, hexanes, and 6% acid (5% Nitric Acid/1% hydrochloric acid). Fosdenopterin HBr (coded as ORGN001 Drug Product) was used for the simulated leachables study.

During the simulated leachables study, a set of CCS each with lyophilized drug product were incubated in an inverted position at 25°C for 1 month (32 days). After the incubation period, the samples were reconstituted with 5 mL of water and held inverted at room temperature for 4 hours, the maximum time allowed for reconstituted drug product. During the final 30 minutes of inverted incubation at room temperature, a portion of plastic tubing was cut and submerged in the reconstituted drug product solution in each vial. A control solution of the liquid drug product that was not exposed to the vial/stopper or tubing was prepared in the same manner as the sample preparation (i.e., it was stored at 25°C for 32 days and then stored at room temperature for 4 hours) to ensure that there was no contamination from the labware or reagents used that could be misinterpreted as a leachable compound.

The analytical evaluation threshold (AET) was calculated based on ICH M7(R1). Leachables of concern were identified at the toxicological threshold of concern (TTC) for chronic exposure to a genotoxic or carcinogenic compound of 1.5 µg/day, used as the Safety Concern Threshold (SCT). This was used to derive an AET of (b) (4) µg/mL. Fosdenopterin HBr is administered daily via intravenous infusion at a recommended dose of 1.2 mg/kg/day (0.9 mg/kg/day of fosdenopterin). Fosdenopterin HBr is lyophilized powder providing 12.5 mg of fosdenopterin HBr per vial (equivalent to 9.5 mg of fosdenopterin). The vial contents should be reconstituted in 5 mL of sterile water.

(b) (4)

The results of the simulated leachables study are presented in Table 1. Six potential (b) (4) leachables and (b) (4) were identified and evaluated for safety. (b) (4)

(b) (4) were detected at concentrations calculated to be greater than the AET of (b) (4) µg/mL, at levels of (b) (4) µg/mL, (b) (4) µg/mL, and (b) (4) µg/mL respectively. Thus, the Applicant conducted a toxicological assessment to further understand the potential risks associated with these impurities and qualify them at the measured levels. However, the Applicant did not evaluate the reported leachables due to lack of toxicological information for them and, instead, they assessed (b) (4) (b) (4) in the toxicological assessment). Further, the derivation of the PDE for (b) (4) is based on occupational exposure in human workers.

Identified Leachables

Analysis	Tentative Identification	CAS#	Est. Concentration (µg/mL)
Direct Injection GC/MS (Semi-Volatile Compounds)			
Headspace GC/MS (Volatile Compounds)			
LC/UV/MS (Non-Volatile Compounds)			
ICP/MS			
*The Applicant identified (b) (4) µg/mL, for a maximum daily exposure of (b) (4) µg (maximum daily dose volume of (b) (4) mL of fosdenopterin HBr). The FDA has established a Reference Daily Intake (RDI) of (b) (4) for adults and children 4 years of age and older (21 CFR 101.9). Considering that the potential daily exposure to (b) (4) is far lower than the RDI set by the FDA, the presence of (b) (4) above the AET at (b) (4) µg/day is of no safety concern.			

Daily doses of (b) (4):

With a maximum daily dose volume of (b) (4) patients would be exposed to (b) (4) µg/day ((b) (4) µg/mL) and (b) (4) µg/day ((b) (4) µg/mL).

At the AET, doses of (b) (4) would be (b) (4) mcg/day ((b) (4) mcg/mL).

Committee Response (All):

The consult request was discussed at the subcommittee meeting on Friday October 30, 2020.

1. Dr. Naomi Kruhlak (Division of Applied Regulatory Sciences) located a consult request from Dr. Jaime D'Agostino (DAAP) in which (b) (4) was identified as leachable and Sponsor proposed the use of (b) (4) as surrogates for general toxicity. Dr. Kruhlak's group confirmed that (b) (4) were structurally acceptable surrogates. I'm attaching the DARS's computational structural [(Q)SAR] analysis of (b) (4)

2. Dr. Jaime D'Agostino's review of the general toxicology information for (b) (4) is attached in Appendix 2. Calculated ADIs for (b) (4) were (b) (4) and (b) (4) mg/day, respectively.

3. Attached in Appendix 3 is a more detailed review of available nonclinical data for (b) (4). The calculated ADI was (b) (4) µg/day.

4. Daily exposures to (b) (4) were below the qualification threshold of (b) (4) µg/day with respect to systemic toxicity for nongenotoxic leachables in parenteral products.

5. Although not part of the consult request, the Subcommittee noted that levels of leachables were obtained from a simulated leachables study rather than long-term stability studies. The Subcommittee noted that experience with simulated leachables studies is limited. It is recommended that the Division consider whether use of a simulated leachables study to determine levels of leachables is appropriate as opposed to use of long-term stability studies.

Final Recommendation:

Overall, the group agreed that it would be acceptable to extrapolate information from (b) (4) to cover (b) (4).

Appendix 1: DARS Consult Request

Adequate repeated dose toxicity studies for the leachable chemical (b) (4) are limited or not available. Therefore, a read across approach was used by the Applicant to extrapolate toxicity data from the surrogates to the leachable chemical to assess its toxicological risk. The Applicant selected (b) (4) and (b) (4) as suitable surrogates for the risk assessment of (b) (4).

To: Jaime D'Agostino (CDER/OND/ON/DPTN)
cc: Jay Chang (CDER/OND/ON/DPTN)
From: CDER/OTS/OCP/DARS: Computational Toxicology Consultation Service
Re: NDA (b) (4)
Date: April 24, 2020

Summary

Adequate repeated dose toxicity studies for the leachable chemical (b) (4) are limited or not available. Therefore, a read-across approach was used by the Applicant to extrapolate toxicity data from the surrogates to the leachable chemical to assess its toxicological risk. The Applicant selected (b) (4) as suitable surrogates for the risk assessment of (b) (4).

The CDER/OTS/OCP/DARS Computational Toxicology Consultation Service was consulted to determine if (b) (4) are acceptable surrogates for (b) (4) and to determine whether any additional surrogates exist. SciFinder Scholar (<https://scifinder.CASRN.org>) was used to confirm the chemical structures of the substances submitted under this consult.

A review of the structural attributes of (b) (4) combined with a search for structural analogs with repeat dose study data confirmed that (b) (4) and (b) (4) are acceptable surrogates. Both surrogates proposed by the Applicant are of comparable, but lower, molecular weight than (b) (4) and would provide a good conservative estimate of its potential toxicity if tested. This is an acceptable approach. Of the publicly available analogs reviewed, (b) (4) is the most structurally similar and has suitable publicly available toxicity data.

No suitable analogs of more similar molecular weight to that of (b) (4) could be found where toxicity could be verified in GLP compliant studies. However, earlier non-GLP studies indicate that most (b) (4) substances were well tolerated in most animal models and most administration routes at doses substantially larger than any likely for this NDA. Any issues noted tended to be with the lower molecular weight preparations (b) (4). Therefore, estimating the toxicity of (b) (4) with results of similar compounds of lesser molecular weight is appropriate.

Leachable	(b) (4)
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Applicant's Surrogates	(b) (4)	

References

(b) (4)

Appendix 2: Review of available toxicity information for (b) (4)

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/

JACKYE R PERETZ
12/10/2020 11:12:11 AM

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY IND ASSESSMENT AND EVALUATION

Application Number*: 117502
Supporting Document Number/s: 39, 47, 53, 100
CDER Receipt Date: SDN 39: 4/17/2015
SDN 47: 8/13/2015
SDN 53: 4/26/2016
SDN 100: 12/12/2019
Sponsor: Origin Biosciences, Inc.
Product: Fosdenopterin hydrobromide as dihydrate
(Fosdenopterin HBr)
Pharmacologic Class: Cyclic pyranopterin monophosphate analog
Indication: Molybdenum Cofactor Deficiency (MoCD)
Type A
Therapeutic area: Inborn Errors of Metabolism
Review Division: Division of Rare Diseases and Medical
Genetics
Reviewer: Jackye Peretz, Ph.D.
Supervisor/Team Leader: Adebayo Laniyonu, Ph.D. (Acting)
Division Director: Mukesh Summan, Ph.D., D.A.B.T. (Acting)
Project Manager: Mimi Phan
Purpose of Review: Study Report Review
Studies submitted under IND 117502 that
have not been reviewed prior to the
submission of NDA 214018
Reviewer Completion Date: October 7, 2020

Template Version: Feb 14, 2020

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Executive Summary (Clinical Relevance)

Nonclinical Regulatory Recommendation:

Clinical Study (ies) Safe to Proceed (Nonclinical Recommendation):

Allow to Proceed

Brief Discussion of Clinically Relevant Findings:

Based on the pharmacology data submitted thusfar, fosdenopterin HBr may be efficacious at restoring molybdenum cofactor biosynthesis in patients with MOCD Type A.

Fosdenopterin HBr was well tolerated in subchronic and chronic toxicity studies in juvenile rats and dogs at doses up to 17.8 the MRHD of 0.9 mg/kg/day (based on HED). Prolonged APTT (20%) was observed in the 39-week toxicity study in juvenile dogs at 6.2x the MRHD (based on HED), but in the absence of other indices that this change negatively affected the health of the animal, it was not considered an adverse effect.

Fosdenopterin HBr was phototoxic at 4.4x the MRHD (based on HED). In conjunction with demonstrating phototoxic potential in vitro, dose-dependent cutaneous skin reactions (erythema, edema, flaking, and eschar) and ophthalmic and histopathologic changes indicative of phototoxicity were observed following intravenous administration of fosdenopterin HBr.

Safety Margins Based on NOAEL from Pivotal Toxicology Studies

Nonclinical				Clinical Safety Margins (Based on HED)
Species/ Duration	NOAEL (mg/kg/day)	HED (mg/kg/day)	AUC ($\mu\text{g}\cdot\text{h}/\text{mL}$)	MRHD 0.9 mg/kg/day
Juvenile Rats 13 weeks	100	16	532 ¹	17.8x
Juvenile Rats 26 weeks	5	0.8	30.5 ¹	0.89x
Juvenile Dogs 39 Weeks	10	5.6	119 ¹	6.2x

NOAEL = no-observed-adverse-effect level; HED = human equivalent dose; AUC = area under the concentration-time curve; MRHD = maximum recommended human dose

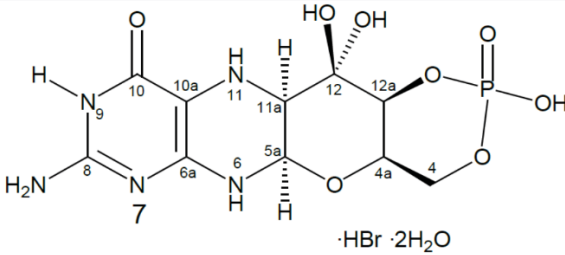
1. AUC values from Sponsor multiplied by specific factors to achieved AUC_{0-24h}. As a result, use of HED to determine clinical safety margins is preferred.

Nonclinical Findings of Potential Clinical Relevance

Toxicity/Finding	Reversible?	Species/ Duration	Dose (mg/kg/day)	HED (mg/kg/day)	Clinical Safety Margin (Based on HED vs MRHD of 0.9 mg/kg/day)
APTT prolongation	Not Assessed	Dog 39 weeks	10	5.6	6.2x
Phototoxicity	Not Assessed	Rats 3 days	25	4.0	4.4x

Nonclinical Summary

Drug Information⁺

Type of Product:	Lyophilized powder for injection
Code/ Generic Name:	Fosdenopterin HBr/ fosdenopterin hydrobromide as dihydrate; ALXN1101; cPMP; (b) (4) ; scPMP
Chemical Name (optional):	Cyclic pyranopterin monophosphate (cPMP) monohydrobromide (HBr) dihydrate; (4a <i>R</i> ,5a <i>R</i> ,11a <i>R</i> ,12a <i>S</i>)-8-Amino-2,12,12-trihydroxy-4,4a,5a,6,9,10,11,11a,12,12a-decahydro-2H-1,3,5-trioxa-6,7,9,11-tetraaza-2λ ⁵ -phosphatetracene-2,10,dione (hydrobromide dihydrate)
CAS# or UNII (if available):	CAS:150829-29-1 (freebase); 2301083-34-9 (fosdenopterin hydrobromide as dihydrate) UNII:
Structure or Biochemical Description:	 Fosdenopterin HBr is a pterin derivative called cyclic pyranopterin monophosphate (cPMP). Fosdenopterin HBr has four chiral centers at positions 5a, 11a, 12a, and 4a.
Molecular Formula/ Molecular Weight:	C ₁₀ H ₁₉ BrN ₅ O ₁₀ P / 480.16 da
Comment on Excipients:	All excipients are covered by levels within the IID and/or qualified by toxicology studies.
Comment on Impurities:	Fosdenopterin HBr drug substance impurity (b) (4) has been qualified for in vivo safety in accordance with the ICH Q3B guideline.
Proposed 505(b)(2):	N
If Yes, Listed Drug:	
Biosimilar:	N
If Yes, Reference Product:	

Pharmacology/ Toxicology SummaryPharmacology (primary & secondary/ MoA)*Key Findings:*

After 8 days of intraperitoneal injection, fosdenopterin HBr at ≥ 1.1 mg/kg/day decreased plasma and brain levels of S-sulfocysteine, decreased urinary uric acid levels, dose-dependently increased liver sulfite oxidase (SO) activity, and dose-dependently increased body weight compared to placebo and untreated MOCS1 KO mice (mouse model of MOCD Type A). Notably, plasma SSC and urinary uric acid may not be adequate biomarkers to guide therapeutic dosing in patients with MOCD Type A because no dose effect was observed with fosdenopterin HBr on these factors. Fosdenopterin HBr had no off-target effects in an in vitro panel of enzyme and radioligand binding assays.

Safety Pharmacology:*Key Findings:*

Fosdenopterin HBr had no effect on neurological function or behavior in the 6- or 9-month toxicity studies in rats (up to 5 mg/kg/day) and dogs (up to 10 mg/kg/day), respectively. Fosdenopterin HBr had no inhibitory effect on a variety of ion channels tested in an in vitro panel, with $IC_{50} > 300$ μ M. In a standalone in vivo CVS study in dogs, fosdenopterin HBr had no effect on body temperature, blood pressure, heart rate, or on qualitative or quantitative ECG parameters. Respiratory safety pharmacology studies with fosdenopterin HBr were not conducted, and direct respiratory assessments were not conducted in the submitted toxicity studies.

PK/ ADME/ TK:*Key Findings:*

Similar systemic exposure to fosdenopterin HBr was observed with subcutaneous and intravenous injections, though C_{max} of fosdenopterin HBr was 36% higher when administered intravenously compared to subcutaneously. No sex differences in exposure were observed with either route of administration. Bioavailability following subcutaneous injection was 97%. Fosdenopterin HBr was low to moderately bound to plasma proteins in vitro, with and without ascorbic acid. (b) (4)

In vitro metabolic profiling indicated that two cPMP-related compounds (C1 and C2) were detected in mouse, rat, dog, and human hepatocytes incubated with 10 μ M E.coli-derived cPMP in the presence of ascorbic acid. Both C1 and C2 correspond to compound Z, a known oxidation product of cPMP. No human-specific metabolites were identified. In vivo metabolite studies have not been conducted with fosdenopterin HBr. E.coli-derived cPMP neither induced nor inhibited CYP enzyme expression or activity, and was not a substrate for P-gp, BCRP, or hepatic uptake and efflux transporters. E.coli-derived cPMP was a substrate for MATE1.

Toxicology

Single-dose Toxicity:

Key Findings:

Fosdenopterin HBr was well tolerated up to 100 mg/kg in rats.

Repeat-dose Toxicity:**Key Findings:**

In the 13-week toxicity study in juvenile rats in which fosdenopterin HBr (coded ALXN1101) was administered at 25, 50, and 100 mg/kg/day once daily, no adverse effects were observed. Animals were dosed subcutaneously from PND 7 to PND 20, and then by intravenous bolus from PND 21 onwards. The NOAEL was 100 mg/kg/day.

In the 26-week toxicity study in juvenile rats in which fosdenopterin HBr (coded ALXN1101) was administered at 1, 3, and 5 mg/kg/day once daily, no adverse effects were observed. Animals were dosed subcutaneously from PND 7 to PND 20, and then by intravenous bolus from PND 21 onwards. The NOAEL was 5 mg/kg/day.

In the 39-week toxicity study in juvenile dogs in which fosdenopterin HBr (coded ALXN1101) was administered at 1, 3, 5, and 10 mg/kg/day once daily, no adverse effects were observed. Prolonged APTT (22%) was observed at the high dose, but in the absence of any other indices that this change negatively affected the health of the animal, it is not considered an adverse effect. Animals administered 1, 3, and 5 mg/kg/day were dosed subcutaneously from PND 5 to PND 25, and then by intravenous bolus from PND 25 onwards. Animals administered 10 mg/kg/day were dosed subcutaneously from PND 5 to PND 25, but then dosed both subcutaneously at 5 mg/kg/day and intravenously at 5 mg/kg/day, for a total dose of 10 mg/kg/day from PND 25 onwards. The maximum feasible intravenous dose was determined to be 5 mg/kg, so these animals were co-administered a subcutaneous dose of fosdenopterin HBr to achieve 10 mg/kg/day. The NOAEL was 10 mg/kg/day.

Species/ Dose/ Duration	Summary	Key Study Findings
Juvenile Rats 25, 50, 100 mg/kg/day 13 Weeks Intravenous ROA	NOAEL: 100 mg/kg/day AUC: 532 $\mu\text{g}\cdot\text{h}/\text{mL}$ Cmax: 181 $\mu\text{g}/\text{mL}$ Tmax: 0.166 h	None
Juvenile Rats 1, 3, 5 mg/kg/day 26 Weeks Intravenous ROA	NOAEL: 5 mg/kg/day AUC: 30.5 $\mu\text{g}\cdot\text{h}/\text{mL}$ (PND 188) Cmax: 16.9 $\mu\text{g}/\text{mL}$ (PND 188) Tmax: 0.083 h (PND 188)	None
Juvenile Dogs 1, 3, 5, 10 mg/kg/day 39 Weeks Subcutaneous (PND1-22) and intravenous (PND 23+; 1, 3, 5 mg/kg/day) or SC+IV (PND23+; 10 mg/kg/day)	NOAEL: 10 mg/kg/day AUC: 119 $\mu\text{g}\cdot\text{h}/\text{mL}$ (Week 39) Cmax: 15.6 $\mu\text{g}\cdot\text{h}/\text{mL}$ (Week 39) Tmax: 0.10 h (Week 39)	- Prolonged APTT at SC+IV dose 10 mg/kg/day - No other treatment-related findings

Genotoxicity**Key Findings:**

Fosdenopterin HBr (b) (4) were all negative in their respective Ames tests, with and without metabolic activation.

Test	Results	Notes
Ames	Negative	Fosdenopterin HBr and (b) (4) up to (b) (4) µg/plate
In vivo chromosomal aberration	Negative	Fosdenopterin HBr; up to 486 µg/mL
in vivo micronucleus	Negative	Fosdenopterin HBr; up to 200 mg/kg/day

Special Studies

Key Findings:

Fosdenopterin HBr is phototoxic. Dose-dependent cutaneous skin reactions (erythema, edema, flaking, and eschar) and ophthalmic and histopathologic changes indicative of phototoxicity were observed following intravenous administration of fosdenopterin HBr.

Nonclinical Assessment and Recommendation

Based on the pharmacology data submitted thusfar, fosdenopterin HBr may be efficacious at restoring molybdenum cofactor biosynthesis in patients with MOCD Type A.

Fosdenopterin HBr was well tolerated in subchronic and chronic toxicity studies in juvenile rats and dogs. The doses used in the 26-week toxicity study in juvenile rats do not provide sufficient margins of safety to the proposed MRHD, but the doses used in the subsequently conducted 13-week toxicity study in juvenile rats are adequate (13.3x the MRHD at the NOAEL, based on HED). Prolonged APTT (20%) was observed in the 39-week toxicity study in juvenile dogs at 4.7x the MRHD (based on HED), but in the absence of other indices that this change negatively affected the health of the animal, it was not considered an adverse effect.

Fosdenopterin HBr was phototoxic at 3.3x the MRHD (based on HED). In conjunction with demonstrating phototoxic potential in vitro, dose-dependent cutaneous skin reactions (erythema, edema, flaking, and eschar) and ophthalmic and histopathologic changes indicative of phototoxicity were observed following intravenous administration of fosdenopterin HBr. The Sponsor is aware of this finding and will label for it accordingly (under NDA 214018).

1 Background

1.1 Regulatory History

During a Type B meeting held with the Sponsor on 4/12/2019, FDA agreed that reproductive toxicity studies and a carcinogenicity study in a single species will be conducted as post-marketing requirements.

NDA 214018 was submitted on 6/29/2020. Multiple nonclinical studies submitted under IND 117502 to support marketing authorization of NDA 214018 were not reviewed. Those studies are reviewed here.

1.2 Relevant INDs, NDAs, BLAs or DMFs

NDA 214018 (NDA for this drug product); DRDMG

1.3 Previous Reviews Referenced

Application	Reviewer	Date in DARRTS	Notes
IND 117502	B. Akinshola	11/25/2013	For studies reviewed, see table under 3.2

2 Supplemental Drug Information

2.1 Drug Formulation

Table 3 Composition of Drug Product Formulations Used in the Nonclinical *in vivo* Toxicology Studies

Component	(b) (4)			
Fosdenopterin hydrobromide				
L-Ascorbic acid				
(b) (4)				
Mannitol				
(b) (4)				
Water for injection				
Hydrochloric acid				
Sodium hydroxide				
(b) (4)				
Use of Material	Cardiovascular safety (dog) (Study 2087-001) Repeat dose toxicity (rat) (Study 2087-002) Repeat dose toxicity (dog) (Study 2087-003)	Repeat dose toxicity (juvenile rats) (Study 902668)	Repeat dose toxicity (dog) (Study 2087-006)	Repeat dose phototoxicity (pigmented rats) (Study 20202895)

Note: Changes from previous formulation are in bold font
QS = quantity sufficient

Table 4 Summary Clinical Development Formulations of Fosdenopterin Drug Product

Component	F5	F6	F7	F8
Fosdenopterin hydrobromide	(b) (4)	(b) (4)	2.5 mg/mL (as HBr dihydrate, equivalent to 1.9 mg/mL free base)	(b) (4)
L-Ascorbic acid			2.0 mg/mL	
Mannitol			37.5 mg/mL	
Sucrose			12.5 mg/mL	
Water for injection			QS	
Hydrochloric acid			QS to adjust pH (b) (4)	
Sodium hydroxide			QS to adjust pH (b) (4)	
(b) (4)			(b) (4)	
Reconstitution solution			WFI	
Use of Material	Clinical Stability Hemolysis assay	Clinical Stability	Clinical Stability <i>Proposed Commercial Formulation</i>	Clinical Stability

Note: Changes from previous formulation are in bold font

QS = quantity sufficient

2.2 Drug Excipients

All excipients are covered by levels within the IID and/or qualified by toxicology studies.

Table 1 Comparison of Excipient Amounts in Reconstituted Drug Product with FDA Inactive Ingredient Database Listing

Excipient	% (w/v) in Reconstituted Drug Product	% (w/v) in IIG ^[1]	
		Lowest	Highest
Mannitol	3.75	2	75
Sucrose	1.25	1	90
Ascorbic acid	0.20	0.088	62.50

[1] Source: <https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm>

2.3 Drug Impurities

Fosdenopterin HBr drug substance impurity (b) (4) has been qualified for *in vivo* safety in accordance with the ICH Q3B guideline.

3 Studies Submitted

3.1 Studies Reviewed

Study	Study Number	SDN	EDR
Primary Pharmacology			
Efficacy in a mouse model MOCSI: biochemical markers	GTR-0483	53	4.2.1.1
Secondary Pharmacology			
Radioligand binding and enzyme screen	TW04-0004059	100	4.2.1.2

Study	Study Number	SDN	EDR
Safety Pharmacology			
Ion Channels	190129	100	4.2.1.3
In vivo in dog	2087-001	39	4.2.1.3
PK-Absorption			
Single dose IV injection in dogs	2087-005	53	4.2.2.7
PK-Distribution			
Plasma protein binding	XS-0485	39	5.3.2.2
PK-Metabolism			
Metabolite profiling	XT134093	39	5.3.2.2
Metabolic stability	XT134092	39	5.3.2.2
CYP induction	XT133120	39	5.3.2.2
CYP inhibition	XT135111	39	5.3.2.2
PK-Excretion			
None			
PK-DDI			
Transporter substrate and inhibitor assays	XT138074	39	5.3.2.2
Single/Repeat Dose Toxicology			
Rats			
ALXN1101: An Acute and 7-Day Intravenous Dose Range-Finding Toxicity and Toxicokinetic Study in Rats	1727-016	53	4.2.3.3.2
A 13-Week Study of the Potential Toxicity of Daily Parenteral Injection of ALXN1101 in Juvenile Rats with a 28-Day Recovery Period	9000563	53	4.2.3.3.2
A 26-Week Study of the Potential Toxicity of Daily Parenteral Injection of ALXN1101 in Juvenile Rats with a 28 Day Recovery Period	902668; Amendment 1	47	4.2.3.2
Dogs			
ALXN1101: A 9-Month Intravenous Toxicity Study in Juvenile Beagle Dogs with a 3-Month Recovery Period	2087-006	53	4.2.3.3.2
Genotoxicity			
Ames test- (b) (4) mcg/plate	9600930	53	4.2.3.3.1
In vitro chrom ab in HPBLs- 486 mcg/mL	9600931	53	4.2.3.3.1
Ames test- (b) (4)	9600806	53	4.2.3.3.1
In vivo rat micronucleus assay- 200 mg/kg	9800235	53	4.2.3.3.2
Carcinogenicity			
Post-approval ¹			
Reproductive and Developmental Toxicity			

Study	Study Number	SDN	EDR
FEED in rats- Waiver Request Intended			
EFD in rats and rabbits: Post-approval ²			
PPND study in rats: Post-approval ²			
Local Toxicity			
Neutral Red Uptake Phototoxicity Assay of ORGN001 in BALB/c 3T3 Mouse Fibroblasts	20181580	100	4.2.3.7.7
Repeat-Dose Phototoxicity Study in Eye and Skin	20202895	100	4.2.3.7.7
1. As agreed with the FDA during the Type B meeting on 12 April 2019, a carcinogenicity study in a single species will be conducted as a post-marketing requirement. 2. Reproductive and developmental toxicity studies with fosdenopterin HBr have not been conducted. As agreed with the FDA during the Type B meeting between the Sponsor and FDA on 12 April 2019, reproductive toxicity studies will be conducted as post-marketing requirements.			

3.2 Studies Not Reviewed

Study	Study Number	Notes
Efficacy in a mouse model of MoCD	GTR-0466	Reviewed previously (B. Akinshola 11/25/2013)
hERG assay	793428	Reviewed previously (B. Akinshola 11/25/2013)
A 2-Week Intravenous Toxicity Study of ALXN1101 Administered Once Daily in Rats with a 4-Week Recovery Period	2087-002	Reviewed previously (B. Akinshola 11/25/2013)
A 2-Week Intravenous Toxicity Study of ALXN1101 Administered Once Daily in Beagle Dogs with a 4-Week Recovery Period	2087-003	Reviewed previously (B. Akinshola 11/25/2013)
Ames test- 500 mcg/plate	964425	Reviewed previously (B. Akinshola 11/25/2013)
In vitro chrom ab in HPBLs- 83.3 mcg/mL	964426	Reviewed previously (B. Akinshola 11/25/2013)
In vivo rat micronucleus assay- 10 mg/kg	980110	Reviewed previously (B. Akinshola 11/25/2013)
ALXN1101: An In Vitro Hemolysis Assay with Human Whole Blood	2087-004	Reviewed previously (B. Akinshola 11/25/2013)

4 Pharmacology

4.1 Primary Pharmacology

Mechanism of action

Fosdenopterin HBr provides an exogenous source of cyclic pyranopterin monophosphate (cPMP), allowing for the enzyme complex MOCS2A/MOCS2B/MOCS3 to convert cPMP to molybdopterin (MPT), which is subsequently converted to molybdenum cofactor, the requisite cofactor necessary for activation of molybdenum-dependent enzymes.

Drug activity related to proposed indication

Fosdenopterin HBr is a cyclic pyranopterin monophosphate (cPMP) analog, restoring molybdenum cofactor biosynthesis and normalizing the level of sulfite oxidase.

Supportive Data

Patients with MoCD type A have mutations in the gene encoding molybdenum cofactor biosynthesis protein 1 (MOCS1), abolishing the formation of cyclic pyranopterin monophosphate (cPMP), the first stable intermediate of the MoCo biochemical pathway. S-sulfocysteine (SSC) is a neurotoxic secondary metabolite that accumulates in MoCD patients, contributes to severe, progressive neurologic damage and early death by inhibiting gamma-glutamylcysteine synthetase in the glutathione synthesis pathway. SSC has been used as a reliable biochemical indicator for MoCD. Fosdenopterin HBr is hypothesized to replace the missing cPMP, restoring molybdenum cofactor biosynthesis and normalizing the level of sulfite oxidase.

MOCS1 knockout (MOCS1^{-/-}) mice have been previously reported to display a severe phenotype that reflects key characteristics of human MoCD Type A ([Lee HJ et al., 2002](#)). MOCS1 KO mice died soon after birth and showed a significantly slower gain of body weight in comparison with their wild type and/or heterozygous littermates.

In [Study GTR-0483](#), fosdenopterin HBr (coded as ALXN1101) at 0.1, 1.1, 2.2, or 4.4 mg/kg/day or placebo was administered to MOCS1 KO mice via intraperitoneal injection once daily from PND 1 to 28. At PND 9, fosdenopterin HBr at ≥ 1.1 mg/kg decreased plasma and brain levels of S-sulfocysteine (SSC) (Figure 3, Figure 7), decreased urinary uric acid levels (Figure 9), increased liver sulfite oxidase (SO) activity (Figure 5), and increased body weight (Figure 1) compared to placebo and untreated MOCS1 KO mice. Beyond PND 9, body weight gains animals administered < 4.4 mg/kg/day were decreased compared to wild-type or heterozygous littermates (Figure 2). A comparison to untreated MOCS1 KO controls beyond PND 9 cannot be made because these mice died or were euthanized by PND 8. Body weights and body weight gains at 4.4 mg/kg/day were maintained similarly to wild-type and heterozygous littermates until PND22. From PND 22 to 28, body weights and body weight gains at 4.4 mg/kg/day were reduced compared to wild-type and heterozygous littermates, though no body weight loss was reported (Figure 2). At PND 28, dose-dependent reductions in brain SSC (Figure 4), decreased plasma SSC levels (Figure 8) and urinary uric acid levels (Figure 10), and dose-dependent increases in liver SO activity (Figure 6) were observed in fosdenopterin HBr-

treated animals. Further, brain SSC levels and liver SO activity at ≥ 2.2 mg/kg/day were comparable to wild-type and heterozygous littermates.

Notably, on PND 9 and PND 28 plasma SSC levels were decreased and urinary uric acid levels were increased ≥ 1.1 mg/kg/day compared to WT and heterozygous littermates, with no obvious dose effect (Figure 8, Figure 10). As a result, the Sponsor concluded that plasma SSC and urinary uric acid may not be adequate biomarkers to guide therapeutic dosing in patients with MOCD Type A.

Figure 1: Body Weights in MOCS1 KO Mice; PND 1-9

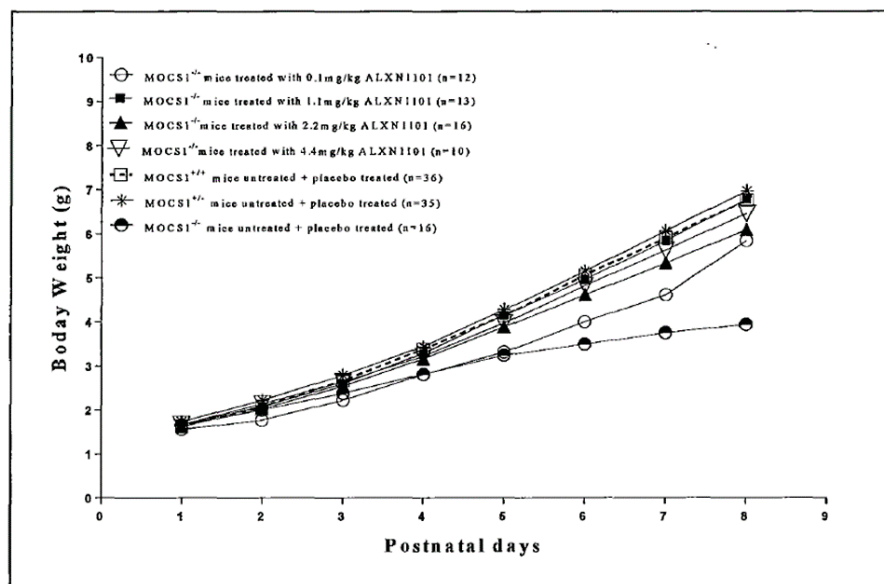


Figure 2: Body Weights in MOCS1 KO Mice; PND 1-28

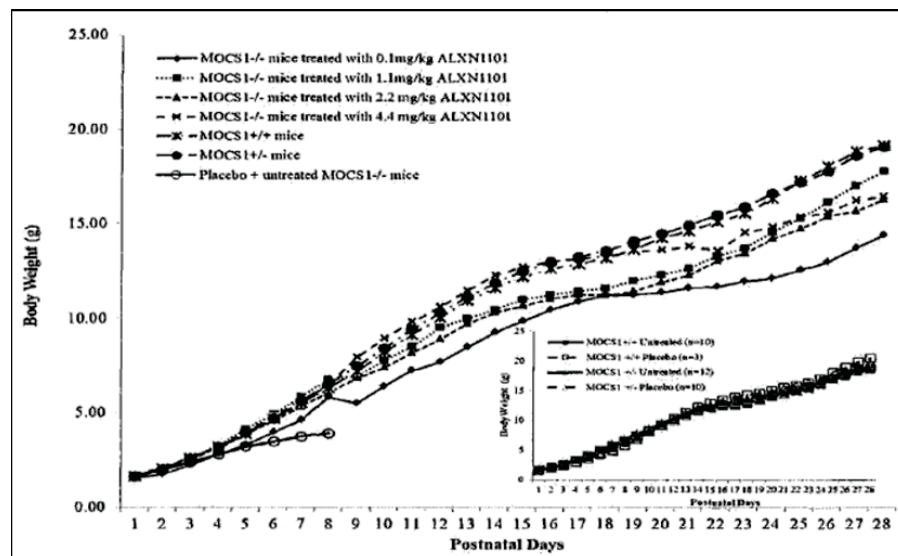


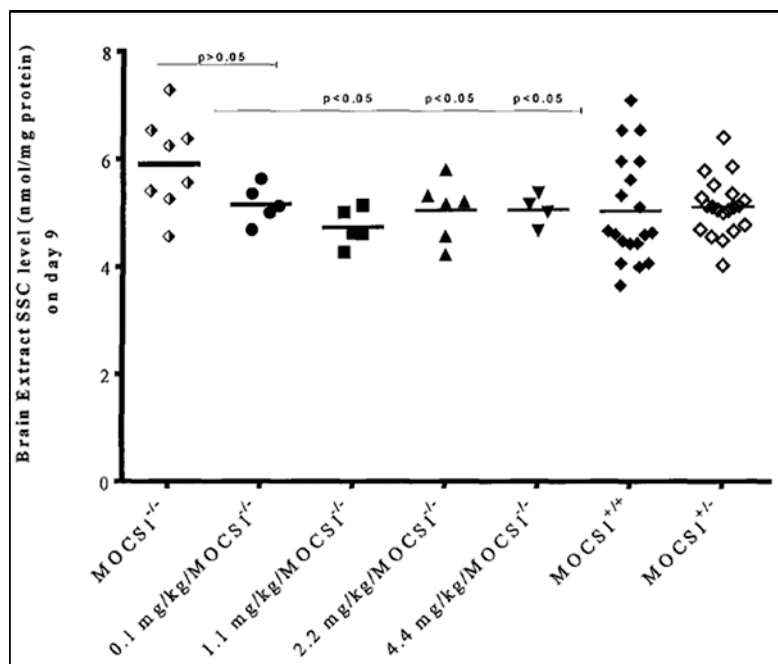
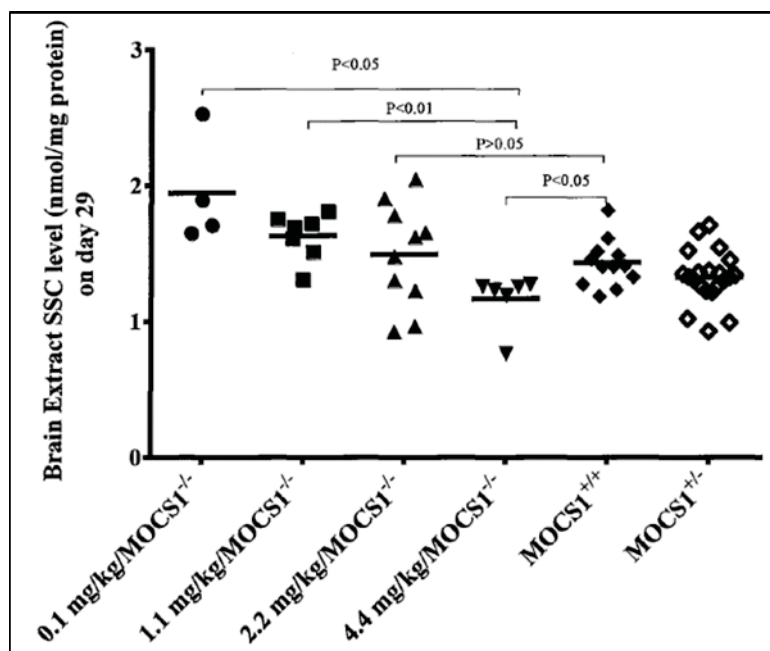
Figure 3: Brain SSC Levels in MOCS1 KO Mice; PND 9**Figure 4: Brain SSC Levels in MOCS1 KO Mice; PND 28**

Figure 5: Liver SO Activity in MOCS1 KO Mice; PND 9

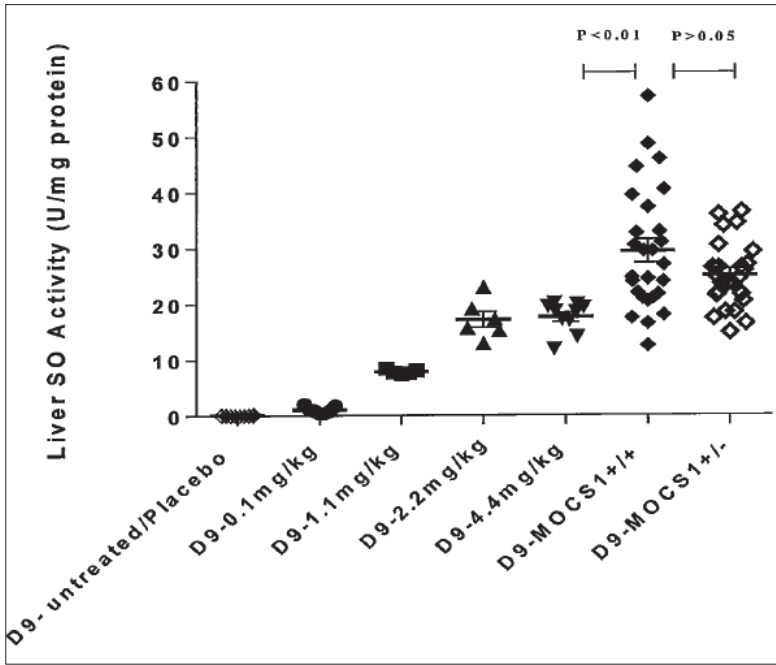


Figure 6: Liver SO Activity in MOCS1 KO Mice; PND 28

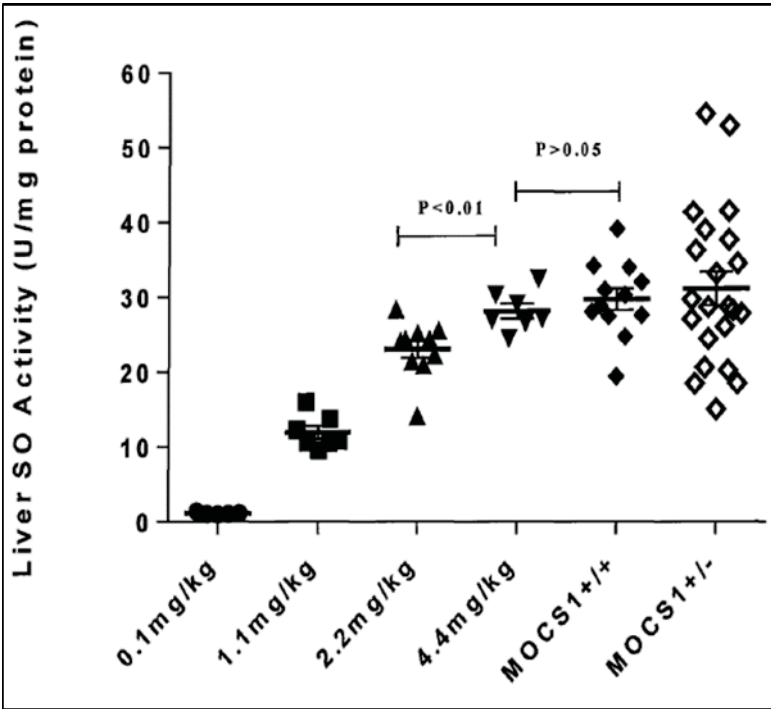


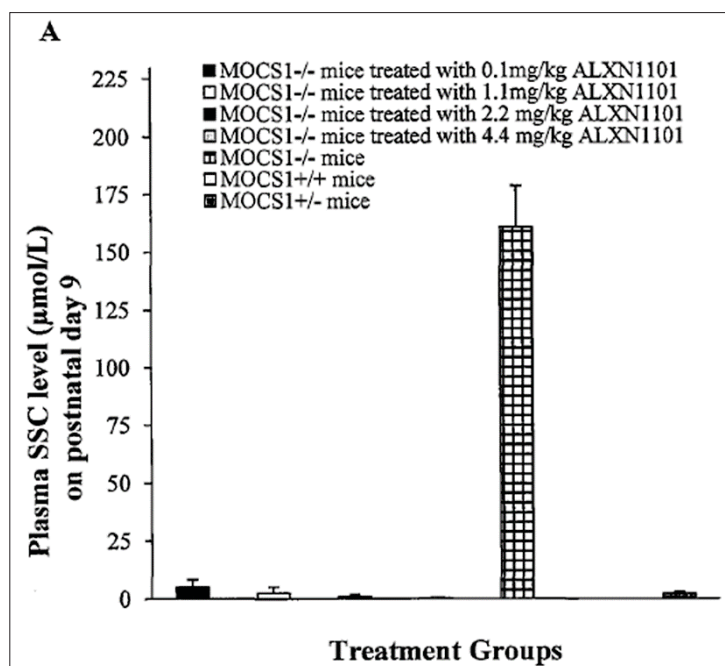
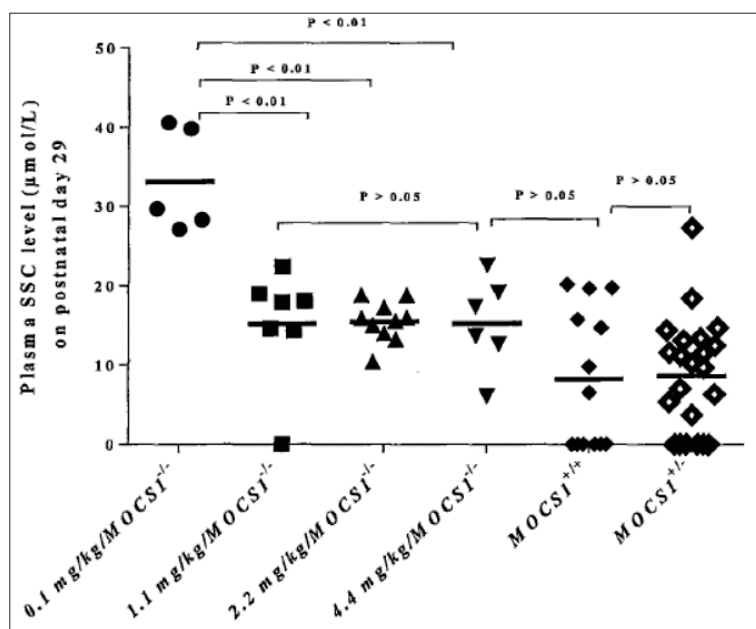
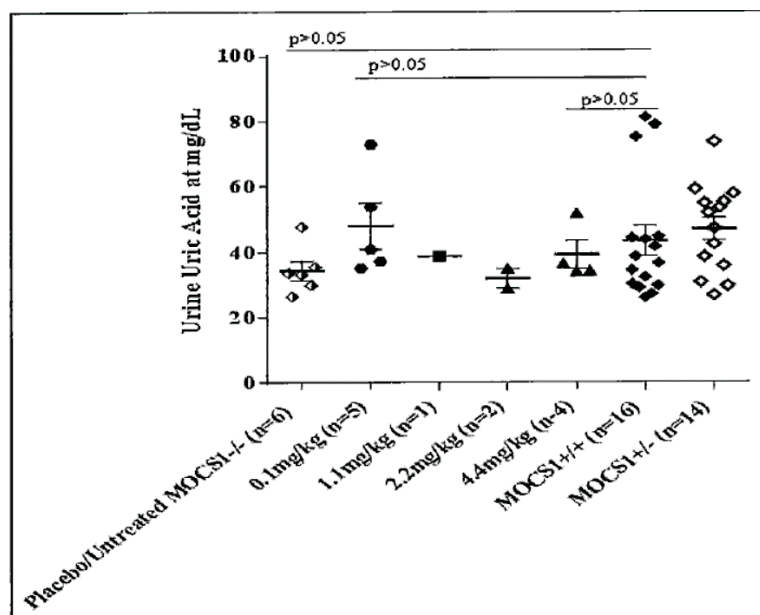
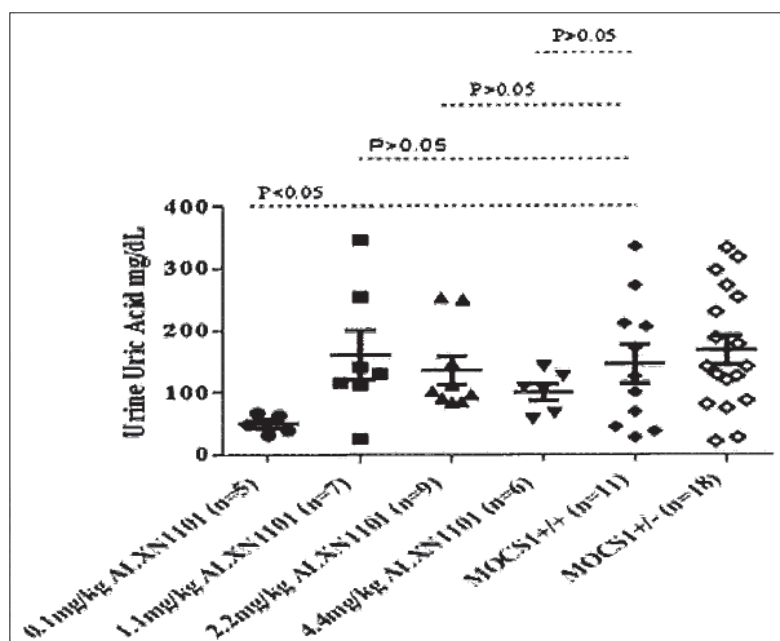
Figure 7: Plasma SSC levels in MOCS1 KO Mice; PND 9**Figure 8: Plasma Levels of SSC in MOCS1 KO Mice; PND 28**

Figure 9: Uric Acid Levels in MOCS1 KO Mice; PND 9**Figure 10: Uric Acid Levels in MOCS1 KO Mice; PND 28**

4.2 Secondary Pharmacology

Fosdenopterin HBr (coded as cPMP/ORG001/ALXN1101) was evaluated in an in vitro panel of enzyme and radioligand binding assays ([Study TW04-0004059](#)). No significant results were observed.

4.3 Safety Pharmacology

Central nervous system

A standalone CNS safety pharmacology study was not conducted. Neurological function and behaviors were evaluated in the 6- and 9-month toxicity studies in rats ([Study 902668](#)) and dogs ([Study 2087-006](#)), respectively. Fosdenopterin HBr had no effect on neurological function or behaviors in either study.

Cardiovascular system

The in vitro effects of fosdenopterin HBr (coded ALXN1101; 10, 30, 100, and 300 μ M) on a variety of ion channels were evaluated using an automatic parallel patch clamp system, the QPatch HT (Sophion Bioscience A/S, Denmark) ([Study 109129](#)). Fosdenopterin HBr had no inhibitory effect on any of the channels tested.

Channel	Cells	Responsibility	IC50
hCav1.2 L-type	CHO	I _{Ca,L} , high threshold calcium current	>300 μ M
hCav3.2 T-type	HEK293	I _{Ca,T} , low threshold calcium current	>300 μ M
hHCN2	HEK	I _f , hyperpolarization-activated cation current	>300 μ M
hHCN4	HEK	I _f , hyperpolarization-activated cation current	>300 μ M
hERG	HEK293	Potassium channel current	>300 μ M
hKir2.1	HEK293	I _{K1} , inwardly rectifying potassium current	>300 μ M
hKir3.1/hKir3.4	HEK293	I _{ACh} , inwardly rectifying potassium current	>300 μ M
Kir6.2/SUR2A	HEK293	I _{KATP} , ATP-sensitive current	>300 μ M
hKv1.5	CHO	I _{Kur} , ultra-rapid delayed rectifier potassium current	>300 μ M
hKv4.3/kChiP2.2	CHO	Potassium channels	>300 μ M
hKvLQT1/hminK	CHO	I _{Ks} , slow delayed rectifier potassium current	>300 μ M
hNav1.5	CHO	Peak and late sodium channel currents	>300 μ M

In a standalone CVS safety pharmacology study, four male beagle dogs were administered a single dose of fosdenopterin HBr (coded ALXN1101) via intravenous infusion at 1, 3, and 10 mg/kg/day, or the vehicle control in a Latin square design with a 7-day washout period between doses ([Study 2087-001](#)). The vehicle consisted of (b) (4)

(b) (4) mannitol (b) (4) mg/mL), and (b) (4) in sterile water for injection, adjusted to pH (b) (4). The animals were instrumented with vascular access ports (VAP) for IV dosing and telemetry transmitters for measurement of body temperature, blood pressure, heart rate, and ECG. Body temperature, systolic, diastolic, and mean arterial blood pressures, heart rate, and ECG parameters (QRS duration and the RR, PR, and QT intervals) were monitored continuously from at least 2 hours pre-dose until at least 24 hours following the start of the 2-hour infusion dose. The QTc interval was calculated using a procedure based on the method described by Spence, et al., and modified by Miyazaki and Tagawa. Notably, the average recovery concentrations determined from samples collected from the dosing formulations were variable, and values ranged from 59.7% to 95.5%. Many of these results were outside of the acceptance criteria for a solution.

No mortalities occurred, and no treatment-related clinical signs or effects on body weight, body temperature, blood pressure, or heart rate were observed. Fosdenopterin HBr had no effect on qualitative or quantitative ECG parameters.

Respiratory system

A standalone respiratory safety pharmacology study was not conducted, and direct respiratory assessments were not conducted in the toxicology studies

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Disproportional Metabolite(s): N

Unique Human Metabolite(s): Unknown

In vivo metabolite studies have not been conducted with fosdenopterin HBr.

Summary of PK/ADME

Pharmacokinetic analysis following a single dose of fosdenopterin HBr (5 mg/kg) administered to juvenile dogs (lactational day 8 or 9) either via intravenous or subcutaneous injections revealed similar systemic exposure to fosdenopterin HBr between the two routes of administration ([2087-005](#)). Bioavailability following subcutaneous injection was 97%. Notably, C_{max} of fosdenopterin HBr was 36% higher when administered via intravenous injection compared to subcutaneous injection. No sex differences in exposure were observed with either route of administration.

Fosdenopterin HBr (coded ALXN1101) was low to moderately bound to plasma proteins in vitro, with and without ascorbic acid (Table 1; [Study XS-0485](#)). (b) (4)

([Study XS-0485](#)).

Table 1: In Vitro Protein Binding of Fosdenopterin HBr

Species	Ascorbic acid 5 mM	Ascorbic acid 25 mM
Mouse	20.7-24.2%	4.1-15.7%
Rat	7.4-17.3%	8.9-14%
Dog	22.6-33.8%	11.8-16.8%
Human	6.0-12.4%	7.2-20.3%
Plasma was spiked with ascorbic acid		(b) (4)

E.coli-derived cPMP was relatively stable when cultured in vitro in mouse, rat, dog, and human hepatocytes without ascorbic acid ([XT134092](#)). In the presence of ascorbic acid, in vitro metabolic profiling indicated that two cPMP-related compounds (C1 and C2) were detected in mouse, rat, dog, and human hepatocytes incubated with 10 µM *E.coli*-derived cPMP ([XT134093](#)). No human-specific metabolites were identified in vitro, and these

compounds were detected in all species tested. Comparison to the analysis of a compound Z-spiked human hepatocyte sample suggests that both C1 and C2 correspond to compound Z, a known oxidation product of cPMP. Notably, loss of fosdenopterin HBr was observed after 240 minutes of incubation in mouse (25%), rat (21%), dog (20%), and human (34%) hepatocytes. Extensive loss of fosdenopterin HBr was observed in boiled/denatured hepatocytes from mouse (71%), rat (65%), dog (65%), and human (55%), suggesting that the metabolism of fosdenopterin HBr/cPMP in hepatocytes occurs predominantly through non-enzymatic degradation processes. In vivo metabolism studies have not been conducted with fosdenopterin HBr.

Treatment of cultured human hepatocytes with *E.coli*-derived cPMP (up to 100 μ M) produced little or no increase in CYP1A2, CYP2B6, or CYP3A4 mRNA and enzyme activity levels. Any measurable increases in fold change for either mRNA levels or enzyme activity were <7.5% as effective as the respective positive controls ([XT133120](#)). Similarly, treatment of human liver microsomes with *E.coli*-derived cPMP produced little or no evidence (IC_{50} : >500 μ M) of direct, time-dependent, or metabolism-dependent inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5 ([XT135111](#)).

E.coli-derived cPMP did not inhibit P-gp, BCRP, OATP1B1, OATP1B3, OCT2, OAT3, MATE1, and MATE2-K (IC_{50} : >200 μ M). *E.coli*-derived cPMP (at 200 μ M) inhibited MATE2-K (25%) and OAT1 (33%). *E.coli*-derived cPMP is not a substrate of P-gp, BCRP, OAT1, OAT3, OATP1B1, OATP1B3, OCT2, or MATE2-K, evidenced by either an efflux ratio below 2 or <2-fold accumulation of cPMP in cells. *E.coli*-derived cPMP is a substrate for MATE1, as accumulation of cPMP into MATE1-expressing cells was >2 in the majority of tested conditions ([XT138074](#)).

MATE1 and MATE2-K (multidrug and toxin extrusion transporters) are primarily expressed on the luminal (apical) membrane of the proximal tubular cells and thought to play a role in the excretion of cations and zwitterions into urine. MATE1 is also expressed in the liver on the canalicular membrane of hepatocytes and mediates the biliary excretion of cationic drugs¹.

6 General Toxicology

6.1 Single-Dose Toxicity

Fosdenopterin HBr (coded ALXN1101) was administered to male and female rats at doses of 20, 50, 75, or 100 mg/kg as a single intravenous injection in a vehicle composed of L-Ascorbic Acid, USP (b) (4) mg/mL, Mannitol USP (b) (4) mg/mL, and Sterile Water for Injection, USP. Fosdenopterin HBr was well tolerated at all doses and did not produce mortality, clinical signs, or changes in body weight ([Study 1727-016](#); Phase A).

¹ Hillgren KM, Keppler D, Zur AA, Giacomini KM, Stieger B, Cass CE and Zhang L (2013) Emerging transporters of clinical importance: An update from the international transporter consortium. *Clinical Pharmacology & Therapeutics* 94(1):52-63.

Fosdenopterin HBr (coded ALXN1101) was administered to male rats at 5 or 10 mg/kg as a single intravenous injection in different two vehicles: 1) L-Ascorbic Acid, USP (2 mg/mL), Mannitol USP ((b) (4) mg/mL), Sterile Water for Injection, USP, or 2) L-Ascorbic Acid, USP ((b) (4) mg/mL), Mannitol USP ((b) (4) mg/mL), and Sterile Water for Injection, USP. Similar PK profiles for fosdenopterin HBr were observed regardless of the vehicle ([Study 1727-016](#); Phase B).

6.2 Repeat-Dose Toxicity⁺

In a dose-range finding study for repeated dose toxicity studies, fosdenopterin HBr (coded ALXN1101) was administered to rats in a bolus IV dose at doses of 30, 60, or 100 mg/kg/day for 7 consecutive days and was well tolerated. There was no mortality, clinical signs, or changes in body weight. The NOEL was determined to be 100 mg/kg/day ([Study 1727-016](#); Phase C).

6.2.1 Study Title: A 13-Week Study of the Potential Toxicity of Daily Parenteral Injection of ALXN1101 in Juvenile Rats with a 28-Day Recovery Period

Study no.:	9000563
Study report location:	EDR 4.2.3.3.2; Study Report
Study initiation date:	April 13, 2015
Conducting laboratory and location:	(b) (4)
Duration:	13
Duration Units:	weeks
GLP compliance:	Y
Drug, lot #, and % purity:	ALXN1101 (fosdenopterin HBr); lot# CA15-0408; 91.79% purity
Target Organ ⁺ :	None

Methods

Doses: 25, 50, 100 mg/kg
 Frequency of dosing: Once daily
 Number/Sex/Group: Main study: 10/sex/group
 Recovery: 5/sex/group
 Toxicokinetic: 3/sex/vehicle;
 12/sex/fosdenopterin HBr groups
 Dose volume: 10 mL/kg
 Formulation/Vehicle: L-Ascorbic Acid, USP ^{(b) (4)} mg/mL; Mannitol
 USP ^{(b) (4)} mg/mL; in Sterile Water for
 Injection, USP, pH ^{(b) (4)}
 Route of administration: SUBCUTANEOUS
 INTRAVENOUS BOLUS
 Species: RAT
 Strain: SPRAGUE-DAWLEY
 Age / Sexual Maturity: Postnatal day 7 / No
 Comment on Study Design and Conduct: Animals were dosed subcutaneously from
 PND 7-20, then by intravenous bolus from
 PND 21 onwards
 Dosing Solution Analysis: Acceptable

Study Design

Experimental Design – Main and Recovery								
Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals			
					Main Study ^a		Recovery Study ^b	
					Males	Females	Males	Females
1	Vehicle Control	0	10	0	10	10	5	5
2	ALXN1101	25	10	2.5	10	10	5	5
3	ALXN1101	50	10	5	10	10	5	5
4	ALXN1101	100	10	10	10	10	5	5

^a 10/sex/group necropsied 24 hours following the last dose (Day 98 pp)
^b 5/sex/group necropsied 4 weeks following the last dose (Day 126 pp)

Experimental Design – Toxicokinetic						
Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	Number of Animals	
					Toxicokinetic Study Day 30 and 97 pp	
					Males	Females
1	Vehicle Control	0	10	0	3	3
2	ALXN1101	25	10	2.5	12	12
3	ALXN1101	50	10	5	12	12
4	ALXN1101	100	10	10	12	12

Observations and Results**NOAEL**

Based on the lack of adverse findings in this study, the NOAEL is 100 mg/kg/day.

Mortality

After weaning, animals were examined twice daily for mortality and moribundity throughout the study. Six female mortalities and one male mortality occurred in this study. However, none of the death appear to be treatment-related, as three of the deaths were in the control group, and no clinical observations or post-mortem evaluations could be attributed to treatment with fosdenopterin HBr. Therefore, no treatment-related mortalities occurred in this study.

Table 2: Clinical Observations; 13-Week Juvenile Rat Study

Dose (mg/kg/day)	Sex	Day	Notes
0	F	22	- No clinical observations prior to death - No gross or microscopic findings to determine cause of death
0	F	23	
25 (TK)	F	31	
50	F	26	
50 (TK)	F	31	
100 (TK)	F	25	Decreased activity, muscle tone decrease, loss of consciousness, lying on side
0 (TK)	M	25	

Clinical Signs

General health and condition of the pups were evaluated daily during the lactation period. After weaning, detailed clinical examinations were conducted weekly throughout the remainder of the study.

Thin appearance and prominent backbone were observed in 100 mg/kg/day females between days 26 and 28. One 50 mg/kg/day male was observed in labored breathing on Day 26. No other treatment-related clinical signs were observed.

Body Weights

The pups were weighed individually on PND 4, 7, 10, 14, 17, and 21. After weaning, body weights were recorded weekly during the study. No adverse changes in body weight were observed. Body weight gain was decreased 5-7% in 100 mg/kg/day males and females compared to controls, but these magnitudes of change were small and not considered biologically relevant in the absence of other clinical signs.

Feed Consumption

Quantitative food consumption was recorded weekly from PND 21 throughout the remainder of the study. No treatment-related effects on food consumption were observed.

Ophthalmoscopy

Ophthalmic examinations were conducted on main study and recovery animals during Week 4 or 5 of treatment. No treatment-related ophthalmic findings were observed. All ocular findings observed in this study were considered to be age-related or expected in this population of animals.

Clinical Pathology

Blood and urine samples were collected during Week 12 of the main study and Week 4 of the recovery period.

Hematology and Coagulation

Neutrophil counts were increased 37% in 100 mg/kg/day males compared to controls at the end of the main study, and remained elevated (28%) following the recovery period. Neutrophil counts were unaffected in 100 mg/kg/day females at the end of the main study, but were increased 97% compared to controls following the recovery period. No treatment-related effects on coagulation parameters were observed.

Clinical Chemistry

No treatment-related effects on clinical chemistry parameters were observed.

Urinalysis

No treatment-related effects on urinalysis parameters were observed.

Gross Pathology

Complete necropsy was conducted on main study and recovery animals at scheduled termination. Dark focal areas of the adrenal glands were observed in two 100 mg/kg/day females, but lacked histopathological correlates. No other treatment-related macroscopic findings were observed.

Organ Weights

Organ weights were recorded from main study and recovery animals at scheduled termination, but not for animals found dead. No treatment-related changes in organ weights were observed.

Histopathology

Adequate Battery?	See pages 31 and 33 of study report A full histopathology battery was assessed for tissue samples collected from vehicle and high dose groups. Histopathology was conducted on tissues with gross lesions in the low and mid dose groups, and recovery animals.
Peer Review?	None described
Histopathological Findings	No treatment-related findings were observed.

Special Evaluation

Sexual Maturation

Main and recovery animals were examined for sexual maturity from PND 26 (females) or PND 35 (males) until they were found to be positive for physical development (vaginal opening for females, preputial separation for males). No effects on the timing of sexual maturation were observed.

Functional Observation Battery (FOB)

A FOB was conducted on main and recovery animals on Days 28 and 85 of the main study. No treatment-related effects on qualitative FOB parameters, grip strength, hind limb splay, or body temperature were observed.

Motor Activity

Locomotor activity assessments were conducted on main and recovery animals on Days 28 and 85 of the main study. No treatment-related effects on motor activity were observed.

Bone Measurements

Right femur and right tibia measurements (length and width) were measured at necropsy for main study and recovery animals. Femoral length was measured from the proximal end of the femoral head to the distal end of the medial condyle (excluding patella), and tibial length from the medial plateau (meniscus removed) to the medial malleolus. Femoral and tibial width were measured at midshaft in medio-lateral orientation. No treatment-related effects on bone measurements were observed.

Toxicokinetics

Blood samples were collected from toxicokinetic animals on Days 30 and 97 at 0 hour (pre-dose), 10 min, 30 min, and 1, 2.5, 4, and 6 hours post-dose.

On Day 30 and Day 97, C_{max} and AUC of AXLN1101 generally increased proportionally to dose over the dose range. Evidence of accumulation of ALXN1101 was observed from Day 30 to Day 97, at all doses. No evidence of sex differences in exposure were observed. T_{max} was 0.166 hours on both Days 30 and 97.

Table 3: TK of Fosdenopterin HBr; 13-Week Juvenile Rat Study

Dose (mg/kg/day)	Day 30			Day 97			
	C _{max} (µg/mL)	AUC _{0-6h} (µg*h/mL)	AUC _{0-24h} (µg*h/mL)	C _{max} (µg/mL)	AUC _{0-6h} (µg*h/mL)	AUC _{0-24h} (µg*h/mL)	Accumul. (AUC _{d97} / AUC _{d30})
25	28.3	18.5	74*	41.2	31.0	124*	1.68
50	60.8	40.9	163.6*	95.7	73.6	294.4*	1.80
100	110	75.3	301.2*	181	133	532*	1.77

* indicates: AUC_{0-6h} multiplied by 4 to calculate AUC_{0-24h}

6.2.2 Study Title: A 26-Week Study of the Potential Toxicity of Daily Parenteral Injection of ALXN1101 in Juvenile Rats with a 28 Day Recovery Period

Study no.: 902668
Study report location: EDR 4.2.3.2; [Study Report](#)
Study initiation date: January 7, 2013
Conducting laboratory and location:  (b) (4)
Duration: 26
Duration Units: weeks
GLP compliance: Y
Drug, lot #, and % purity: ALXN1101 (fosdenopterin HBr); lot#
856/cPMP/PD/002 and
856/cPMP/PD/003; 93.17% and 95.64%
purity, respectively.
Target Organ⁺: None

Methods

Doses: 1, 3, and 5 mg/kg
Frequency of dosing: Once daily
Number/Sex/Group: 3-month interim sacrifice:
Main study: 10/sex/group
Recovery: 5/sex/group

Full 26-week duration:
Main study: 10/sex/group
Recovery: 5/sex/group
Toxicokinetic: 3/sex/vehicle,
9/sex/fosdenopterin HBr groups

Dose volume: 10 mL/kg
Formulation/Vehicle: L-Ascorbic Acid USP (2mg/ml), Mannitol (b)
(4)
(b) (4) Water for Injection, USP; pH
(b) (4)

Route of administration: SUBCUTANEOUS
INTRAVENOUS BOLUS

Species: RAT
Strain: SPRAGUE-DAWLEY
Age / Sexual Maturity: PND 7 / No

Comment on Study Design and Conduct:

- Animals were dosed subcutaneously from PND 7-20, then by intravenous bolus from PND 21 onwards
- Toxicokinetic animals were administered a single dose of vehicle or fosdenopterin HBr on Day 1 of dosing (PND 7): 3/sex/vehicle, 18/sex/treatment groups

Dosing Solution Analysis: Acceptable

Study Design

Experimental Design – Main and Recovery								
Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals			
					Main Study ^a (Subsets A and B)		Recovery Study ^b (Subsets C and D)	
					Males	Females	Males	Females
1	Vehicle Control	0	10	0	10 + 10	10 + 10	5 + 5	5 + 5
2	ALXN1101	1	10	0.1	10 + 10	10 + 10	5 + 5	5 + 5
3	ALXN1101	3	10	0.3	10 + 10	10 + 10	5 + 5	5 + 5
4	ALXN1101	5	10	0.5	10 + 10	10 + 10	5 + 5	5 + 5

^a 10/sex/group necropsied 24-hours following 84 doses (Subset A: Day 91 pp); 10/sex/group necropsied 24-hours (Subset B: Day 189 pp) following the last dose.

^b 5/sex/group (recovery) necropsied 4 weeks following 84 doses (Subset C: Day 119 pp), the remaining 5/sex/group (recovery) necropsied 4 weeks following the last dose (Subset D: Day 217 pp).

Text Table 5
Experimental Design – Toxicokinetic

Group No.	Test Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals			
					Toxicokinetic Study Day 7 pp (Subsets F and G)		Toxicokinetic Study Day 188 pp (Subset E)	
					Males	Females	Males	Females
1	Vehicle Control	0	10	0	3 + 3	3 + 3	3	3
2	ALXN1101	1	10	0.1	18 + 18	18 + 18	9	9
3	ALXN1101	3	10	0.3	18 + 18	18 + 18	9	9
4	ALXN1101	5	10	0.5	18 + 18	18 + 18	9	9

Subset G replaced Subset F (refer to [Study Plan Amendment No. 9](#)).

Observations and Results

NOAEL

Based on the lack of adverse findings in this study, the NOAEL is 5 mg/kg/day.

Mortality

After weaning, animals were examined twice daily for mortality and moribundity throughout the study. No treatment-related mortalities were observed.

Clinical Signs

General health and condition of the pups were evaluated daily during the lactation period. After weaning, detailed clinical examinations were conducted weekly throughout the remainder of the study. No treatment-related clinical signs were observed.

Body Weights

The pups were weighed individually on PND 4 and daily from PND 7 to PND 20. After weaning, body weights were recorded weekly during the study. No treatment-related effects on body weights were observed.

Feed Consumption

Quantitative food consumption was recorded weekly from PND 21 throughout the remainder of the study. No treatment-related effects on food consumption were observed.

Ophthalmoscopy

Ophthalmic examinations were conducted on main study and recovery animals during Weeks 5 and 26. No treatment-related ophthalmic findings were observed

Clinical Pathology

Blood samples were collected during Weeks 12, 25, and 16 of the main study, and Week 4 of the recovery period. Urine samples were collected from main study animals during Weeks 12 and 25.

Hematology and Coagulation

No treatment-related effects on hematology or coagulation parameters were observed

Clinical Chemistry

No treatment-related effects on clinical chemistry parameters were observed

Urinalysis

No treatment-related effects on urinalysis parameters were observed

Gross Pathology

Complete necropsy was conducted on main study and recovery animals at scheduled termination. No treatment-related macroscopic findings were observed.

Organ Weights

Organ weights were recorded from main study and recovery animals at scheduled termination, but not for animals found dead or terminated in poor condition. Only the brain, heart, kidney, liver, lung, ovary, spleen, thymus, uterus, testis, adrenals, pituitary, prostate, and thyroid were weighed. No treatment-related changes in organ weights were observed.

Histopathology

Adequate Battery?	See pages 32 of study report A full histopathology battery was assessed for tissue samples collected from vehicle and high dose groups.
Peer Review?	None described
Histopathological Findings	No treatment-related histopathological findings were observed.

Special EvaluationSexual Maturation

Main study animals were examined for sexual maturity from PND 26 (females) or PND 35 (males) until they were found to be positive for physical development (vaginal opening for females, preputial separation for males). No effects on the timing of sexual maturation were observed.

Functional Observation Battery (FOB)

A FOB was conducted on main and recovery animals on Days 28, 85, and 183 of the main study. No treatment-related effects on qualitative FOB parameters, grip strength, hind limb splay, or body temperature were observed.

Motor Activity

Locomotor activity assessments were conducted on main and recovery animals on Days 28, 85, and 183 of the main study. No effects on motor activity were observed.

Bone Measurements

Right femur and right tibia measurements (length and width) were measured at necropsy for main study and recovery animals. Femoral length was measured from the proximal end of the femoral head to the distal end of the medial condyle (excluding patella), and tibial length from the medial plateau (meniscus removed) to the medial malleolus. Femoral and tibial width were measured at midshaft in medio-lateral orientation. No treatment-related effects on bone measurements were observed.

Toxicokinetics

Blood samples were collected from toxicokinetic animals on Days 7 at 0 hour (pre-dose), 15 min, 30 min, and 1, 2, and 4 hours post-dose, and on Days 90 and 188 at 0 hour (pre-dose), 5 min, 15 min, 30 min, and 1, 2, 3, and 4 hours post-dose.

Cmax and AUC of fosdenopterin HBr generally increased proportionally to dose following either SC or IV dosing. Notably, exposure to fosdenopterin HBr (AUC) was greater in the PND 7 animals following SC dosing than PND 90 or PND 188 animals following IV dosing. These differences in AUC may be due to a difference in elimination and clearance (CLss/F vs. CLss) between the SC and IV dosing. It is unclear whether the difference in AUC with dose route is due to differences in drug metabolism or renal clearance. No evidence of sex differences in exposure or accumulation was evident.

Table 4: TK of Fosdenopterin HBr; 26-Week Juvenile Rat Study

Day	Dose (mg/kg/day)	Cmax (ng/mL)	AUC _{0-t} (ng*h/mL)	AUC _{0-24h} (ng*h/mL)	Tmax (h)	CL/F (mL/h/kg)	CLss (mL/h/kg)
PND 7 SC	1	1180	2470 ^a	14,820	0.5	379	-
	3	3910	7330 ^a	43,980	0.5	386	-
	5	5580	11,200 ^a	67,200	1.0	NR	-
PND 90 IV	1	3220	1190 ^b	14,820	0.083	-	835
	3	7880	3490 ^b	41,880	0.083	-	840
	5	12,700	5490 ^b	65,880	0.083	-	894
PND 188 IV	1	3690	1620 ^b	6480	0.083	-	605
	3	11,200	5170 ^c	20,680	0.083	-	577
	5	16,900	7630 ^c	30,520	0.083	-	653

a. AUClast= AUC0-4h
b. AUClast= AUC0-2h
c. AUClast= AUC0-3h
1. AUC0-4h multiplied by 6 to calculate AUC0-24h
2. AUC0-2h multiplied by 12 to calculate AUC0-24h
3. AUC0-3h multiplied by 4 to calculate AUC0-24h

6.2.3 Study Title: ALXN1101: A 9-Month Intravenous Toxicity Study in Juvenile Beagle Dogs with a 3-Month Recovery Period

Study no.: 2087-006
Study report location: EDR 4.2.3.3.2; [Study Report](#)
Study initiation date: August 12, 2013
Conducting laboratory and location: (b) (4)
Duration: 9
Duration Units: months
GLP compliance: Y
Drug, lot #, and % purity: ALXN1101 (fosdenopterin HBr); lot#
856/cPMP/PD/004; 93.5% purity; lot#
856_GMP/cPMP/003, 94.7% purity; lot#
856_GMP/cPMP/004, 95.4% purity
Target Organ⁺: BLOOD

Methods

Doses:	1, 3, 5, 10 mg/kg (see Table 5 below)
Frequency of dosing:	Once daily
Number/Sex/Group:	Main study: 4-6/sex/group Recovery: 3/sex/group
Dose volume:	10 mL/kg/dose
Formulation/Vehicle:	L-Ascorbic Acid, USP (2 mg/mL), Mannitol USP ((b) (4) mg/mL), and Sterile Water for Injection, USP; pH (b) (4)
Route of administration:	SUBCUTANEOUS INTRAVENOUS BOLUS
Species:	DOG
Strain:	BEAGLE
Age / Sexual Maturity:	Lactation Day 5-6 / No
Comment on Study Design and Conduct:	- Animals were dosed SC from PND 5-25, then by either IV bolus (Groups 1-4) or both IV bolus and SC injection (Groups 5 and 6) from PND 25 onwards - The maximum feasible IV dose was determined to be 5 mg/kg (10 mL/kg). To achieve a dose of 10 mg/kg/day, animals in Groups 6 were co-administered an additional 5 mg/kg (10 mL/kg) SC dose of fosdenopterin HBr (total of 20 mL fosdenopterin HBr/kg/animal). - A second placebo group (Group 5) was included to act as a control for co-administration of a SC dose with an IV dose, as administered to Group 6.
Dosing Solution Analysis:	The homogeneity of dosing preparations were out of specification on 8/27/2013, 2/25/2014, and 5/8/2014. All remaining preparations were within <5% of the RSD. The concentration of dosing preparations were out of specification on 8/27/2013 (87-88% of nominal concentration). All remaining preparations were within 10% of the nominal concentration.

Table 5: Study Design of 9-Month Juvenile Dog Study

Group Assignments					
Group Number	Dose Level (mg/kg/day)	Number of Animals		Animal Numbers	
		Male	Female	Male	Female
1 ^a	0	8 ^c	8 ^c	609 – 616	601 – 608
2 ^a	1	5	5	622 – 626	617 – 621
3 ^a	3	5	6	632 – 636	627 – 631, 10629
4 ^a	5	7 ^c	9 ^c	646 – 652	637 – 644, 10645
5 ^b	0	8 ^c	8 ^c	661 – 668	653 – 660
6 ^{b, d}	10	8 ^c	8 ^c	677 – 684	669 – 676

^aDoses were administered by subcutaneous (SC) injection on Days 1 through 21, and by intravenous (IV) injection thereafter.

^bAll doses were administered by SC injection on Days 1 through 21, and by a combination of IV injection and SC injection thereafter. Groups 5 and 6 received a total dose of approximately 20 mL/kg; during the split dosing phase, 10 mL/kg was administered SC and 10 mL/kg was administered by IV injection during the same dosing session.

^cThree animals/sex/group were designated for recovery.

^dDosing for Group 6 began on LD 6 rather than LD 5 as required.

Observations and Results**NOAEL**

APTT was prolonged up to 22% at the highest dose tested (10 mg/kg/day). In the absence of any other indices that this change negatively affected the health of the animal, it is not considered an adverse effect. Thus, based on the lack of adverse findings in this study, the NOAEL is 10 mg/kg/day (intravenous + subcutaneous dosing).

Mortality

After weaning, animals were examined twice daily for mortality and moribundity throughout the study. No treatment-related mortalities occurred in this study. However, three animals were terminated early and one found dead during the study. Two deaths (0 mg/kg/day male and 3 mg/kg/day female) appear related to an inflammatory response independent of treatment with fosdenopterin HBr. One 5 mg/kg/day male was observed with a physical deformity, pectus excavatum, and was terminated. One 10 mg/kg/day male was found dead on Day 78, and the Sponsor determined the cause of death to intra-arterial dosing, though this could not be confirmed microscopically. Further details for this animal are provided under Clinical Signs.

Table 6: Clinical Observations; 39-Week Juvenile Dog Study

Dose (mg/kg/day)	Sex	Day	Notes
0 (SC)	Male	18 Euthanized in extremis	On Day 17, observations of swelling at the SC injection site, hypersensitivity to touch, and vocalization

			- On Day 18, previous clinical signs persisted, with decreased activity, prostration, skin warm to touch, abdominal pain, and weight loss. - Clinical pathology changes associated with an inflammatory response. - Necropsy findings included red discoloration and red fluid in the subcutis, subcutaneous mixed inflammation and hemorrhage at the injection site, and edema in the lungs, with no evidence of an active infection.
3 (IV)	Female	12 Euthanized in extremis	- On Day 11, clinical signs of thin appearance and decreased activity. - On Day 12, previous clinical signs persisted, with inappetence, hypersensitivity to touch, and vocalization. - Clinical pathology changes associated with an inflammatory response, as well as increased platelet counts and prolonged APTT. - Necropsy findings of lung inflammation, with evidence of infection.
5 (IV)	Male	7 Euthanized	Physical abnormality (pectus excavatum) not observed during initial examination
10 (IV+SC)	Male	78 Found dead	- On Day 77, clinical signs of decreased activity, shallow breathing, vocalization, and vomiting. - Post-dosing observations of severe neurologic and respiratory impairment, ventral recumbency and cold to touch. - Brain lesions (hemorrhage, edema, and neuronal necrosis) and substantial lung hemorrhage and edema

Clinical Signs

General health and condition of the juvenile animals were evaluated daily for 3 weeks, 3x per week from 3-8 weeks of age, and weekly thereafter. Swelling, edema, erythema, scabbed area, discolored (red), and warm skin, scars, hypersensitivity to touch, prostration, vocalization, and decreased activity were observed in Groups 5 and/or 6; animals administered placebo or fosdenopterin HBr via SC injection for the duration of the study. Occasionally following intravenous administration, fosdenopterin HBr-treated animals were observed with decreased activity (males: 3 and 5 mg/kg/day; females: ≥ 1 mg/kg/day), lacrimation (males: 3 and 5 mg/kg/day; females: ≥ 1 mg/kg/day), hypersensitivity to touch (females: 3 mg/kg/day), vocalization (females: 3 mg/kg/day), audible breathing and coughing (females: 5 mg/kg/day), and enlarged vulva (females: 1 and 5 mg/kg/day).

Following dosing on Day 80, one 5 mg/kg/day female was observed with had clinical signs of vocalization, prostration, and hypersalivation, dull mentation, generalized weakness, mild tachypnea, moderate dyspnea, and pale pink mucus membranes. Heart rate and lung sounds were normal, but increased air sounds were observed over the trachea. The animal was unconscious when provided with supplemental oxygen, and SC administered 0.2 mg/kg Metacam. The Sponsor reported that the animal had stable vitals over the next few hours and was rousable, but resumed unconsciousness. Neurologic signs of severe ataxia (with assisted standing) and minimal reflexes were observed, with no indication of pain or distress. The animal slowly improved over the next 24 hours, was capable of standing, slightly disoriented, exhibited mild to moderate ataxia, and decreased reactivity to stimulus, but was improved since the last evaluation. The animal continued to improve and was normal within 72 hours. This animal was not dosed on Day 81 and was administered the dose by SC injection on Days 82 and 83. IV dosing resumed on Day 84. During necropsy, mild brain dilation which correlated with microscopic ventricular dilation, leading the Sponsor to suggest that the cause of these findings was procedural, related to inadvertent intra-arterial dosing.

A 10 mg/kg/day male was found dead on Day 78. Following dosing on Day 77, this animal was observed with adverse clinical signs of decreased activity, shallow breathing, vocalization, ventral recumbency, skin cold to touch, and vomiting, as well as severe neurologic and respiratory impairment, characterized by minimal response of obtunded constricted pupils and ventral rotation of the eyes. Veterinary intervention with fluids for supportive care and treatment for potential pain/inflammation were provided. Approximately 1.5 hours post-dose, the animal had continued to display signs of decreased activity, vocalization, shallow breathing, as well as noted in ventral recumbency, had mucoid feces, and the skin was cold to touch. At necropsy, brain lesions, including hemorrhage, edema, and neuronal necrosis, and substantial lung hemorrhage and edema were observed. The Sponsor suggested that the cause of these findings was procedural, related to inadvertent intra-arterial dosing.

Body Weights

Body weights were recorded for treated animals at birth (LD 0), daily from LD 1 to 3 weeks of age (Day 21 or 23), 3x per week from 3-8 weeks of age, and weekly thereafter. No adverse effects on body weights were observed.

Feed Consumption

Qualitative food consumption was observed twice daily but not reported.

Ophthalmoscopy

Ophthalmic examinations were conducted on all treated animals during Weeks 3 and 26, and once prior to scheduled termination. No treatment-related ophthalmic findings were observed.

ECG

Electrocardiographic examinations were conducted on all treated animals during Weeks 3 and 26, and once prior to scheduled termination. 6 lead ECGs were recorded at Week

3 and 10 lead ECGs were recorded thereafter. RR, PR, and QT intervals, and QRS duration were measured and heart rate was calculated from the number of ECG complexes in 10 seconds. Corrected QT (QTc) interval was calculated using a procedure based on the method described by Fridericia. No adverse changes in quantitative or qualitative ECG parameters were observed.

Clinical Pathology

Hematology and Coagulation

Blood samples were collected for hematology and coagulation evaluations during Weeks 5, 13, and 26, and once prior to scheduled termination.

Beginning at Week 5 and continuing throughout the study, increased neutrophils (100%) were observed in 10 mg/kg/day males compared to controls, and were associated with increased total leukocytes (68%) and increased globulin (14%). However, neutrophils were decreased up to 32% at 1, 3, and 5 mg/kg/day males compared to controls, most prominently decreased at the terminal blood sample collection. Similar findings were observed following the recovery period. Red cell mass (erythrocyte, hemoglobin, and hematocrit) was decreased 10% and absolute reticulocytes were decreased 49% in 5 mg/kg/day females compared to controls. APTT was prolonged up to 22% (2.37 seconds) in 10 mg/kg/day males compared to controls, and was most prominently prolonged at the terminal blood sample collection. Prothrombin was minimally decreased (<5%) in 10 mg/kg/day females compared to controls.

Clinical Chemistry

Blood samples were collected for serum chemistry evaluations during Weeks 1, 5, 9, 13, and 26, and once prior to scheduled termination. Animals were not fasted during Weeks 1, 5, or 9.

Beginning at Week 1 and continuing throughout the study, ALP levels were increased up to 64% in ≥ 1 mg/kg/day males compared to controls, but did not progressively increase over time. Over the course of the study, lipase levels were increased up to 143% in 10 mg/kg/day compared to controls. At Week 39, increased globulin (14%) and decreased albumin/globulin ratio (-18%) were observed in 10 mg/kg/day males compared to controls.

Urinalysis

Urine was collected for urinalysis evaluations during Weeks 13 and 26, and once prior to scheduled termination. No treatment-related effects on urinalysis parameters were observed.

Gross Pathology

Complete necropsy was conducted on all F₁ animals terminated early, found dead or at scheduled termination.

Moderate red focus/foci of the brain and moderate red discoloration of the lung were observed in one male at 10 mg/kg/day (animal number 679) that was found dead on Day

78. These findings were correlated with brain hemorrhage/necrosis/cerebellar herniation and lung hemorrhage/edema microscopically. Though not confirmed microscopically, the Sponsor considered these findings related to inadvertent intra-arterial dosing.

Mild brain dilation was observed in one 5 mg/kg/day female after the recovery period. Brain dilation was correlated with microscopic ventricular dilation. Potential intra-arterial dosing was noted in this animal during the dosing period. Ventricular dilation, brain lesions, gliosis, spongiosis, and foamy macrophages were also noted in this animal. Though not confirmed microscopically, the Sponsor considered these findings related to inadvertent intra-arterial dosing.

No other treatment-related macroscopic findings were observed.

Organ Weights

Organ weights were recorded from all surviving treated animals at scheduled termination.

Relative spleen weight was increased 59% and 73% in 10 mg/kg/day females compared to controls at the end of the main study and recovery period, respectively. No histopathological correlates in the spleen were observed.

Histopathology

Adequate Battery?	See page 1854 of study report (Appendix A of Pathology Report) The brain and spleen were considered potential target organs and examined from all treated animals.
Peer Review?	Yes
Histopathological Findings	No definitive treatment-related histopathological findings were observed. <u>Brain</u> Gliosis, foamy macrophages, and spongiosis were observed in one 5 mg/kg/day female after the recovery period. Necrosis/degeneration, edema, and hemorrhage were observed in a 10 mg/kg/day male found dead. <u>Bone marrow</u> Mixed cell hyperplasia was observed in the femur, rib, and sternum of one 3 mg/kg/day female that was euthanized. Bone marrow hyperplasia was not observed in other animals. <u>Injection site</u> Microscopic findings at the last injection sites (intravenous and/or subcutaneous) were of similar type (crust, hemorrhage, inflammation, degeneration,

	fibrosis, edema), magnitude, and incidence across all groups, including concurrent controls.
--	--

Special Evaluation

Neurological Examinations

Neurological examinations (cranial nerves, postural reactions, spinal reflexes, and sensations) were conducted on all treated animals during Weeks 13 and 26, and once prior to scheduled termination. No treatment-related effects on neurological function was observed.

Bone Measurements

Bone length measurements of the right femur and right tibia were documented at the terminal and recovery necropsies. No treatment-related effects on bone lengths were observed.

Toxicokinetics

Blood samples were collected from all treated animals on Day 24 and during Week 39 at 0.083, 0.25, 0.5, 1, 2, 3, 4, and 6 hours post-dose.

On Day 24 and Week 39, AUC of fosdenopterin HBr generally increased proportionally to dose over the dose range. However, C_{max} increased proportionally to dose from 1-5 mg/kg/day, but less than proportionally to dose from 5 mg/kg/day to 10 mg/kg/day. Evidence of accumulation of fosdenopterin HBr was observed from Day 24 to Week 39, independent of dose. No evidence of sex differences in exposure were observed.

Table 7: TK of Fosdenopterin HBr; 39-Week Juvenile Dog Study

Dose (mg/kg/day)	Day 24			Week 39			
	C _{max} (ng/mL)	AUC _{0-4h} (ng*h/mL)	AUC _{0-24h} (ng*h/mL)	C _{max} (ng/mL)	AUC _{0-6h} (ng*h/mL)	AUC _{0-24h} (ng*h/mL)	Accumul. (AUC _{w39} / AUC _{d24})
1 (IV)	2100	2090	12,540	3370	2990	11,960	1.43x
3 (IV)	5960	5500	33,000	9980	9460	37,840	1.72x
5 (IV)	9730	9870	59,220	16,900	14,600	58,400	1.48x
10 (SC)	10,300	19,100	114,600	15,600	29,700	118,800	1.55x
1. AUC0-4h multiplied by 6 to calculate AUC0-24h 2. AUC0-6h multiplied by 4 to calculate AUC0-24h							

7 Genetic Toxicology

7.1 In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Fosdenopterin HBr (coded ALXN1101), up to 5000 µg/plate, was negative in the Ames test, with and without metabolic activation ([Study 9600930](#)).

The impurity, (b) (4) were all negative in the Ames test (up to (b) (4) µg/plate), with and without metabolic activation ([Study 9600806](#)).

7.2 *In Vitro* Assays in Mammalian Cells

Fosdenopterin HBr (coded ALXN1101; up to 486 µg/mL) did not induce chromosomal damage (chromatid or chromosome gaps, or polyploidy) when cultured with human peripheral blood lymphocytes, with and without metabolic activation ([Study 9600931](#)).

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Fosdenopterin HBr (coded ALXN1101), administered to rats at 50, 100, or 200 mg/kg via IV infusion for two days, did not induce bone marrow cytotoxicity, had no clastogenic effects, and did not induce micronuclei formation in bone marrow polychromatic erythrocytes ([Study 9800235](#)).

8 Carcinogenicity

As agreed with the FDA during the Type B meeting on April 12, 2019, a carcinogenicity study in a single species will be conducted post-approval.

9 Reproductive and Developmental Toxicology

Reproductive and developmental toxicity studies with fosdenopterin HBr have not been conducted. As agreed with the FDA during the Type B meeting between the Sponsor and FDA on 12 April 2019, reproductive toxicity studies will be conducted post-approval. The Sponsor intends to request a waiver for the fertility and early embryonic development study in rats.

10 Special Toxicology Studies

10.1 Study Title⁺: Neutral Red Uptake Phototoxicity Assay of ORGN001 in BALB/c 3T3 Mouse Fibroblasts

Study no.: 20181580

Study report (Electronic) location: EDR 4.2.3.7.7; [Study Report](#)

Type of Study: Phototoxicity

The objective of this study was to evaluate the phototoxic potential of fosdenopterin HBr (coded ORGN001) as measured by the relative reduction in viability of BALB/c 3T3 mouse fibroblasts exposed to fosdenopterin HBr and ultraviolet radiation (+UVR), as compared with the viability of fibroblasts exposed to fosdenopterin HBr in the absence of ultraviolet radiation (-UVR). Promethazine was used as the positive control.

The assays were valid, and fosdenopterin HBr demonstrated phototoxic potential.

Table 8: In Vitro Phototoxicity Summary

Compound	IC50 (µg/mL) -UVR	IC50 (µg/mL) +UVR	PIF	MPE	Phototoxic Potential	UVR % Survival	OD ₅₄₀
Pos. Control	98.802	1.238	80.739	0.550	Yes	91	0.803
Fosdenopterin HBr		19.85	>5.043	0.326	Yes	84	0.762
Fosdenopterin HBr		18.16	>5.503	0.380	Yes	89	0.789

PIF: Photoirritancy Factor (phototoxic= PIF> 5)
MPE: Mean Photo Effect (phototoxic= MPE> 0.15)
UVR Percent Survival criterion= >80%
OD540 criterion= ≥0.400
PIF≥ 1: The test article was phototoxic (+UVR) but not cytotoxic (-UVR) , and the PIF could not be calculated, although it is clear that some level of phototoxic potential exists In this case, a “>PIF” was calculated and the highest testable test article concentration (-UVR) was used for calculation

10.2 Study Title*: Repeat-Dose Phototoxicity Study to Determine the Effects of Intravenous Administration of ORGN001 on Eyes and Skin in Pigmented Rats

Study no.: 20202895

Study report (Electronic) location: EDR 4.2.3.7.7; [Study Report](#)

Type of Study: Phototoxicity

The objective of this study was to determine the potential phototoxic effects of fosdenopterin HBr (coded ORGN001), when administered intravenously via the tail vein, once daily for 3 consecutive days, on the eyes and skin of female Crl:LE (Long-Evans) pigmented rats (aged 96 days old), followed by exposure to ultraviolet B, ultraviolet A, and visible light from a xenon lamp.

Table 9: Phototoxicity Study Design

Group	Number of Rats	Descriptor	Dose Level (mg/kg)	Concentration (mg/mL)	Dose Volume (mL/kg)	UVA Dose, J/cm ²	Interval Between Dose and UVR Exposure ^a
1	5	Vehicle	0	0	10	10 ± 1	Within 10 minutes
2	5	ORGN001	25	2.5	10	10 ± 1	
3	5	ORGN001	50	5	10	10 ± 1	
4	5	ORGN001	100	10	10	10 ± 1	
5	5	ORGN001	100	10	10	0	N/A ^b

^a Interval based on the T_{max} of the test article. The actual exposure time of the group was based on the individual dosing time of the group. For deviations, see [Appendix 1](#) – Protocol and Deviations.
^b Sham exposure; rats were sedated and restrained but not exposed to UVR.
N/A = Not applicable

Dose-dependent cutaneous skin reactions (erythema, edema, flaking, and eschar), ophthalmic findings (corneal lesions), and histopathologic changes in ocular anterior

chamber, ciliary body, cornea, and lens indicative of phototoxicity were observed in this study.

After a single exposure to UVR, erythema grade 1 (barely perceptible redness), erythema grade 2 (distinct redness), edema grade 1 (mild, raised <1 mm), and/or skin flaking (eschar) grade 1 (barely perceptible scales) were observed at 25, 50, and 100 mg/kg/day. No dermal observations occurred in the vehicle or 100 mg/kg/day sham UVR control groups. At 25 mg/kg/day, erythema grade 1 was observed in both the pigmented and non-pigmented sites of all five animals within 1 hour post-UVR exposure on Day 3. In the non-pigmented site, these observations persisted up to Day 6 in 4/5 animals. In the pigmented site, only 1/5 animals was observed with erythema grade 1 on Day 6. At 50 mg/kg/day, erythema grades 1 or 2 was observed in both the pigmented and non-pigmented sites of all five animals within 1-4 hours post-UVR exposure on Day 3, and persisting in all animals by Day 6. Edema was observed in the pigmented sites in 4/5 animals and non-pigmented sites in 2/5 animals. Skin flaking was observed in three animals in both pigmented and non-pigmented sites. At 100 mg/kg/day, erythema grades 1 or 2, edema, and skin flaking were observed in non-pigmented sites of all five animals post-UVR exposure on Day 3. Erythema grade 1 or 2 and edema were observed in pigmented sites of all five animals post-UVR exposure on Day 3. Erythema and edema persisted on Day 6 in non-pigmented sites of all animals and in pigmented sites on one animal. Skin flaking was observed in four animals in both pigmented and non-pigmented sites.

Corneal lesions were dose-dependently observed at 50 and 100 mg/kg/day. The mildest form of the lesion was a deposit of white material diffuse within the cornea. The more severely affected eyes had a thick deposit of white material within the cornea that prohibited examination beyond the lesion. Some eyes had both the thick deposit surrounded by diffuse white material. In some eyes, the diameter of the white deposit within the cornea prevented complete examination of the fundi. In a few eyes, the white deposit in the cornea covered such a large area that the fundi could not be examined at all. This white deposit of material, whether the diffuse form or large deposit form, is different in appearance from the white material associated with corneal dystrophy. The deposit has a more cellular appearance compared with the more crystalline appearance in corneal dystrophy.

Histopathological findings in the ocular anterior chamber, ciliary body, cornea, and lens were observed at 50 and/or 100 mg/kg/day following UVR exposure. In the anterior chamber and ciliary body, minimal mixed inflammatory cell infiltrates were observed at 100 mg/kg/day. In the cornea, the severity of neutrophilic infiltrates in corneal stroma dose-dependently increased compared to controls, with edema, corneal endothelial hypertrophy, and cell loss. Single cell necrosis and hyperplasia of the corneal epithelium was observed at 100 mg/kg/day. In the lens, epithelial hyperplasia was observed at 50 mg/kg/day.

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/s/

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**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number: IND 117,502
Supporting document/s: SD 001
Sponsor's letter date: May 15, 2013
CDER stamp date: May 16, 2013
Product: ALXN1101
Indication: Treatment of Molybdenum Cofactor Deficiency
(MoCD) Type A
Sponsor: Alexion Pharmaceuticals, Inc.
Review Division: Division of Gastroenterology and Inborn Errors
Products (DGIEP)
Reviewer: B. Emmanuel Akinshola, Ph.D.
Supervisor/Team Leader: Sushanta K. Chakder, Ph.D.
Division Director: Donna Griebel, M.D.
Project Manager: Jessica M. Benjamin, MPH.

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1 Executive Summary

1.1 Introduction

ALXN1101 is a synthetic pterin derivative known as cyclic pyranopterin monophosphate (cPMP), formulated as a powder for reconstitution for intravenous (IV) use in the treatment of molybdenum cofactor deficiency (MoCD). MoCD is a rare, severe, neonatal-onset disorder with autosomal recessive inheritance caused by defects in the biosynthesis of molybdenum cofactor (MoCo). MoCD type A affects two thirds (66 %) of MoCD patients and is caused by a mutation in the MOCS1 gene (Reiss, J. and Johnson, J.L. Hum. Mutat. 21:569-576, 2003). MoCD is also characterized by the functional loss of sulfite oxidase (SO), a MoCo-dependent enzyme. MoCo is synthesized through a complex biochemical pathway involving the conversion of cPMP and molybdopterin (MPT) intermediates to the cofactor. The loss of SO activity leads to sulfite accumulation and the formation of S-sulfocysteine (SSC), which induces neurotoxicity and is an established biomarker for MoCD. The sponsor is proposing to use ALXN1101 as a substitute in the MoCo biosynthesis pathway to restore SO level and reduce the levels of sulfite and SSC, thereby alleviating the MoCD disease severity.

1.2 Brief Discussion of Nonclinical Findings

Nonclinical studies with ALXN1101 have been conducted in rats and dogs and in a knockout mice model of molybdenum cofactor deficiency. In *in vitro* pharmacology studies using bacterial (*E. coli*) and human enzyme systems, ALXN1101 at 50 to 1000 picomoles (pM) concentrations promoted molybdopterin synthesis and restored function to reconstituted human sulfite oxidase enzyme. Treatment of Molybdenum cofactor knockout mice (MOCS1^{-/-}) displaying the MoCD type A disorder with 2-4 µg of ALXN1101 per mouse at 3 doses/week for 107 days, rescued the mice from premature death and decreased plasma SSC levels. Intravenous (IV) infusion of ALXN1101 (for 2 hr) at doses of 0, 1, 3 or 10 mg/kg did not produce any adverse effects on cardiovascular function in Beagle dogs, and no adverse effects on neurobehavioral or pulmonary functions in Sprague-Dawley rats. Similarly, ALXN1101 at concentrations up to 0.5 mg/ml did not show any effect on the human ether a-go-go (hERG) channels in Chinese hamster ovary (CHO) cells. In pharmacokinetic studies in rats and dogs, ALXN1101 was administered by 2hr IV infusions, once daily for 14 days at doses of 0, 1, 3, or 10 mg/kg. The increase in C_{max} and AUC were dose proportional, with estimated terminal half-life (at the 10 mg/kg dose) of 0.603 hr and 0.706 hr on days 1 and 14 in the rat, and 0.820 hr and 0.756 hr on days 1 and 14 in the dog. There were no accumulations of the drug at any dose level, and there were no gender differences in exposure in rats or dogs. ALXN1101 did not bind to plasma proteins, but was bound to human erythrocyte (with a blood to plasma ratio of 0.695) and rat erythrocyte (with a blood to plasma ratio of 0.791). Five ALXN1101 metabolites were identified in the S9 fractions and hepatocytes of mouse, rat, dog, and human without any species-related differences. ALXN1101 is not an inhibitor of CYP450 isozymes, and did not induce any CYP450 enzyme activity in *in vitro* assays.

In repeat dose 2-week toxicology studies in rats and dogs, ALXN1101 administration at dose levels of 0, 1, 3, or 10 mg/kg/day by 2-hr IV infusion was well tolerated, with a NOAEL of ≥ 10 mg/kg/day. ALXN1101 was not genotoxic in the *in vitro* bacterial reverse

mutation (Ames) test, *in vitro* mammalian (human peripheral blood lymphocytes) chromosomal aberration test, or the *in vivo* mammalian (rat bone marrow) erythrocyte micronucleus test.

The incubation of human red blood cells (RBCs) with ALXN1101 at concentrations ranging from 0.045mg/ml to 0.25 mg/ml for 10 min did not result in hemolysis, precipitation, or turbidity.

1.3 Recommendations

None

1.3.1 Clinical Study Safe to Proceed: Yes

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)

677-24-7

Generic Name

N/A

Code Name

ALXN1101

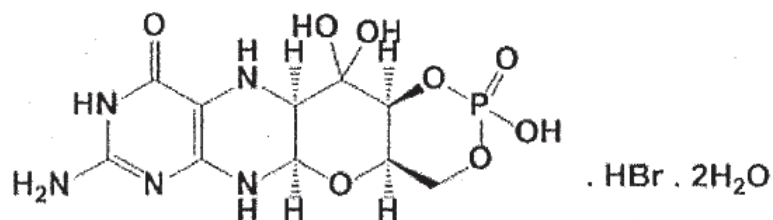
Chemical Name

Cyclic Pyranopterin Monophosphate (cPMP) Monohydrobromide Dihydrate

Molecular Formula/Molecular Weight

C₁₀H₁₉BrN₅O₁₀P /480.16

Structure or Biochemical Description



Pharmacologic Class

Enzyme cofactor replacement therapy

2.2 Relevant INDs, NDAs, BLAs and DMFs

N/A

2.3 Drug Formulation

Composition of the ALXN1101 Drug Product

Name of Ingredient	Quantity per vial	Function	Compendial Quality Standard(s)
ALXN1101	(b) (4)	(b) (4)	In-House Standard
L-Ascorbic acid			USP
D-Mannitol			USP
Hydrochloric acid (HCl)		pH adjustment	NF
Sodium hydroxide (NaOH)		pH adjustment	NF

¹ Q.S. added to adjust the pH to (b) (4)

2.4 Comments on Novel Excipients

There are no novel excipients in ALXN1101 drug product.

2.5 Comments on Impurities/Degradants of Concern

(b) (4)

2.6 Proposed Clinical Protocol

The sponsor proposed a randomized, blinded, placebo-controlled, single-dose, sequential-cohort, dose-escalation first-in-human (FIH) study to evaluate the safety, tolerability, and pharmacokinetics (PK) of a single intravenous (IV) dose of ALXN1101 in healthy adult male and female subjects. Thirty two study subjects with ages between 18 and 60 and weighing between 55 and 100 kg will be randomized into 4 dose cohorts of 0.10, 0.32, 0.90, and 1.50 mg/kg, with 8 subjects/cohort consisting of six active drug subjects and 2 placebo subjects. In this phase 1 dose escalation study, each study

cohort will complete 5 days of study visit before the next dosing cohort initiation, and all subjects will be followed for safety and PK assessments from the day of dosing through 30 days after dosing. Safety and tolerability of ALXN1101 will be assessed by physical examination, vital signs, electrocardiogram (ECG), laboratory and adverse events analysis.

2.7 Previous Clinical Experience

No prior clinical trials with ALXN1101 have been conducted. The sponsor (Alexion Pharma Intl., SARL) acquired assets related to *Eschericia coli*-derived cyclic pyranopterin monophosphate (rcPMP) from (b) (4) currently provide rcPMP to pediatric patients with Molybdenum Cofactor Deficiency (MoCD). ALXN1101 is a synthetic form of cPMP.

2.8 Regulatory Background

At a pre-IND (type B) meeting held on March 26, 2013, the sponsor was asked to conduct an *in vitro* study to assess the hemolytic potential of ALXN1101 before the initiation of a clinical trial. The sponsor was also asked to conduct a 6-month chronic juvenile rat toxicology study prior to the proposed 6-month clinical trial of ALXN1101 in pediatric patients with MoCD.

3.1 Studies Reviewed

Pharmacology

Study/GLP Status/Section	Species	Dose(s)	Dosing Regimen/ROA	Results
<i>In vitro</i> biological activity/non-GLP/ Section 4.1.1.1	n/a	n/a	n/a	ALXN1101 and rcPMP displayed similar <i>in vitro</i> activities in terms of MPT synthesis and SO reconstitution.
Pharmacodynamic Study (GTR-0466 v1.0/ non-GLP)/ Section 4.1.2.1)	MOCS1 ^{-/-} Mice	See Table 5	Repeat dose administration 3 times a week over 107 days IH and IP	ALXN1101 effectively rescued MOCS1 ^{-/-} mice from premature death. ALXN1101 effectively decreased the plasma SSC level in MOCS1 ^{-/-} mice.
Cardiovascular Effects Study (2087-001/GLP)/ Section 4.1.3.2.1)	Beagle dogs	0, 1, 3, 10 mg/kg/day	Repeat dose administration over 14 days IV	ALXN1101 produced no effects on cardiovascular function in beagle dogs
Neurobehavioral and Pulmonary Effects Study (2087-002/GLP)/ Section 4.1.3.2.2)	Sprague-Dawley rats	0, 1, 3, 10 mg/kg/day	Repeat dose administration over 14 days IV	ALXN1101 produced no effects on neurobehavioral or pulmonary function in rats

Pharmacokinetics

Overview			Test Article:			
Type of Study	Test System	Method of Administration	Testing Facility	Study Number	Location in IND	
Absorption: IV Administration						
Multiple-dose Absorption and Excretion	Rat	IV Infusion	(b) (4)	2087-002	4.2.3.2	
Multiple-dose Absorption and Excretion	Dog	IV Infusion		2087-003	4.2.3.2	
Distribution						
Plasma Protein Binding	Plasma	<i>In Vitro</i>		HU-0025-DV-HC	4.2.2.3	
Blood-to-plasma Ratio	Whole Blood and Plasma	<i>In Vitro</i>		HU-0025-DV-HC		
Metabolism						
Single Exposure <i>In Vitro</i> Metabolism	Liver S9 Fractions	<i>In Vitro</i>		HU-0025-DV-HC	4.2.2.3	
Single Exposure <i>In Vitro</i> Metabolism	Cryopreserved Hepatocytes	<i>In Vitro</i>		HU-0025-DV-HC		
Metabolite Identification	Liver S9 Fractions and Hepatocytes	<i>In Vitro</i>		HU-0025-DV-HC		
Excretion						
N/A	N/A	N/A		N/A	N/A	
Pharmacokinetic Drug Interactions						
Cytochrome P450 Inhibition	SUPERSOMES	<i>In Vitro</i>		HU-0025-DV-HC	4.2.2.3	
Cytochrome P450 Induction	Hepatocytes	<i>In Vitro</i>		HU-0025-DV-HC	4.2.2.3	
Other						
N/A	N/A	N/A		N/A	N/A	

Toxicology

Overview						Test Article: ALXN1101		
Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	GLP Compliance	Testing Facility	Study Number	Location in IND
Repeat-Dose Toxicity	Sprague Dawley Rat	Intravenous	14 days	0, 1, 3, 10	Yes	(b) (4)	2087-002	4.2.3.2
Repeat-Dose Toxicity	Beagle Dog	Intravenous	14 days	0, 1, 3, 10	Yes		2087-003	4.2.3.2
Genotoxicity – Bacterial Reverse Mutation Test	S. typhimurium, E. coli	Incubation	65 hrs, 45min	Up to 500µg/plate	Yes		964425	4.2.3.3.1
Genotoxicity-Mammalian Chromosomal Aberration Test	Human Peripheral Blood Lymphocytes	Incubation	4 hrs, and 21 hrs	500µg/mL concentration	Yes		964426	4.2.3.3.1
Genotoxicity-Rat Erythrocyte Micronucleus Test	Sprague Dawley rat	Intravenous	24 hrs (2 doses)	0, 1, 3, 10	Yes		980110	4.2.3.3.2
Hemolysis Assay - Human Whole Blood	Human	Incubation	10 minutes	Up to 0.25mg/mL	Yes		2087-004	4.2.3.7.7 when available
hERG tail current assay	CHO K-1 cells	Perfusion into cell culture media	10-15 minutes	2µM concentration	Yes		793428	To be provided when available

All the studies reviewed are shown in a tabulated format in the sponsor's table above.

3.2 Studies Not Reviewed

N/A

3.3 Previous Reviews Referenced

N/A

4 Pharmacology

ALXN1101 is a synthetic form of cyclic pyranopterin monophosphate (cPMP), a natural compound found in all healthy individuals, and it is derived from the enzymatic conversion of guanosine triphosphate (GTP). cPMP is converted to molybdopterin (MPT), which is further converted to the molybdenum cofactor (MoCo). MoCo is essential for the function of the enzymes sulfite oxidase (SO), xanthine dehydrogenase, and aldehyde oxidase in their biosynthetic pathway. Specifically, SO detoxifies the sulfite that would otherwise accumulate in the central nervous system in molybdenum cofactor deficiency (MoCD), leading to neuronal cell death, seizures and premature death. The sponsor proposed that by ALXN1101 administration, cPMP will be made available for MoCo biosynthesis, and subsequently, SO and all the other molybdenum-dependent enzymes will be reconstituted, resulting in a reduction in sulfite levels followed by a decline in SSC accumulation.

4.1 Primary Pharmacology

Pharmacodynamics

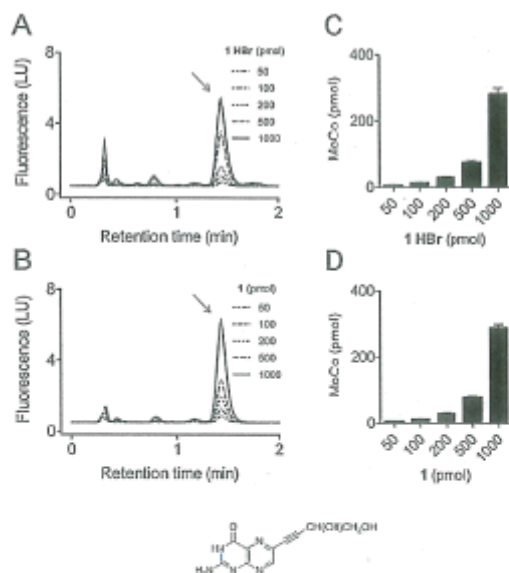
Synthesis of Cyclic Pyranopterin Monophosphate, A Biosynthetic Intermediate in the Molybdenum Cofactor Pathway. Keith Clinch et al., *J. Med. Chem.* 2013; 56:p1730-1738.

The primary pharmacodynamic effect of ALXN1101 is the alleviation of molybdenum cofactor deficiency by promoting molybdopterin (MPT) biosynthesis and restoring sulfite oxidase (SO) function. The ability of ALXN1101 to promote the synthesis of MPT and SO enzyme reconstitution was evaluated in this *in vitro* study. In the MPT synthesis study, 50 – 1000 picomoles of ALXN1101 or *E. coli* derived cPMP was incubated with MPT synthesizing enzyme subunits (MoaE and MoaD) to assess the enzymatic conversion of ALXN1101 or cPMP to MPT. The level of MPT synthesis was determined by HPLC quantification of the MPT oxidation product dephosphorylated forms shown in figures A and B below.

In the SO reconstitution study, a mixture of MoaE, MoaD, gephyrin C4, MgCl₂, ATP and molybdate was also incubated with 50 – 1000 picomoles of cPMP and human apo-SO. The efficacy of molybdenum cofactor (MoCo) insertion was evaluated as a function of the activity of the reconstituted sulfite oxidase enzyme as shown in figures C and D below.

In summary, ALXN1101 at concentrations of 50 to 1000 picomoles promoted MPT synthesis in a bacterial (*E.coli*) enzyme system, and restored sulfite oxidase activity in a human (apo-SO) enzyme system.

In Vitro Synthesis of MPT and SO Reconstitution by ALXN1101



Legend: *In vitro* biological activity of ALXN1101 ("1 HBr" as in charts A and C) and *E. coli* derived cPMP ("1" as in charts B and D).

(A-B) Enzymatic conversions (MPT synthesis): MPT was synthesized *in vitro* using the MPT-synthase subunits MoaE (*E. coli* MPT-synthase large subunit) and thiocarboxylated MoaD (*E. coli* MPT-synthase small subunit). The reaction was started by adding different amounts of ALXN1101 and *E. coli* derived cPMP (50 to 1000 pmol) and after incubation, MPT amounts were assessed by HPLC quantification of the MPT oxidation product Dephospho-Form A (arrowed).

(C-D) Reconstitution of human apo-SO: Mixture of MoaE, MoaD, geophyrin C4, MgCl₂, ATP and molybdate was prepared and the reaction was started by addition of different amounts of cPMP (0.05 to 1 nmol). Following the incubation, apoSO was added and finally, Moco insertion efficacy was evaluated as a function of the activity of reconstituted SO. Values shown in charts C and D are averages of three independent experiments, and error bars indicate standard deviation.

In Vivo Efficacy of ALXN1101 and *E. coli* Derived cPMP in MOCS1^{-/-} Mice (Study GTR-0466 v1.0).

Methods: The objective of this study was to compare the efficacies of *E. coli* derived cPMP and ALXN1101 in the rescue of MOCS1^{-/-} (molybdenum cofactor deficient) knockout mice from early death.

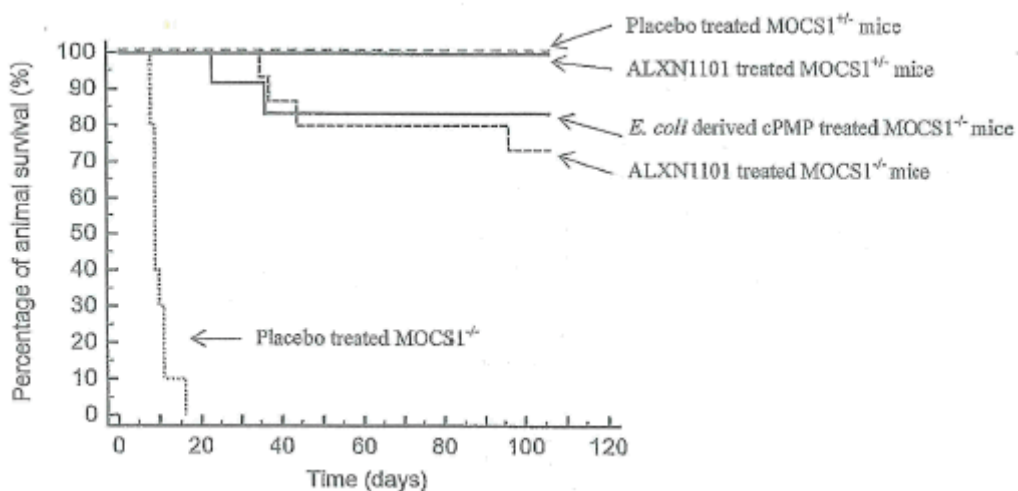
Male and female knockout MOCS1^{-/-} mice pups were treated with placebo, ALXN1101 or *E. coli* derived cPMP (rcPMP) at 2 µg/mouse (3 times /week) from postnatal day 1 to 21, and at 4 µg/mouse (3 times /week) from days 22 to 107, while MOCS1^{+/-} heterozygous control mice pups received 20 to 50 µL of the placebo solution. The study articles were administered intrahepatically (IH) from day 1 to 58, and intraperitoneally (IP) from days 59 to 107.

From postnatal day 1, the body weights of animals were measured 3 times/week, and the activities and food consumption of the mice were also observed 3 times/week.

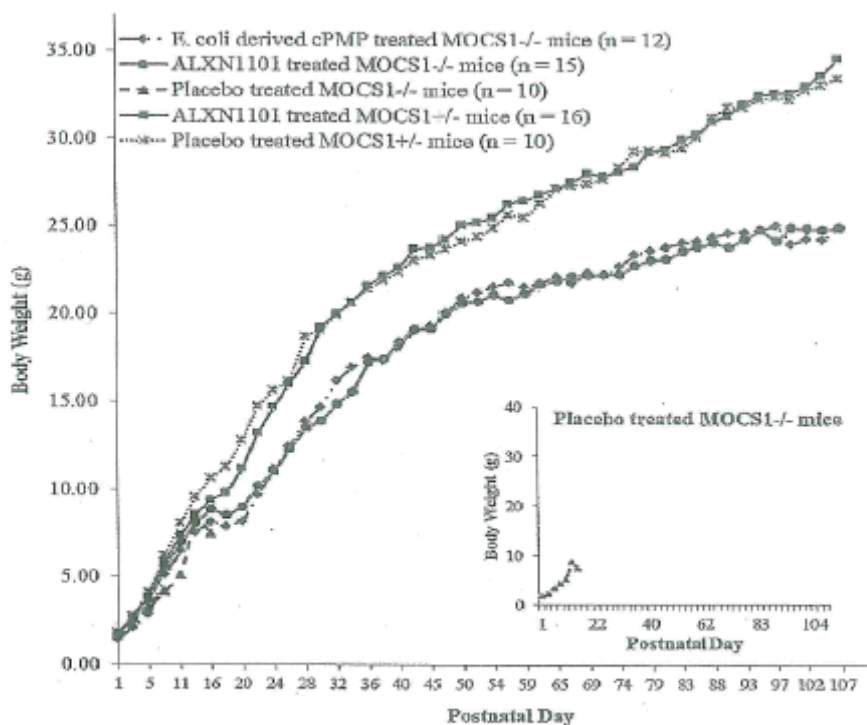
Results: The administration of 2 – 4 µg of rcPMP or ALXN1101 effectively rescued MOCS1^{-/-} mouse pups from early death as they appeared agile, alert, and indistinguishable from the heterozygous control mice. However, MOCS1^{-/-} mice treated with placebo showed severe abnormalities such as reduced body size, dehydration, slow movement and limb paralysis, and death within 8 to 16 days after birth. All of the

MOCS^{+/−} mice treated with either placebo or ALXN1101 were alive and agile during the course of study. MOCS1^{−/−} mice treated with rcPMP had an average body weight of 24.92 ± 1.10 g similar to ALXN1101 treated mice with an average body weight of 24.91 ± 1.10 g. MOCS1^{−/−} mice treated with rcPMP had a plasma SSC level of 60.59 ± 9.55 $\mu\text{mol/L}$ at 24 hr post administration, while the SSC level in the ALXN1101 group was 55.62 ± 6.20 $\mu\text{mol/L}$ at the same time point. ALXN1101 administration restored SO activity and led to the reduction of sulfite and SSC levels in the mutant mice to alleviate MoCD disease condition, as shown in the figures and the sponsor's table below.

Rescue of MOCS1^{−/−} Mice treated with rcPMP and ALXN1101



Body Weights (in g) of MOCS1^{-/-} Mice Treated with ALXN1101 or rcPMP



Legend: Body weight of MOCS1^{-/-} mice under treatment. Figure inset shows the mean body weight of placebo treated MOCS1^{-/-} mice throughout the course of study. All animals in this group died between postnatal day 8 and 16.

Mean Plasma SSC levels of MOCS1^{-/-} Mice treated with ALXN1101 or rcPMP

Treatment	Genotype	SSC ($\mu\text{mol/L}$)			Mean SSC ($\mu\text{mol/L}$) in all three time points
		24 hrs	48 hrs	72 hrs	
<i>E. coli</i> derived cPMP	MOCS1 ^{-/-}	60.59 $\pm$ 9.55 (n=10)	97.07 $\pm$ 10.76 (n=10)	117.32 $\pm$ 9.72 (n=10)	16.03 $\pm$ 2.32 (n=30)
ALXN1101	MOCS1 ^{-/-}	55.62 $\pm$ 6.20 (n=10)	77.71 $\pm$ 5.47 (n=11)	105.98 $\pm$ 7.97 (n=11)	
Placebo	MOCS1 ^{+/-}	6.64 $\pm$ 2.90 (n=10)	14.71 $\pm$ 3.14 (n=10)	26.76 $\pm$ 3.29 (n=10)	

Legend: Measurement of the plasma SSC level at three time points post cPMP treatment. Plasma samples were collected at the following time points: (1) 24 hours post cPMP injection on postnatal day 103; (2) 48 hours post cPMP injection on postnatal day 95; (3) 72 hours post cPMP injection on postnatal day 100. SSC levels at the three timepoints in placebo-treated MOCS1^{-/-} were in the normal range of variations in this group as confirmed by a more comprehensive follow-up study (mean SSC level of non-treated MOCS1^{+/-} mice was 5.66 $\pm$ 0.85 $\mu\text{mol/L}$, ranging from 0 to 27.30 $\mu\text{mol/L}$, n=52, across ages on postnatal day 8, day 28, day 317 and day 340, GTR-0483 v1.0). RD Data Binder# 256-Section V

In conclusion, study results demonstrated that ALXN1101 had similar effects as rcPMP on MOCS1^{-/-} mice's lifespan, body weight, and plasma S-sulfocysteine (SSC). In a murine model of MoCD type A, the study article rescued mice from early death and did not affect the body weight of the study animals.

4.3 Safety Pharmacology

Neurobehavioral and Pulmonary Effects of ALXN1101 in Sprague-Dawley Rats (Study # 2087-002)

Methods: Five to 6 weeks old male (130-210 g) and female (100-170 g) Sprague-Dawley rats were used for functional observation battery (FOB) and pulmonary function tests to assess the safety of ALXN1101. Ten (10) female rats/group were used in the FOB test whereas 8 male rats/group were used in the pulmonary function test. The animals were assigned to one of 4 groups and each group was administered ALXN1101 daily by 2-hr IV infusion for 14 days at 0 (vehicle control), 1, 3, or 10 mg/kg. All animals were observed for morbidity or mortality twice daily, and detailed clinical observations were performed weekly. Pulmonary endpoints of respiratory rate, tidal and minute volumes were measured along with neurobehavioral endpoints of autonomic, sensorimotor, neuromuscular and activity/arousal in the functional observation battery (FOB).

Results: FOB (Activity/arousal, neuromuscular, sensorimotor, autonomic, and physiologic) measurements did not show any test article-related changes or dose-response relationships different from control animal values, in the treatment group animals. There were also no test article-related changes in respiratory rate, tidal volume, or minute volume in the treated animals during the pulmonary evaluations. In conclusion, ALXN1101 did not produce any neurobehavioral or pulmonary changes in rats following daily IV administration at doses of 1, 3, and 10 mg/kg for 14 days.

Potential Cardiovascular Effects of ALXN1101 in the Beagle Dog (Study # 2087-001)

Methods: Four 9 to 31 month old male Beagle dogs weighing 8.20 to 8.75 kg were administered vehicle control solution and ALXN1101 at doses of 1, 3, and 10 mg/kg, with one animal/treatment each week followed by a 7-day washout period between dosing, until each dog received all the treatments. The vehicle and the test article were administered by IV infusion over 2 hours at a dose volume of 20 ml/kg.

Animals were observed for clinical signs, body weight, body temperature, heart rate, blood pressure (systolic, diastolic, mean arterial pressure). Electrocardiographic (ECG) recordings were also conducted for qualitative and quantitative ECG (QRS duration, QT, RR, PR intervals) parameter changes as a result of test article treatment.

Results: There was no ALXN1101-related morbidity, mortality, or changes in body weight, body temperature, and blood pressure. There were also no qualitative or quantitative changes in ECG parameters in dogs administered ALXN1101 at doses up to 10 mg/kg/day.

In conclusion, IV administration of ALXN1101 at doses of 0, 1, 3, and 10 mg/kg/day for 14 days produced no effects on cardiovascular function in Beagle dogs.

5 Pharmacokinetics/ADME

5.1 PK/ADME

Absorption

Multiple-Dose Absorption and Excretion of ALXN1101 in Sprague-Dawley Rats (Study # 2087-002)

Methods: Male and female rats (3/sex vehicle control, and 6/sex in treatment groups) were randomly assigned to one of four groups and administered IV infusions of ALXN1101 daily at doses of 0, 1, 3, or 10 mg/kg for 14 days. Measurement of plasma concentration of ALXN1101 was conducted on day 1 and day 14 at pre-dose, and at 5, 15, 30 min, 1 hr, 2 hr, 4 hr, and 8 hours following the end of the 2-hr infusion period by liquid chromatography-mass spectrometry (LC-MS/MS). Whole blood was collected from the jugular vein and mixed with 0.05 ml of (30 mg/ml) ascorbic acid solution.

Results: The mean maximum plasma concentrations (C_{max}) of ALXN1101 were similar for day 1 and day 14 at each dose level, and ranged from 220 ± 14.9 ng/ml to 2720 ± 300 ng/ml on day 1 and from 233 ± 7.07 ng/ml to 2660 ± 209 ng/ml on day 14 in male and female rats. Male rats dosed on day 14 had slightly lower mean ALXN1101 plasma concentrations than female rats dosed on day 14 at the 10 mg/kg dose, and slightly lower mean ALXN1101 levels than the male rats dosed on day 1. The C_{max} increased with increasing dose, however, two rats treated with ALXN1101 were observed to have plasma levels of the test article below the quantifiable level (BQL).

In general, exposures (AUC) to ALXN1101 were similar for days 1 and 14, with increased exposure in a dose proportional manner with a mean exposure (AUC_{0-3}) range of 339 to 4100 ng•h/ml on day 1 and from 349 to 3890 ng•h/ml on day 14 in male and female rats. However, exposure in female rats appeared to increase in a greater than dose proportional manner at the 10 mg/kg dose group. Terminal half-life of ALXN1101 also increased with increasing dose in male and female rats, but the estimates for the 3 mg/kg dose groups for day 1 and day 14 were made using a sampling time less than twice the half-life, causing the estimates for the 3 mg/kg dose group to be less reliable. The mean half-life ranged from 0.603 hr to 0.630 hr on day 1 or from 0.503 hr to 0.706 hr on day 14 in male and female rats. A summary of the PK parameters is shown in the sponsor's table below.

Pharmacokinetic Parameters for ALXN1101 for each Dose on Day 1 and Day 14 in Male and Female Rats Combined

Day	Dose (mg/kg)	Administered Dose	C _{max} (ng/mL)	C _{max} SE (ng/mL)	C _{max} /D ^a	T _{max} (hr)	AUC _{last} (hr*ng/mL)	AUC _{last} SE (hr*ng/mL)	AUC _{last} /D ^b	AUC(0-3) (hr*ng/mL)	AUC(0-3) /D ^b	Half-life (hr)
1	1	0.701	220	14.9	314	2.08	339	18.7	483	339	483	0.630
1	3	2.34	548	130	234	2.08	804	167	344	804	344	0.395 ^c
1	3 ^d	2.34	657	84.8	281	2.08	965	106	412	965	412	0.395 ^c
1	10	8.12	2720	300	334	2.08	4500	369	555	4100	505	0.603
14	1	0.736	233	7.07	317	2.08	349	10.1	475	349	475	0.503
14	3	2.31	616	127	266	2.08	903	163	391	903	391	0.340 ^c
14	3 ^d	2.31	739	38.0	320	2.08	1060	52.1	459	1060	459	0.332 ^c
14	10	8.27	2660	209	321	2.08	4270	311	516	3890	471	0.706

D = Dose; SE = Standard Error

a. Units for C_{max}/Dose: (kg*ng/mL/mg)

b. Units for all AUC/Dose: (hr*ng/mL)/(mg/kg)

c. Note that terminal half-life was estimated over a sampling time less than 2x the half-life.

d. 3mg/kg dose group excluding 2 rats with all ALXN1101 concentrations BQL.

Multiple-Dose Absorption and Excretion of ALXN1101 in Beagle Dogs (Study # 2087-003)

Methods: Male and female dogs (5/sex vehicle control and 10 mg/kg dose groups; 3/sex in 1 and 3 mg/kg dose groups) were randomly assigned to one of four groups and administered IV infusions of ALXN1101 daily at doses of 0, 1, 3, or 10 mg/kg for 14 days. LC-MS/MS determination of plasma concentrations of ALXN1101 were conducted on day 1 and day 14 at pre-dose, and at 5, 15, 30 min, 1 hr, 2 hr, 4 hr, and 8 hours following the end of the 2-hr infusion period. Whole blood was collected from the jugular vein and mixed with 0.05 ml of (30 mg/ml) ascorbic acid solution.

Results: On day 1, the mean ALXN1101 C_{max} values increased with dose from 1 to 10 mg/kg. The increase in C_{max} was dose proportional from 1 to 3 mg/kg and slightly less than proportional to dose from 3 to 10 mg/kg with mean C_{max} of 613 ± 49.9 ng/ml, 2078 ± 181 ng/ml, and 5898 ± 1040 ng/ml for 1, 3, and 10 mg/kg dose levels, respectively. The estimated terminal half-lives were 0.726 h, 0.683 h, and 0.820 h at 1, 3, and 10 mg/kg dose groups, respectively. The mean exposure (AUC₀₋₃) to ALXN1101 increased with increasing dose, in a dose proportional manner with mean exposure values of 1023 ± 96.9 ng•h/ml, 3421 ± 347 ng•h/ml, and 9794 ± ng•h/ml for 1, 3, and 10 mg/kg dose levels, respectively.

On day 14, the mean ALXN1101 C_{max} values increased with dose from 1 to 10 mg/kg. The increase in C_{max} was generally proportional to dose with mean values of 660 ± 43, 2172 ± 223 and 6352 ± 1260 ng/ml for 1, 3, and 10 mg/kg dose levels, respectively. The estimated terminal half-lives (t_{1/2}) were 0.723 h, and 0.756 h at the 3 and 10 mg/kg dose groups, respectively. All the terminal t_{1/2}s in the 1 mg/kg dose group were estimated over a period less than 2 times the estimated t_{1/2}; so the results were excluded. The mean exposure (AUC₀₋₃) to ALXN1101 increased with increasing dose, in a dose proportional manner with mean exposure values of 1066 ± 79.9 ng•h/ml, 3487 ± 395 ng•h/ml, and 10,402 ± 2160 ng•h/ml for 1, 3, and 10 mg/kg dose levels, respectively. There was no accumulation of ALXN1101 with 14 days of dosing, and no differences in exposure between male and female animals. A summary of the PK parameters is shown in the Sponsor's tables below.

Pharmacokinetic Parameters for ALXN1101 for each Dose on Day 1 in Male and Female Dogs Combined

Day	Dose Group	Actual Dose (mg/kg)	Statistics	Cmax (ng/mL)	Tmax (h)	t½ (h)	AUC0-3 (h*ng/mL)	AUClast (h*ng/mL)	Cmax/Dose ^a	AUC0-3/Dose ^b	AUClast/Dose ^b
1	1 mg/kg	0.792	N	6	6	2	6	6	6	6	6
			Mean	613	2.08	0.726	1023	1151	773	1291	1453
			SD	49.9	0	NC	96.9	195	63.0	122	246
			CV%	8.2	0	NC	9.5	16.9	8.2	9.5	16.9
			Min	532	2.08	0.721	874	874	671	1103	1103
			Median	633	2.08	0.726	1058	1246	798	1335	1572
			Max	656	2.08	0.731	1111	1312	828	1402	1656
			Geo Mean	611	2.08	0.726	1019	1136	771	1286	1434
			CV% Geo Mean	8.4	0	NC	9.8	18.2	8.4	9.8	18.2
			NC	6	6	3	6	6	6	6	6
1	3 mg/kg	2.55	Mean	2078	2.08	0.683	3421	3965	816	1343	1557
			SD	181	0	NC	347	442	71.2	136	173
			CV%	8.7	0	NC	10.1	11.1	8.7	10.1	11.1
			Min	1880	2.08	0.621	3032	3458	738	1190	1357
			Median	2045	2.08	0.711	3332	3911	803	1308	1535
			Max	2370	2.08	0.718	4011	4712	930	1575	1850
			Geo Mean	2072	2.08	0.682	3407	3946	813	1338	1549
			CV% Geo Mean	8.6	0	NC	9.9	10.9	8.6	9.9	10.9
			NC	10	10	10	10	10	10	10	10
			Mean	5898	2.1	0.82	9794	12478	660	1096	1396
1	10 mg/kg	8.94	SD	1040	0.0527	0.0829	1630	2150	116	183	241
			CV%	17.6	2.5	10.1	16.7	17.2	17.6	16.7	17.2
			Min	3790	2.08	0.737	6672	8979	424	746	1005
			Median	5820	2.08	0.8	9790	12657	651	1095	1416
			Max	7390	2.25	0.982	12186	15635	826	1363	1749
			Geo Mean	5809	2.1	0.816	9664	12305	650	1081	1377
			CV% Geo Mean	19.1	2.4	9.8	17.7	18.0	19.1	17.7	18.0
			NC	10	10	10	10	10	10	10	10
			Mean	5898	2.1	0.82	9794	12478	660	1096	1396
			SD	1040	0.0527	0.0829	1630	2150	116	183	241

NC: Not Calculable
a. Cmax/Dose units: (ng/mL)/(mg/kg)
b. AUCs/Dose units: (h*ng/mL)/(mg/kg)

Pharmacokinetic Parameters for ALXN1101 for each Dose on Day 14 in Male and Female Dogs Combined

Day	Dose Group	Actual Dose (mg/kg)	Gender	Cmax (ng/mL)	Tmax (h)	t½ (h)	AUC0-3 (h*ng/mL)	AUClast (h*ng/mL)	Cmax/Dose ^a	AUC0-3/Dose ^b	AUClast/Dose ^b
14	1 mg/kg	0.666	N	6	6	0	6	6	6	6	6
			Mean	660	2.08	NA	1066	1160	990	1599	1740
			SD	43.0	0	NA	79.9	176	64.5	120	264
			CV%	6.5	0	NA	7.5	15.2	6.5	7.5	15.2
			Min	602	2.08	NA	946	946	903	1419	1419
			Median	658	2.08	NA	1070	1154	987	1605	1732
			Max	727	2.08	NA	1183	1383	1091	1775	2075
			Geo Mean	659	2.08	NA	1063	1148	989	1595	1723
			CV% Geo Mean	6.5	0	NA	7.6	15.4	6.5	7.6	15.4
			NC	6	6	6	6	6	6	6	6
14	3 mg/kg	2.31	Mean	2172	2.08	0.723	3487	4065	939	1509	1758
			SD	223	0	0.081	395	658	96.5	171	284
			CV%	10.3	0	11.2	11.3	16.2	10.3	11.3	16.2
			Min	1950	2.08	0.659	3138	3601	844	1358	1558
			Median	2080	2.08	0.701	3305	3767	900	1429	1629
			Max	2500	2.08	0.883	4090	5261	1081	1769	2276
			Geo Mean	2162	2.08	0.72	3470	4024	935	1501	1741
			CV% Geo Mean	10.1	0	10.5	11.0	15.2	10.1	11.0	15.2
			NC	10	10	10	10	10	10	10	10
			Mean	6352	2.08	0.756	10402	12926	869	1422	1767
14	10 mg/kg	7.31	SD	1260	4.68E-16	0.105	2160	3270	172	296	447
			CV%	19.8	0	13.9	20.8	25.3	19.8	20.8	25.3
			Min	3660	2.08	0.579	6119	6994	500	837	956
			Median	6295	2.08	0.74	10065	12406	861	1376	1696
			Max	8030	2.08	0.916	13767	18583	1098	1882	2541
			Geo Mean	6223	2.08	0.749	10180	12527	851	1392	1713
			CV% Geo Mean	22.5	0	14.3	22.9	27.7	22.5	22.9	27.7
			NC	10	10	10	10	10	10	10	10
			Mean	6352	2.08	0.756	10402	12926	869	1422	1767
			SD	1260	4.68E-16	0.105	2160	3270	172	296	447

NC: Not Calculable
a. Cmax/Dose Units: (ng/mL)/(mg/kg)
b. AUCs/Dose Units: (h*ng/mL)/(mg/kg)

Determination of Aqueous Solubility, CYP450 Inhibition, CYP450 Induction, Plasma Stability, Plasma Protein Binding and Red Blood Cell Partitioning, Metabolic Stability and Metabolite Profiling of ALXN1101 (cPMP) (Study # HU-0025-DV-HC)

Plasma Protein Binding:

Methods: The protein binding of ALXN1101 (2 μ M) was determined in mouse, rat, dog, and human plasma supplemented with 3 mg/ml ascorbic acid (to promote test article stability) by equilibrium dialysis. ALXN1101 or warfarin control article was diluted 250-fold from stock solution into plasma for a final concentration of 2 μ M and an adjusted pH of 7.4. Triplicate 400 μ l aliquots of spiked plasma were dialyzed against blank plasma for the equilibrium control condition. Prior to the end of the dialysis period, calibration standard curves were prepared at concentrations ranging from 0.00206 to 2.5 μ M and matrix matched and quenched. For ALXN1101 assay, samples were added to 280 μ l quench solution, and for the control article, 50 μ l aliquots from the assay incubation plate were added to 200 μ l quench solution. The percentage of protein bound and recovered ALXN1101 were measured from the samples and the equilibrium ratio determined by LC/MS/MS.

Results: ALXN1101 at a concentration of 2 μ M did not bind to human, beagle dog, SD rat, or CD-1 mouse plasma proteins, as indicated by the negative values of the % protein bound (see table below). However, the control drug, warfarin demonstrated 96 %, 93.7 % 99.3 % and 70.7 % binding to human, dog, rat and mouse plasma protein, respectively, at an assay concentration of 2 μ M. The equilibrium ratio values for ALXN1101 ranged from 1.3 (dog) to 1.7 (human), in contrast to warfarin where the equilibrium ratio values ranged from 1.4 (mouse) to 23.2 (human). The recoveries of ALXN1101 ranged from 40.4 % to 57 %, as shown in the sponsor's table below.

ALXN1101 Plasma Protein Binding in Different Species

Species	Matrix (Supplemented with 3 mg/mL Ascorbic Acid)	% Protein Bound	% Recovery (vs. Nominal Conc.)	Equilibrium Ratio
CD-1 Mouse	Lithium Heparin Plasma	-15.0	57.0	1.5
SD Rat	Lithium Heparin Plasma	-16.3	55.8	1.5
Beagle Dog	K2EDTA Plasma	-75.5	40.4	1.3
Human	K2EDTA Plasma	-29.3	49.5	1.7

Blood to plasma ratio of ALXN1101 at 2 μ M was determined using fresh whole blood and plasma from rat and human supplemented with 3 mg/mL ascorbic acid (b) (4)

(b) (4) The blood to plasma ratio was 0.791 in rat and 0.695 in human, indicating ALXN1101 only modestly binds to human and rat erythrocytes.

Metabolism

Methods: In an *in vitro* study, the matrix stability of ALXN1101 (2 μ M) was tested at 37°C in pooled plasma from human, dog, rat, and mouse. The ALXN1101 or propantheline control article was diluted 250-fold from the 500 μ M intermediate stock solution into plasma for a final concentration of 2 μ M. The time points for the assay incubation mixtures were 0, 10, 20, 30, 60, 120, 240, and 360 minutes. For quenching the ALXN1101 assay samples, 50 μ l of the test article incubation plate was added to 140 μ l quench solution (Acetonitrile (ACN) containing internal standard (IS)), and for quenching the propantheline assay samples, 50 μ l of the control article incubation plate was added to 200 μ l ACN containing IS. In addition, 150 μ l of 0.25 mg/ml ascorbic acid solution was added to each well of the test article incubation plate to promote test article stability.

Results: The ALXN1101 matrix stability half-lives in the plasma of species tested were, 3.6 minutes (dog), 10.6 minutes (human), 21.3 minutes (mouse), and 34.8 minutes (rat). In the presence of ascorbic acid, the half-life increased significantly to > 360 minutes, showing that ascorbic acid was required for compound stability as depicted in the Sponsor's table below. The control article (propantheline) showed half-lives ranging from 28.3 minutes (human) to > 360 minutes (rat). However, in the control assay, the addition of 0.3 mg/ml ascorbic acid to human plasma showed only a modest increase in half-life from 28.3 min to 65.4 minutes.

Half-Lives ($T_{1/2}$) for ALXN1101 and Propantheline Plasma Stability Assay Results

Compound ID	Species	Matrix	$T_{1/2}$ (min)	% Remaining at 60 min
ALXN1101 (cPMP)	Human	K ₂ EDTA Plasma	10.6	0.0
ALXN1101 (cPMP)	Human	K ₂ EDTA Plasma + 0.3 mg/ml Ascorbic Acid	>360 (2310)	100.9
ALXN1101 (cPMP)	Dog	K ₂ EDTA Plasma	3.6	0.0
ALXN1101 (cPMP)	CD-1 Mouse	Lithium Heparin Plasma	21.3	14.0
ALXN1101 (cPMP)	SD Rat	Lithium Heparin Plasma	34.8	28.8
Control:				
Propantheline	Human	K ₂ EDTA Plasma	28.3	48.5
Propantheline	Human	K ₂ EDTA Plasma + 0.3 mg/ml Ascorbic Acid	65.4	66.8
Propantheline	Dog	K ₂ EDTA Plasma	161	81.1
Propantheline	CD-1 Mouse	Lithium Heparin Plasma	36.7	27.3
Propantheline	SD Rat	Lithium Heparin Plasma	>360 (462)	97.1

[Note: Expected ranges for Propantheline $T_{1/2}$ in plain K₂EDTA plasma matrix are: 50±30 minutes (human), >150 minutes (dog), 55±25 minutes (mouse), and >150 minutes (rat).]

Metabolic Stability of ALXN1101 in Liver S9 Fractions

Methods: In an *in vitro* study, the metabolic stability of ALXN1101 (2 μ M) was studied in pooled liver S9 fractions diluted in potassium phosphate buffer, pH 7.4 at 37 °C to a concentration of 2 mg/ml (2X solution). The test and control articles were diluted from the 500 μ M intermediate stock solution to a 2X solution. 75 μ l of the 2X compound solution (containing the phase 1 and 2 metabolic cofactors) was added to an equal volume of 2 mg/ml liver S9 diluted with potassium phosphate buffer in polypropylene 96-well plates, with a final concentration of 2 μ M of the test and control articles. The assay time points were 0, 15, 30, 60, 90 and 120 minutes. For quenching the ALXN1101 assay samples, 100 μ l of acetonitrile (ACN) was added to 280 μ l quench solution, and 150 μ l of 0.25 mg/ml ascorbic acid solution was added to each plate well prior to LC/MS/MS bioanalysis. For the control article, 100 μ l aliquots from the assay incubation plate were added to 400 μ l quench solution prior to LC/MS/MS analysis.

Results: The half-lives of ALXN1101 (in the presence of liver S9 with phase 1 and 2 cofactors) were 141 minutes (human), 61.9 minutes (beagle dog), 144 minutes (SD rat), and 73.7 minutes (CD-1 mouse). In the presence of 3 mg/ml ascorbic acid, the half-life of ALXN1101 in human plasma increased significantly to > 360 minutes. The calculated intrinsic clearance values (CL_{int}) were 16.2, 46.2, 35.6, and 136 ml/min/kg, respectively, and the percent of ALXN1101 remaining at 1 hour was 93.5 %, 57.5 %, 79.3 %, and 70.6 %, respectively. The control article, imipramine showed half-lives of 52.9, 5.7, 2.9 and 7.3 minutes for human, dog, rat, and mouse S9, respectively. The CL_{int} values ranged from 43.2 ml/min/kg in humans to 1783 ml/min/kg in rats, as shown in the sponsor's table below.

Liver S9 Metabolic Stability Results Summary

Compound ID	Species	$T_{1/2}$ (min)	% Remaining at 60 min	CL_{int} (ml/min/kg)
ALXN1101 (cPMP)	Human	141	93.5	16.2
ALXN1101 (cPMP)	Beagle Dog	61.9	57.5	46.2
ALXN1101 (cPMP)	S-D Rat	144	79.3	35.6
ALXN1101 (cPMP)	CD-1 Mouse	73.7	70.6	136
Imipramine	Human	52.9	45.6	43.2
Imipramine	Beagle Dog	5.7	0.2	500
Imipramine	S-D Rat	2.9	0.0	1783
Imipramine	CD-1 Mouse	7.3	0.4	1364

Hepatocyte Metabolic Stability

Methods: In an *in vitro* study, cryopreserved hepatocytes obtained from ≥ 10 donors were resuspended in media and adjusted to 1×10^6 cells/ml at 37°C . The test and control articles were diluted from 500 μM intermediate stock solutions to a 2X concentration of 4 μM . 100 μl of the 2X compound solution was added in triplicate to an equal volume of the 1×10^6 cells/ml at 37°C so that the final compound concentration in the assay incubations were 2 μM . The assay time points were 0, 30, 60, 120 and 180 minutes. For quenching the ALXN1101 assay samples, 50 μl of ACN was added to 140 μl quench solution, and 150 μl of 0.25 mg/ml ascorbic acid solution was added to each plate well prior to LC/MS/MS bioanalysis. For the control articles, 50 μl aliquots from the assay incubation plate were added to 200 μl quench solution prior to LC/MS/MS bioanalysis.

Results: ALXN1101 had metabolic half-lives of 161, 124, 59.7, and 20.4 minutes when incubated with human, beagle dog, SD rat and CD-1 mouse hepatocytes, respectively. The calculated intrinsic clearance values (CL_{int}) in hepatocytes were 23.2 (human), 37.8 (dog), 141 (rat), and 801 (mouse) ml/min/kg, respectively. The percent parent ALXN1101 remaining at 1 hr ranged from 18.6 % (mouse) to 77.8 % (human). Based on the CL_{int} values, the rank order between the species were generally similar for S9 and hepatocytes. For the control articles, imipramine showed metabolic half-lives of 110 minutes (human), 36.5 minutes (dog), 54.1 minutes (rat) and 52.9 minutes (mouse), while the half-lives for propranolol were 289, 52.5, 56.8 and 204 minutes, respectively. The human and mouse values were extrapolated beyond 180 minutes, the longest time point tested in the *in vitro* assay. A summary of the metabolite stability result is presented in the sponsor's table below.

Hepatocyte Metabolite Stability Results Summary

Compound ID	Species	T1/2 (min)	% Remaining at 60 min	CL _{int} (ml/min/kg)
ALXN1101 (cPMP)	Human	161	77.8	23.2
ALXN1101 (cPMP)	Beagle Dog	124	75.3	37.8
ALXN1101 (cPMP)	S-D Rat	59.7	49.3	141
ALXN1101 (cPMP)	CD-1 Mouse	20.4	18.6	801
Imipramine	Human	110	65.2	34.0
Imipramine	Beagle Dog	36.5	32.4	128
Imipramine	S-D Rat	54.1	44.7	156
Imipramine	CD-1 Mouse	52.9	45.2	309
Propranolol	Human	289	107.9	13.0
Propranolol	Beagle Dog	52.5	54.1	89.1
Propranolol	S-D Rat	56.8	75.8	148
Propranolol	CD-1 Mouse	204	103.5	80.3

Metabolite Identification

Based on the metabolite stability data from the S9 fractions and hepatocyte samples of mouse, rat, dog and human, 5 metabolites of ALXN1101 were observed following the LC/MS scans. Three of the metabolites M1 (1.8 %), M2 (2.2 %) and M3 (0.5 %) were phase 1 oxidative metabolites, and the other 2 metabolites M4 (3.9 %, glucosylation) and M5 (7.7 %, glucuronidation and sulfonation) were phase 2 metabolites. All the metabolites were observed in all species tested, and only the M3 phase 1 metabolite could not be quantified in any of the species tested. A summary of the data and structures of the identified metabolites are presented below from the sponsor's submission.

Metabolites of ALXN1101 from S9 and Hepatocyte Assay Samples

ID	Biotransformation	Mass Shift	Expected m/z	Q1 / Q3	R.T. (min)	% Area
	Parent	0	363.9	363.9/166.0	0.9*	NA
M1	Oxidation	+16	379.9	379.9/166.0	0.9*	1.8
M2	Oxidation	+16	379.9	379.9/89.9	1.0*	2.2
M3	Oxidation + OH + H	+34	397.7	397.7/166.0	6.7	0.5
M4	Glucosylation	+162	525.9	525.9/166.0	2.7	3.9
M5	Glucuronidation + Sulfonation	+256	619.9	619.9/443.9	5.8	7.7

LC/MS Chromatographic Data of ALXN1101 Metabolites

Analyte	MRM	Liver S9	Hepatocytes
Parent	363.9/166.0	Detected	Detected
Dehydration	345.9/166.0	ND	ND
Oxidation-1 (M1)	379.9/166.0	ND	ND
Oxidation-2 (M2)	379.9/98.7	ND	Peak detected but not quantifiable vs. background
Oxidation+OH+H (M3)	397.9/166.0	Peak detected but not quantifiable vs. background	Peak detected but no time-dependence and present at T _{zero}
Glucosylation (M4)	525.9/166.0	Peak detected but no time-dependence and present at T _{zero}	ND
Sulfonation-1	443.9/166.0	ND	ND
Sulfonation-2	443.9/246.0	ND	ND
Glucuronidation	539.9/363.9	ND	ND
Glucuronidation+Sulfonation (M5)	619.9/443.9	Peak detected but levels at T _{zero} higher than at subsequent time points	ND
Compound Z [ND: not detected]	344.0/166.0	ND	ND

Structures of Identified Metabolites of ALXN1101

M1	Oxidation
MRM	379.9/166.0
Structure	

M2	Oxidation
MRM	379.9/98.7
Structure	

M4	Glucosylation
MRM	525.9/166.0
Structure	

M5	Glucuronidation + Sulfonation
MRM	619.9/443.9
Structure	

PK Drug Interaction (CYP450 Enzyme Inhibition, Induction)

ALXN1101 was tested at multiple concentrations (0.00271 to 25 µM) using CYP450 supersomes for inhibition of human CYP450 isozymes (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4). None of the CYP450 isozymes were inhibited at the highest concentration (25 µM) tested; except for CYP2B6 which showed 9.9 % inhibition at 25 µM. Assays of CYP1A2, CYP2C8 and CYP2D6 isozymes demonstrated negative values at the 25 µM concentration test. ALXN1101 was also tested for potential induction of CYP450 enzyme activity in human hepatocytes from one donor. The potential induction of CYP450 isozymes 1A2, 2B6, 2C9 and 3A4 were assessed using phenacetin, bupropion, diclofenac and testosterone as substrates, respectively, with control inducers omeprazole, phenobarbital, and rifampicin, respectively. The maximal fold induction (relative to vehicle control) was 1.1 (CYP1A2) at 10 µM ALXN1101, showing that ALXN1101 did not induce any of the CYP450 enzyme activities tested. The positive control articles demonstrated induction ranging from 1.7-fold (CYPs 2B6, 2C9) to 5.3-fold (CYP3A4), thereby validating the assay.

Excretion

No studies to determine ALXN1101 excretion were submitted.

6 General Toxicology

6.1 Single-Dose Toxicity

No single-dose toxicology studies were submitted.

6.2 Repeat-Dose Toxicity

Study title: A 2-Week Intravenous Toxicity Study of ALXN1101 Administered Once Daily in Rats with A 4-Week Recovery Period.

Study no.:	2087-002
Study report location:	Volume 4, Pages 1 to 1036
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 8, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	ALXN1101, lot # 856/cPMP/PD/001, and 91.3 % purity; Vehicle, lot #s 952-4-23, and 952-4-26

Key Study Findings

The administration of ALXN1101 as a daily 2 hour intravenous infusion at 3 and 10 mg/kg/day doses to rats for 2 weeks followed by a 4-week recovery period was well tolerated. Clinical signs of transiently scabbed areas, sparse hair, abrasions, and red

discharge at the catheter exteriorization sites were observed in main study and recovery animals. A dose-responsive increase in the mean absolute and relative adrenal gland weights (to body and brain weights) was observed in female rats dosed at ≥ 1 mg/kg/day when compared to controls. However, these organ weight differences were not statistically significant, had no microscopic correlates, and did not show similar trends in male animals. The NOEL or NOAEL dose for ALXN1101 in rats was 10 mg/kg/day.

Methods

Doses:	0 (vehicle), 3, and 10 mg/kg/day
Frequency of dosing:	Once daily (2-hour continuous) IV infusion for 14 days.
Route of administration:	Intravenous (IV)
Dose volume:	20 ml/kg
Formulation/Vehicle:	(b) (4) mannitol (b) (4) Water for injection. pH adjusted to (b) (4)
Species/Strain:	Rats/Sprague Dawley
Number/Sex/Group:	15/sex/group for vehicle control and 10 mg/kg/day dose group, 10/sex/group for 1 and 3 mg/kg/day dose groups.
Age:	5 to 6 weeks
Weight:	Males: 130 to 210 g, females: 100 to 170 g.
Satellite groups:	5/sex/group from control and 10 mg/kg dose group were designated as 4-week recovery animals. 4/sex/group (control) and 7/sex/group (1, 3, and 10 mg/kg/dose) were designated as Toxicokinetic (TK) animals.
Unique study design:	None
Deviation from study protocol:	A control female animal was dosed via the tail vein due to femoral catheter displacement; Animals had dose accountability outside of ± 10 %; Dosing syringes were maintained at room temperature for shorter duration than protocol required.

Observations and Results

All animals were observed twice daily for morbidity, mortality, injury, and availability of food and water.

Mortality

There was no animal mortality attributed to the test article administration. All main study animals survived to study termination and scheduled necropsies.

Clinical Signs

Detailed clinical examination of each animal was performed during the acclimation period, 7 days before dosing initiation and weekly for each main study animal.

At the end of dosing on day 1, 3 of 8 group 1 (control), 1 of 8 group 2 (1 mg/kg/day), and 3 of 8 group 4 (10 mg/kg/day) animals were considered to have clonic convulsions, with 3 group 1 control males and 1 of the 3 group 4 males noted to be transiently apneic. The clinical signs were not considered adverse because apnea is known to occur in normal rats, and the convulsion episodes are considered to be incidental stereotypy behavior in rats.

Transient clinical observations include, white discoloration and red material around the eyes/nose, swelling of the face/cranial region/exteriorization site, salivation, abrasions, scabbed areas at the incision site, sparse or absent hair at the face/forelimb/shoulder, and red discharge at the catheter exteriorization site. The incidences of these clinical signs were not dose responsive and not suggestive of test article administration. Many of these observations were also not present in recovery animals, with the exception of transiently scabbed areas, sparse hair, abrasions, red discharge at the catheter exteriorization site.

Body Weights

Body weights for all animals were measured and recorded prior to dosing initiation and weekly during the study.

There were no test article-related effects on body weight. Slight increases and/or decreases in body weight were observed in animals from the control and treatment groups, and were considered to be normal variations in the animal species.

Feed Consumption

Food consumption was measured and recorded for each main study animal weekly during the study.

There were no test article-related effects on food consumption.

Ophthalmoscopy

Ophthalmoscopic examinations were conducted on all animals pretest, and prior to the terminal and recovery necropsies.

There were no effects of the test article observed upon ophthalmoscopic examinations. The observed transient findings of hemorrhage-iris, iritis, and corneal opacity during the terminal examination were attributed to expected pathology in rats of the age, sex, and strain being studied.

ECG

ECG recordings were not conducted.

Hematology

Blood samples (3 to 4 ml) were collected from the animals at the end of infusion on days 1 and 14 for the evaluation of hematology parameters.

There were no test article-related hematological effects noted in treated animals. At study termination, all animal groups including controls had moderate to marked elevations in neutrophils (relative to expected ranges) that were considered related to catheter and/or infusion procedures. These changes had resolved by the end of the recovery period. Statistically significant differences observed in coagulation times between treated animals when compared to control animals were not considered meaningful because they were small in magnitude and not dose-responsive.

Summary of Hematology Values in Male Rats

Endpoint	Study Interval	0 mg/kg/day Mean ± SD	1 mg/kg/day Mean ± SD	3 mg/kg/day Mean ± SD	10 mg/kg/day Mean ± SD
Platelets 10 ³ /μL	Terminal	953.8 ± 363.82	1207.0 ± 320.48	1216.0 ± 255.58	*1328.9 ± 185.98
Neutrophils 10 ³ /μL	Terminal	6.630 ± 4.2014	4.270 ± 0.9713	*3.686 ± 1.0626	5.261 ± 2.3072
Other Cells 10 ³ /μL	Terminal	0.311 ± 0.2013	0.199 ± 0.1212	0.182 ± 0.0985	0.379 ± 0.2049
Platelets 10 ³ /μL	Recovery	1182.4 ± 125.55	-----	-----	1034.2 ± 116.35
Neutrophils 10 ³ /μL	Recovery	1.924 ± 1.0259	-----	-----	1.944 ± 0.6343
Other Cells 10 ³ /μL	Recovery	0.218 ± 0.0327	-----	-----	#0.294 ± 0.0288

* Significantly different from control; (p < 0.05)

Significantly different from control; (p < 0.01)

----- No recovery animals from dose group

Summary of Hematology Values in Female Rats

Endpoint	Study Interval	0 mg/kg/day Mean ± SD	1 mg/kg/day Mean ± SD	3 mg/kg/day Mean ± SD	10 mg/kg/day Mean ± SD
Neutrophils 10 ³ /μL	Terminal	2.037 ± 0.7845	3.873 ± 2.1168	4.295 ± 3.0352	3.153 ± 2.1021
MCH (picogram)	Terminal	19.22 ± 0.520	18.90 ± 0.579	*18.57 ± 0.653	19.05 ± 0.510
Absolute reticulocytes 10 ³ /μL	Terminal	213.58 ± 37.404	198.69 ± 57.069	212.59 ± 77.002	210.54 ± 48.990
Neutrophils 10 ³ /μL	Recovery	1.358 ± 0.4064	-----	-----	1.332 ± 0.3339
MCH (picogram)	Recovery	19.48 ± 0.277	-----	-----	19.64 ± 0.817
Absolute reticulocytes 10 ³ /μL	Recovery	144.78 ± 12.841	-----	-----	*187.12 ± 36.384

* Significantly different from control; (p < 0.05)

----- No recovery animals from dose group

Clinical Chemistry

Blood samples were collected from main study animals at the end of infusion on days 1 and 14 for the evaluation of clinical chemistry parameters.

There were no test article-related effects on clinical chemistry parameters in the treated animals. All mean and individual values measured were considered within an acceptable range.

Urinalysis

There were no definitive test article-related alterations observed in urinalysis parameters studied in animals at any dose level. There was a tendency for increased urine occult blood in individual animals dosed at ≥ 3 mg/kg/day relative to control animals, but since the findings were not generally accompanied by an increase in erythrocytes, the likelihood of false positives makes these findings of uncertain relevance to the test article. There were occasional differences observed in urine volume and specific gravity that were not considered adverse because of their sporadic incidence. A summary of the findings on urinalysis are presented in the Sponsor's tables below.

Summary of Urinalysis Values in Male Rats

Endpoint	Study Interval	0 mg/kg/day			1 mg/kg/day			3 mg/kg/day			10 mg/kg/day		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Volume mL	Terminal	14.40	5.873	10	11.85	6.298	10	9.50	2.953	10	10.55	3.730	10
Specific Gravity	Terminal	1.0307	0.01954	10	1.0276	0.01231	9	1.0358	0.00835	10	1.0357	0.01347	10
pH	Terminal	7.65	0.474	10	7.45	0.497	10	7.45	0.369	10	7.30	0.537	10

Summary of Urinalysis Values in Male Rats

Endpoint	Study Interval	0 mg/kg/day			10 mg/kg/day		
		Mean	SD	N	Mean	SD	N
Volume mL	Recovery	9.20	6.676	5	6.30	1.987	5
Specific Gravity	Recovery	1.0490	0.01304	5	1.0556	0.01240	5
pH	Recovery	7.00	0.354	5	7.00	0.000	5

Summary of Urinalysis Values in Female Rats

Endpoint	Study Interval	0 mg/kg/day			1 mg/kg/day			3 mg/kg/day			10 mg/kg/day		
		Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Volume mL	Terminal	6.50	3.464	10	8.30	5.067	10	8.05	4.963	10	12.35	9.411	10
Specific Gravity	Terminal	1.0428	0.01410	9	1.0406	0.01709	10	1.0365	0.01249	10	1.0397	0.02984	10
pH	Terminal	7.39	0.651	9	7.45	0.550	10	7.35	0.412	10	7.35	0.626	10

Summary of Urinalysis Values in Female Rats

Endpoint	Study Interval	0 mg/kg/day			10 mg/kg/day		
		Mean	SD	N	Mean	SD	N
Volume mL	Recovery	5.90	5.561	5	3.40	2.535	5
Specific Gravity	Recovery	1.0550	0.02736	4	1.0642	0.01545	5
pH	Recovery	6.90	0.418	5	6.90	0.418	5

Gross Pathology

After 2 weeks of dosing, the main study animals were sacrificed and necropsied. Recovery animals were also sacrificed and necropsied at the end of the 4 weeks recovery period.

There were no apparent test article-related macroscopic findings in male or female animals at the terminal or recovery necropsies.

Macroscopic observations such as swelling/thickening (correlated microscopically with subacute/chronic inflammation, edema, and/or abscess formation), or abrasions/scabs seen at the infusion site were considered directly or indirectly associated with catheterization or infusion procedure. Other observations such as, a subcutaneous black nodule within the inguinal area (correlated microscopically with a hematoma), tan focus/foci and tan discoloration in the kidneys (correlated microscopically with pyelonephritis), tan discoloration of the prostate or enlargement of the salivary gland (correlated microscopically with acute inflammation), and enlargement of the spleen (correlated microscopically with increased extramedullary hematopoiesis) or enlargement of the iliac and renal lymph nodes were not considered adverse findings due to the lack of a dose response.

Organ Weights

Organ weights of main study and recovery animals were recorded after necropsy.

The following organs or tissues were weighed and preserved:

Organs or Tissues Weighed, Preserved, and Microscopically Examined

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination		
			1, 4	2, 3	Rec ^a
Adrenal glands	X	X	X		
Aorta		X	X		
Bone with bone marrow, femur		X	X		
Bone with bone marrow, sternum		X	X		
Bone marrow smear ^b		X			
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	X		
Epididymides (males)		X	X		
Esophagus		X	X		
Eyes (with optic nerve)		X	X		
GALT ^c		X	X		
Heart	X	X	X		
Infusion site(s) ^d		X	X		
Joint, tibiofemoral		X	X		
Kidneys	X	X	X		
Lacrimal glands, exorbital		X	X		
Large intestine, cecum		X	X		
Large intestine, colon		X	X		
Large intestine, rectum		X	X		
Larynx		X	X		
Liver	X	X	X		
Lung with bronchi	X	X	X		
Lymph node, mandibular		X	X		
Lymph node, mesenteric		X	X		

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination		
			1, 4	2, 3	Rec ^a
Vagina (females)		X	X		
Gross lesions		X	X	X	X
Tissue masses with regional lymph node ^b		X	X		

Organs or Tissues Weighed, Preserved, and Microscopically Examined

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination		
			1, 4	2, 3	Rec ^a
Mammary gland (process females only)		X	X		
Nerve, sciatic		X	X		
Ovaries with oviducts (females)	X	X	X		
Pancreas		X	X		
Pituitary gland	X	X	X		
Prostate gland (males)	X	X	X		
Salivary gland, mandibular ^e	X	X	X		
Salivary gland, parotid		X	X		
Salivary gland, sublingual		X	X		
Seminal vesicles (males)	X	X	X		
Skeletal muscle, biceps femoris		X	X		
Skin (thigh)		X	X		
Small intestine, duodenum		X	X		
Small intestine, ileum		X	X		
Small intestine, jejunum		X	X		
Spinal cord, cervical		X	X		
Spinal cord, lumbar		X	X		
Spinal cord, thoracic		X	X		
Spleen	X	X	X		
Stomach, glandular		X	X		
Stomach, nonglandular		X	X		
Target Organs ^f		X	X	X	X
Testes (males)	X	X	X		
Thymus	X	X	X		
Thyroid gland (with parathyroid) ^g	X	X	X		
Tongue		X	X		
Trachea		X	X		
Ureters		X	X		
Urinary bladder		X	X		
Uterus with cervix (females)	X	X	X		

There were no apparent test article-related organ weight changes in male or female animals at the terminal or recovery necropsies.

After necropsy in main study animals, the mean absolute and relative adrenal gland weights (to body and brain weights) in females at ≥ 1 mg/kg/day were dose-dependently greater than that of control female animals. However, these weight differences were not statistically significant, and did not have any microscopic correlates

Histopathology

Animal tissues for microscopic examination were fixed in hematoxylin and eosin-stains and paraffin sectioned for observations.

Adequate Battery

Yes

Peer Review

Yes

Histological Findings

There were no apparent test article-related microscopic findings in male or female animals at the terminal or recovery necropsies.

Histopathological findings at the infusion sites were typical to the effects commonly seen with catheterization procedure, were not dose responsive and therefore not considered to be test article-related.

Multiple histopathology findings were considered secondary to catheterization and/or the infusion procedure, including inflammation, hemorrhage, necrosis in multiple tissues; bone marrow hypercellularity; extramedullary hematopoiesis in the liver and spleen; Kupffer cell hypertrophy and liver focal necrosis; vegetative and valvular endocarditis in the heart; pyelonephritis in the kidneys; perivascular eosinophilic infiltration in the lungs; epidermal hyperplasia in the skin; cavitation, edema, fibrosis, and hematoma formation in the subcutis were not considered to be test article related. A summary of the microscopic findings are presented in the tables below.

Summary of Microscopic Observations in Male Rats

Tissue Observation: Skin, Subcutis	Severity	0 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
No. of animals examined		3	1	1	4
Cavitation		2	0	0	0
	- Minimal	1	0	0	0
	- Mild	1	0	0	0
Edema	Severe	0	0	0	0
Hematoma	Mild	0	1	0	0
Hemorrhage	Minimal	2	0	0	1
Inflammation, subacute, chronic		3	0	1	4
	- Moderate	1	0	1	4
	- Severe	2	0	0	0

Summary of Microscopic Observations in Female Rats

Tissue Observation: Skin, Subcutis	Severity	0 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
No. of animals examined		0	2	3	4
Cavitation		0	0	2	2
	- Mild	0	0	2	1
	-Moderate	0	0	0	1
Fibrosis	Mild	0	0	0	1
Hemorrhage	Minimal	0	0	1	2
Inflammation, subacute,chronic		0	2	3	4
	- Minimal	0	0	0	1
	- Mild	0	0	1	0
	-Moderate	0	2	2	3

In conclusion, the administration of ALXN1101 as daily 2 hour intravenous infusion doses of 1, 3, and 10 mg/kg/day to rats for 2 weeks did not produce any test article-related effects on clinical signs, body weight, food consumption, ophthalmoscopic examination, hematology, clinical chemistry, urinalysis parameters, organ weights, macroscopic or microscopic observations. The NOEL dose was 10 mg/kg/day.

Toxicokinetics

Blood samples for toxicokinetics (TK) analysis were collected from all animals on dosing days 1 and 14. Three rats/sex/group (0, control) and 6 rats/sex/group (1, 3, and 10 mg/kg designated as TK animals were sampled predose, and either at 15 min, 1 and 4 hours, or 5 and 50 min, and 2 and 8 hours following ALXN1101 infusion.

No quantifiable concentrations of ALXN1101 were found in any control rats at 5 min after the end of infusion, or treated rats at predose on day 1 or day 14. The mean plasma ALXN1101 concentrations were similar for day 1 and day 14 at each dose level assessed.

In general, exposure to ALXN1101 increased in a dose proportional manner. In the 1, 3, and 10 mg/kg dose groups, all but two rats had quantifiable concentrations of ALXN1101 in collected samples at the end of the IV infusion.

The C_{max} , T_{max} , $AUC_{(0-3)}$, and AUC_{last} all increased with increasing dose. While the exposure to ALXN1101 in male rats increased in a dose proportional manner, the exposure in female rats appeared to increase in a greater than dose proportional manner at the 10 mg/kg dose. The T_{max} in male and female animals on day 1 and day 14 was steady at 2.08 hr.

Male to female ratios showed no consistent differences in exposure between males and females, and C_{max} ratios ranged from 0.606 to 1.42 while $AUC_{(0-3)}$ ratios ranged from 0.635 to 1.31.

Daily dosing of ALXN1101 did not result in accumulation of ALXN1101 from day 1 to day 14. The mean accumulation ratios for C_{max} and $AUC_{(0-3)}$ ranged from 0.949 to 1.12.

A summary of the toxicokinetic data is shown in the sponsor's tables below.

Table 7-7. ALXN1101 Toxicokinetic Parameters by Sex

Sex	Dose (mg/kg)	Day	Actual Dose	C_{max} (ng/mL)	C_{max} SE (ng/mL)	C_{max} /D ^a	T_{max} (hr)	AUClast (hr*ng/mL)	AUClast SE (hr*ng/mL)	AUClast /D ^b	AUC(0-3) (hr*ng/mL)	AUC(0-3) /D ^b	Half-life (hr)
Female	1	1	0.701	232	28.9	331	2.08	352	36.1	502	352	502	0.668
Female	1	14	0.736	240	10.7	326	2.08	359	14.8	488	359	488	0.492
Female	3	1	2.34	563	87.5	240	2.08	819	119	350	819	350	0.389 ^c
Female	3	14	2.31	766	59.0	332	2.08	1100	82.7	478	1100	478	0.313
Female	10	1	8.12	3110	112	383	2.08	4940	180	609	4580	564	0.540
Female	10	14	8.27	3010	122	364	2.08	4920	217	595	4490	543	0.632
Male	1	1	0.701	208	11.4	297	2.08	283	14.3	404	NC ^d	NC ^d	NC ^d
Male	1	14	0.736	226	9.29	307	2.08	298	11.9	405	NC ^d	NC ^d	NC ^d
Male	3	1	2.34	532	276	227	2.08	789	353	337	789	337	0.405 ^e
Male	3	14	2.31	465	233	201	2.08	702	300	304	702	304	0.379 ^e
Male	10	1	8.12	2320	528	285	2.08	4080	633	502	3630	447	0.700
Male	10	14	8.27	2300	273	278	2.08	3300	364	399	3300	399	0.368

a. Units for C_{max}/D : ((ng/mL)/(mg/kg))

b. Units for all AUC/D: (hr*ng/mL)/(mg/kg)

c. Note that terminal half-life was estimated over a sampling time less than 2x the half-life.

d. NC = Not Calculable for AUC(0-3) and AUC(0-3)/D due to all samples from male animals at 3 hours being BQL.

e. NC = Not Calculable for half-life due to insufficient quantifiable samples in terminal phase.

Table 7-8. ALXN1101 Toxicokinetic Parameters by Sex, Excluding 2 Rats

Sex	Dose (mg/kg)	Day	Actual Dose	C_{max} (ng/mL)	C_{max} SE (ng/mL)	C_{max} /D ^a	T_{max} (hr)	AUClast (hr*ng/mL)	AUClast SE (hr*ng/mL)	AUClast /D ^b	AUC(0-3) (hr*ng/mL)	AUC(0-3) /D ^b	Half-life (hr)
Female	1	1	0.701	232	28.9	331	2.08	352	36.1	502	352	502	0.668
Female	1	14	0.736	240	10.7	326	2.08	359	14.8	488	359	488	0.492
Female	3	1	2.34	563	87.5	240	2.08	859	114	367	859	367	0.416 ^c
Female	3	14	2.31	766	59.0	332	2.08	1100	82.7	478	1100	478	0.313
Female	10	1	8.12	3110	112	383	2.08	4940	180	609	4580	564	0.540
Female	10	14	8.27	3010	122	364	2.08	4920	217	595	4490	543	0.632
Male	1	1	0.701	208	11.4	297	2.08	283	14.3	404	NC ^d	NC ^d	NC ^d
Male	1	14	0.736	226	9.29	307	2.08	298	11.9	405	NC ^d	NC ^d	NC ^d
Male	3	1	2.34	799	125	341	2.08	1130	166	482	1130	482	0.379 ^e
Male	3	14	2.31	697	33.0	302	2.08	1000	48.7	433	1000	433	0.357
Male	10	1	8.12	2320	528	285	2.08	4080	633	502	3630	447	0.700
Male	10	14	8.27	2300	273	278	2.08	3300	364	399	3300	399	0.368

a. Units for C_{max}/D : ((ng/mL)/(mg/kg))

b. Units for all AUC/D: (hr*ng/mL)/(mg/kg)

c. Note that terminal half-life was estimated over a sampling time less than 2x the half-life.

d. NC = Not Calculable for AUC(0-3) and AUC(0-3)/D due to all samples from male animals at 3 hours being BQL.

e. NC = Not Calculable for half-life due to insufficient quantifiable samples in terminal phase.

Table 7-5. ALXN1101 Toxicokinetic Parameters (All Males and Females Combined)

Day	Dose (mg/kg)	Actual Dose	C _{max} (ng/mL)	C _{max} SE (ng/mL)	C _{max} /D ^a	T _{max} (hr)	AUC _{last} (hr*ng/mL)	SE AUC _{last} (hr*ng/mL)	AUC _{last} /D ^b	AUC(0-3) (hr*ng/mL)	AUC(0-3) /D ^b	Half-life (hr)
1	1	0.701	220	14.9	314	2.08	339	18.7	483	339	483	0.630
1	3	2.34	548	130	234	2.08	804	167	344	804	344	0.395 ^c
1	10	8.12	2720	300	334	2.08	4500	369	555	4100	505	0.603
14	1	0.736	233	7.07	317	2.08	349	10.1	475	349	475	0.503
14	3	2.31	616	127	266	2.08	903	163	391	903	391	0.340 ^c
14	10	8.27	2660	209	321	2.08	4270	311	516	3890	471	0.706

D = Dose; SE = Standard Error

a. Units for C_{max}/D: ((ng/mL)/(mg/kg))

b. Units for all AUC/D: (hr*ng/mL)/(mg/kg)

c. Note that terminal half-life was estimated over a sampling time less than 2x the half-life.

Table 7-6. ALXN1101 Toxicokinetic Parameters (Males and Females Combined, Excluding 2 Rats)

Day	Dose (mg/kg)	Actual Dose	C _{max} (ng/mL)	C _{max} SE (ng/mL)	C _{max} /D ^a	T _{max} (hr)	AUC _{last} (hr*ng/mL)	SE AUC _{last} (hr*ng/mL)	AUC _{last} /D ^b	AUC(0-3) (hr*ng/mL)	AUC(0-3) /D ^b	Half-life (hr)
1	1	0.701	220	14.9	314	2.08	339	18.7	483	339	483	0.630
1	3	2.34	657	84.8	281	2.08	965	106	412	965	412	0.395 ^c
1	10	8.12	2720	300	334	2.08	4500	369	555	4100	505	0.603
14	1	0.736	233	7.07	317	2.08	349	10.1	475	349	475	0.503
14	3	2.31	739	38.0	320	2.08	1060	52.1	459	1060	459	0.332 ^c
14	10	8.27	2660	209	321	2.08	4270	311	516	3890	471	0.706

D = Dose; SE = Standard Error

a. Units for C_{max}/D: ((ng/mL)/(mg/kg))

b. Units for all AUC/D: (hr*ng/mL)/(mg/kg)

c. Note that terminal half-life was estimated over a sampling time less than 2x the half-life.

Table 7-13. Accumulation Ratios by Dose (Males and Females Combined)

Dose (mg/kg)	C _{max} , Day 14 (ng/mL)	C _{max} , Day 1 (ng/mL)	C _{max} Accumulation Ratio	AUC(0-3), Day 14 (hr*ng/mL)	AUC(0-3), Day 1 (hr*ng/mL)	AUC(0-3) Accumulation Ratio
1	233	220	1.06	349	339	1.03
3	616	548	1.12	903	804	1.12
10	2660	2720	0.978	3890	4100	0.949

Table 7-14. Accumulation Ratios by Dose (Males and Females Combined, Excluding 2 Rats)

Dose (mg/kg)	C _{max} , Day 14 (ng/mL)	C _{max} , Day 1 (ng/mL)	C _{max} Accumulation Ratio	AUC(0-3), Day 14 (hr*ng/mL)	AUC(0-3), Day 1 (hr*ng/mL)	AUC(0-3) Accumulation Ratio
1	233	220	1.06	349	339	1.03
3	739	657	1.12	1060	965	1.10
10	2660	2720	0.978	3890	4100	0.949

Table 7-15. Accumulation Ratios by Dose and Sex

Sex	Dose (mg/kg)	C _{max} , Day 14 (ng/mL)	C _{max} , Day 1 (ng/mL)	C _{max} Accumulation Ratio	AUC(0-3), Day 14 (hr*ng/mL)	AUC(0-3), Day 1 (hr*ng/mL)	AUC(0-3) Accumulation Ratio
Female	1	240	232	1.03	359	352	1.02
	3	766	563	1.36	1100	819	1.35
	10	3010	3110	0.968	4490	4580	0.982
Male	1	226	208	1.09	NC ^a	NC ^a	NC ^a
	3	465	532	0.873	762	789	0.869
	10	2300	2320	0.991	3300	3630	0.909

a. NC: Not calculable for AUC(0-3) due to all samples from male animals at 3 hours being BQL.

Table 7-16. Accumulation Ratios by Dose and Sex, Excluding 2 Rats

Sex	Dose (mg/kg)	C _{max} , Day 14 (ng/mL)	C _{max} , Day 1 (ng/mL)	C _{max} Accumulation Ratio	AUC(0-3), Day 14 (hr*ng/mL)	AUC(0-3), Day 1 (hr*ng/mL)	AUC(0-3) Accumulation Ratio
Female	1	240	232	1.03	359	352	1.02
	3	766	563	1.36	1100	859	1.28
	10	3010	3110	0.968	4490	4580	0.982
Male	1	226	208	1.06	NC ^a	NC ^a	NC ^a
	3	697	799	0.873	1000	1130	0.887
	10	2300	2320	0.991	3300	3630	0.909

a. NC: Not calculable for AUC(0-3) due to all samples from male animals at 3 hours being BQL.

Dosing Solution Analysis

The test article concentrations were determined for all dose levels for each preparation. The average recovery values determined from the dosing formulations ranged from 80.6 % to 90.6 %. Summary of the dosing solution analysis is shown in the sponsor's table below.

Concentration					
Dose Level (mg/kg)	Nominal Concentration (mg/mL)	Nominal Concentration Adjusted for Purity (mg/mL)	Range of Average Calculated Concentration ^a (mg/mL)	Range of Average %Recovery ^{a, b}	Range of %Relative Standard Deviation ^a
0	0.00	0.00	none detected	NA	NA
1	0.05	0.0457	0.0368-0.0380	80.6-83.2	2.2-7.0
3	0.15	0.137	0.116-0.117	84.4-85.6	0.3-2.6
10	0.5	0.457	0.400-0.414	87.6-90.6	0.7-1.8

^aResults are the range of values determined during Days 1 and 14
^bAverage %recovery was calculated from the nominal concentration after adjustment for purity.
 NA – Not Applicable

Study title: A 2-Week Intravenous Toxicity Study of ALXN1101 Administered Once Daily in Beagle Dogs with A 4-Week Recovery Period

Study no.: 2087-003
 Study report location: Volume 6, Pages 1 to 805
 Conducting laboratory and location: (b) (4)
 Date of study initiation: July 19, 2012
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: ALXN1101, lot # 856/cPMP/PD/001, 91.3 % purity; Vehicle, lot #s 952-4-23, and 952-4-26

Key Study Findings

The administration of ALXN1101 as daily 2 hour IV infusion doses of 0 (control), 3, and 10 mg/kg/day to dogs for 2 weeks followed by a 4-week recovery period was well tolerated. Clinical signs of soft/mucoid feces, vomiting, twitching, aggressive behavior, and vocalization were not dose-responsive. A significantly longer PR interval (+ 12.5 %) was observed on day 14 in the 1 mg/kg/day male group prior to dosing. Discolorations and scabbed areas at the infusion sites were correlated histopathologically to hemorrhage, but were not dose-responsive. Various types of inflammation were seen on the skin overlying the infusion vein across the dose groups. Organ weight changes were observed in the thyroid/parathyroid, pituitary gland, spleen and uterus of animals in the 10 mg/kg/day dose groups at recovery were absent at terminal necropsy. The NOEL dose for ALXN1101 in dogs was 10 mg/kg/day.

Methods

Doses: 0 (vehicle), 3, and 10 mg/kg/day
 Frequency of dosing: Once daily (2-hour continuous) IV infusion for 14 days.
 Route of administration: Intravenous (IV) catheter via the cephalic vein.
 Dose volume: 20 ml/kg
 Formulation/Vehicle: (b) (4)
 Water for injection. pH adjusted to (b) (4)
 Species/Strain: Dog/Beagle
 Number/Sex/Group: 5/sex/group for vehicle control and 10 mg/kg/day dose group, 3/sex/group for 1 and 3 mg/kg/day dose groups
 Age: 5 to 6 months
 Weight: Males: 5.5 to 12 kg; females: 5 to 10 kg
 Satellite groups: 2/sex/group from the control and 10 mg/kg/dose groups were designated as 4-week recovery animals. Main study and recovery animals were used for toxicokinetic (TK) analysis.
 Unique study design: No
 Deviation from study protocol: 2 female animals were not within the 5 to 10 kg specified body weight range; Several animals were older than the 6 months age specified; TK blood samples were collected 24 to 49 seconds late for two animals; TK blood sample was not collected on day 1 in a male control animal due to aggressive behavior; Three animals were dosed for longer than 2 hrs; TK samples were collected outside of nominal proposed time in 5 animals. The deviations did not affect the study outcome.

Observations and Results

All animals were observed twice daily for morbidity, mortality, injury, and availability of food and water.

Mortality

There was no animal mortality attributed to the test article administration. All main study animals survived to study termination and scheduled necropsies.

Clinical Signs

Detailed clinical examination of each animal was performed during the acclimation period, 7 days before dosing initiation and weekly for each main study animal.

There were no clinical signs that could be related to administration of ALXN1101.

Transient and sporadic observations in dogs included, soft/mucoid feces, vomitus, twitching, head movements (from side to side), increased activity, vocalization, aggressive behavior, salivation, and thin body condition. The observed clinical changes were considered to be incidental because they did not show a dose-response relationship and were not suggestive of a relationship to test article administration. There were also transient observations of lacerations (face, ear), scabbed area (ear), abrasions (tail), red discharge (hind foot, ear, and mouth), swelling (forefoot), and impaired limb function (forelimb). These observations are consistent with minor injuries common in canines housed in steel cages, or also as a result of study procedures such as physical limb restraint, and percutaneous peripheral catheter placement/removal in the IV infusion process.

Body Weights

Body weights of all animals were measured and recorded prior to randomization for dosing, and weekly during the dosing period.

There were no test article-related effects on body weight. The slight changes observed in body weight in control and treated animals were considered to be within normal variations for the species.

The mean body weights of the male dogs were 8.34 (0, mg/kg, control), 8.65 (1 mg/kg), 9.0 (3 mg/kg), and 8.64 kg (10 mg/kg), respectively at the start of the study, and were 8.53 (0 mg/kg), 8.33 (1 mg/kg), 9.10 (3 mg/kg) and 8.48 kg (10 mg/kg) respectively, for the different dose groups at the end of the 2 week dosing period. The mean body weights of the female dogs were 5.82 (0 mg/kg), 6.08 (1 mg/kg), 5.84 (3 mg/kg), and 5.89 kg (10 mg/kg) respectively at the start of the study, and were 5.90 (0 mg/kg), 6.15 (1 mg/kg), 5.68 (3 mg/kg), and 5.87 kg (10 mg/kg) respectively, for the different dose groups at the end of the 2 week dosing period.

Feed Consumption

Food consumption in animals was measured and recorded daily during the study.

There were no test article-related effects on food consumption in study animals.

Ophthalmoscopy

Ophthalmoscopic examinations were conducted on all animals pretest, and prior to terminal and recovery necropsy.

There were no test article related effects observed on the ophthalmoscopic examinations.

ECG

Electrocardiographic (ECG) recordings were performed on all animals pretest, predose, and 1 to 2 hours postdose on treatment days 1 and 14, and prior to the recovery necropsy.

There was no effect of ALXN1101 on qualitative or quantitative ECG parameters. A significantly longer PR interval (+ 12.5 %) was observed in the 1 mg/kg/day male animals at the day 14 predose interval when compared to male control animals. Because the changes were observed only in low dose male animals prior to dosing, the observation was not considered test article-related.

Hematology

Blood samples for hematology were collected from all animals via the jugular vein predose, and at 5, 15, and 30 minutes, and 1, 2, 4, and 8 hours after the end of infusion on days 1 and 14.

There were no effects of ALXN1101 on hematology parameters measured in male or female animals at the day 14 or recovery intervals.

Clinical Chemistry

Blood samples for clinical chemistry were collected from the study animals pretest, prior to the day 14 terminal necropsy, and prior to the recovery necropsy.

There were no test article-related effects on the clinical chemistry parameters in male and female animals at day 14 or recovery intervals.

Urinalysis

Urine samples were collected from study animals for at least 16 hours pretest, prior to the day 14 terminal necropsy, and prior to the recovery necropsy.

There were no test article-related effects observed in the urinalysis parameters in main study or recovery animals. There were occasional sporadic differences in urine volume and specific gravity between control and treated animals, but were not considered adverse due to the variability of the results.

There were also occasional minor variations observed in urinary physical (appearance), biochemical (protein, ketones), and microscopic (erythrocytes, crystals) components between treatment groups, but were considered to be within acceptable biologic variability range.

Gross Pathology

After 2 weeks of dosing, the main study animals were sacrificed and necropsied. Recovery animals were also sacrificed and necropsied at the end of the 4 weeks recovery period.

There were no test article-related macroscopic findings.

Red discoloration at the infusion site observed in most study animals was not dose-responsive. The finding correlated microscopically to hemorrhage and was considered to occur as a result of the dosing procedure and not as a result of the test article.

Organ Weights

Organ weights of main study and recovery animals were recorded after necropsy.

The following organs or tissues were weighed and preserved:

Organs or Tissues Weighed, Preserved, and Microscopically Examined

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination	
			Terminal	Recovery
Adrenal glands	X	X	X	
Aorta		X	X	
Bone with bone marrow, femur		X	X	
Bone with bone marrow, rib		X	X	
Bone with bone marrow, sternum		X	X	
Bone marrow smear ^a		X		
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	X	
Epididymides (males)		X	X	
Esophagus		X	X	
Eyes (with optic nerve)		X	X	
Gallbladder		X	X	
GALT ^b		X	X	
Heart	X	X	X	
Infusion site(s) ^c		X	X	
Joint, tibiofemoral		X	X	

^a Latendresse JR, Warbritton AR, Jonassen H, Creasy DM. Fixation of testes and eyes using a modified Davidson's fluid: comparison with Bouin's fluid and conventional Davidson's fluid. Toxicol Pathol. 2002 Jul-Aug;30(4):524-33.

Organs or Tissues Weighed, Preserved, and Microscopically Examined

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination	
			Terminal	Recovery
Kidneys	X	X	X	
Large intestine, cecum		X	X	
Large intestine, colon		X	X	
Large intestine, rectum		X	X	
Larynx		X	X	
Liver	X	X	X	
Lung with bronchi	X	X	X	
Lymph node, mandibular		X	X	
Lymph node, mesenteric		X	X	
Mammary gland (females)		X	X	
Nerve, sciatic (peripheral nerve)		X	X	
Ovaries (females)	X	X	X	
Oviducts (females)		X	X	
Pancreas		X	X	
Parathyroid glands ^c	X	X	X	
Pituitary gland	X	X	X	
Prostate gland (males)	X	X	X	
Salivary gland, mandibular ^d	X	X	X	
Salivary gland, parotid		X	X	
Salivary gland, sublingual		X	X	
Skeletal muscle, biceps femoris		X	X	
Skin (thigh)		X	X	
Small intestine, duodenum		X	X	
Small intestine, ileum		X	X	
Small intestine, jejunum		X	X	
Spinal cord, cervical		X	X	
Spinal cord, lumbar		X	X	
Spinal cord, thoracic		X	X	
Spleen	X	X	X	
Stomach, cardia		X	X	

Organs or Tissues Weighed, Preserved, and Microscopically Examined

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination	
			Terminal	Recovery
Stomach, fundus		X	X	
Stomach, pylorus		X	X	
Target Organs ^e		X		X
Testes (males)	X	X	X	
Thymus	X	X	X	
Thyroid gland	X	X	X	
Tongue		X	X	
Trachea		X	X	
Ureters		X	X	
Urinary bladder		X	X	
Uterus with cervix (females)	X	X	X	
Vagina (females)		X	X	
Gross lesions		X	X	X
Tissue masses with regional lymph node ^f		X	X	

^aBone marrow smears will be prepared only for animals necropsied at scheduled intervals. Evaluation will be performed at the discretion of the Study Director and/or Sponsor (additional cost).

^bGut-Associated Lymphoid Tissue

^cParathyroid glands will be collected and weighed with the thyroid gland.

^dOnly the right mandibular salivary gland will be weighed.

^ePotential additional target organs (not included on this list, except for bone marrow) and potential target organ gross lesions will be designated by the Study Director, Pathologist and/or Sponsor based on experimental findings (additional cost).

^fA regional lymph node drains the region where a tissue mass is located. A regional lymph node may not always be grossly identified when a mass is present.

^gThe infusion site(s) will be collected to include vessel approximately 1 cm distal and proximal to the catheter tip. The location of the catheter tip will be marked on the unfixed specimen. After fixation the specimen will be trimmed and processed to slide to provide transverse histologic sections representing the catheter tip and surrounding tissues both proximal and distal to the catheter tip. Relative to the catheter tip, "proximal" shall be defined as closer to the heart and "distal" shall be defined as further from the heart. Surrounding tissues will be identified as vessel, vessel stroma, and potential subcutaneous tissue; skin shall be included only if it aids the trimming

There were no test article-related organ weight changes.

The following organ weights (absolute and relative to body and brain) were increased at terminal necropsy, when compared to controls: prostate in male dogs dosed at 10 mg/kg/day, thymus in males at 3 and 10 mg/kg/day, pituitary gland weights in female dogs dosed at 3 mg/kg/day, and mandibular salivary gland in females dosed at 1 mg/kg/day. Since these changes were not dose-responsive and have no microscopic correlates, they were considered normal biologic variations.

A summary of the organ weights are provided in the table below. There were no statistical analyses performed.

Summary of Prostate Gland Weight Values in Male Dogs

Endpoint	Study Interval	0 mg/kg/day Mean $\pm$ SD	1 mg/kg/day Mean $\pm$ SD	3 mg/kg/day Mean $\pm$ SD	10 mg/kg/day Mean $\pm$ SD
Body weight (Kg)	Terminal	8.12 $\pm$ 0.88	8.18 $\pm$ 0.74	9.07 $\pm$ 0.94	8.10 $\pm$ 0.38
Prostate gland weight (g)	Terminal	0.675 $\pm$ 0.122	0.695 $\pm$ 0.090	0.625 $\pm$ 0.175	0.605 $\pm$ 0.118
Prostate gland /Bwt %	Terminal	0.2576 $\pm$ 0.0590	0.2454 $\pm$ 0.0428	0.2085 $\pm$ 0.0560	0.2346 $\pm$ 0.0492
Prostate gland /BrWt ratio	Terminal	0.3538 $\pm$ 0.0737	0.3601 $\pm$ 0.0526	0.3125 $\pm$ 0.0856	0.3178 $\pm$ 0.0761
Body weight (Kg)	Recovery	9.23 $\pm$ 0.32	-----	-----	8.95 $\pm$ 0.99
Prostate gland weight (g)	Recovery	1.290 $\pm$ 0.283	-----	-----	1.225 $\pm$ 0.175
Prostate gland /Bwt %	Recovery	0.2983 $\pm$ 0.0722	-----	-----	0.2926 $\pm$ 0.0459
Prostate gland /BrWt ratio	Recovery	0.6129 $\pm$ 0.1276	-----	-----	0.6086 $\pm$ 0.0947

Summary of Thymus Weight Values in Male Dogs

Endpoint	Study Interval	0 mg/kg/day Mean $\pm$ SD	1 mg/kg/day Mean $\pm$ SD	3 mg/kg/day Mean $\pm$ SD	10 mg/kg/day Mean $\pm$ SD
Body weight (Kg)	Terminal	8.12 $\pm$ 0.88	8.18 $\pm$ 0.74	9.07 $\pm$ 0.94	8.10 $\pm$ 0.38
Thymus weight (g)	Terminal	0.442 $\pm$ 0.159	0.453 $\pm$ 0.113	0.470 $\pm$ 0.065	0.449 $\pm$ 0.175
Thymus/BWt %	Terminal	0.1635 $\pm$ 0.0508	0.1568 $\pm$ 0.0299	0.1568 $\pm$ 0.0215	0.1687 $\pm$ 0.0564
Thymus/BrWt ratio	Terminal	0.2283 $\pm$ 0.0760	0.2337 $\pm$ 0.0573	0.2363 $\pm$ 0.0399	0.2350 $\pm$ 0.0952
Body weight (Kg)	Recovery	9.23 $\pm$ 0.32	-----	-----	8.95 $\pm$ 0.99
Thymus weight (g)	Recovery	0.539 $\pm$ 0.131	-----	-----	0.546 $\pm$ 0.115
Thymus/BWt %	Recovery	0.1236 $\pm$ 0.0263	-----	-----	0.1306 $\pm$ 0.0298
Thymus/BrWt ratio	Recovery	0.2578 $\pm$ 0.0663	-----	-----	0.2715 $\pm$ 0.0619

Summary of Mandibular Salivary Gland Values in Female Dogs

Endpoint	Study Interval	0 mg/kg/day Mean $\pm$ SD	1 mg/kg/day Mean $\pm$ SD	3 mg/kg/day Mean $\pm$ SD	10 mg/kg/day Mean $\pm$ SD
Body weight (Kg)	Terminal	5.47 $\pm$ 0.55	6.03 $\pm$ 1.07	5.63 $\pm$ 0.37	5.70 $\pm$ 1.00
Sal.gl,mand./sub.,rt (g)	Terminal	0.202 $\pm$ 0.028	0.230 $\pm$ 0.021	0.223 $\pm$ 0.022	0.249 $\pm$ 0.089
Sal.gl,mand./sub.,rt /BWt %	Terminal	0.1052 $\pm$ 0.0171	0.1120 $\pm$ 0.0106	0.1090 $\pm$ 0.0103	0.1258 $\pm$ 0.0442
Sal.gl,mand./sub.,rt /BrWt ratio	Terminal	0.1195 $\pm$ 0.0159	0.1290 $\pm$ 0.0135	0.1260 $\pm$ 0.0119	0.1368 $\pm$ 0.0349
Body weight (Kg)	Recovery	7.15 $\pm$ 0.07	-----	-----	6.40 $\pm$ 0.71
Sal.gl,mand./sub.,rt (g)	Recovery	0.227 $\pm$ 0.031	-----	-----	0.249 $\pm$ 0.037
Sal.gl,mand./sub.,rt /BWt %	Recovery	0.0840 $\pm$ 0.0102	-----	-----	0.0974 $\pm$ 0.0137
Sal.gl,mand./sub.,rt /BrWt ratio	Recovery	0.1225 $\pm$ 0.0213	-----	-----	0.1389 $\pm$ 0.0223

Summary of Pituitary Gland Weight Values in Female Dogs

Endpoint	Study Interval	0 mg/kg/day Mean $\pm$ SD	1 mg/kg/day Mean $\pm$ SD	3 mg/kg/day Mean $\pm$ SD	10 mg/kg/day Mean $\pm$ SD
Body weight (Kg)	Terminal	5.47 $\pm$ 0.55	6.03 $\pm$ 1.07	5.63 $\pm$ 0.37	5.70 $\pm$ 1.00
Pituitary Gland/ (g)	Terminal	0.044 $\pm$ 0.017	0.050 $\pm$ 0.007	0.070 $\pm$ 0.025	0.050 $\pm$ 0.001
Pituitary Gland/Bwt %	Terminal	0.0008 $\pm$ 0.0002	0.0008 $\pm$ 0.0000	0.01013 $\pm$ 0.0005	0.0009 $\pm$ 0.0001
Pituitary Gland/Brwt/ratio	Terminal	0.0007 $\pm$ 0.0002	0.0007 $\pm$ 0.0001	0.0010 $\pm$ 0.0004	0.0007 $\pm$ 0.0000
Body weight (Kg)	Recovery	9.23 $\pm$ 0.32	-----	-----	8.95 $\pm$ 0.99
Pituitary Gland/ (g)	Recovery	0.054 $\pm$ 0.021	-----	-----	0.057 $\pm$ 0.003
Pituitary Gland/Bwt %	Recovery	0.0006 $\pm$ 0.0002	-----	-----	0.0006 $\pm$ 0.0000
Pituitary Gland/Brwt/ratio	Recovery	0.0007 $\pm$ 0.0002	-----	-----	0.0008 $\pm$ 0.0000

At terminal necropsy, the uterus weights (absolute and relative to body and brain) were lower in female dogs dosed at 1 mg/kg/day when compared to controls, but the change was considered to be related to variation in sexual maturity and not test article-related. At the recovery necropsy, decreases in the following organ weights (absolute and relative to body and brain) were observed in animals dosed at 10 mg/kg/day in comparison to control animals: thyroid/parathyroid gland weights in males, pituitary gland weights in females, spleen and uterus weights in females. The observed changes were considered to be due to normal biologic variations because similar findings were not observed in the animals at terminal necropsy. A summary of the organ weights are provided in the table below. There were no statistical analysis performed and only the control and high dose data for the recovery animals were presented.

Summary of Thyroid/Parathyroid Weight Values in Male Dogs

Endpoint	Study Interval	0 mg/kg/day Mean $\pm$ SD	1 mg/kg/day Mean $\pm$ SD	3 mg/kg/day Mean $\pm$ SD	10 mg/kg/day Mean $\pm$ SD
Body weight (Kg)	Terminal	8.12 $\pm$ 0.88	8.18 $\pm$ 0.74	9.07 $\pm$ 0.94	8.10 $\pm$ 0.38
Thyroid/Para. Gland (g)	Terminal	0.680 $\pm$ 0.100	0.616 $\pm$ 0.089	0.538 $\pm$ 0.101	0.499 $\pm$ 0.051
Thyroid/Para. Gl/Bwt %	Terminal	0.0095 $\pm$ 0.0019	0.0130 $\pm$ 0.0040	0.0129 $\pm$ 0.0046	0.0099 $\pm$ 0.0014
Thyroid/Para. Gl/Brwt ratio	Terminal	0.0099 $\pm$ 0.0010	0.0143 $\pm$ 0.0056	0.0152 $\pm$ 0.0072	0.0107 $\pm$ 0.0017
Body weight (Kg)	Recovery	9.23 $\pm$ 0.32	-----	-----	8.95 $\pm$ 0.99
Thyroid/Para. Gland (g)	Recovery	1.069 $\pm$ 0.089	-----	-----	0.720 $\pm$ 0.087
Thyroid/Para. Gl/Bwt %	Recovery	0.0116 $\pm$ 0.0014	-----	-----	0.0081 $\pm$ 0.0019
Thyroid/Para. Gl/Brwt ratio	Recovery	0.0140 $\pm$ 0.0022	-----	-----	0.0098 $\pm$ 0.0017

Summary of Pituitary Gland Weight Values in Female Dogs

Endpoint	Study Interval	0 mg/kg/day Mean $\pm$ SD	1 mg/kg/day Mean $\pm$ SD	3 mg/kg/day Mean $\pm$ SD	10 mg/kg/day Mean $\pm$ SD
Body weight (Kg)	Terminal	5.47 $\pm$ 0.55	6.03 $\pm$ 1.07	5.63 $\pm$ 0.37	5.70 $\pm$ 1.00
Pituitary Gland/ (g)	Terminal	0.044 $\pm$ 0.017	0.050 $\pm$ 0.007	0.070 $\pm$ 0.025	0.050 $\pm$ 0.001
Pituitary Gland/Bwt %	Terminal	0.0008 $\pm$ 0.0002	0.0008 $\pm$ 0.0000	0.01013 $\pm$ 0.0005	0.0009 $\pm$ 0.0001
Pituitary Gland/Brwt/ratio	Terminal	0.0007 $\pm$ 0.0002	0.0007 $\pm$ 0.0001	0.0010 $\pm$ 0.0004	0.0007 $\pm$ 0.0000
Body weight (Kg)	Recovery	9.23 $\pm$ 0.32	-----	-----	8.95 $\pm$ 0.99
Pituitary Gland/ (g)	Recovery	0.054 $\pm$ 0.021	-----	-----	0.057 $\pm$ 0.003
Pituitary Gland/Bwt %	Recovery	0.0006 $\pm$ 0.0002	-----	-----	0.0006 $\pm$ 0.0000
Pituitary Gland/Brwt/ratio	Recovery	0.0007 $\pm$ 0.0002	-----	-----	0.0008 $\pm$ 0.0000

Summary of Spleen Weight Values in Female Dogs

Endpoint	Study Interval	0 mg/kg/day Mean $\pm$ SD	1 mg/kg/day Mean $\pm$ SD	3 mg/kg/day Mean $\pm$ SD	10 mg/kg/day Mean $\pm$ SD
Body weight (Kg)	Terminal	5.47 $\pm$ 0.55	6.03 $\pm$ 1.07	5.63 $\pm$ 0.37	5.70 $\pm$ 1.00
Spleen (g)	Terminal	40.842 $\pm$ 17.241	43.591 $\pm$ 12.914	47.621 $\pm$ 11.782	33.330 $\pm$ 13.846
Spleen /Bwt %	Terminal	0.7318 $\pm$ 0.2288	0.7134 $\pm$ 0.0823	0.8442 $\pm$ 0.2026	0.5939 $\pm$ 0.2807
Spleen/Brwt/ratio	Terminal	0.6059 $\pm$ 0.2119	0.6307 $\pm$ 0.1845	0.6774 $\pm$ 0.1572	0.4762 $\pm$ 0.1871
Body weight (Kg)	Recovery	7.15 $\pm$ 0.07	-----	-----	6.40 $\pm$ 0.71
Spleen (g)	Recovery	64.415 $\pm$ 8.071	-----	-----	30.951 $\pm$ 24.018
Spleen /Bwt %	Recovery	0.9015 $\pm$ 0.1218	-----	-----	0.4657 $\pm$ 0.3238
Spleen/Brwt/ratio	Recovery	0.8032 $\pm$ 0.0177	-----	-----	0.4263 $\pm$ 0.3222

Summary of Uterus/Cervix Weight Values in Female Dogs

Endpoint	Study Interval	0 mg/kg/day Mean $\pm$ SD	1 mg/kg/day Mean $\pm$ SD	3 mg/kg/day Mean $\pm$ SD	10 mg/kg/day Mean $\pm$ SD
Body weight (Kg)	Terminal	5.47 $\pm$ 0.55	6.03 $\pm$ 1.07	5.63 $\pm$ 0.37	5.70 $\pm$ 1.00
Uterus w/cervix (g)	Terminal	1.552 $\pm$ 0.756	0.624 $\pm$ 0.224	1.916 $\pm$ 2.026	1.509 $\pm$ 0.685
Uterus w/cervix/ Bwt %	Terminal	0.0286 $\pm$ 0.0150	0.0110 $\pm$ 0.0053	0.0344 $\pm$ 0.0371	0.0282 $\pm$ 0.0153
Uterus w/cervix/ Brwt ratio	Terminal	0.0234 $\pm$ 0.0117	0.0090 $\pm$ 0.0032	0.0274 $\pm$ 0.0292	0.0219 $\pm$ 0.0104
Body weight (Kg)	Recovery	7.15 $\pm$ 0.07	-----	-----	6.40 $\pm$ 0.71
Uterus w/cervix (g)	Recovery	5.988 $\pm$ 5.183	-----	-----	2.300 $\pm$ 0.457
Uterus w/cervix/ Bwt %	Recovery	0.0834 $\pm$ 0.0717	-----	-----	0.0366 $\pm$ 0.0112
Uterus w/cervix/ Brwt ratio	Recovery	0.0785 $\pm$ 0.0728	-----	-----	0.0321 $\pm$ 0.0073

Histopathology**Adequate Battery**

Yes

Peer Review

Yes

Histological Findings

There were no test article-related microscopic findings.

The following, non dose-responsive, histopathological changes were observed at the infusion sites across the dose groups: hemorrhage, subacute inflammation (with perivascular distribution), intimal proliferation (with increased number of endothelial cells without vascular wall alteration), and vascular degeneration/regeneration (proliferation or remodeling of the vascular wall with endothelial cell proliferation), and thrombus. The changes are commonly encountered in infusion studies and were considered related to the dosing procedures and not to the test article.

At the site of the infusion vein, various types of inflammation as well as erosion/ulcer and epidermal exudate were also observed in the overlying skin. These changes were observed across dose groups with no dose-response effects and were therefore not considered to be test article related.

In conclusion, the administration of ALXN1101 as a daily 2 hour intravenous infusion at dose levels of 1, 3, and 10 mg/kg/day to dogs for 2 weeks did not produce any test article-related effects on clinical signs, body weight, food consumption, ophthalmoscopic and electrocardiographic evaluations, hematology, clinical chemistry, urinalysis, organ weights, macroscopic or histopathologic observations. ALXN1101 was well tolerated in dogs with a NOEL of 10 mg/kg/day.

Toxicokinetics

Plasma samples were collected on day 1 and day 14 prior to dosing, and at 5, 15, and 30 minutes, and 1, 2, 4, and 8 hours after the end of ALXN1101 infusion from 3 dogs/sex/group.

No quantifiable concentrations of ALXN1101 were found prior to dosing in samples from the study animals or in postdose samples from the control animals. However, the first samples collected on day 1 and day 14 in ALXN1101 treated animals was quantifiable after (2.08 hr) dosing.

Plasma concentrations of ALXN1101 increased as the dose increased from 1 to 10 mg/kg. The variability of plasma concentration between the animal dose groups (CV %) ranged from 6.5 % to 15 % at the 1 mg/kg dose group, from 8.7 % to 23.8 % in the 3 mg/kg dose group, and from 15.5 % to 74.8 % in the 10 mg/kg dose groups on days 1 and 14.

The C_{max} , t_{max} , $AUC_{(0-3)}$, and AUC_{last} were relatively dose proportional and all increased with increasing dose from 1 to 10 mg/kg/day on day 1 and day 14. On sampling days 1 and 14, there were no apparent differences in exposure between male and female dogs in all dose groups. The mean male to female exposure ratios for C_{max} , $AUC_{(0-3)}$, and AUC_{last} ranged from 0.86 to 1.23 on day 1 and from 0.98 to 1.09 on day 14.

The median t_{max} value on day 1 and day 14 after the start of infusion for all the dose groups was 2.08 hours, with the exception of one animal in the 10 mg/kg/day dose group on day 1 which had a t_{max} value of 2.25 hours postdose.

Were there any TK data other than accumulation ratios??

No accumulation in exposure was observed at the dose levels studied, and the observed mean accumulation ratios for C_{max} , $AUC_{(0-3)}$, and AUC_{last} ranged from 1.01 to 1.08.

Summaries of the Toxicokinetic parameters are presented in the sponsor's tables below.

Toxicokinetic Parameters on Day 1 for ALXN1101 in male and Female Dogs Combined

Day	Dose Group	Actual Dose (mg/kg)	Statistics	C_{max} (ng/mL)	T_{max} (h)	$t\frac{1}{2}$ (h)	AUC_{0-3} (h*ng/mL)	AUC_{last} (h*ng/mL)	$C_{max}/Dose^a$	$AUC_{0-3}/Dose^a$	$AUC_{last}/Dose^a$
1	1 mg/kg	0.792	N	6	6	2	6	6	6	6	6
			Mean	613	2.08	0.726	1023	1151	773	1291	1453
			SD	49.9	0	NC	96.9	195	63.0	122	246
			CV%	8.2	0	NC	9.5	16.9	8.2	9.5	16.9
			Min	532	2.08	0.721	874	671	874	1103	1103
			Median	633	2.08	0.726	1058	1246	798	1335	1572
			Max	656	2.08	0.731	1111	1312	828	1402	1656
			Geo Mean	611	2.08	0.726	1019	1136	771	1286	1434
			CV% Geo Mean	8.4	0	NC	9.8	18.2	8.4	9.8	18.2
1	3 mg/kg	2.55	N	6	6	3	6	6	6	6	6
			Mean	2078	2.08	0.683	3421	3965	816	1343	1557
			SD	181	0	NC	347	442	71.2	136	173
			CV%	8.7	0	NC	10.1	11.1	8.7	10.1	11.1
			Min	1880	2.08	0.621	3032	3458	738	1190	1357
			Median	2045	2.08	0.711	3332	3911	803	1308	1535
			Max	2370	2.08	0.718	4011	4712	930	1575	1850
			Geo Mean	2072	2.08	0.682	3407	3946	813	1338	1549
			CV% Geo Mean	8.6	0	NC	9.9	10.9	8.6	9.9	10.9
1	10 mg/kg	8.94	N	10	10	10	10	10	10	10	10
			Mean	5898	2.1	0.82	9794	12478	660	1096	1396
			SD	1040	0.0527	0.0829	1630	2150	116	183	241
			CV%	17.6	2.5	10.1	16.7	17.2	17.6	16.7	17.2
			Min	3790	2.08	0.737	6672	8979	424	746	1005
			Median	5820	2.08	0.8	9790	12657	651	1095	1416
			Max	7380	2.25	0.982	12186	15635	826	1363	1749
			Geo Mean	5809	2.1	0.816	9564	12305	650	1081	1377
			CV% Geo Mean	19.1	2.4	9.8	17.7	18.0	19.1	17.7	18.0

NC: Not Calculable

a. $C_{max}/Dose$ units: (ng/mL)/(mg/kg)

b. $AUCs/Dose$ units: (h*ng/mL)/(mg/kg)

Toxicokinetic Parameters on Day 14 for ALXN1101 in male and Female Dogs Combined

Day	Dose Group	Actual Dose (mg/kg)	Gender	Cmax (ng/mL)	Tmax (h)	t½ (h)	AUC0-3 (h*ng/mL)	AUClast (h*ng/mL)	Cmax/Dose ^a	AUC0-3/Dose ^a	AUClast/Dose ^a
14	1 mg/kg	0.666	N	6	6	0	6	6	6	6	6
			Mean	660	2.08	NA	1066	1160	990	1599	1740
			SD	43.0	0	NA	79.9	176	64.5	120	264
			CV%	6.5	0	NA	7.5	15.2	6.5	7.5	15.2
			Min	602	2.08	NA	946	946	903	1419	1419
			Median	658	2.08	NA	1070	1154	987	1605	1732
			Max	727	2.08	NA	1183	1383	1091	1775	2075
			Geo Mean	659	2.08	NA	1063	1148	989	1595	1723
			CV% Geo Mean	6.5	0	NA	7.6	15.4	6.5	7.6	15.4
14	3 mg/kg	2.31	N	6	6	6	6	6	6	6	6
			Mean	2172	2.08	0.723	3487	4065	939	1509	1758
			SD	223	0	0.081	395	658	96.5	171	284
			CV%	10.3	0	11.2	11.3	16.2	10.3	11.3	16.2
			Min	1950	2.08	0.659	3138	3601	844	1358	1558
			Median	2080	2.08	0.701	3305	3767	900	1429	1629
			Max	2500	2.08	0.883	4090	5261	1081	1769	2276
			Geo Mean	2162	2.08	0.72	3470	4024	935	1501	1741
			CV% Geo Mean	10.1	0	10.5	11.0	15.2	10.1	11.0	15.2
14	10 mg/kg	7.31	N	10	10	10	10	10	10	10	10
			Mean	6352	2.08	0.756	10402	12926	869	1422	1767
			SD	1260	4.68E-16	0.105	2160	3270	172	296	447
			CV%	19.8	0	13.9	20.8	25.3	19.8	20.8	25.3
			Min	3660	2.08	0.579	6119	6994	500	837	956
			Median	6295	2.08	0.74	10065	12406	861	1376	1696
			Max	8030	2.08	0.916	13767	18583	1098	1882	2541
			Geo Mean	6223	2.08	0.749	10180	12527	851	1392	1713
			CV% Geo Mean	22.5	0	14.3	22.9	27.7	22.5	22.9	27.7

NC: Not Calculable

a. Cmax/Dose Units: (ng/mL)/(mg/kg)

b. AUCs/Dose Units: (h*ng/mL)/(mg/kg)

Accumulation Ratios by Dose in Male and Female Dogs Combined

ALXN1101 Dose ^a	PK Parameter	Day 14 Mean	Day 1 Mean	Accumulation Ratio ^b
1 mg/kg	AUC0-3	1023	1066	1.04
	AUClast	1151	1160	1.01
	Cmax	613	660	1.08
3 mg/kg	AUC0-3	3421	3487	1.02
	AUClast	3965	4065	1.02
	Cmax	2078	2172	1.04
10 mg/kg	AUC0-3	9794	10402	1.06
	AUClast	12478	12926	1.04
	Cmax	5898	6352	1.08

a: Actual doses on Day 1: 0.792 mg/kg (1 mg/kg), 2.55 mg/kg (3 mg/kg), 8.94 mg/kg (10 mg/kg) Actual doses on Day 14: 0.666 (1 mg/kg), 2.31 (3 mg/kg), 7.31 mg/kg (10 mg/kg)

b: Accumulation Ratio = (Day 14 Mean Value)/(Day 1 Mean Value)

Dosing Solution Analysis

The test article concentrations were determined for all dose levels for each preparation. The average recovery values determined from the dosing formulations ranged from 73 % to 98.7 %. A summary of the dosing solution analysis is presented in the sponsor's table below.

Dosing Solution Analysis Summary

Concentration					
Dose Level (mg/kg)	Nominal Concentration (mg/mL)	Nominal Concentration Adjusted for Purity (mg/mL)	Range of Average Calculated Concentration ^a (mg/mL)	Range of Average %Recovery ^{a, b}	Range of %Relative Standard Deviation ^a
0	0.00	0.00	none detected	NA	NA
1	0.05	0.0457	0.0334-0.0402	73.0-88.0	2.1-3.1
3	0.15	0.137	0.116-0.127	84.4-93.0	0.4-1.7
10	0.5	0.457	0.366-0.451	80.1-98.7	1.9-5.8

^aResults are the range of values determined during Days 1 and 14
^bAverage %recovery was calculated from the nominal concentration after adjustment for purity.
NA – Not Applicable

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Bacterial Reverse Mutation Test in *Salmonella typhimurium* and *Eschericia coli*

Study no.: 964425
Study report location: Volume 8, pages 1 to 75
Conducting laboratory and location: (b) (4)
Date of study initiation: October 26, 2012
GLP compliance: The OECD principle of GLP compliance was followed.
QA statement: Yes
Drug, lot #, and % purity: ALXN1101, lot # 856/cPMP/PD/002 and 93.1 % purity.

Key Study Findings

ALXN1101 did not show any evidence of genotoxicity in the *Salmonella typhimurium* and *Eschericia coli* in the *in vitro* mutagenicity assay.

Methods

Strains: TA 1535, TA 1537, TA 98, TA 100, WP2
uvrA

Concentrations in definitive study: 0, 40 and 500 µg/ml

Basis of concentration selection: The maximum solubility of the test article in the vehicle and the maximum amount of vehicle that can be added to the test system or the five highest levels below the toxic concentration.

Negative control: Dimethyl sulfoxide (DMSO)

Positive control:  (b) (4)

Formulation/Vehicle:  (b) (4) L-
Ascorbic Acid (2 mg/ml); Mannitol
 (b) (4)
mg/ml); Water; pH adjusted to  (b) (4)

Incubation & sampling time: 65 hours and 15 minutes with and without metabolic activation

Study Validity

For study validity, the mean revertant colony counts of the reference item for each strain should be close to or within the current historical control range of the laboratory. All positive reference items (with S9 mix where required) should produce increases in revertant colony numbers to at least twice the concurrent controls.

Results

Following exposure to ALXN1101, no incomplete or absent background lawns of non-revertant bacteria, or substantial reductions in revertant colony counts were obtained, indicating that the test article was non-toxic to the bacteria at the levels (40 – 500 µg/ml) tested. No substantial increases in revertant colony numbers were obtained with any tester strains, following exposure to ALXN1101 at any dose level, in the presence or absence of S9 mix. Summary tables of study design and assay results from the sponsor's submission are presented below.

ALXN1101 – Pre-Incubation Assay Study Design

Dose No.	Formulation Conc. (µg/mL)	Dose Volume (µL/plate)	Final Conc. (µg/plate)	No. of Replicates		No. of Strains
				0S9	+S9	
0/ Vehicle control	-	128	-	3	3	5
0/ Vehicle control	-	256	-	3	3	5
0/ Vehicle control	-	512	-	3	3	5
0/ Vehicle control	-	1000	-	3	3	5
1/ ALXN1101	40	25	1.00	3	3	5
1/ ALXN1101	40	50	2.00	3	3	5
1/ ALXN1101	40	100	4.00	3	3	5
2/ ALXN1101	500	16	8.00	3	3	5
2/ ALXN1101	500	32	16.0	3	3	5
2/ ALXN1101	500	64	32.0	3	3	5
2/ ALXN1101	500	128	64.0	3	3	5
2/ ALXN1101	500	256	128	3	3	5
2/ ALXN1101	500	512	256	3	3	5
2/ ALXN1101	500	1000	500†	3	3	5
Positive controls	‡		‡	3	3	5

† Where the high level was the maximum practical, based on the solubility of the Test Item in the vehicle and the maximum amount of vehicle that could be added to the test system.

‡ Dose depends on the test organism and the positive controls.

ALXN1101 – Pre-Incubation Assay Result in the Absence of S9 Mix

Strain	Conc. (µg/plate)	S9	No. of Revertants					Plate Observations *			Fold Response †
			x ₁	x ₂	x ₃	mean	SD	x ₁	x ₂	x ₃	
TA1535	Vehicle A ^a	0	13	30	12	18	10				1.0
		32	0	16	14	15	1				0.8
		64	0	23	24	21	5				1.1
		128	0	20	15	19	4				1.1
		256	0	16	19	20	5				1.1
		500	0	28	19	24	5				1.3
TA1537	Vehicle A ^a	0	7	18	13	13	6				1.0
		32	0	25	22	21	4				1.7
		64	0	17	12	16	3				1.2
		128	0	16	14	15	1				1.2
		256	0	18	18	20	1				1.5
		500	0	23	18	20	3				1.6
TA98	Vehicle A ^a	0	22	35	27	28	7				1.0
		32	0	28	29	26	4				0.9
		64	0	24	25	26	3				0.9
		128	0	26	27	31	7				1.1
		256	0	24	37	29	7				1.0
		500	0	16	20	18	2				0.6
TA100	Vehicle A ^a	0	108	123	105	112	10				1.0
		32	0	108	117	119	12				1.1
		64	0	131	138	123	8				1.2
		128	0	136	116	119	11				1.1
		256	0	121	129	143	11				1.2
		500	0	129	104	118	13				1.0
WP2 uvrA	Vehicle A ^a	0	48	49	48	48	1				1.0
		32	0	40	48	47	6				1.0
		64	0	52	59	49	5				1.1
		128	0	52	46	47	5				1.0
		256	0	61	45	40	11				1.0
		500	0	45	61	51	8				1.1

* Comments on the plate or background lawn: no comments.

† Fold response in mean revertants compared to concurrent vehicle control.

SD Sample standard deviation.

a Vehicle dose volume of 1000 µL/plate.

ALXN1101 – Pre-Incubation Assay Result in the Presence of S9 Mix

Strain	Conc. (µg/plate)	S9	No. of Revertants					Plate Observations *			Fold Response †
			x_1	x_2	x_3	mean	SD	x_1	x_2	x_3	
TA1535	Vehicle A ^a	+	18	23	23	21	3				1.0
	32	+	17	18	24	20	4				0.9
	64	+	22	24	20	22	2				1.0
	128	+	22	28	25	25	3				1.2
	256	+	16	19	22	19	3				0.9
	500	+	16	11	12	13	3				0.6
TA1537	Vehicle A ^a	+	10	18	21	16	6				1.0
	32	+	19	25	26	23	4				1.4
	64	+	18	31	10	20	11				1.2
	128	+	15	17	19	17	2				1.0
	256	+	12	26	15	18	7				1.1
	500	+	12	18	23	18	6				1.1
TA98	Vehicle A ^a	+	43	37	49	43	6				1.0
	32	+	48	34	28	37	10				0.9
	64	+	41	44	41	42	2				1.0
	128	+	44	42	39	42	3				1.0
	256	+	33	39	43	38	5				0.9
	500	+	39	39	32	37	4				0.9
TA100	Vehicle A ^a	+	121	103	117	114	9				1.0
	32	+	145	147	151	148	3				1.3
	64	+	146	157	170	158	12				1.4
	128	+	123	139	154	139	16				1.2
	256	+	148	135	143	142	7				1.2
	500	+	133	128	125	129	4				1.1
WP2 uvrA	Vehicle A ^a	+	48	47	58	51	6				1.0
	32	+	65	61	53	60	6				1.2
	64	+	59	54	48	54	6				1.1
	128	+	46	45	43	45	2				0.9
	256	+	48	50	50	49	1				1.0
	500	+	61	43	41	48	11				0.9

* Comments on the plate or background lawn: no comments.

† Fold response in mean revertants compared to concurrent vehicle control.

SD Sample standard deviation.

a Vehicle dose volume of 1000 µL/plate.

ALXN1101 – Pre-Incubation Assay Positive Controls

Strain	Treatment	Conc. (µg/plate)	S9	No. of Revertants					SD	Fold Response †
				x_1	x_2	x_3	mean			
TA1535	(b) (4)	0.5	0	318	314	318	317	2		17
TA1537		50	0	477	426	384	429	47		34
TA98		1	0	153	155	171	160	10		5.7
TA100		0.5	0	510	461	446	472	33		4.2
WP2 uvrA		0.5	0	925	972	964	954	25		20
TA1535		5	+	257	249	241	249	8		12
TA1537		5	+	94	84	93	90	6		5.5
TA98		5	+	203	228	233	221	16		5.1
TA100		5	+	719	783	785	762	38		6.7
WP2 uvrA		15	+	115	124	138	126	12		2.5

† Fold response in mean revertants compared to concurrent vehicle control.

SD Sample standard deviation.

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: (b) (4) **Bacterial Reverse Mutation Test in Salmonella Typhimurium and Eschericia Coli**

Study no.: 9600210

Study report location: Volume 8, pages 1 to 49

Conducting laboratory and location:

(b) (4)

Date of study initiation: March 7, 2013

GLP compliance: The OECD principle of GLP compliance was followed.

QA statement: Yes

Drug, lot #, and % purity:

(b) (4)

Key Study Findings

(b) (4) at dose levels tested in the presence or absence of S9 mix did not show any genotoxic activity in the Salmonella typhimurium and Eschericia coli in *in vitro* mutagenicity assay.

Methods

Strains: TA 1535, TA 1537, TA 98, TA 100, WP2
uvrA

Concentrations in definitive study:

(b) (4)

µg/ml

Basis of concentration selection: Standard limit dose maximum

(b) (4)

, and the maximum practical solubility of the test item in the vehicle

(b) (4)

Negative control: Dimethyl sulfoxide (DMSO)

Positive control:

(b) (4)

Vehicle: 0.2 % L-ascorbic acid in water, pH

(b) (4)

Incubation & sampling time: 64 hours and 30 minutes with and without metabolic activation

Study Validity

For study validity, the mean revertant colony counts of the vehicle control for each strain should be close to, or within the current historical control range of the laboratory. All positive controls (with S9 mix where required) should produce increases in revertant colony numbers to at least twice the concurrent vehicle control levels with the appropriate bacterial strain (b) (4) x for TA 100).

Results

Following exposure to (b) (4) (impurities of ALXN1101), the mean revertant colony counts for the vehicle and the test items were within the laboratory historical control range, and the background lawns were normal.

The sterility check plate for the vehicle was contaminated (1 colony), but the sterility check plates for the formulations of the test item were not. The contamination was observed only on a few plates but the formulations were sterile and did not obscure the colony counts, nor affect the outcome of the study.

The appropriate positive controls (with S9 mix where required) induced increases in revertant colony numbers to at least twice the concurrent vehicle levels with the appropriate bacterial strain (b) (4) x for TA 100), confirming the sensitivity of the test system and activity of the S9 mix.

An invalid result was however obtained when WP2 uvrA was treated with (b) (4) in the presence of S9. The assay was still considered valid because the sensitivity of WP2 uvrA was confirmed in the absence of S9 using (b) (4), while the effectiveness of the S9 mix was confirmed by the positive results for the other bacterial strains in the presence of S9.

When exposed to (b) (4), there were no incomplete or absent background lawns of non-revertant bacteria, and there were no substantial reductions in revertant colony counts. This result indicated that the test items were non-toxic to the bacteria at the levels tested. There was also no precipitation observed in the assay.

The exposure to (b) (4) at all dose levels tested in the presence or absence of S9 mix did not result in any increases in revertant colony numbers with any of the tester strains. A summary of the assay results are presented in the sponsor's table below.

(b) (4)

7.2 *In Vitro* Assays in Mammalian Cells

Study title: In Vitro Mammalian Chromosome Aberration Test in Human Peripheral Blood Lymphocytes

Study no.:	964426
Study report location:	Volume 8, pages 1 to 69
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 23, 2012
GLP compliance:	The OECD principle of GLP compliance was followed.
QA statement:	Yes
Drug, lot #, and % purity:	ALXN1101, lot # 856/cPMP/PD/002 and 93.17 % purity.

Key Study Findings

ALXN1101 did not induce chromosome damage in human peripheral blood lymphocytes at the concentrations used in this *in vitro* study.

Methods

Cell line: Human Peripheral Blood Lymphocytes

Concentrations in definitive study: 0.05, 0.10, 0.20, 2.60, 3.90, 4.0, 5.85, 6.0, 8.0, 8.78, 13.2, 19.8, 27.9, 40.8, 58.8, and 83.3 µg/ml

Basis of concentration selection: The maximum solubility of the test article in the vehicle and the maximum amount of vehicle that can be added to the test system, or the highest level below the toxic concentration.

Negative control: Formulation vehicle (A)

Positive control: (b) (4)

Formulation/Vehicle: (b) (4) L-
 Ascorbic Acid (2 mg/ml); Mannitol (b) (4)
 mg/ml); Water; pH adjusted to (b) (4)

Incubation & sampling time: 4 hr (In the absence and presence of S9) and 21 hr (In the absence of S9 mix)

Study Validity

Assay validity is based on the vehicle/negative control findings being within or close to the historical control range, while the positive control reference items should produce a substantial increase in the incidence of aberrant cells (at least twice) compared with the concurrent control with values remaining beyond the 99 % upper limit of the historical negative/vehicle control range. Based on these criteria, the study was valid.

Results

ALXN1101 did not cause any substantial increases in the proportion of aberrant metaphases at any of the experimental points as shown in the summary result table below. The proportion of aberrant metaphases for all the vehicle control and test item groups was within the laboratory negative historical control range as shown in the historical control values figure below.

There were also no substantial increases in the incidence of chromatid or chromosome gaps or polyploidy observed at any experimental point. No precipitation or change in culture medium color was observed.

However, the appearance of a ring-like structure between chromatids was observed during the 21 hour incubation period at the highest dose level (83.3 µg/ml) tested. The ring-like structure was not classified as a chromosomal aberration and the biological significance of the finding is unknown. Summary tables of study design and assay results from the sponsor's submission are presented below.

ALXN1101 Chromosomal Aberration Test Study Design

Dose No.	Material	Formulation Conc. (µg/mL)	Dose Volume (µL)	Final Conc. (µg/mL)	No. of Cultures		
					4 Hours (0S9)	4 Hours (+S9)	21 Hours (0S9)
1	Vehicle control	-	198	-	2	2	2
			296	-	2	2	2
			444	-	2	2	2
			667	-	2	2	2
			1000	-	2	2	2
	ALXN1101	500	26.0	2.60	2	2	2
			39.0	3.90	2	2	2
			58.5	5.85	2	2	2
			87.8	8.78	2	2	2
			132	13.2	2	2	2
			198	19.8	2	2	2
			296	27.9	2	2	2
			444	40.8	2	2	2
			667	58.8	2	2	2
			1000	83.3†	2	2	2
			5	0.05	2	-	2
			10	0.10	2	-	2
			20	0.20	2	-	2
			400	4.0	-	2	-
			600	6.0	-	2	-
			800	8.0	-	2	-

† Where the high level was the maximum practical, based on the solubility of the Test Item in the vehicle and the maximum amount of vehicle that could be added to the test system.

(b) (4)

ALXN1101 Chromosomal Aberration Test Result

Treatment	Conc. (µg/mL)	RMI MI	No. Cells (%) Examined	% Aberrant	No. of Aberrations						Incidental Observations †			
					b	e	B	E	other	(g	G	P	C)	
4-hour treatment in the absence of S9 (0S9)														
Vehicle A	-	8.6	100	200	0.0	0	0	0	0	0	1	0	0	0
ALXN1101	40.8	9.1	106	200	0.5	1	0	0	0	0	3	0	0	0
	58.8	11.4	133	200	0.0	0	0	0	0	0	1	0	0	0
	83.3	9.0	105	200	1.0	2	0	0	0	0	2	1	0	0
(b) (4)	0.10	8.4	98	200	4.0*	6	0	2	0	0	3	0	0	0
4-hour treatment in the presence of S9 (+S9)														
Vehicle A	-	6.8	100	200	1.5	3	0	0	0	0	2	1	1	0
ALXN1101	40.8	5.9	87	200	0.5	1	0	0	0	0	4	0	0	0
	58.8	7.3	106	200	0.0	0	0	0	0	0	1	0	1	1
	83.3	7.3	108	200	1.5	1	1	2	0	0	2	0	1	0
(b) (4)	6.0	6.4	94	200	23.0*	33	9	16	0	0	21	1	0	0
21-hour treatment in the absence of S9 (0S9)														
Vehicle A	-	2.1	100	200	0.5	0	0	0	0	1	3	0	0	1
ALXN1101	40.8	4.0	190	200	2.5	3	0	2	0	0	6	0	0	0
	58.8	4.3	201	200	0.5	1	0	0	0	0	5	0	0	0
	83.3	2.9	137	200	1.0	1	0	1	0	0	1	0	0	44*
(b) (4)	0.05	3.3	153	200	6.0*	7	2	3	0	0	6	0	0	0

MI, RMI Mitotic Index, Relative Mitotic Index (vehicle = 100%).

b, e, g Chromatid break, exchange, gap.

B, E, G Chromosome break, exchange, gap.

other Includes pulverized chromosomes and cells with > 8 aberrations.

P Polyploidy and endoreduplication.

C Centromeric disruption or other abnormal observation.

a Appearance of a ring-like structure between chromatids.

† g, G, P and C are excluded from the calculation of % aberrant cells.

N/A Not assessable (insufficient cells available due to toxicity).

* Substantial increase compared to concurrent vehicle control.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: (b) (4) In Vitro Mammalian Chromosome Aberration Test in Human Peripheral Blood Lymphocytes

Study no.: 9600211
 Study report location: Volume 8, pages 1 to 51
 Conducting laboratory and location: (b) (4)
 Date of study initiation: March 4, 2013
 GLP compliance: The OECD principle of GLP compliance was followed.
 QA statement: Yes
 Drug, lot #, and % purity: (b) (4)

Key Study Findings

(b) (4) did not induce chromosome damage in human peripheral blood lymphocytes at the concentrations used in this study.

Methods

Cell line: Human Peripheral Blood Lymphocytes
 Concentrations in definitive study: (b) (4) µg/ml
 Basis of concentration selection: Standard limit of 1 mM, as recommended by the ICH guidelines.
 Negative control: Formulation vehicle
 Positive control: (b) (4)
 Vehicle: 0.2 % L-Ascorbic Acid, water, pH (b) (4)
 Incubation & sampling time: 4 hr (In the absence and presence of S9) and 21 hr (In the absence of S9 mix)

Study Validity

Assay validity is based on the vehicle/negative control result remaining within or close to the historical control range, while the positive controls should produce a substantial increase in the incidence of aberrant cells (at least twice) compared with the concurrent control with values remaining beyond the (b) (4) % upper limit of the historical negative/vehicle control range.

Results

Neither (b) (4) caused any substantial increases in aberrant metaphases at any experimental point. The proportion of aberrant metaphases for all

the vehicle control and test item groups was within the laboratory negative historical control range.

The positive control reference items caused substantial increases in the proportion of aberrant metaphases in each phase of the study.

There were no substantial increases in the incidence of chromatid or chromosome gaps and no polyploidy was observed at any dose level. A slight increase in chromatid gaps was observed with (b) (4) in the 21-hour incubation time at (b) (4) µg/ml. However, this increase was not consistent within the cultures. Summaries of assay results are presented in the sponsor's table below.

(b) (4)



(b) (4)

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Mammalian Erythrocyte Micronucleus Test in Rat Bone Marrow

Study no:	980110
Study report location:	Volume 8, pages 1 to 105
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 9, 2012
GLP compliance:	The OECD principle of GLP compliance was followed.
QA statement:	Yes
Drug, lot #, and % purity:	ALXN1101, lot # 856/cPMP/PD/002-10 and 93.17 % purity.

Key Study Findings

The intravenous administration of ALXN110 at doses of 2.5, 5, and 10 mg/kg/day for 2 days did not induce immature or mature micronucleated erythrocytes in the Sprague Dawley rat bone marrow cytogenetic study.

Methods

Doses in definitive study: 2.5, 5, and 10 mg/kg/day
 Frequency of dosing: Daily
 Route of administration: Intravenous
 Dose volume: 20 ml/kg
 Formulation/Vehicle: (b) (4) L-Ascorbic Acid (2 mg/ml); Mannitol (b) (4) Water; pH adjusted to (b) (4)
 Species/Strain: Rat/Sprague Dawley
 Number/Sex/Group: 5/sex/group (control, ALXN1101); 3/sex/group (positive control)
 Satellite groups: N/A
 Basis of dose selection: The maximum practical dose based on solubility and stability limitations of the test article.
 Negative control: Formulation vehicle
 Positive control: Cyclophosphamide monohydrate (CP)

Study Validity

The incidence of micronucleated immature erythrocytes for the vehicle control group should be close to or within the laboratory historical vehicle/negative control range. In addition, the positive control group should show clear unequivocal positive responses.

Results

ALXN1101 treated animals did not show any increases in the number of micronucleated immature erythrocytes (MIE). Individual and group mean values for animals treated with the vehicle or the test article are all within the historical range for control animals. However, cyclophosphamide caused clear, unequivocal increases in the frequency of MIE.

ALXN1101 treated animals did not show increases in the incidence of micronucleated mature erythrocytes (MME). The incidence of MME for all groups was uniformly low, confirming the absence of micronucleus-like artifacts.

ALXN1101 treated animals did not show any decreases in the proportion of immature erythrocytes, indicating that the test article did not cause bone marrow toxicity. A summary of the study findings is presented in the sponsor's table below.

ALXN1101 Rat Micronucleus Test Result Summary

Sampling Time - 24 Hours (±30 minutes) After the Final Administration							
Group 1 - Vehicle control ^a				Group 4 - ALXN1101 10 mg/kg/day ^a			
Group 2 - ALXN1101 2.5 mg/kg/day ^a				Group 5 - Cyclophosphamide 20 mg/kg ^b			
Group 3 - ALXN1101 5 mg/kg/day ^a							
Group	% IE/(IE+ME) Males	% IE/(IE+ME) Females	% IE/(IE+ME) Males & Females	Incidence MIE Males	Incidence MIE Females	Incidence MIE Males & Females	Incidence MME Males & Females
1	48.4	52.0	50.2	1.8	1.2	1.5	0.0
2	54.0	50.1	52.1	2.0	1.6	1.8	0.0
3	58.2	50.0	54.1	2.4	1.2	1.8	0.0
4	52.2	51.1	51.6	1.8	1.4	1.6	0.0
5	47.2	40.4	43.8	32.3	28.7	30.5	0.5
%IE/(IE+ME)	Proportion of immature erythrocytes						
MIE	Number of micronucleated cells observed per 2000 immature erythrocytes examined.						
MME	Number of micronucleated mature erythrocytes observed (mean value expressed per 2000 mature erythrocytes examined, see results for individual animals in appendices for actual numbers of cells examined)						
a	Two doses were administered 24 hours apart (±15 minutes)						
b	Positive control (20 mg of the monohydrate per kg) dosed once only, by oral gavage, 24 hours prior to sampling						

In conclusion, ALXN1101 did not show any evidence of genotoxicity in this *in vivo* test for induction of chromosome damage.

10 Special Toxicology Studies

ALXN1101: An *In Vitro* Hemolysis Assay with Human Whole Blood (Study # 2087-004)

Methods: The objective of this study was to evaluate the potential of a range of concentrations of ALXN1101 to hemolyze human red blood cells *in vitro*. Pooled blood was collected from a fasting non-smoker donor with no known medication or recreational drug use, and 32 aliquot vials of 0.5 ml of whole blood samples were labeled as Blood A, B, C, and D, consisting of 4 groups of 8 vials each. The appropriate volume of negative control (0.9 % NaCl), test article (ALXN1101), positive control (sterile water), and vehicle (ALXN1101 IV vehicle) were mixed with Blood A thru D vials as a (v/v) ratio, respectively. The concentrations of the test article added to Blood vials B were 0.25, 0.14, 0.08, and 0.045 mg/ml, at a dilution ratio of 1:1, 1:2.5, 1:5, and 1:10, respectively. From the separated plasma, 0.5 ml each of plasma was also aliquoted into 3 vials labeled Plasma A, B, and C, and the plasma visually inspected for hemolysis. The appropriate volume of the negative control (0.9 % NaCl), test article (0.25 mg/ml ALXN1101), and vehicle was added as a ratio to the plasma (v/v) of 1:1 and was added to the plasma vials A, B, and C, respectively. All the blood and plasma vials were gently mixed, and incubated at 37 °C for 10 min. Following incubation, the plasma samples were visually inspected for turbidity whereas plasma free hemoglobin levels in the blood samples were measured.

Results

The incubation of plasma with saline, vehicle, or formulated ALXN1101 (0.25 mg/ml) at a 1:1 dilution did not produce any turbidity or precipitation.

The incubation of red blood cells with vehicle or formulated ALXN1101 (0.045 to 0.25 mg/ml) did not increase the concentration of free hemoglobin, when compared to the saline (negative controls) and therefore did not produce any appreciable hemolysis of red blood cells. The positive control (sterile water), produced an increase in the concentration of free hemoglobin, hence hemolysis of red blood cells. The findings are summarized in the sponsor's tables below.

ALXN1101: In Vitro Hemolysis Assay with Human Whole Blood.

Hemolytic Potential and Blood Compatibility - Human			
Sample	Endpoint	Visual Evaluation (Plasma)	
	Plasma Free Hemoglobin (Whole Blood) mg/dL	Precipitation	Turbidity
<u>Blood A/Plasma A</u>			
Sample 1: 0.9% NaCl (Negative Control) (Aliquot 1)	10.1	Negative	Negative
Sample 1: 0.9% NaCl (Negative Control) (Aliquot 2)	10.5	NA	NA
Sample 2: 0.9% NaCl (Negative Control) (Aliquot 1)	26.3	NA	NA
Sample 2: 0.9% NaCl (Negative Control) (Aliquot 2)	28.1	NA	NA
Sample 3: 0.9% NaCl (Negative Control) (Aliquot 1)	28.4	NA	NA
Sample 3: 0.9% NaCl (Negative Control) (Aliquot 2)	32.1	NA	NA
Sample 4: 0.9% NaCl (Negative Control) (Aliquot 1)	53.2	NA	NA
Sample 4: 0.9% NaCl (Negative Control) (Aliquot 2)	49.6	NA	NA
<u>Blood B/Plasma B</u>			
Sample 1: 0.25 mg/mL ALXN1101 (Aliquot 1)	0.0*	Negative	Negative
Sample 1: 0.25 mg/mL ALXN1101 (Aliquot 2)	0.1	NA	NA
Sample 2: 0.14 mg/mL ALXN1101 (Aliquot 1)	6.2	NA	NA
Sample 2: 0.14 mg/mL ALXN1101 (Aliquot 2)	9.1	NA	NA
Sample 3: 0.08 mg/mL ALXN1101 (Aliquot 1)	26.0	NA	NA
Sample 3: 0.08 mg/mL ALXN1101 (Aliquot 2)	24.7	NA	NA
Sample 4: 0.045 mg/mL ALXN1101 (Aliquot 1)	48.6	NA	NA
Sample 4: 0.045 mg/mL ALXN1101 (Aliquot 2)	40.4	NA	NA
0.9% NaCl – 0.9% Sodium Chloride for Injection, USP Water – Sterile Water for Injection, USP NA – Not Applicable			
		*Value is below the limit of quantitation	

ALXN1101: In Vitro Hemolysis Assay with Human Whole Blood.

Hemolytic Potential and Blood Compatibility - Human			
Sample	Endpoint	Visual Evaluation (Plasma)	
	Plasma Free Hemoglobin (Whole Blood) mg/dL	Precipitation	Turbidity
Blood C			
Sample 1: Water (Positive Control)	6030.0	NA	NA
Sample 1: Water (Positive Control)	9550.0	NA	NA
Sample 2: Water (Positive Control)	510.0	NA	NA
Sample 2: Water (Positive Control)	95.4	NA	NA
Sample 3: Water (Positive Control)	239.0	NA	NA
Sample 3: Water (Positive Control)	213.0	NA	NA
Sample 4: Water (Positive Control)	319.0	NA	NA
Sample 4: Water (Positive Control)	344.0	NA	NA
Blood D/Plasma C			
Sample 1: ALXN1101 IV Vehicle (Vehicle Control)	2.0	Negative	Negative
Sample 1: ALXN1101 IV Vehicle (Vehicle Control)	0.1	NA	NA
Sample 2: ALXN1101 IV Vehicle (Vehicle Control)	6.3	NA	NA
Sample 2: ALXN1101 IV Vehicle (Vehicle Control)	8.1	NA	NA
Sample 3: ALXN1101 IV Vehicle (Vehicle Control)	12.0	NA	NA
Sample 3: ALXN1101 IV Vehicle (Vehicle Control)	12.4	NA	NA
Sample 4: ALXN1101 IV Vehicle (Vehicle Control)	25.9	NA	NA
Sample 4: ALXN1101 IV Vehicle (Vehicle Control)	40.5	NA	NA

0.9% NaCl – 0.9% Sodium Chloride for Injection, USP

Water – Sterile Water for Injection, USP

NA –Not Applicable

In conclusion, ALXN1101 at concentrations of 0.045 to 0.25 mg/ml did not produce any precipitation, turbidity, or hemolysis when incubated with human blood.

11 Integrated Summary and Safety Evaluation

ALXN1101 is a synthetic pterin derivative known as cPMP, formulated as a powder for reconstitution for IV use in the treatment of molybdenum cofactor deficiency (MoCD). MoCD is a rare, severe, neonatal-onset disorder with autosomal recessive inheritance caused by defects in the biosynthesis of molybdenum cofactor (MoCo). MoCD type A affects two thirds (66 %) of MoCD patients and is caused by a mutation in the MOCS1 gene. The sponsor proposed to use ALXN1101 as a substitute in the MoCo biosynthesis pathway to restore SO level and reduce the levels of sulfite and SSC, thereby alleviating the MoCD disease severity.

The sponsor proposed a randomized, blinded, placebo-controlled, single-dose, sequential-cohort, dose-escalation first-in-human (FIH) study to evaluate the safety, tolerability, and pharmacokinetics (PK) of a single intravenous (IV) dose of ALXN1101 in healthy adult male and female subjects. Thirty two study subjects between the ages of 18 and 60 years and weighing between 55 and 100 kg will be randomized into 4 dose cohorts of 0.10, 0.32, 0.90, and 1.50 mg/kg. Each dose cohort will consist of 8 subject cohorts of six active drug subjects and 2 placebo subjects.

The sponsor submitted preclinical pharmacology, safety pharmacology, pharmacokinetic, genotoxicity and toxicology studies in support of the IND.

The effectiveness of ALXN1101 was demonstrated in an *in vitro* assay in which it caused an increase in molybdopterin (MPT) synthesis and functional restoration of sulfite oxidase (SO) activity.

The pharmacodynamic effect of ALXN1101 was also demonstrated *in vivo* in a mouse model of molybdenum cofactor deficiency (MoCD) where 2 – 4 µg of rcPMP or ALXN1101, administered 3 times/week effectively rescued knockout MOCS1^{-/-} mice pups from premature death.

In safety pharmacology studies, daily intravenous administration of ALXN1101 at dose levels of 1 - 10 mg/kg/day to Sprague Dawley rats for 14 days did not adversely affect neurobehavioral and pulmonary functions. Similarly, administration of ALXN1101 at 1-10 mg/kg/day in Beagle dogs for 7 days did not affect cardiovascular functions.

ALXN1101, 0, 1, 3, or 10 mg/kg, was administered for 14 days to Sprague Dawley rats and Beagle dogs, followed by a 4-week recovery period to study its pharmacokinetics and metabolism of the drug.

There was no accumulation of ALXN1101 at any of the tested doses in either species, and there was no difference in the exposure between males and females at any tested dose in either species. The mean ALXN1101 C_{max} values in male and female rats ranged from 220 ± 14.9 ng/ml to 2720 ± 300 ng/ml on day 1 and from 233 ± 7.07 ng/ml to 2660 ± 209 ng/ml on day 14, and the mean exposure (AUC₀₋₃) level ranged from 339 to 4100 ng•h/ml on day 1 and from 349 to 3890 ng•h/ml on day 14 in male and female rats. The mean ALXN1101 C_{max} values ranged from 613 ± 49.9 ng/ml, to 5898 ± 1040 ng/ml on day 1 and from 660 ± 43 ng/ml, to 6352 ± 1260 ng/ml on day 14 in male and female dogs; and the mean exposure (AUC₀₋₃) level ranged from 1023 ± 96.9 ng•h/ml to 9794 ± 1630 ng•h/ml on day 1 and from 1066 ± 79.9 ng•h/ml to 10,402 ± 2160 ng•h/ml on day 14 in male and female dogs.

In rats, the estimated terminal half-lives could not be accurately estimated in the 1 or 3 mg/kg dose group, but were 0.603 h and 0.706 h in the 10 mg/kg dose group on days 1 and 14, respectively.

In dogs, the estimated terminal half-lives on day 1 were 0.726 h, 0.638 h, and 0.820 h in 1, 3, and 10 mg/kg dose groups, respectively. On day 14, the estimated half-lives were 0.723 h and 0.756 h in the 3 and 10 mg/kg dose groups, respectively, and could not be estimated in the 1 mg/kg dose group.

Five metabolites of ALXN1101 were identified in all the species (mouse, rat, dog and human) studies, without any species-related differences.

ALXN1101 (0.00271 to 25 µM) was not an inhibitor of human CYP450 isozymes or was not an inducer of CYP450 isozymes.

ALXN1101 only modestly binds to rat and human erythrocytes as shown by a blood-to-plasma ratio of 0.791 in rats and 0.695 in humans.

ALXN1101 was studied in repeat-dose (1 to 10 mg/kg/day) toxicology studies in Sprague-Dawley rats and Beagle dogs following repeated dosing for 14 days with a 4-

week recovery period. ALXN1101 did not show any adverse effects in rats and dogs, and the NOAEL dose was determined to be 10 mg/kg/day in both rats and dogs.

No evidence of genotoxicity was observed with ALXN1101 in the *in vitro* Ames test, *in vitro* mammalian chromosomal aberration test, or *in vivo* mammalian erythrocyte micronucleus test. There were also no evidence of genotoxicity with the major impurities of ALXN1101 (b) (4) in the Ames test or the chromosomal aberration test.

In an *in vitro* hemolysis test, ALXN1101 at a concentration range of 0.045 to 0.25 mg/ml did not show any hemolytic activity, precipitation or turbidity following incubation with human blood.

Recommendation:

It is safe to proceed with the proposed clinical dose of ALXN1101 in the study subjects.

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/s/

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